



MMIN
2026

Program Book

Hotel Casa, Amsterdam

September 7-10, 2026

monitoringmolecules.org

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MESSAGE FROM THE CONFERENCE CO-CHAIRS

Welcome to Amsterdam for the **20th International Conference on Monitoring Molecules in Neuroscience (MMiN) 2026**. This year's meeting celebrates more than four decades of scientific history since Professor Charles Marsden and his colleagues established the first "in vivo meeting" in Nottingham, UK, in 1982. What began as a highly specialized methods meeting focused on pioneering in vivo microdialysis and voltammetric techniques has continuously adapted to the frontiers of science across 44 years expanding its scope to embrace technologies such as intracellular neurochemical monitoring, functional imaging, microfluidic sampling tools, electro-enzymatic biosensors, and genetically engineered optical-receptor sensing developments.

We are privileged to anchor our scientific program with an extraordinary lineup of four world-renowned plenary lecturers who represent the absolute vanguard of our field. Over the conference, their presentations will sit alongside a diverse selection of symposia and posters highlighting the latest technological and biological breakthroughs in neurochemistry. We encourage you to actively engage in these sessions, participate in our lively discussions, and take full advantage of the networking opportunities to forge new cross-disciplinary collaborations. Beyond the lecture halls, we hope you find time to explore the historic canals, world-class museums, and unique charm of this beautiful city.

We extend our deepest gratitude to our program committee for their time, expertise, and thoughtful curation in assembling such a diverse scientific lineup. We also want to express our sincere appreciation to our generous sponsors and exhibitors, whose vital support has made this gathering possible. Their collective commitment heavily contributes to advancing our understanding of brain chemistry in health and disease. Finally, we would like to officially recognize and thank Dr. Stephanie Cragg, President of the International Society for Monitoring Molecules in Neuroscience. Her outstanding leadership, guidance, and continuous dedication to our global scientific community have been instrumental in bringing this meeting to fruition.

Thank you for bringing your expertise, history, and passion to Amsterdam. We wish you an inspiring, productive, and memorable conference.

Warmest regards,

Ingo Willuhn and Paul Phillips

Conference Co-Chairs, Monitoring Molecules in Neuroscience 2026

PROGRAM COMMITTEE

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GENERAL INFORMATION

Venue

MMiN 2026 is taking place at **Hotel Casa Amsterdam**. Located in the vibrant east side of Amsterdam, Hotel Casa offers modern amenities, comfortable rooms, and easy access to public transportation.

Conference Hotel: **Hotel Casa Amsterdam**

Meeting Rooms: University of Amsterdam 1,2,3 and 4 (**UvA1-4**) for Plenary and Symposia

Erasmus 1-2 for Poster & Exhibitors. Lunches and breaks are served in the same area.

All rooms are on the Conference Level (1st Floor). See map below.

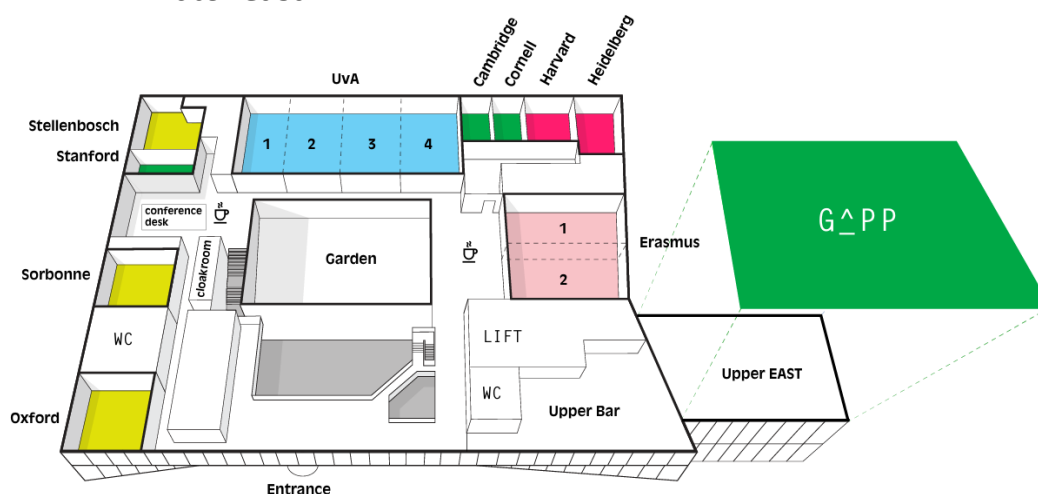
Address: Eerste Ringdijkstraat 4, 1097 BC Amsterdam, Netherlands

Phone: +31 20 665 1171

Website: <https://hotelcasa.nl/>

Check-in/out: Check-in after 3:00 PM, check-out by 11:00 AM

Wi-Fi: Hotel-Casa



Registration

Registration is open daily at the **Conference Desk** on the first floor. Please note that an MMiN Conference badge is required to attend all sessions, so please stop by the registration desk to pick up your badges and banquet tickets.

Date	Hours
Monday, September 7	10:00 AM – 6:00 PM
Tuesday, September 8	8:00 AM – 6:00 PM
Wednesday, September 9	8:00 AM – 6:00 PM
Thursday, September 10	8:00 AM – 3:00 PM

Meals

Welcome reception, dinner on September 9, daily lunch and coffee breaks are included in your conference registration fees.

- September 7, 2026: coffee break, welcome reception (canapes & drinks)
- September 8, 2026: coffee breaks, lunch
- September 9, 2026: coffee breaks, lunch, reception dinner (dinner & drinks)
- September 10, 2026: coffee breaks, lunch

Please note: Food and beverages offered have been selected to accommodate a variety of dietary preferences and needs. Guests with severe allergies are asked to speak with the catering team regarding suitable options, as food is prepared in shared environments where cross-contact with allergens may occur.

Reception Dinner at House of Watt

Join us for dinner at House of Watt – just steps away from the conference venue. You should have indicated your attendance on the registration form or talk to our staff if you interested in attending. Guest passes are available at additional cost.

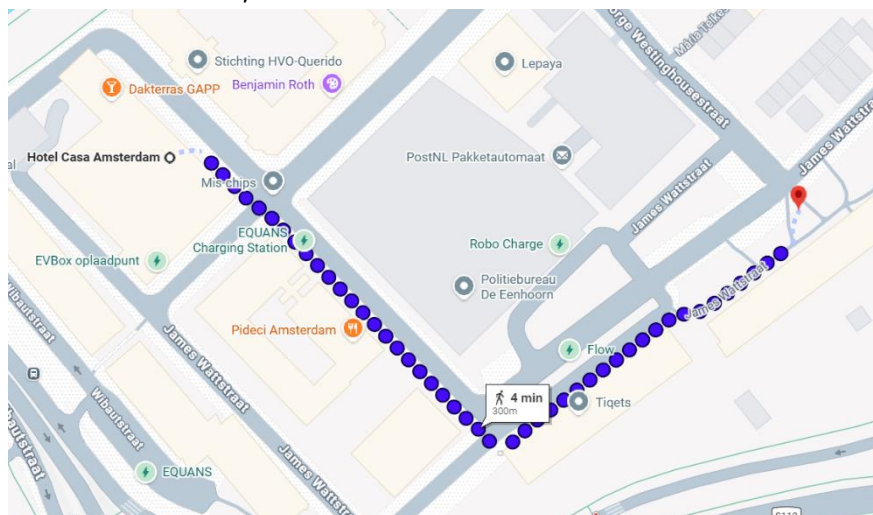
Date: September 9, 2026

Time: 7:00 PM – 10:00 PM

Address: House of Watt, James Wattstraat 73, 1097 DL Amsterdam

Getting there: About 4 minutes' walk from Hotel Casa ([Google map link](#))

1. Head toward Stephensonstraat for 160m
2. Turn left onto James Wattstraat and the venue will be on your right in 140m



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Thank you to our sponsor and exhibitors for their support and participation to make MMiN 2026 possible!

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PROGRAM

Day 1: Monday, September 7, 2026

Time	Program Room	Chairs / Speakers
1:30 PM – 2:00 PM	MMiN 2026 Opening <i>UvA 3-4</i>	Ingo Willuhn , Amsterdam UMC & Netherlands Institute for Neuroscience Paul Phillips , University of Washington Stephanie J. Cragg , University of Oxford
2:00 PM – 3:00 PM	Plenary 1 <i>UvA 3-4</i> <i>Two-Photon Holographic Manipulation of Neuronal Circuits</i>	Valentina Emiliani , Vision Institute, Paris Introduction by: Stephanie J. Cragg , University of Oxford
3:00 PM – 3:30 PM	Break <i>Erasmus 1-2</i>	
3:30 PM – 5:00 PM	Symposium 1 <i>UvA 1-2</i> <i>Bioanalytical Technologies for Multimodal Recording</i>	Chair: Leslie Sombers , University of Florida Speakers: Anders Borgkvist , Karolinska Institute Tracy Cui , University of Pittsburgh Ann-Sofie Cans , Chalmers University of Technology Leslie Sombers , University of Florida
	Symposium 2 <i>UvA 3-4</i> <i>Dopamine Signaling Across Spatiotemporal Domains</i>	Chair: Garret Stuber , University of Washington Speakers: Lucy Tian , University of Washington Vijay Nambodiri , University of California, San Francisco Stephen Zhang , New York University Kauê Costa , University of Alabama at Birmingham
5:00 PM – 6:30 PM	Opening Reception <i>Erasmus 1-2</i>	

Day 2: Tuesday, September 8, 2026

Time	Program Room	Chairs / Speakers
9:00 AM – 10:00 AM	Plenary 2 UvA 3-4 <i>Food for Thought – Neuronal Activity and Energy Provision in the Brain</i>	Martyn Boutelle , Imperial College London Introduction by: Parastoo Hashemi , Imperial College London
10:00 AM – 10:30 AM	Break Erasmus 1-2	
10:30 AM – 12:00 PM	Symposium 3 UvA 1-2 <i>Fluorescent Carbon Nanotube-Based Tools</i>	Chair: Sebastian Kruss , Ruhr University Bochum Speakers: Sebastian Kruss , Ruhr University Bochum Laurent Cognet , University of Bordeaux Chandima Bulumulla , Howard Hughes Medical Institute Natsumi Komatsu , University of Illinois Urbana-Champaign
	Symposium 4 UvA 3-4 <i>Brain Metabolism</i>	Chair: Eugene Mosharov , Columbia University Speakers: Sally Gowers , Imperial College London Eugene Mosharov , Columbia University Melissa Hexter , Center for Neuroscience Research of Lyon Eliana Fernandes , University of Coimbra
12:00 PM – 12:30 PM	Lunch Erasmus 1-2	
12:30 PM – 1:30 PM	Lunch & Discuss UvA 3-4	
1:30 PM – 3:00 PM	Symposium 5 UvA 1-2 <i>Monitoring the Brain Proteome and Metabolome In Vivo</i>	Chair: Robert Kennedy , University of Michigan Speakers: John Melchior , Pacific Northwest National Laboratory Barbara Bojko , Nicolaus Copernicus University Per Andren , Uppsala University Robert Kennedy , University of Michigan
	Symposium 6 UvA 3-4 <i>Proteomic and Molecular Approaches to Mapping Neural Circuits of Motivated Behavior</i>	Chair: Ream Al-Hasani , Washington University in St. Louis Speakers: Eileen Brouwer , University Medical Center Utrecht Olivia Drake , University of Alabama at Birmingham Jordan McCall , Washington University in St. Louis Lindsay Schwarz , St. Jude Children's Research Hospital
3:00 PM – 3:15 PM	Break Erasmus 1-2	

3:15 PM – 4:45 PM	Symposium 7 <i>UvA 1-2</i> Nucleic Acid and Enzyme Based Biosensors	Chair: Tod Kippin , University of California, Santa Barbara Speakers: Ajay Pradhan , Chalmers University of Technology Faridat Abdulsalami , Imperial College London Charles Rezaei , École des Mines Saint-Étienne Tod Kippin , University of California, Santa Barbara
	Symposium 8 <i>UvA 3-4</i> Dopamine Signaling Across Multiple Timescales	Co-chairs: Rebecca Smausz & Mark Walton , University of Oxford Speakers: Bart Lodder , Harvard University Ulrik Gether , University of Copenhagen Rebecca Smausz , University of Oxford Ali Mohebi , University of Wisconsin - Madison
4:45 PM – 6:15 PM	Poster Session I & Exhibit <i>Erasmus 1-2</i>	

Day 3: Wednesday, September 9, 2026

Time	Program Room	Chairs / Speakers
9:00 AM – 10:00 AM	Plenary 3 <i>UvA 3-4</i> What Genetically Engineered Sensors Tell Us About the Mind	Lin Tian , Max Planck Florida Institute for Neuroscience & University of California, Davis Introduction by: Tommaso Patriarchi , University of Zürich
10:00 AM – 10:30 AM	Break <i>Erasmus 1-2</i>	
10:30 AM – 12:00 PM	Symposium 9 <i>UvA 1-2</i> GPCR-Based Optical Sensors for Novel Analytes	Chair: Michael Bruchas , University of Washington Speakers: Tommaso Patriarchi , University of Zürich Andre Berndt , University of Washington Zhaofa Wu , Chinese Academy of Sciences Gunnar Sorensen , H. Lundbeck A/S
	Symposium 10 <i>UvA 3-4</i> Harnessing Cutting-Edge Analytical Methods to Decipher Mechanisms of Human Disease	Chairs: Michael Johnson , University of Kansas Speakers: Michael Johnson , University of Kansas Parastoo Hashemi , Imperial College London Jan Booij , Amsterdam UMC Jan Stegemann , Fraunhofer Institute for Microelectronic Circuits and Systems
12:00 PM – 12:30 PM	Lunch <i>Erasmus 1-2</i>	

12:30 PM – 1:30 PM	Lunch & Discuss <i>UvA 3-4</i>	
1:30 PM – 3:00 PM	Symposium 11 <i>UvA 1-2</i> <i>Data Mining and Machine Learning to Analyze Neurochemical Data</i>	Co-chairs: Marion Rivalan , Université Paris-Saclay & Sandrine Parrot , University of Lyon Speakers: Sandrine Parrot , University of Lyon Marion Rivalan , Université Paris-Saclay Eliana Lousada , GHU Paris Psychiatrie & Neurosciences Kenneth Kishida , Wake Forest University
	Symposium 12 <i>UvA 3-4</i> <i>Neurochemical Correlates of Opioid Use</i>	Chair: B. Jill Venton , University of Virginia Speakers: B. Jill Venton , University of Virginia Jones Griffith Parker , Northwestern University Aidan Evans-Strong , Washington University in St. Louis Yunhee So , Hanyang University
3:00 PM – 3:15 PM	Break <i>Erasmus 1-2</i>	
3:15 PM – 4:45 PM	Symposium 13 <i>UvA 1-2</i> <i>Monitoring the Unmonitorable</i>	Chair: Simon Bossi , University of Oxford Speakers: Jonathan Marvin , Howard Hughes Medical Institute Bethan O'Connor , University of Oxford Ségolène Bompierre , Donders Institute for Brain, Cognition & Behaviour Simon Bossi , University of Oxford
	Symposium 14 <i>UvA 3-4</i> <i>Pharmacological Consequences of Addictive Substances</i>	Chair: Lillian Brady , University of Alabama at Birmingham Speakers: Lillian Brady , University of Alabama at Birmingham Hannah Reiley , Vanderbilt University Nathan Marchant , Amsterdam UMC Samantha Webster , University of Virginia
4:45 PM – 6:15 PM	Poster Session II & Exhibit <i>Erasmus 1-2</i>	
7:00 PM – 10:00 PM	Reception Dinner <i>House of Watt (offsite)</i>	

Day 4: Thursday, September 10, 2026

Time	Program Room	Chairs / Speakers
9:00 AM – 10:00 AM	Plenary 4 UvA 3-4 <i>New Birds on the Block for Multicomponent Optogenetic Brain Studies</i>	Peter Hegemann , Humboldt University of Berlin Introduction by: Andre Berndt , University of Washington - Seattle
10:00 AM – 11:30 AM	Poster Session III & Exhibit Erasmus 1-2	
11:30 AM – 12:30 PM	MMiN Business Meeting (open to all) UvA 3-4	
12:30 PM – 1:30 PM	Lunch & Professional Development Session Erasmus 1-2 & UvA 3-4	More details are coming soon.
1:30 PM – 3:00 PM	Symposium 15 UvA 1-2 <i>From Measurement to Meaning: Theory and Analysis of High-Dimensional Neural and Neurochemical Data</i>	Co-chairs: Alfredo Elhazaz-Fernandez , Netherlands Institute for Neuroscience & Isis Alonso-Lozares , Netherlands Institute for Neuroscience Speakers: Devika Narain , Radboud University Felice Veen , Netherlands Institute for Neuroscience J. Alexander Heimel , Netherlands Institute for Neuroscience Philip Jean-Richard-Dit-Bressel , University of New South Wales
	Symposium 16 UvA 3-4 <i>Deciphering the Role of Astrocytes in Behaving Animals</i>	Chair: Matthew Wanat , University of Texas at San Antonio Speakers: Matthew Wanat , University of Texas at San Antonio Xinzhu Yu , University of Texas Health Science Center at Houston Michael Scofield , Medical University of South Carolina Olena Bukalo , National Institutes of Health
3:00 PM – 3:15 PM	Break Erasmus 1-2	
3:15 PM – 4:45 PM	Symposium 17 UvA 1-2 <i>Implantable Microfluidics for In-Vivo Neurochemical Monitoring</i>	Chair: Yi Zhang , University of Connecticut Speakers: Yi Zhang , University of Connecticut Michael J. Cima , Massachusetts Institute of Technology Ian Bain , University of Michigan Julie Stenken , University of Arkansas
	Symposium 18 UvA 3-4 <i>From Sensors to Signals: Overcoming</i>	Co-chairs: James McCutcheon , UiT The Arctic University of Norway & Jamie Roitman , University of Illinois Chicago

	Challenges in Measuring Spontaneous Dopamine Transients	Speakers: Rachel Donka , University of Illinois Chicago Florian Schoukroun , Washington University in St. Louis Emanuel Lopes , Wake Forest University Jinsung Lee , Hanyang University
4:45 PM – 4:50 PM	Closing UvA 1-2 & UvA 3-4	

PLENARY ABSTRACTS

Plenary 1: Two-Photon Holographic Manipulation of Neuronal Circuits

Valentina Emiliani, *Vision Institute, Paris*

The genetic targeting of neuronal cells with activity reporters, such as calcium or voltage indicators, has driven a paradigmatic shift in neuroscience, where photons have replaced electrons in reading large-scale brain activities at cellular resolution. Simultaneously, optogenetics has shown that targeting neuronal cells with photosensitive microbial opsins enables the transduction of photons into electrical currents of opposing polarities. This allows for the activation or inhibition of neuronal signals in a minimally invasive manner.

These advances have, in turn, spurred the development of sophisticated wavefront-shaping techniques to enable "all-optical" interrogation of deep brain circuits with high spatial and temporal resolution across large volumes¹.

In this presentation, we will discuss the most recent approaches that we have recently proposed to enhance the capacity for patterned all-optical circuit manipulation. These approaches enable efficient in vivo two-photon multitarget optogenetic photostimulation² and voltage imaging^{3,4} in both head-fixed and freely moving mice^{5,6}. As an example of patterned optogenetics, we will present a recent experiment demonstrating high-throughput connectivity mapping in the mouse visual cortex⁷.

1. V. Emiliani et al. Optogenetics for light control of biological systems. *Nature Rev Methods Prime*. (2022)
2. G.Faini*, D.Tanese*, et al. Ultrafast Light Targeting for High-Throughput Precise Control of Neuronal Networks *Nature Comm*, 14, 1888 (2023)
3. R. R. Sims*, I. Bendifallah*, et al. Scanned voltage imaging. *Nature Comm*, 15, 5095 (2024)
4. C. Grimm*, R. R. Sims*, et al. Two-photon voltage imaging with rhodopsin-based sensors. *Neuron* (2026)
5. N. Accanto*, F. Blot *, A. Lorca* et al. A flexible two-photon fiberscope for fast activity imaging and precise optogenetic photostimulation of neurons in freely moving mice. *Neuron*, Jan 18;111(2):176-189.e6 (2023)
6. F. Blot *, D. Decombe, et al. 2P-FENDO-II: a fiber bundle microscope for all optical brain study on large field of view in freely moving mice. *Cell Reports Methods*, 6 101305 (2026)
7. I-W. Chen*, C. Y. Chan*, et al. High-throughput synaptic connectivity mapping using two-photon holographic optogenetics and compressive sensing in vivo. *Nat Neurosci* 28, 2141–2153 (2025)

Plenary 2: Food for Thought – Neuronal Activity and Energy Provision in the Brain

Martyn Boutelle, *Imperial College London*

Since Roy and Sherrington's initial 1890 hypothesis, increasing insight into how the brain regulates its local blood supply in response to local neuronal activity has been gained including the control of capillary diameter, and hence local blood flow, by pericytes. Local blood flow in turn controls the delivery of energy molecules to neurons either directly as glucose, or it has been suggested, indirectly as lactate produced by astrocytes. In vivo monitoring of these energy molecules over time together with neuronal activity in the intact brain provides a lens to study this relationship. We have developed a range of real-time on-line analysis techniques for energy metabolites that can be coupled with the in vivo sampling technique of microdialysis. Using a combination of microfluidic chips and biosensors a temporal resolution can be achieved sufficient to resolve changes in extracellular levels of energy molecules over a period of days. Importantly, we can use the same measurement systems in humans patients and experimental systems.

In this presentation I will discuss the use of online microdialysis to study the relationship between neuronal activity and brain energy supply from mild physiological stimulation to complex pathophysiological situations such as stroke, cardiac arrest and human traumatic brain injury.

Plenary 3: What Genetically Engineered Sensors Tell Us About the Mind

Lin Tian, *Max Planck Florida Institute for Neuroscience & University of California, Davis*

To study the neural circuitry, the action of one cell in the context of others, one would precisely measure and perturb specific neuronal populations and molecules in behaving animals who are specifically engaged in performing the computation or function of interest. The dataset of millions of neurons firing together underlying a behavior is required to develop and refine theories (hypotheses) explaining animal behavior in terms of brain physiology. The focus of the lab is to develop novel genetically encoded indicators based on fluorescence proteins and chemical dyes, especially focusing on direct and specific measurement of myriad input signals with needed spatial and temporal resolutions. In this talk, I will discuss our recent progress in developing genetically encoded indicators for neuromodulators using machine-learning-based single-cell screening. I will discuss the design, characterization, and applications of these genetically encoded indicators for both in vivo imaging and drug discovery. In combination with calcium imaging and optogenetics, these sensors are well poised to permit direct functional analysis of how the spatiotemporal coding of neural input signaling mediates the plasticity and function of target circuits.

Plenary 4: New Birds on the Block for Multicomponent Optogenetic Brain Studies

Peter Hegemann, *Humboldt University of Berlin*

The discovery of the light-activated ion channels, Channelrhodopsins, revolutionized in concert with optical reporter systems the neurosciences due to the new option to stimulate and monitor neuronal activity solely by using light. This new field of Optogenetics is now challenged by the desire to control not only membrane voltage but also transcription, translation and protein activity by light. We are currently working on two component rhodopsin-tandems that comprise a rhodopsin linked to a adenylyl or guanylate cyclase (R-GCs, R-ACs) or phosphodiesterase (R-PDEs) that modulate neuronal cAMP and cGMP concentrations to promote or inhibit signalling processes involved in metabolic regulation or gene expression. In addition, we are characterizing red sensitive rhodopsins with maximal absorption beyond 600nm to regulate G-protein signalling with far-red light that is preferentially transmitted by the brain tissues. We are concentrating on bidirectionally photo-switchable rhodopsins of Crustaceans as for example Mantis Shrimps and other Crustaceans. Bidirectional switching between red and green sensitivities will allow better control of G-protein signalling compared to the so far established rhodopsins that are switchable between blue and UV or green and UV states.

References:

- Broser et al. (2023) Diversity of Rhodopsin-cyclases in zoospore forming fungi. *Proc. Natl. Acad. Sci USA* 120(44) e2310600120. doi/10.1073/pnas.2310600120
- Spreen et al. (2025) Optogenetic silencing by combining a rhodopsin cyclase with an engineered cGMP-gated potassium channel. *Sci Adv.* 28;11(48): eadx1195. doi: 10.1126/sciadv.adx1195..PMID: 41313760
- Brouillon et al. (2025) Spectral tuning and signaling of diverse and most red sensitive visual rhodopsins that drive high colour discrimination in Mantis Shrimp eyes. *bioRxiv*, doi.org/10.1101/ 2025.11.25.690411

SYMPOSIA ABSTRACTS

Symposium 1: Bioanalytical Technologies for Multimodal Recording

Chair: **Leslie Sombers**, *University of Florida*

Speakers:

Anders Borgkvist, *Karolinska Institute*

Tracy Cui, *University of Pittsburgh*

Ann-Sofie Cans, *Chalmers University of Technology*

Leslie Sombers, *University of Florida*

This symposium details advances in bioanalytical technologies that are enabling unprecedented quantitative evaluation of neurotransmitters, neuropeptides, and pharmacological agents that act in and on brain circuits. The presentations highlight the unique strengths of a range of bioanalytical strategies, and how each is being used to advance understanding of function. First, **Anders Borgkvist** (Karolinska Institute) will discuss using two-photon imaging combined with vesicle probes and monoamine biosensors to study synaptic selection in the substantia nigra reticulata, with new evidence for synaptic plasticity in this region of a mouse model of Parkinson's disease. Next, X. **Tracy Cui** (University of Pittsburgh) will report on a multimodal microelectrode array technology developed to monitor interactions between neurotransmitter release and neuronal activity in freely-moving animals, as well as innovative strategies to tackle signal degradation challenges associated with long-term use. **Ann-Sofie Cans** (Chalmers University of Technology) will discuss ultrafast electrochemical nanotip biosensors for monitoring acetylcholine and glutamate release at single cells. The technology has demonstrated sub-millisecond resolution of individual acetylcholine and glutamate events displaying diverse kinetic profiles, revealing dynamic regulation of the fusion pore. Finally, **Leslie Sombers** (University of Florida) will discuss a workflow combining co-detection of catecholamines and met-enkephalin using fast-scan cyclic voltammetry with peptidomic analysis using mass spectrometry to investigate the temporal complexity and chemical diversity of peptide participants in opioid signaling. Overall, the talks will detail how distinct analytical tools for molecular monitoring can be applied, and strategically combined, for multi-perspective insight into function.

Symposium 2: Dopamine Signaling Across Spatiotemporal Domains

Chair: **Garret Stuber**, *University of Washington*

Speakers:

Lucy Tian, *University of Washington*

Vijay Namboodiri, *University of California, San Francisco*

Stephen Zhang, *New York University*

Kauê Costa, *University of Alabama at Birmingham*

Dopamine has long been described as a unitary teaching signal, yet this framing sits uneasily with the anatomical and kinetic heterogeneity of the mesostriatal system. Release varies across micrometers of striatal tissue and across timescales spanning milliseconds to minutes, and the postsynaptic consequences of that release depend on the concurrent activity state of the target circuit. Resolving how these domains relate to one another requires measurement approaches that span them. This session brings together four investigators using complementary optical and electrochemical methods to sample dopamine signaling at distinct spatial and temporal resolutions. **Lucy Tian** (University of Washington) will present simultaneous recordings of ventral striatal dopamine dynamics and medium spiny neuron activity during Pavlovian conditioning, addressing how release relates to the firing of the cells that read it. **Vijay Namboodiri** (University of California, San Francisco) will describe the spatial structure of dopamine release, combining two-photon imaging with fiber photometry to compare microscale release heterogeneity against bulk population signals. **Stephen Zhang** (New York University) will discuss methods for monitoring dopamine across multiple timescales during naturalistic behavior, where the relevant dynamics are not constrained by trial structure. **Kauê Costa** (University of Alabama at Birmingham) will present evidence that striatal dopamine encodes prediction errors across distinct informational domains, extending the error signal beyond reward value. Together these talks ask whether a single dopamine signal is being sampled at different resolutions, or whether distinct signals occupy separable spatiotemporal domains. Discussion will focus on how measurement technique constrains interpretation and on the standards needed to reconcile findings across methods.

Symposium 3: Fluorescent Carbon Nanotube-Based Tools

Chair: **Sebastian Kruss**, *Ruhr University Bochum*

Speakers:

Sebastian Kruss, *Ruhr University Bochum*

Laurent Cognet, *University of Bordeaux*

Chandima Bulumulla, *Howard Hughes Medical Institute*

Natsumi Komatsu, *University of Illinois Urbana-Champaign*

Protein-based indicators have revolutionized neurotransmitter imaging but rely on visible-spectrum fluorescence and transfection. This symposium highlights the emergence of fluorescent Single-Walled Carbon Nanotubes (SWCNTs) operating in the near-infrared, including both the NIR and SWIR windows, as class of synthetic nanosensors enabling imaging of neuromodulator release and diffusion of neurochemicals including dopamine, serotonin, and oxytocin. These non-photobleaching probes fluoresce in the 900-1700 nm tissue transparency window, which minimizes autofluorescence and scattering, enabling high-resolution and long-timescale neurochemical monitoring. This symposium will feature advances in engineering of SWCNTs-based sensors to create brighter, ratiometric, or lifetime fluorescence signals. It will also feature advances uniquely enabled by these probes in understanding neurotypical function and diseases such as dementias. These nanosensors have also enabled unique insights into the spatiotemporal regulation of neurotransmitter signaling at the single-cell level for immune cells, and at the single-varicosity level for neurons – particularly for dopamine. Lastly, SWCNTs have served as mechanical probes of the extracellular space in brain tissue to measure differences in the extracellular space density and diffusivity, again with synaptic-scale resolution. This session brings together experts in materials science and neurobiology to bridge molecular engineering with systems-level neuroscience, catalyzing new tools and insights for decoding chemical communication in the living brain.

Symposium 4: Brain Metabolism

Chair: **Eugene Mosharov**, *Columbia University*

Speakers:

Sally Gowers, *Imperial College London*

Eugene Mosharov, *Columbia University*

Melissa Hexter, *Center for Neuroscience Research of Lyon*

Eliana Fernandes, *University of Coimbra*

The majority of *in vivo* neurochemical studies have focused on monitoring neurotransmitters during behaviors, disease states, and their physiological regulation. However, brain metabolism serves as the foundation for all neural activity and functions. It both regulates and is regulated by the neural status, including behavioral, emotional, and motivational states as well as brain pathology. This panel will feature four speakers discussing neurochemical studies pertaining to brain metabolism. **Sally Gowers** (Imperial College London) will discuss the development of advanced 3D-printed microfluidic biosensors and wearable bioinstrumentation for the continuous, real-time monitoring of human brain metabolism. These devices establish direct connections to clinical microdialysis catheters and incorporate micro-scale electrochemical biosensors capable of simultaneously monitoring critical metabolic indicators, including glucose, lactate, pyruvate, and glutamate, along with ionic shifts such as potassium. She will evaluate the practical applications of these devices in detecting delayed secondary brain injuries following traumatic brain injury (TBI). **Eugene Mosharov** (Columbia University) will present the comprehensive brain-wide mapping of the human brain's molecular energetic landscape (MitoBrainMap). To accomplish this objective, they fragmented frozen human brain tissue into samples comparable to the size of MRI voxels, which were subsequently analyzed to map biochemical markers such as oxidative phosphorylation enzyme capabilities, mitochondrial DNA levels, and mitochondria-specific respiratory capacity. This research revealed a remarkable diversity of mitochondrial phenotypes optimized for energy transformation. This platform serves as a foundation for exploring the molecular energetic landscape. **Melissa Hexter** (Center for Neuroscience Research of Lyon) will discuss the impact of ketogenic diet on the excitatory-inhibitory balance. This research employs a multimodal approach that integrates NMR-based metabolomics with *in vivo* glutamate biosensing during spreading depolarization in Sprague-Dawley rats. This study provides evidence for the ketogenic diet inducing a global metabolic rewiring, resulting in a shift in the excitation-inhibition balance. This shift is attributed to reduced releasable glutamate and enhanced GABA levels, which favour inhibition. **Eliana Fernandes** (University of Coimbra) will describe the development and *in vivo* application of carbon fiber microelectrodes for the simultaneous real-time amperometric monitoring of glucose, lactate, and oxygen, along with the recording of local field potentials-related currents in the rat cerebral cortex. These microelectrode arrays enable the simultaneous characterization of neurometabolic responses associated with individual ictal events induced by local application of 4-aminopyridine across all sensors.

Symposium 5: Monitoring the Brain Proteome and Metabolome In Vivo

Chair: **Robert Kennedy**, *University of Michigan*

Speakers:

John Melchior, *Pacific Northwest National Laboratory*

Barbara Bojko, *Nicolaus Copernicus University*

Per Andren, *Uppsala University*

Robert Kennedy, *University of Michigan*

Classically in vivo monitoring experiments have focused on a few known, targeted compounds at a time. This work has been dramatically pushed forward with spatially resolved sensors and imaging technologies. A complement to these technologies that is emerging is deep analysis of the living brain, i.e. use of proteomics and metabolomics to identify and monitor hundreds of compounds collected from the living brain by sampling methods. These new tools can help understand large shifts in protein signaling and metabolome that accompany different behavioral and disease states. For this symposium, we will include speakers who have used such technology to identify metabolome and proteome rearrangements associated with sleep states, drug use, and traumatic brain injury. New sampling approaches that can better track such changes will also be highlighted. Studies described include evaluation of lipoproteins in the brain (**John Melchior**), use of solid phase microextraction in vivo for metabolomic and lipidomic brain monitoring (**Barbara Bojko**), tissue spatially resolved omic analysis of brain (**Per Andren**), and microdialysis dialysis coupled with LC-MS/MS for metabolomic and proteomic analysis of the brain extracellular space in response to cocaine (**Robert Kennedy**).

Symposium 6: Proteomic and Molecular Approaches to Mapping Neural Circuits of Motivated Behavior

Chair: **Ream Al-Hasani**, Washington University in St. Louis

Speakers:

Eileen Brouwer, University Medical Center Utrecht

Olivia Drake, University of Alabama at Birmingham

Jordan McCall, Washington University in St. Louis

Lindsay Schwarz, St. Jude Children's Research Hospital

This symposium brings together work spanning distinct behavioral domains of feeding, pain, reward, and anxiety. Each speaker will describe their unique approach in dissecting the molecular and circuit-level identity of neurons that drive these motivated and emotional behavior.

Behavioural and transcriptomic evidence for hypothalamic circuit remodelling during anorexia-like states:

Eileen Brouwer opens by examining hypothalamic circuit remodeling in a mouse model of anorexia nervosa, combining activity-dependent genetic labeling (TRAP2) with single-nucleus transcriptomics to reveal the neuronal ensembles recruited during food anticipation under negative energy balance.

A Novel Cellular Target for Opioids in the Nucleus Accumbens: **Olivia Drake** identifies a transcriptionally distinct, μ -opioid-receptor-enriched neuronal subtype in the nucleus accumbens, marked by Chst9, that is disproportionately responsive to opioid agonism despite its small population size, implicating this subtype as a key cellular target underlying opioid reward.

Monitoring Molecules ex vivo to identify new in vivo strategies for neural circuit-selective pharmacology:

Jordan McCall, PhD presents a novel ex vivo calcium-imaging framework for discovering non-genetic, pharmacological strategies to selectively modulate neural circuits, demonstrated through an agonist cocktail that inhibits pain-promoting locus coeruleus neurons projecting to prefrontal cortex, a translational alternative to optogenetic and chemogenetic approaches.

Intersectional Dissection of Catecholaminergic Neural Circuits to Uncover Functional Heterogeneity: **Lindsay**

Schwarz, PhD uses intersectional genetic tools to uncover a previously unrecognized role for brainstem C1 adrenergic neurons in promoting anxiety-like behavior through brain-body signal integration, positioning this population as a potential therapeutic target.

Together, these talks illustrate how activity-dependent labeling, single-cell transcriptomics, proteomics and intersectional circuit-mapping tools are converging to reveal the cellular architecture of motivated behavior—and how its disruption underlies feeding, pain, addiction, and anxiety disorders.

Symposium 7: Nucleic Acid and Enzyme Based Biosensors

Chair: **Tod Kippin**, *University of California, Santa Barbara*

Speakers:

Ajay Pradhan, *Chalmers University of Technology*

Faridat Abdulsalami, *Imperial College London*

Charles Rezaei, *École des Mines Saint-Étienne*

Tod Kippin, *University of California, Santa Barbara*

Neurochemical methods often suffer from temporal limitations due to sequential sample collection and analysis stages. While *in situ* detection has been achieved through approaches such as *in vivo* electrochemistry, these methods often trade off chemical precision for temporal resolution, with approaches like fast-scan cyclic voltammetry lacking the analytical resolution or selectivity of their slower counterparts. In contrast, biosensors utilize a biological recognition element to selectively detect chemicals while simultaneously enabling high temporal *in situ* sampling. These biological recognition elements manifest in various forms, including enzymes and antibodies that possess inherent binding selectivity, as well as aptamers—artificial, single-stranded DNA or RNA oligonucleotides—engineered to adopt intricate three-dimensional shapes. The integration of the biological recognition elements into a recording device can also present its own challenges, with solutions including the incorporation onto the surface of traditional electrodes, the use of silicon-based platforms and the utilization of organic conductive polymers. This panel will showcase research conducted by four speakers employing diverse biosensors. **Ajay Pradhan** (Chalmers University of Technology) will present a data on the utilization of carbon fiber-based nanometric enzyme-modified amperometric glutamate biosensors. The primary objective of this research is to characterize presynaptic glutamate release at the single-vesicle resolution of human iPSC-derived neurons. The findings obtained from these investigations indicate that these neurons possess the capability of reliable non-evoked glutamate release and exhibit a spectrum of intricate fusion pore dynamics that are responsive to intracellular calcium levels. By employing this approach, they have successfully modeled early stages of Alzheimer's disease with elevated amyloid- β oligomers, and observed a notable augmentation in the glutamate release frequency. **Faridat Abdulsalami** (Imperial College London) will present the development of a continuous neurochemical monitoring unit coupled with cerebral microdialysis. This device is designed to detect glutamate and GABA in the injured brain following traumatic brain injury (TBI). The device incorporates an enzyme-based selective glutamate biosensor and a second biosensor that detects both GABA and glutamate. The simultaneous tandem recordings enable the subtraction of the glutamate signal from the combined signal, thereby providing independent concentration assessments of glutamate and GABA. **Charles Rezaei** (École des Mines Saint-Étienne) will explore how conjugated polymers can expand the capabilities of bioelectronic interfaces. He will highlight the advantages of organic materials for mechanically conformable and biocompatible neural interfaces and then discuss the potential of conjugated polymers for electrochemical detection of neurotransmitters, focusing on dopamine and

voltammetric approaches such as fast-scan cyclic voltammetry. Finally, he will introduce our ongoing work towards using PEDOT-MA as a sensing interface, with the longer-term goal of integrating molecular detection capabilities into flexible neural probes. **Tod Kippin** (University of California, Santa Barbara) will present an overview of the development of electrochemical aptamer-based biosensors for *in vivo* molecular monitoring at high temporal resolution. These devices employ nucleic acids specifically engineered to selectively detect a wide range of analytes, encompassing small molecules to proteins. Consequently, they afford substantial potential applications. Notably, one specific implementation of these biosensors involves the quantification of addictive substances *in vivo*.

Symposium 8: Dopamine Signaling Across Multiple Timescales

Co-chairs: **Rebecca Smausz**, *University of Oxford* & **Mark Walton**, *University of Oxford*

Speakers:

Bart Lodder, *Harvard University*

Ulrik Gether, *University of Copenhagen*

Rebecca Smausz, *University of Oxford*

Ali Mohebi, *University of Wisconsin - Madison*

How does the brain dynamically regulate motivational states and adaptive behaviour? Neuromodulators such as dopamine play a key role in reward-guided behaviour, yet the underlying mechanisms remain contentious. As both dopamine signalling and behaviour unfold across multiple timescales, uncovering the complex relationship between them has been challenging, in part because of longstanding limitations in tools for quantifying signalling across timescales in freely-behaving animals. However, recent methodological advances in optical biosensors offer promising ways to address this.

This symposium aims to bring together scientists at the forefront of these advances to discuss the state of the field. **Bart Lodder** will provide insights into how fluorescent lifetime photometry at high temporal resolution (FLIPR) enables quantitative, absolute readouts of dopamine and calcium signalling across timescales during motivated behaviour. **Ulrik Gether** will discuss evidence for distinctions between fast and slow dopamine signals across striatal regions, and their influence on dopamine receptor and transporter activity. **Rebecca Smausz** will then show evidence that the reward-rate-dependent changes in slow dopamine signals are independent of dopamine transient dynamics in the ventral striatum, providing a distinct signal to shape engagement with reward seeking. Finally, **Ali Mohebi** will extend this framework beyond the striatum, presenting simultaneous multi-region recordings that compare how dopamine release dynamics differ between the striatum and prefrontal cortex during learning and motivated behaviour. He will argue that slow and fast dopamine signals are shaped by distinct mechanisms in each region, with implications for how reward history is translated into learning rate and behavioural engagement.

This symposium, featuring speakers across career stages and expertise, will offer new perspectives on dopamine signalling across timescales, motivated behaviour, and emerging techniques to further explore these questions.

Symposium 9: GPCR-Based Optical Sensors for Novel Analytes

Chair: **Michael Bruchas**, *University of Washington*

Speakers:

Tommaso Patriarchi, *University of Zürich*

Andre Berndt, *University of Washington*

Zhaofa Wu, *Chinese Academy of Sciences*

Gunnar Sorensen, *H. Lundbeck A/S*

Neuromodulatory signaling unfolds across multiple spatial and temporal scales to shape brain state, physiology, and behavior. Recent advances in genetically encoded fluorescent sensors, protein engineering, and in vivo neurochemical monitoring are rapidly expanding our ability to directly measure these signals in the intact brain. This symposium will highlight emerging approaches for monitoring diverse extracellular and intracellular signaling molecules and demonstrate how these tools can reveal the molecular dynamics underlying sleep, arousal, stress, and behavior. Speakers will present new strategies for engineering and optimizing genetically encoded sensors using structure-guided design, directed evolution, machine learning, computational approaches, and high-throughput screening. These efforts are producing increasingly sensitive and selective tools for monitoring classical neuromodulators, neuropeptides, purines, hormones, reactive signaling molecules, and intracellular second-messenger dynamics. Particular emphasis will be placed on expanding sensor palettes to enable multicolor measurements and the simultaneous observation of interacting molecular signals in awake and freely moving animals. The symposium will also illustrate how complementary neurochemical approaches can connect molecular dynamics with physiological state. Studies of histamine release using microdialysis and fiber photometry will examine how distinct wake-promoting pharmacological mechanisms alter extracellular histamine during sleep-wake regulation. New fluorescent sensors for purinergic signaling will reveal interactions among extracellular and intracellular purines, cellular metabolism, and neuromodulation during sleep-wake transitions and stress. Finally, advances in sensors for norepinephrine, opioids, calcium, hydrogen peroxide, hormones, neuropeptides, and related signaling systems will demonstrate how molecular tool development can be integrated with circuit and behavioral neuroscience. Together, these approaches provide an increasingly powerful framework for monitoring the chemical language of the brain in real time.

Symposium 10: Harnessing Cutting-Edge Analytical Methods to Decipher Mechanisms of Human Disease

Chairs: **Michael Johnson**, *University of Kansas*

Speakers:

Michael Johnson, *University of Kansas*

Parastoo Hashemi, *Imperial College London*

Jan Booij, *Amsterdam UMC*

Jan Stegemann, *Fraunhofer Institute for Microelectronic Circuits and Systems*

Recent advances in analytical science are transforming our ability to probe complex biological systems and unravel the mechanisms underlying human diseases. This symposium highlights research that leverages high-resolution, high-sensitivity methods to link molecular dynamics with disease phenotypes and open new avenues for diagnosis and therapy. **Michael Johnson** is a Professor of Chemistry in the Ralph N. Adams Institute for Bioanalytical Chemistry at the University of Kansas, Lawrence. Dr. Johnson's research employs combined bioanalytical approaches—electrochemical detection, fluorescence microscopy, and caged compound photorelease—to study neuronal communication and oxidative stress in neurodegenerative disorders like Alzheimer's disease and Parkinson's disease. **Parastoo Hashemi** is a Professor in Brain Diagnostics and Therapeutics at Imperial College, London. Dr. Hashemi specializes in electroanalytical methods for real-time in vivo quantification of neurotransmitters such as serotonin and histamine in the brain and body. Her work elucidates dynamic chemical changes associated with neurological and mood disorders, offering mechanistic understanding of how altered neurotransmission contributes to disease and pointing toward novel diagnostic biomarkers and therapeutic targets. **Jan Booij** is a Professor of Radiology and Nuclear Medicine at Amsterdam UMC whose research centers on molecular neuroimaging using positron emission tomography (PET) and single-photon emission computed tomography (SPECT). His work focuses on developing and applying neuroreceptor and dopamine transporter imaging to study Parkinson's disease, dementia with Lewy bodies, schizophrenia, addiction, major depression, and related neurological disorders, while also advancing new radiotracers for preclinical and clinical imaging. **Jan Stegemann** is a researcher at the Fraunhofer Institute for Microelectronic Circuits and Systems (IMS) whose work combines optical sensing, near-infrared (NIR) imaging, and nanotechnology to develop innovative biosensing platforms. Dr. Stegemann's research includes high-speed hyperspectral NIR imaging (HyperNIR), carbon nanotube-based optical nanosensors for detecting biomolecules such as neurotransmitters, and rapid diagnostic technologies for applications ranging from environmental monitoring to antimicrobial resistance testing. Collectively, this diverse group of speakers conduct research that is timely and relevant.

Symposium 11: Data Mining and Machine Learning to Analyze Neurochemical Data

Co-chairs: **Marion Rivalan**, *Université Paris-Saclay* & **Sandrine Parrot**, *University of Lyon*

Speakers:

Sandrine Parrot, *University of Lyon*

Marion Rivalan, *Université Paris-Saclay*

Eliana Lousada, *GHU Paris Psychiatrie & Neurosciences*

Kenneth Kishida, *Wake Forest University*

The field of neuroscience is well acquainted with both the accumulation and analysis of complex and multi-dimensional data. Machine Learning (ML) is commonly used in the analysis of multi-dimensional data with neural network models used in neuroimaging, electrophysiology, or even to predict clinical outcomes. Often associated with data mining, these ML approaches learn from empirical data. In the monitoring of neurochemicals, ML has been shown to be powerful for the validation and advancement of high precision measurement tools, such as its application in in vivo voltammetry. However, the utility of ML in neurochemical monitoring research is not limited to just neurochemical detection. ML is beginning to provide new insights into the connection between neurochemical changes and complex phenotypes in a range of experimental settings.

Here, we aim to show the potentials of data mining and ML in neurochemical data analysis by answering a range of neuroscience questions. The symposium gathers recent examples of studies in which data mining and ML were used to bring additional results and new insights in experimental (**Sandrine Parrot**, **Marion Rivalan**, **Kenneth Kishida**) and clinical research (**Eliana Lousada**, **Kenneth Kishida**).

For instance, can we explain a genotype or a behavior exhibited by animals through neurochemical data? What type of new information could we learn and interpret from the individual differences in behavior vs their neurochemical substrates? Is the new information really new as compared to the current data analysis based on statistical hypothesis testing?

How can we extract neurochemicals from human data during surgeries using FSCV on carbon fiber electrodes or platinum-iridium sEEG electrodes? Can we connect those measurements to human behavior including complex human behavior quantified using ML tools?

Symposium 12: Neurochemical Correlates of Opioid Use

Chair: **B. Jill Venton**, *University of Virginia*

Speakers:

B. Jill Venton, *University of Virginia*

Jones Griffith Parker, *Northwestern University*

Aidan Evans-Strong, *Washington University in St. Louis*

Yunhee So, *Hanyang University*

This symposium will concentrate on new methods to detect neurochemical changes in the brain after opioids and in opioid use disorder. **B. Jill Venton** will present on Focused-Ultrasound modulation of dopamine as a treatment for relapse in fentanyl use disorder. Her lab has combined fast-scan cyclic voltammetry (FSCV) and low intensity focused ultrasound (LIFU) to determine LIFU patterns for dopamine excitation and inhibition in the NAcc, and then applied LIFU during abstinence periods in Fentanyl Use Disorder rats to prevent relapse. **Jones Griffith Parker** will present on modulation of striatal patch/matrix ensemble dynamics by morphine and buprenorphine. His lab uses dual-color, in vivo two-photon imaging to determine how morphine alters the levels and dynamics of calcium activity in mu-opioid expressing and non-expressing spiny-projection neurons. **Aidan Evans-Strong** will present on real-time dynorphinergic and kappa opioid receptor dynamics in the nucleus accumbens shell during fentanyl exposure and withdrawal. He uses in vivo fiber photometry to monitor dynorphinergic neuron calcium activity and kappa opioid receptor binding and compares it to withdrawal symptoms. He will show work investigating dynorphin and kappa opioid receptor dynamics in distinct nucleus accumbens shell subregions in food reward, fentanyl administration, and fentanyl withdrawal. **Yunhee So** will present on investigation of phasic and tonic dopamine changes induced by stimulants, opioids, and hallucinogens in mice nucleus accumbens core. He will report on work using FSCV and multiple cyclic square wave voltammetry in the mouse nucleus accumbens core to determine how stimulants, an opioid, and hallucinogens alter evoked phasic dopamine release kinetics as well as tonic dopamine levels. In addition, he will correlate conditioned place preference testing behavior results with the observed dopamine responses. Overall, this symposium highlights how advanced neurochemical tools are revealing the brain adaptations that drive opioid use disorder and identifying potential therapeutic strategies to reduce relapse.

B. Jill Venton: Focused-Ultrasound Modulation of dopamine as a treatment for relapse in Fentanyl Use Disorder

Jones Griffith Parker: Modulation of striatal patch/matrix ensemble dynamics by morphine and buprenorphine

Aidan Evans-Strong: Real-time dynorphinergic and kappa opioid receptor dynamics in the nucleus accumbens shell during fentanyl exposure and withdrawal.

Yunhee So: Investigation of phasic and tonic dopamine changes induced by stimulants, opioids, and hallucinogens in mice nucleus accumbens core

Symposium 13: Monitoring the Unmonitorable

Chair: **Simon Bossi**, *University of Oxford*

Speakers:

Jonathan Marvin, *Howard Hughes Medical Institute*

Bethan O'Connor, *University of Oxford*

Ségolène Bompierre, *Donders Institute for Brain, Cognition & Behaviour*

Simon Bossi, *University of Oxford*

Recent advances in experimental tools have provided unprecedented abilities to monitor previously hidden aspects of neurochemicals and neurophysiology. However, many aspects of brain signalling remain “unmonitorable”. Emerging discoveries in neurotransmission have highlighted the need for further innovative approaches, to tackle the elusive properties of brain signalling. This symposium will highlight speakers from a range of career stages, demonstrating the cutting-edge strategies to study emerging aspects of brain physiology, and, areas that require additional novel tools to be developed.

Jonathan Marvin (Senior scientist) will present work on novel sensors for real-time detection of molecules that are challenging to monitor. The presentation will cover established sensors for aspartate, ATP, and glucose, as well as emerging sensors currently under development for molecules including glucose-6-phosphate, cysteine, succinate, and GTP. **Bethan O'Connor** (Junior Post-doc) will present new insights into the physiology and regulation of dopamine axon signalling, gained using the recently developed genetically encoded voltage sensor ASAP5. Her work highlights the role of dopamine transporters in shaping neuronal signalling, providing unprecedented access to aspects of dopaminergic function that have previously been difficult to monitor. **Ségolène Bompierre** (Junior Post-doc) will present her recent findings on how dopamine and norepinephrine regulate the formation of new inhibitory synaptic boutons through PKA signalling in hippocampal inhibitory neurons. By combining FRET-FLIM PKA biosensors with live imaging, her work reveals how dynamic changes in PKA activity drive inhibitory bouton formation. Finally, **Simon Bossi** (Intermediate Career Development Fellow) will highlight recent transformative discoveries in glycinergic transmission, emphasizing the need for new tools that permit direct monitoring to better understand this emerging form of neurotransmission. Collectively, these talks will showcase the latest advances, illustrate the potential of innovative methodologies, and stimulate discussion on the development of next-generation tools for investigating complex brain function.

Symposium 14: Pharmacological Consequences of Addictive Substances

Chair: **Lillian Brady**, *University of Alabama at Birmingham*

Speakers:

Lillian Brady, *University of Alabama at Birmingham*

Hannah Reiley, *Vanderbilt University*

Nathan Marchant, *Amsterdam UMC*

Samantha Webster, *University of Virginia*

Addictive substances produce complex pharmacological effects that reshape neurotransmission, receptor function, and neural circuit activity to drive persistent behavioral adaptations. Understanding these processes requires analytical approaches capable of monitoring neurochemical signaling across multiple spatial and temporal scales while linking molecular mechanisms to circuit function and behavior. This session brings together complementary expertise in electrochemical sensing, analytical chemistry, pharmacology, and systems neuroscience to showcase how classical and innovative molecular monitoring technologies are revealing the diverse neurochemical consequences of addictive substances, from acute drug action to the neural adaptations that promote addiction and relapse.

Dr. Lillian J. Brady (University of Alabama at Birmingham) will present ex vivo electrochemical studies defining how nicotine engages nicotinic acetylcholine receptors to regulate dopamine release in nucleus accumbens circuits, revealing sex-dependent mechanisms that contribute to addiction vulnerability. **Dr. Hannah B. Reiley** (Vanderbilt University) will describe in vivo measurements demonstrating how cocaine-induced disruption of dopamine clearance reshapes neurochemical signaling during reward and aversion learning. Expanding beyond psychostimulants, **Dr. Nathan Marchant** (Amsterdam UMC) will examine the neural circuits that govern choice between alcohol and non-alcohol rewards, providing new insight into the mechanisms underlying alcohol-related decision making. **Samantha Webster** (University of Virginia) will present ex vivo electrochemical measurements of cocaine-induced changes in dopamine release and reuptake within the *Drosophila* mushroom body, demonstrating how complementary experimental models can advance our understanding of the neurochemical actions of addictive substances.

Together, these presentations highlight complementary approaches for measuring the pharmacological consequences of addictive substances across molecular, circuit, and behavioral levels. By integrating analytical chemistry, electrochemical sensing, and systems neuroscience, this session will provide attendees with a broad perspective on how neurochemical measurements illuminate the mechanisms underlying addiction. Collectively, these talks underscore the value of molecular monitoring for advancing both basic discovery and translational research while identifying new opportunities for therapeutic intervention in substance use disorders.

Symposium 15: From Measurement to Meaning: Theory and Analysis of High-Dimensional Neural and Neurochemical Data

Co-chairs: **Alfredo Elhazaz-Fernandez**, *Netherlands Institute for Neuroscience* & **Isis Alonso-Lozares**, *Netherlands Institute for Neuroscience*

Speakers:

Devika Narain, *Radboud University*

Felice Veen, *Netherlands Institute for Neuroscience*

J. Alexander Heimel, *Netherlands Institute for Neuroscience*

Philip Jean-Richard-Dit-Bressel, *University of New South Wales*

Recent developments in time-resolved neurochemical monitoring, including fast-scan cyclic voltammetry, genetically encoded fluorescent sensors, fiber photometry, and large-scale calcium imaging, improved the simultaneous measurement of multiple neural and neurochemical signals across behavioral timescales. These advances have also exposed a growing gap between data acquisition and interpretation. This panel will focus on the analytical and theoretical challenges posed by high-dimensional, temporally structured neurochemical datasets.

We will discuss how latent variable models, dimensionality reduction, and dynamical systems approaches shape the interpretation of neural population activity and neurochemical dynamics, and what assumptions these frameworks impose on mechanistic inference. Particular emphasis will be placed on novel statistical frameworks for analysing population-level neural activity and neurochemical release in awake, behaving animals. These methods provide unbiased post-hoc analytical approaches for time-series data, with the potential to capture richer relationships between neural signals and behavior.

By bringing together perspectives from experimental measurement and theory-driven analysis, this discussion aims to clarify current limitations in analyzing large-scale neurochemical data, highlight emerging analytical strategies, and outline open challenges in linking high-dimensional measurements to interpretable models of neural computation and behavior.

Symposium 16: Deciphering the Role of Astrocytes in Behaving Animals

Chair: **Matthew Wanat**, *University of Texas at San Antonio*

Speakers:

Matthew Wanat, *University of Texas at San Antonio*

Xinzhu Yu, *University of Texas Health Science Center at Houston*

Michael Scofield, *Medical University of South Carolina*

Olena Bukalo, *National Institutes of Health*

Brain research has traditionally focused on studying neuronal populations. However, it has become increasingly clear that glia, such as astrocytes, play a critical role in regulating brain function and neuronal activity. Standard techniques for probing neuronal function (e.g. electrophysiology and optogenetics) are not ideally suited for studying astrocytes given that glia do not fire action potentials. This challenge has spurred the development of strategies to interrogate, characterize, and record from astrocytes to understand how they contribute to brain function and behavior. In this panel we will highlight recent research findings using contemporary tools to study what astrocytes do in the brain and how they regulate behavior. **Dr. Matthew Wanat** (University of Texas at San Antonio) will discuss how astrocytes in the ventral tegmental area regulate reward learning. **Dr. Xinzhu Yu** (University of Texas Health Science Center at Houston) will talk about the role of astrocytes in regulating emotional and prosocial behaviors. **Dr. Mike Scofield** (MUSC) will talk about prelimbic cortical astrocytes and how they actively shape heroin-seeking circuits rather than merely supporting them. **Dr. Olena Bukalo** (NIAAA) will present recent data on monitoring calcium activity during emotional memory retrieval and updating, in vivo, in astrocytes and neurons using fiber photometry and single and multi-photon microendoscopic imaging. In summary, the talks in this panel will highlight various contemporary approaches used to record, characterize and manipulate astrocytes, which has furthered our knowledge regarding how astrocytes function in vivo to regulate behavior.

Keywords: Astrocytes, calcium, behavior

Symposium 17: Implantable Microfluidics for In-Vivo Neurochemical Monitoring

Chair: **Yi Zhang**, *University of Connecticut*

Speakers:

Yi Zhang, *University of Connecticut*

Michael J. Cima, *Massachusetts Institute of Technology*

Ian Bain, *University of Michigan*

Julie Stenken, *University of Arkansas*

Microdialysis-based probes have been widely used in both clinical and fundamental neuroscience. Unlike fast-scan cyclic voltammetry and genetically encoded sensors, microdialysis enables multiplexed neurochemical analysis when coupled with liquid chromatography–mass spectrometry (LC–MS). However, conventional microdialysis is constrained by relatively large probe diameters, low temporal resolution, and poor recovery of neuropeptides and proteins. This symposium will highlight emerging implantable microfluidic systems and experimental approaches that overcome these limitations. Topics will include membrane-free push–pull microsampling, wireless operation in freely moving animals, miniaturized probe architectures, and integration with advanced mass spectrometry for ultrasensitive neurochemical analysis. Emphasis will be placed on approaches enabling improved sampling of both small molecules and neuropeptides in behaving animals, as well as prospects for multimodal and closed-loop operation. By bringing together researchers across micro/nanofabrication, analytical chemistry, and neuroscience, this symposium aims to define current challenges and identify future opportunities for implantable microfluidics in molecular neuroscience.

Symposium 18: From Sensors to Signals: Overcoming Challenges in Measuring Spontaneous Dopamine Transients

Co-chairs: **James McCutcheon**, *UiT The Arctic University of Norway* & **Jamie Roitman**, *University of Illinois Chicago*

Speakers:

Rachel Donka, *University of Illinois Chicago*

Florian Schoukroun, *Washington University in St. Louis*

Emanuel Lopes, *Wake Forest University*

Jinsung Lee, *Hanyang University*

For years, fast-scan cyclic voltammetry (FSCV) was the leading method for measuring rapid neurotransmitter transients in vivo. Its development involved rigorous experiments to ensure electrochemical changes were correctly attributed to the target molecule, not to metabolites, pH

changes, or artifacts. Labs using FSCV were highly aware of potential errors, fostering collaboration between analytical chemistry and behavioral neuroscience.

Recently, fiber photometry has overtaken FSCV as the primary method for measuring neurotransmitters in behavioral neuroscience. Its ease of use has driven widespread adoption. While photometry offers features to enhance signal specificity, its readout remains indirect, with factors like receptor kinetics and optics potentially distorting measurements. A growing disconnect between labs using photometry and those developing sensors underscores the need for careful validation. In addition, electrochemical techniques continue to evolve and contribute important insights related to both tonic and phasic neurotransmitter levels.

This symposium features four early-career researchers addressing the methodological challenges associated with estimation of phasic and tonic neurotransmitter. **Rachel Donka** (University of Illinois Chicago) will compare three fluorescent dopamine sensors in response to opioid agonists, showing differences between them in capturing spontaneous dopamine transients, and one inconsistent with FSCV data. **Florian Schoukroun** (Washington University in St. Louis) will use another sensor to identify dopamine deficits linked to obesity and how pharmacological restoration of dopamine transmission can be directly visualized in real time. **Emanuel Lopes** (Wake Forest University) will discuss a method to assess DA uptake kinetics, and its application in exploring interactions between the kappa opioid receptor and the dopamine transporter. **Jinsung Lee** (Hanyang University) will introduce MCEWV, a novel electrochemical method to quantify basal dopamine levels, overcoming the limitation of current electrochemical tools in tracking tonic dynamics.

Together, these four talks will outline multiple challenges faced when estimating tonic and phasic levels of neurotransmitter and will show that open discussion and collaboration can propel the field forward.

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P2-39	Monitoring substance P dynamics in vitro and in vivo with a genetically encoded fluorescent sensor	Vittorio Sandri

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Poster #	Abstract Title	Presenter
P3-41	Effects of a muscarinic M4-PAM on striatal neurochemical dynamics	Gunnar Sorensen
P3-42	Fiber photometry reveals reduced dopamine signaling in diet-induced obesity	Florian Schoukroun
P3-43	Fast and sensitive analysis of dopamine, serotonin and other neurotransmitters in microdialysis samples with high time resolution, using the ALEXYS™ Neurotransmitter Analyzer	Martin Eysberg
P3-44	Translating FSCV to human model systems: real-time serotonin sensing in 3D iPSC-derived spheroids	Bettina Bohl
P3-45	A spatially and transcriptionally defined mu-opioid receptor-enriched MSN population mediates opioid reward learning	Olivia Drake
P3-46	Striatal lactate shapes dopamine release through cholinergic interneurons	Tony Barbay
P3-47	Investigating the representation of outcome controllability across valences in dopamine and serotonin signaling in the ventromedial striatum	Ipek Bolukbasi
P3-48	Real-time monitoring of dopamine dynamics in the Drosophila mushroom body in vivo using fast-scan cyclic voltammetry	Cheonho Park
P3-49	Green and Red GRAB Sensors for Real-Time Monitoring of Endocannabinoid Dynamics	Weijian Teo
P3-50	Cost shapes striatal dopamine value signals during sequentially revealed cost-benefit offers in rats	Lauren Burgeno
P3-52	A decrease in phasic dopamine precedes the rapid satiation of sodium appetite	James McCutcheon

Poster #	Abstract Title	Presenter
P3-53	Surface Functionalization of Carbon Nanotube Yarn Electrodes for Enhanced Dopamine Adsorption Kinetics and Tonic Dopamine Detection	Jaehyun Jang
P3-54	Development of a multi-analyte neurochemical monitoring unit	Faridat Abdulsalami
P3-55	Glycation-induced Dopaminergic Dysfunction in Parkinson's Disease Model Zebrafish	Keshala Palliyaguruge
P3-57	Tachykinin control of striatal neuromodulator release during reward learning	Michael Seibert

Poster Abstracts

Poster Session I | September 8

P1-1 - Histamine dynamics in wild type and orexin/ataxin-3 mice

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Histamine (HA) plays a crucial role in the regulation of sleep-wake cycles. However, the underlying mechanism behind this regulation remains unknown. By studying how HA release is modulated with wake-inducing compounds, we can understand its role in controlling sleep/wake cycles better. We investigated three different classes of wake-inducing compounds orexin receptor 2 (Ox2R) agonists, histamine-3 receptor (H3R) antagonists/inverse agonists and dopamine transporter/norepinephrine transporter inhibitors. Through these three distinct mode-of-actions we analyzed the compound-specific effects on extracellular histamine in wild-type mice using microdialysis and fiber photometry. We implanted male/female C57Bl/6J mice or orexin/ataxin-3 mice with either a microdialysis probe or with a GPCR-activation based GRAB-HA1h biosensor and optic fiber, targeting the hypothalamus. The experiments were performed in either the light or dark phase (inactive or active period). During the experiment the mice were administered with one of the wake-modulatory compounds or vehicle. In wild-type mice an Ox2R agonist increased HA levels in hypothalamus dose-dependently, a H3R antagonists/inverse agonist increased HA levels in hypothalamus dose-dependently, and a dopamine transporter/norepinephrine transporter inhibitor did not alter HA levels in hypothalamus dose-dependently. In orexin/ataxin-3 mice, an Ox2R agonist increased HA levels in hypothalamus more than in wild-type mice, whereas a H3R antagonists/inverse agonist increased histamine similarly in both genotypes. Both Ox2R agonists and H3R antagonists influence histamine release whereas compounds like dopamine transporter/norepinephrine transporter inhibitors do not

impact histamine release dose-dependently. The effects of a H3R antagonist appear independent of orexin signaling, showing similar profiles across both genotypes tested. In contrast, an Ox2R agonist elicits a genotype-dependent histaminergic response, suggesting heightened sensitivity in the absence of endogenous orexin in the orexin/ataxin-3 mice. These findings underscore distinct mechanisms by which these compounds influence arousal circuits.

P1-2 - Real-time, Voltammetric Co-Detection of Serotonin and Glucose at Carbon-Fiber Microbiosensors

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Serotonin plays an important role in the human gut-brain axis and has been implicated in diverse neuropsychiatric disorders including mood disorders (anxiety and depression), schizophrenia, substance abuse, and Parkinson's disease. Glucose is the major fuel source for the brain. As such, glucose availability and metabolism are undoubtedly linked to serotonin signaling and, thus, to circuit function. However, the understanding of the direct relationship between local glucose availability and serotonin transmission at discrete brain recording sites is lacking, largely due to challenges in the co-detection of these analytes. Historically, serotonin dynamics have been monitored using real-time electrochemical techniques. Glucose, which is not inherently electroactive, has been studied using microdialysis sampling with subsequent chemical analysis. These measurement strategies differ substantially in terms of spatial and temporal resolution, precluding direct correlation of the two chemical signals. In this work, carbon-fiber microbiosensors were used for simultaneous voltammetric detection of serotonin and glucose in rat striatum. Serotonin is naturally electroactive and generates a distinctive voltammogram that can be used for identification. In the presence of glucose, glucose oxidase immobilized on the sensor surface produces electroactive hydrogen peroxide, which generates its own signature cyclic voltammogram. Thus, the voltammetric strategy allows for simultaneous quantification of serotonin and glucose in live brain tissue, at the same place and time. The voltammetric biosensors have been characterized for sensitivity to both analytes and assessed for stability over the course of several hours. Selectivity against a range of potential interferents including dopamine, norepinephrine, and 5-hydroxyindole acetic acid has been thoroughly assessed. In vivo measurements demonstrate a direct relationship between evoked serotonin release and glucose availability at the same micron-scale recording sites in striatum, as both analytes scale with stimulation duration. Pharmacological validation using escitalopram, a selective serotonin reuptake inhibitor, prolongs the lifetime of both serotonin and glucose in the extracellular space. Overall, the tight temporal and spatial correlation between these signals demonstrates that glucose serves as a critical first responder that becomes immediately available to energetically support local serotonin signaling. These data, which directly link serotonin neurotransmission with local energetic availability, suggest that dysfunction in brain glucose metabolism could exacerbate the potential for neuropsychiatric disorders in which serotonin signaling is altered.

P1-3 - Dopamine transporters shape striatal dopamine axon activation and converge with GABA receptors to influence dopamine release

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Dopamine (DA) transmission plays a key role in reward, motivation, and action selection, and becomes dysregulated in substance-use disorders and Parkinson's disease. Mesostriatal DA neurons form extensive axonal arbours in the striatum, where DA signalling is regulated by diverse interacting mechanisms. DA transporters (DATs) not only govern the extracellular lifetime of DA, but also regulate striatal DA release probability and its short-term plasticity. DATs have been demonstrated as electrogenic in dissociated preparations and at cell bodies, but whether DATs exhibit electrogenic properties on striatal DA axons is poorly understood. If striatal DATs are electrogenic, it is not known what the functional consequences of these conductances might be for axonal spiking. We explored the impact that DATs exert on axonal membrane potential using a newly-developed, genetically-encoded fluorescent voltage indicator (ASAP5), which enabled us to measure changes in membrane potential in a population of DA axons in acute mouse striatal slices. We demonstrate that striatal DATs mediate an afterdepolarisation following axonal action potentials in dorsal striatum. We found that DAT-mediated changes to axonal membrane potential are greater in dorsal versus ventral striatum. DAT-mediated depolarisations limit subsequent axonal action potential size, consistent with DATs promoting short-term depression in striatal DA release. DAT-mediated currents are independent of DA D2 receptors, but might converge with γ -aminobutyric acid (GABA) receptor-mediated currents, because GABA receptors, like DATs, promote the afterdepolarisation following axonal action potentials in dorsal striatum. Furthermore, at the level of DA release, activity at GABA receptors supports DAT-mediated changes to DA release probability in dorsal but not ventral striatum, without changing DA uptake rates. Experiments are underway to understand whether DAT-mediated currents are dependent on DA release. Overall, these data indicate that DATs regulate DA axon activation in the striatum, and that the roles of DATs might intersect with other axonal regulatory partners, such as GABA receptors.

P1-4 - Decoding the Function of Cannabinoid Receptor 1 (CB1R)-Expressing GABAergic Interneurons in the Basolateral Amygdala During Fear Extinction

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Endocannabinoid signaling in the basolateral amygdala (BLA) is a key regulator of fear extinction, yet its effects on local inhibitory microcircuits remain poorly understood. While prior work has shown that

endocannabinoid signaling modulates glutamatergic cortical inputs to the BLA during extinction learning, it is unclear how cannabinoid signaling influences GABAergic interneurons that shape BLA output. Here, we identify and functionally characterize a molecularly defined population of CB1R-expressing GABAergic interneurons in the BLA and monitor their contribution to extinction-related circuit dynamics. Using RNAscope and immunohistochemistry, we found that gamma-synuclein (Snca) is selectively enriched in BLA interneurons that co-express GABAergic and CB1R markers and have minimal overlap with neurons expressing excitatory markers. This molecular profile enabled an intersectional viral strategy to target Snca+ GABAergic interneurons with improved specificity. Optogenetic inhibition of these cells during extinction impaired extinction learning, indicating that this interneuron population is required for normal extinction acquisition. In parallel, CRISPR-mediated disruption of CB1R in GABAergic interneurons impaired retrieval of extinction memory, demonstrating that cannabinoid signaling in inhibitory BLA circuits is necessary for adaptive fear suppression. To directly probe circuit output at the molecular level, we used fiber photometry-based monitoring of GABA release at BLA cortical output pathways. These recordings revealed that inhibitory signaling is dynamically regulated during extinction and is sensitive to endocannabinoid control. Together, these findings identify a molecularly defined CB1R-expressing interneuron population in the BLA and establish a framework for monitoring how endocannabinoid signaling shapes inhibitory transmission during fear extinction.

P1-5 - Distinct neuron-population activity dynamics in subregions of the medial prefrontal cortex during adaptive action control

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The inability to inhibit unwanted behavior is a recurrent trait in psychiatric conditions such as obsessive-compulsive disorder and addictions, suggesting dysfunctional promotion and inhibition of appetitive actions. The prelimbic (PL) and infralimbic (IL) cortices are thought to play complementary roles in action regulation, with evidence drawn from studies of addiction, fear conditioning, and habit formation that broadly support a canonical dichotomy in which PL generally promotes and IL generally suppresses or automatizes learned behavior. However, how individual neurons and their population activity in these regions encode behavioral promotion and suppression in real time on a trial-by-trial basis remains unclear. To address this question, we used one-photon calcium imaging to record neuronal activity in the PL and IL of rats performing a Go/No-go task. This task also incorporated a rule switch to a Differential Reinforcement of Omission (DRO) contingency, in which the previously reinforced Go (lever-press) response had to be omitted to earn a reward. Neuronal activity was measured using GCaMP6s expressed via AAV-DJ-CaMKIIa in 10 animals, yielding approximately 200 single units in each region across task phases. Our results show that while both PL and IL recruited similar relative numbers of behaviorally responsive neurons across task epochs and phases, population-level analyses revealed distinct dynamics between these regions, specifically

during DRO, despite largely overlapping representations during well-established behavioral performance. Chemogenetic inhibition further demonstrated a functional dissociation: simultaneous inhibition of both PL and IL during stable performance after task acquisition significantly impaired Go responses, whereas inhibition of either region alone had no effect. This indicates that PL and IL make redundant but collectively necessary contributions to action initiation once behavior is well established. In contrast, during DRO acquisition, selective inhibition of PL, but not IL, disrupted the suppression of the previously reinforced response, pointing to a specific role for PL in enforcing newly required behavioral policies under contingency change. Together, these results indicate that PL and IL exhibit convergent population dynamics during overtrained/asymptotic behavioral performance but dissociate during contingency-change-induced relearning, with PL playing a critical role in enforcing the updated relevant behavioral policy when active control is required. These findings challenge the canonical PL-IL dichotomy derived from studies of fear, habit and addiction, suggesting that functional differentiation within medial prefrontal cortex is not fixed but dynamically gated by the demands of the current behavioral context.

P1-6 - Simultaneous Monitoring of Dopamine and Serotonin Release in the Ventromedial Striatum during Reward Learning

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Dopamine and serotonin are key neuromodulators that shape motivated behavior and reward learning, and they represent major pharmacological targets in a wide range of psychiatric disorders, including addiction, depression, and anxiety disorders. Both neurotransmitter systems have been extensively studied in isolation, yet their coordinated activity and interactions remain poorly understood due to previous technical limitations. The ventromedial striatum (VMS), a central hub in reward processing, receives dense dopaminergic input from the Ventral Tegmental Area and serotonergic projections from the Dorsal Raphe nucleus (DRN). However, it is unknown whether the relationship between dopamine and serotonin in VMS is competitive or complementary, and whether this relationship stays consistent across time and contexts. To address this question, we utilized fiber photometry to simultaneously record dopamine and serotonin release in VMS, using genetically encoded sensors rGRAB-DA3m and GRAB-5HT. We validated serotonin sensing by optogenetically stimulating serotonergic neurons in the DRN in anesthetized TPH2-Cre rats while recording neuromodulator activity in VMS. To further validate signal specificity, recordings were performed before and after administration of the selective-serotonin reuptake-inhibitor escitalopram. Optogenetic stimulation of the DRN reliably increased both dopamine and serotonin release, consistent with the functional connectivity of these two neuromodulator systems. Importantly, escitalopram selectively enhanced serotonin activity. In our second experiment, we used fiber photometry to record dopamine and serotonin activity in freely behaving rats performing an appetitive Pavlovian-conditioning task. Animals were trained to associate one conditioned stimulus (CS+) with the delivery of a food reward, while a second stimulus (CS-) was not reinforced. Over the

course of multiple training sessions, serotonin activity in VMS emerged selectively in response to the CS+, exhibited a ramping profile during cue presentation, and an additional increase following reward delivery. Dopamine signals displayed the canonical learning-related shift from reward-evoked responses early in training to mainly cue-evoked responses as cue-reward associations were acquired. These findings suggest that during Pavlovian-reward learning, the relationship between striatal dopamine and serotonin might not reflect opposition, but complementary aspects of predictive reward learning, because reward produces increased release of both transmitters and because of the differential time course. Similar to dopamine, serotonin activity seems to be modulated across time, as a function of learning. Together, these findings establish a framework for simultaneously monitoring dopamine and serotonin activity in vivo and reveal distinct yet overlapping patterns of activity during reward learning.

P1-7 - Region-specific relationship between striatal dopamine and temporal-difference reinforcement-learning during temporal discrimination

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Dopamine release across striatal subregions is widely implicated in reinforcement learning, action selection, and behavioral control, yet the extent to which regional dopamine dynamics conform to classical temporal-difference reward-prediction error signals remains debated. This issue is particularly important because the ventromedial striatum (VMS), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) are associated with partially distinct Pavlovian and instrumental functions and may therefore exhibit different dopamine dynamics during the same behavior. To address this, we measured dopamine release using fast-scan cyclic voltammetry in rats performing a temporal discrimination task, in which animals had to estimate elapsed time and switch between levers depending on whether a short or long interval was presented. Rats performed the task accurately and showed temporally structured switching behavior consistent with noisy internal timing. We developed a temporal-difference reinforcement learning model in which the agent's internal clock speed varied across trials, allowing the model to capture both the animals' timed behavior and the temporal structure of reward prediction errors. We then transformed the model-derived temporal-difference prediction errors into predicted dopamine release traces, which were compared with the experimentally recorded dopamine dynamics in the VMS, DMS, and DLS. The model captured key features of dopamine dynamics in the VMS and DMS, suggesting that population-level dopamine release in these regions is well approximated by classical reward prediction error-like computations during temporal discrimination. In contrast, dopamine dynamics in the DLS were only partially explained by the model. Across task variants, apparent qualitative differences in dopamine responses between VMS and DMS could be partly explained by quantitative differences in discounting and variability in internal clock speed. We further found that asymmetric scaling of positive and negative

temporal-difference errors improved the correspondence between model predictions and dopamine release, particularly under probabilistic reward conditions. Trial-by-trial variation in internal clock speed also generated distinct temporal-difference error trajectories, which were associated with corresponding variation in dopamine dynamics, with the strongest empirical relationship observed in the VMS. Together, our results suggest that temporal-difference learning provides a strong framework for understanding striatal dopamine release, while also highlighting specific cases in which model predictions deviate from the observed dopamine dynamics.

P1-8 - Electrochemical detection of serotonin in sert mutant *Drosophila* larvae

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Drosophila melanogaster (fruit fly) is an excellent model to study neurotransmitter dynamics in neurological disorders, such as depression and Parkinson's disease. 5-Hydroxytryptamine (5-HT), or serotonin, is an electroactive neurotransmitter strongly associated with these conditions, and its signaling is tightly regulated by the serotonin transporter (SERT), which mediates reuptake from the synaptic cleft. Dysregulation of SERT can contribute to disease development by not allowing the reuptake of serotonin to occur efficiently. Selective serotonin reuptake inhibitors (SSRIs), the most widely prescribed antidepressants, act by blocking SERT to help increase the amount of circulating serotonin. To investigate these dynamics in real time, we utilized fast-scan cyclic voltammetry (FSCV), a technique that enables real-time detection of neurotransmitter changes on a sub-second timescale. In parallel, optogenetics was used to precisely control serotonin release. This combined technique provides a powerful approach to evaluate the role of SERT in regulating serotonin signaling. This study aims to investigate the impact that genetic alterations in the SERT gene have on serotonin release and reuptake. Two mutant *Drosophila* lines were examined: dSERT16, a partial loss-of-function mutant with deletion of the promoter region that reduces dSERT expression, and dSERTΔ3.9, a complete loss-of-function mutant missing 3.9 kb of the dSERT coding sequence. The dSERTΔ3.9 mutant exhibited reduced serotonin signaling. Although serotonin levels slowly decreased towards baseline following stimulation, the total amount released was significantly diminished, suggesting impaired serotonin recycling due to loss of transporter function, which in turn leads to depletion of presynaptic serotonin available. In contrast, dSERT16 displayed a moderate slowing of reuptake kinetics but retained some transporter activity to maintain releasable serotonin pools, resulting in signals that returned to baseline with less reduction of available serotonin. To further test SERT-dependent regulation, escitalopram was evaluated across wild-type and mutant lines. We observed that escitalopram significantly inhibits serotonin reuptake in wild-type flies, prolonging extracellular serotonin signals, while producing lessened effects in dSERT16 and no impact on dSERTΔ3.9, where transporter function is already reduced or absent. Chronic escitalopram exposure via feeding over a 24, 48, and 72-hour period, revealed no effect on serotonin reuptake; however, with a 72-hour exposure, there was minimal serotonin being released. Thus, chronic escitalopram depletes serotonin in larvae. Future

studies could examine an adult fly brain model. With the adult fly, we will be able to assess even longer-term administration, anywhere between one week to one-month. Thus, providing insight into the efficiency of SSRIs in the presence of SERT dysregulation and the role these mutations play in neuropsychiatric disease.

P1-9 - Insulin receptors and acetylcholine are required for insulin's effects on striatal cholinergic interneurons, dopamine release, and dopamine-dependent nutrient sensing

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Insulin enters the brain from the periphery after ingestion to act as a satiety signal in the hypothalamus. Our previous studies, however, showed that insulin also amplifies evoked dopamine (DA) release in ex vivo rat striatal slices through a local mechanism involving striatal cholinergic interneurons (ChIs) and activation of nAChRs on DA axons. Pharmacological studies indicated these effects were mediated by insulin receptors (InsRs). Here we used patch-clamp recordings and fast-scan cyclic voltammetry in transgenic mouse lines to confirm specific involvement of InsRs on ChIs and to explore mechanisms underlying insulin's effects on ChIs. First, we established mouse striatal ChIs express InsR mRNA using RNAscope, as well as InsR protein using immunohistochemistry. We then generated a mouse line that selectively lacks InsRs in cholinergic neurons (conditional InsR knockout, InsR-cKO) to test whether InsRs in ChIs was sufficient for the boosting effect of insulin on striatal DA release in slices. We found that the effect of physiological concentrations of insulin (30 nM) on evoked DA release was lost in InsR-cKOs. In patch-clamp recordings, insulin increased action potential number in ChIs during 3 s depolarizing current injections, indicative of increased cell excitability. Unexpectedly, the effect of insulin on ChI excitability was absent in mice lacking forebrain choline acetylcholinesterase (ChAT-cKO mice) and thus unable to synthesize ACh. These data indicate involvement of a self-regulatory mechanism by which ACh is necessary to propagate insulin's ability to enhance ChI excitability. Our current evidence points to the possible involvement of K⁺-channel closure as the underlying ionic mechanism in insulin-induced enhancement of ChI excitability. We are testing two candidate channels that are regulated by ACh, expressed in ChIs, and are known to govern ChI excitability. 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-cKO mice and their littermate controls. Mice were given 1 h access to a solution containing either 6% glucose (nutritious) or equally sweet 0.25% saccharin (non-nutritious) on alternating days. As predicted, consumption of glucose escalated over pairs of sessions in controls, whereas escalation of saccharin intake was less pronounced. In ChAT-cKO mice, however, we found no preference for glucose versus saccharin over the same period. Importantly, a similar deficit in nutrient sensing was seen when InsR-cKO mice were tested in the same paradigm. 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. Moreover, this local circuit plays an important role in nutrient sensing, providing further evidence that insulin is a nutrient reward signal.

P1-10 - 3D paper device for neuronal cell culture and neurochemical analyses

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In vitro cell culture is a well-known technique that has enabled and continues to enable the study of biological phenomena at the cellular level. Understanding cell metabolism and functions is key to our understanding of diseases and the development of new treatments. However, classic 2D cell culture techniques are limited in their description of biological systems and networks, as cell interactions and tissue architecture are inherently 3D. Such 3D architecture is crucial for the brain, in which large, complex 3D networks are controlled by neurotransmitters and other chemicals. Paper is a powerful substrate to develop new, easy-to-use techniques for 3D cell culture. Biocompatible, with a natural extracellular matrix-like fiber structure and available in all laboratories, paper cell culture can represent an economical 3D cell culture model. We first tested the hypothesis that by modifying the paper fiber surface with protein, the adherence of cells can be enhanced, thus increasing their survival. With this substrate, we were able to maintain murine primary cortical and mesencephalic neuron cultures for more than 14 days, and primary cortical glial cell cultures for more than 21 days. To validate the protocol, we used confocal microscopy, showing that glial cells are colonizing the paper and that neurons establish a complex network of connexions in 3D (Fig.1). We have also used GCaMP, a genetically encoded intracellular calcium indicator, to validate that the neurons in this model are physiologically active. Using GRABDA, a genetically encoded dopamine fluorescent sensor, we also validate that dopamine release can be detected in such 3D mesencephalic neuron cultures. We conclude that the device is compatible with fluorescent microscopy and with the use of various fluorescent cellular sensors. We propose that this device will be helpful to facilitate the study of neuronal networks in 3D in controlled and highly reproducible conditions. As a material, paper can be made conductive with chemical treatments or physical alterations. With this improvement of the electrical properties of paper, we hypothesize that we will be able to produce electrochemical devices that can monitor molecules including electroactive neurotransmitters such as dopamine and serotonin. With techniques derived from printed electronics, we plan to print circuits and develop paper chips compatible with cell culture and the use of fluorescence microscopy. We have already been able to characterize paper electrodes for allowing the detection of dopamine and serotonin with cyclic voltammetry. Modifying the electrode fabrication protocol by varying the conductive ink used can change its electrical properties, making it possible to control the resistive/capacitive properties of paper electrodes. This work established the basis for a new approach to study neuronal dynamics in vitro in a 3D model, an environment more faithful to biological systems compared to classic 2D cell culture.

P1-11 - Cocaine behavioral and neurochemical phenotype of CDase knock-out mice

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Lipid topology modifies both pre- and post-synaptic morphological specializations and regulates the membrane localization, function and affinity of certain neuronal ion channels and neurotransmitter receptors, as well as the vesicular exocytotic machinery within the active zone. One regulator of lipid topology is ceramidase (CDase), an enzyme that cleaves ceramides into bioactive sphingolipids. Herein, we examined the cocaine behavioral and neurochemical phenotype of CDase knock-out (KO) mice. Relative to wild-type (WT) controls, KO mice exhibited a larger cocaine-conditioned place-preference (5 and 10 mg/kg), with no difference in the expression of sensitized locomotor activity. No net-flux in vivo microdialysis conducted in the nucleus accumbens failed to detect genotypic differences in basal dopamine content. However, KO mice exhibited blunted cocaine-induced increases in both dopamine and serotonin, with no change in DAT or SERT expression detected. These data reveal a key role for CDase in gating stimulated vesicular monoamine release within the nucleus accumbens to influence the conditioned rewarding properties of cocaine.

P1-12 - Impact of mitochondrial calcium uniporter (MCU) knockout on dopamine axon physiology

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The motor symptoms of Parkinson's disease arise from degeneration of substantia nigra dopamine neurons projecting to the dorsolateral striatum, whereas neighbouring ventral tegmental area neurons projecting to the nucleus accumbens are relatively resistant. While both populations exhibit autonomous pacemaker activity, distinct spike-associated Ca²⁺ dynamics and buffering are thought to underlie their differential vulnerability. Mitochondrial Ca²⁺ uptake via the mitochondrial Ca²⁺ uniporter (MCU) plays a central role in coupling activity-dependent Ca²⁺ oscillations to ATP production and in buffering cytosolic Ca²⁺. However, its contribution to presynaptic function and dopamine release across vulnerable and resistant striatal territories remains unclear. We used a dopaminergic neuron-specific MCU knockout mouse line and performed multimodal ex vivo recordings in striatal slices, combining voltage imaging (ASAP5f) to monitor membrane depolarisation, calcium imaging (jRCaMP1.2) to measure intracellular Ca²⁺ dynamics, and fast-scan cyclic voltammetry (FSCV) to quantify evoked dopamine release. Despite loss of MCU, evoked dopamine release was preserved, while spike waveform properties and Ca²⁺ signalling showed modest, region-specific alterations. In the dorsolateral striatum, MCU KO mice exhibited larger spike amplitudes, increased afterdepolarisations, and greater Ca²⁺ transient amplitudes and integrals relative to age- and sex-matched controls. In contrast, in the nucleus accumbens, evoked spike and Ca²⁺ transient

amplitudes and integrals were reduced. Together, our findings indicate that mitochondrial Ca^{2+} uptake in dopamine axons is not strictly required to sustain dopamine transmission, yet its deletion elicits region-specific compensatory adaptations, suggesting an important functional role. The distinct intrinsic Ca^{2+} dynamics of vulnerable substantia nigra and resistant ventral tegmental area dopaminergic neurons suggest that these populations face different physiological demands in maintaining basal dopamine transmission. MCU knockout likely imposes additional challenges related to Ca^{2+} buffering and cellular energetics, thereby revealing mechanisms on which these populations differentially rely. Further elucidation of these mechanisms may provide insight into the selective vulnerability observed in Parkinson's disease.

P1-13 - A critical role for midbrain dopamine neuron inhibition in punishment learning

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Midbrain dopamine neurons have been ascribed a key role in modifying behaviour on the basis of its consequences, including learning to avoid harmful actions ("punishment learning"). Canonical accounts argue pauses in ventral midbrain dopamine neuron (VTADA) activity as a mechanism for punishment learning. However, there has been limited direct evidence for this account. In a series of studies using optogenetics, chemogenetics, in vivo pharmacology, and fibre photometry, we show that inhibition of VTADA neurons is both necessary and sufficient for punishment learning. We show that response-elicited (but not response-independent) pauses in VTADA activity drives punishment-like avoidance. Conversely, blocking VTADA inhibition (via GABA-A antagonist bicuculline) or chemogenetically activating VTADA (via excitatory designer receptor hM3Dq) during initial punishment training causes enduring deficits in punishment avoidance. Interestingly, these manipulations did not drive enduring deficits if given after initial training, indicating a specific role for VTADA pauses in learning-mediated plasticity. Using fibre photometry to record VTADA neuron activity and inhibitory GABA input, we show dissociated neural dynamics to action-elicited outcomes that conform with reinforcement- versus punishment-coding. Together, these findings show inhibitory dynamics within VTADA circuits plays a critical role in promoting adaptive avoidance of harmful outcomes.

P1-14 - Dissecting cortical control over palatable food intake: role for dopamine-receptive projections to the nucleus accumbens and the basolateral amygdala

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Background: Stressful events can drive towards impulsive actions and increased consumption of palatable foods. The prefrontal cortex (PFC) plays a critical role in regulating palatable food intake and responses to stress, although its precise mechanism is still unclear. Dopamine fluctuations might play an important role. Notably, dopamine-sensitive PFC neurons project to various brain regions, including the basolateral amygdala (BLA) and the nucleus accumbens (NAc). We hypothesize that these distinct PFC projections play differential roles in stress-induced overeating and impulsivity. Experimental design: Using viral vectors, we enabled pathway-specific chemogenetic manipulation and photometric recordings in male C57BL/6 and DrD1-cre mice. Palatable food intake was measured by consumption of high-fat food, while impulsive behaviors were assessed using the 5-choice serial reaction time task. Stress was induced through social defeat or yohimbine administration. Results: Photometric recordings of dopamine dynamics in the PFC revealed increased dopamine release in response to both rewarding stimuli (e.g., fat intake) and stressful experiences (e.g., social defeat and foot shock). The mPFC D1 neurons project most abundantly to the BLA and NAC. Incubation of dopamine increased excitability in the PFCD1 → BLA pathway, while it reduced PFCD1 → NAC excitability. In-vivo recordings of the pathways revealed an inhibition of the PFCD1 → NAC pathway during palatable food intake, while the activity of the PFCD1 → BLA pathway was not altered. In contrast, both pathways were activated during a foot shock. Chemogenetic activation of dopamine-sensitive PFC → BLA projections increased fat intake, while stress-induced impulsivity remained unaffected. In contrast, activation of PFC → NAc projections reduced impulsive actions induced by stress, without affecting food intake. Conclusions: Our findings demonstrate that distinct PFC projections to the NAc and BLA differentially modulate dopamine and palatable food intake. These results contribute to the development of targeted interventions for stress-related disorders.

P1-15 - Activation of nicotinic receptors on striatal dopamine axons attenuates axonal spiking via prolonged depolarization and Na⁺-channel inactivation

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Striatal dopamine (DA) and acetylcholine (ACh) play key roles in reward prediction signalling and goal-directed behaviour and also directly interact. Activation of nicotinic ACh receptors (nAChRs) on DA axons can depolarise DA axons and if reaching a sufficient threshold, can ectopically generate axonal action potentials and DA release events in mouse brain slices ex vivo, as well as in vivo in some

cases. However, activation of nAChR on DA axons has also been shown to cause a subsequent short-term depression (STD) of DA release that is not due to vesicular depletion. To understand underlying mechanisms of the nAChR-mediated STD of DA release, we tested whether nAChR activation compromised subsequent spiking and Ca²⁺ transients in DA axons, by imaging genetically encoded fluorescent reporters for membrane voltage (ASAP5) and intracellular calcium (jRCaMP1.2) in populations of DA axons in acute slices of mouse striatum. Brief stimulation of ACh input to nAChRs depolarized DA axons for a more prolonged duration (~140 ms) than directly activated intrinsic spike-like depolarizations (~a few ms). During nAChR-mediated depolarization, there was significant attenuation of successive intrinsic spikes and associated calcium transients, indicating that spike attenuation by nAChRs underlies the STD of ensuing DA release. This spike attenuation was not present in absence of nAChRs control. Because depolarization recruits voltage-gated sodium channels (VGSCs), we tested whether STD in DA release arises from a subsequent limitation to the pool of VGSCs available to mediate axon reactivation. Using fast-scan cyclic voltammetry to detect DA release, we found that its STD was more sensitive to modest inactivation of VGSCs after nAChR activation. In summary, these data show that extended depolarization of DA axons by nAChRs attenuates subsequent direct spikes, Ca²⁺ transients and DA release by transiently limiting the VGSCs available for reactivation. This mechanism of axonal refractoriness could underpin a dynamic, analogue scaling inhibition of spike-evoked DA release level according to recent history of activity in cholinergic interneurons.

P1-16 - NEURO-INFLAMMATION markers studied with push pull microdialysis in mouse prefrontal cortex using MSD and shotgun proteomics workflows

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Background: Neuroinflammation is a key event in many neurodegenerative diseases. Frequently, neuroinflammation is studied after systemic administration of lipopolysaccharide (LPS) or benzoyl ATP (Bz-ATP). The systemic effects of these drugs might obscure the central cascades that are relevant in neurodegenerative diseases. In the current study, we evaluated local effects of neuroinflammation without interference of systemic effects. For this purpose, neuroinflammation markers were studied using gravitational push pull microdialysis with local administration of LPS and Bz-ATP in the intracerebral fluid (ICF). Methods: Mice (C57 Black6) were implanted with microdialysis guides in the prefrontal cortex and an ICV cannula. The animals were allowed to recover for at least 2 weeks. 24 hours prior to the experiment, a push pull microdialysis probe (PES 2 mm open surface, 200 kDa, Micron Bio BV) was slowly inserted into the guide. Push pull probes were connected the following day with PEEK tubing as inlet and FEP tubing as outlet. The collector was placed below the cage to ensure the correct gravitational pull. Microdialysis probes were perfused at 0.5 µL/min with aCSF containing 0.2% albumin. After a 2 hours stabilization period, perfusion flows were checked and samples were collected at hourly intervals. After the collection of one basal sample, LPS was administered in the ICF and followed after two hours by Bz-ATP infusion. Perfusion was continued for an additional 5 hours to

monitor local inflammatory markers. Analysis: Inflammatory markers TNF-alpha, TNF-gamma, IL1-beta, IL-6, IL-10 & KC/GRO were analyzed using a custom S-PLEX Proinflammatory Panel 1 kit (MSD Catalog # K152AKV). Additionally, samples were analyzed with an unbiased proteomics workflow. Proteins were extracted, cleaned-up and digested with trypsin. The tryptic digest was then injected onto an analytical column (Pepsep C18 15 cm x 150 µm x 1.5 µm) with an Evosep One nanoflow LC run at 30 SPD, detection was done by a high-resolution mass spectrometry (Bruker TimsTOF HT) in DIA-PASEF acquisition mode. Data analysis was performed using Spectronaut software. Results: Significant increases of all measured neuroinflammation markers were induced by the local administration of LPS and Bz-ATP. These observations allow us to modulate and study local neuroinflammatory effects. The study shows differences in the dynamics of the monitored markers, where some markers increased instantly upon LPS administration, other inflammatory markers only showed effect when combined with Bz-ATP administration. Shotgun proteomics allowed central monitoring of a wide array of proteins, which were affected by central administration of LPS and Bz-ATP. This could shed further light on the cascades of neuroinflammation and the pathology of neurodegenerative diseases.

P1-17 - Regulation of subsecond extracellular purine signaling by nucleoside transporters in reactive astrocytes

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Significance/Objectives: Reactive astrocytes are increasingly recognized as active regulators of brain inflammation and repair, rather than passive support cells. However, the molecular signals that define their functional state have not been fully elucidated. Purinergic signaling has come to the forefront as a key process in the regulatory network. Specifically, adenosine and guanosine influences inflammation, cell survival, and intercellular communication. These purines signals are highly dynamic therefore tools that can monitor extracellular purine changes in real time are essential for understanding how astrocytes respond to injury and therapeutic modulation. Nucleoside transporters, including equilibrative and concentrative transporter families, help control extracellular purine availability by regulating nucleoside movement across the astrocyte membrane. We hypothesize that guanosine administration modulates the phenotype and purinergic signaling of reactive astrocytes, promoting a more anti-inflammatory state. Methods: Primary cultured astrocytes were induced into a reactive phenotype utilizing inflammatory agents and treated with guanosine. Flow cytometry was used to assess astrocyte phenotype, quantitative PCR was used to measure transporter- and inflammation-related gene expression, and fast-scan cyclic voltammetry was used to monitor rapid extracellular purine dynamics. Results: Preliminary data suggest that guanosine treatment alters the phenotype of reactive astrocytes and shifts purine release dynamics. Early analyses indicate changes in inflammatory marker expression and differences in extracellular purine signaling following guanosine administration. Ongoing transporter expression analyses will help determine whether these functional effects are associated with altered nucleoside transport. Conclusion: These preliminary findings suggest that guanosine modulates reactive astrocyte behavior

and may reshape purinergic signaling in ways that favor an anti-inflammatory phenotype. Ongoing studies will clarify nucleoside transporters contribute to these effects and help define how guanosine influences molecular signaling in reactive astrocytes.

P1-18 - MAVEN: a compact, integrated platform for real-time multimodal neurochemical and electrophysiological monitoring

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Advancing our understanding of neurological disorders requires the ability to monitor neurochemical and electrical dynamics simultaneously. To bridge the gap between benchtop research and clinical needs, we developed MAVEN (Multifunctional Apparatus for Voltammetry, Electrophysiology, and Neuromodulation), a compact and portable platform for integrated multimodal sensing and modulation. As a comprehensive research tool, MAVEN supports various voltammetry waveforms, including the previously developed MCSWV (Multiple Cyclic Square Wave Voltammetry) for absolute concentration of dopamine sensing and N-MCSWV (N-shaped MCSWV) for absolute concentration of serotonin monitoring, and enables concurrent electrophysiological recording with synchronization for both electrical stimulation and external devices. Engineered with robust safety features to facilitate transition into clinical research, the platform is currently being validated in various studies related to psychiatric disorders, such as addiction and depression. These efforts focus on leveraging MAVEN's integrated electrochemical sensing, electrophysiological monitoring, and neuromodulation. Furthermore, recent data demonstrate its robust performance in real-time monitoring of various pharmacological agents, highlighting its potential for broader point-of-care diagnostics and human clinical applications. These findings position MAVEN as a next-generation platform for developing personalized, closed-loop clinical strategies across a wide range of bio-sensing and therapeutic contexts.

Poster Session II | September 9

P2-21 - Serotonin Activation in a Perceptual Decision Task: Large-Scale Additive Impact on Neural Population Activity with Preserved Behavioral Performance

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Serotonin (5-HT) is a central neuromodulator which is implicated in, amongst other functions, cognitive flexibility. 5-HT is released from the dorsal raphe nucleus (DRN) throughout nearly the entire forebrain. Little is known, however, about how serotonin affects downstream populations of neurons and how this modulation might support its cognitive functions. Here, we optogenetically stimulated serotonergic neurons in the DRN while recording large parts of the brain with Neuropixels during quiet wakefulness and performance of a perceptual decision-making task. During quiet wakefulness, 5-HT stimulation induced a rapid switch in internal state, as indicated by dilation of the pupil, suppression of hippocampal sharp wave ripples, and increased exploratory behaviors, such as whisking. To elucidate the brain-wide effect of optogenetic activation of serotonergic neurons on single neuron spiking activity, we performed acute Neuropixels recordings across seven target trajectories, recording 7,478 neurons across 17 mice. We found that 5-HT stimulation significantly modulated neural activity in all recorded brain regions, both during quiet wakefulness and task performance. During task performance, however, activation of serotonergic neurons had no effect on behavior. Using a generalized linear model, we show that serotonergic modulation combines additively with choice-related activity, as opposed to a multiplicative gain modulation; the choice-by-stimulation interaction was near zero across regions and animals. As a geometric consequence of this additivity, 5-HT modulation was confined to a subspace orthogonal to the choice axis. These findings suggest that serotonin can reconfigure distributed neural dynamics without interfering with ongoing task computations.

P2-22 - Co-Detection of Striatal DA and Glutamate In Vivo at a Single Microbiosensor using Fast-Scan Cyclic Voltammetry

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FDA-approved pharmacological treatments for stimulant-use disorders are essentially non-existent, as the mechanisms by which these drugs of abuse drive maladaptive behaviors remain unclear. This gap in our understanding limits treatments to symptomatic relief rather than targeting the underlying mechanisms that drive the dysfunction itself. A large body of research has explored the individual role(s) played by a wide range of neurochemicals in driving addictive pathologies. However, neurochemical dynamics are spatially and temporally heterogeneous, and it is likely that coordinated signaling by multiple species working together is important to influence circuit function. Dopamine

(DA) and glutamate, for example, are two essential neurotransmitters implicated in the development and maintenance of substance use disorders. Both are released in major projections to the ventral striatum, an important brain region central to regulating reward-seeking behaviors and motivation. Precisely timed DA transients in the nucleus accumbens contribute to experience-dependent plasticity by signaling unexpected positive or negative outcomes to shape learning. Similarly, glutamate signals associated with drug-related cues are thought to strengthen drug-related memory to influence decision-making and impulse control, contributing to compulsive drug-seeking and relapse. The timing of these signals is key to driving functional output; however, there is a critical gap in understanding the dynamics of DA and glutamate, relative to one another, at specific recording sites. Therefore, this work uses fast-scan cyclic voltammetry coupled with glutamate microbiosensors to characterize the simultaneous release and reuptake of DA and glutamate at single micron-scale recording sites in anesthetized rat striatum. Initial experiments provide an unprecedented look at reciprocal modulation in real-time by pharmacological manipulation with either a DA or glutamate reuptake inhibitor—cocaine or DL-TBOA, respectively. Since the timing of these neurochemical signals is likely essential in shaping reward and memory circuits in an experience-dependent manner, quantitative characterization of these signals will provide fundamental insight for developing targeted treatment strategies that extend beyond symptom relief.

P2-23 - Imaging of Catecholamine Uptake and Release by Immune cells using near infrared fluorescent nanosensors

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Near infrared (NIR, 800 nm -1700 nm) fluorescence imaging promises ultra-low background and scattering (tissue transparency window). We use nanomaterials such as NIR fluorescent single-walled carbon nanotubes (SWCNTs) as building blocks to create advanced sensors/probes and use them to understand chemical communication between cells. Here, I will present novel optical approaches and sensors that extract or provide more information. One example is fluorescence lifetime imaging microscopy (FLIM) of SWCNT-based sensors as absolute and calibration-free NIR imaging method for dopamine. This technique is enabled by laser scanning confocal microscopy (LSCM) optimized for NIR signals (>800 nm) and time correlated single photon counting (TCSPC). Another example are ratiometric fluorescent sensors for catecholamine neurotransmitters, which increase the overall robustness of imaging. Finally, I will show that catecholamines are also important in the immune system⁵. Neutrophils are key effector cells of the innate immune system that respond to small signaling molecules regulating immune responses. For a long time, similarities between neuronal and immune cells have been discussed. Here, we show that human neutrophils rapidly take up, package and use catecholamine neurotransmitters such as dopamine or epinephrine via the machinery known from neurons. Uptake and release of catecholamines as well as trafficking and packaging into MPO/VMAT2-positive primary vesicles is visualized with false fluorescent neurotransmitters. We also directly image the fast (> 10 s) and transient release of catecholamines from neutrophils with near

infrared fluorescent nanosensors. Serotonin or activated platelets trigger calcium (Ca²⁺) signaling and consequently exocytosis of catecholamines. They reduce NET-formation but increase platelet aggregation. Thus, we establish similarities between neurons and neutrophils and identify a paracrine neutrophil-platelet feedback loop relevant for inflammatory and coagulatory conditions.

P2-24 - Monitoring real-time dopamine release in vivo - Comparison of electrochemical and fluorescent methods

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Striatal dopamine release is essential for motivation, movement, and reward processing. The most studied regions in the striatum, the limbic ventromedial striatum (VMS) and the sensorimotor dorsolateral striatum (DLS), are thought to have different neurotransmission profiles and distinct behavioral functions. Currently, two state-of-the-art techniques are utilized to monitor real-time dopamine release in the brain: fiber photometry (FP) and fast-scan cyclic voltammetry (FSCV). Measurements obtained with these techniques diverge frequently, even when collected in similar behavioral tasks. It is imperative to understand the source for these discrepancies in order to integrate findings and to generate a more complete picture of dopamine function. Here, we systematically investigate the differences between in-vivo FSCV and FP measurements by combining behavioral and anesthetized recordings – the latter enabling more precisely controlled dopamine release. Our results confirm that both techniques are capable of measuring dopamine. However, when we increase the delivered reward magnitude during recordings in a behavioral dose-response experiment, FSCV signals lengthen and exhibit increased release peaks, whereas FP signals only lengthen without increased peak release. However, anesthetized recordings show that it is possible to electrically elicit and measure increased dopamine peaks with FP, albeit not in a linear dose-response manner. Aversive stimuli elicit decreases in dopamine measures by both techniques, but decreases are more sustained in FSCV and more immediate for FP. The pharmacological inhibition of dopamine transporters affects FSCV measurements substantially more compared to FP. Similarly, the indirect manipulation of dopamine transmission through antagonizing nicotinic acetylcholine receptors increased dopamine peaks measured with FSCV, while the effect on FP recordings was small. Antagonizing dopamine D2 receptors increases dopamine peaks sensed with both FSCV and FP, but the effect is weak in FP and more prolonged in FSCV recordings. Taken together these findings imply that FSCV and FP are not interchangeable measures of dopamine neurotransmission and may reflect distinct signaling aspects. Dopamine signals quantified with FSCV capture a wider dynamic range, since signals are proportional to reward magnitude, return to baseline concentrations slower, and are more sensitive to dopamine transporter inhibition and autoreceptor blockade. By comparison, FP recordings displayed faster temporal dynamics with limited peak scaling and less sensitivity than expected to pharmacological manipulations targeting dopamine transmission via different

mechanisms. Together, this supports the idea that FSCV recordings reflect volume transmission of dopamine, while FP recordings capture dopamine release more proximal to release sites.

P2-25 - How temporal predictability and reward value interact to shape dopamine reward prediction errors

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Adaptive behaviour relies on learning from experience and predicting outcomes. Environmental cues become predictive signals for rewards, enabling behavioural updates when predictions are violated. These reward prediction errors (RPEs) are encoded by striatal dopamine release in the ventromedial striatum (VMS). During Pavlovian conditioning, dopamine release transfers from reward delivery to reward-predictive cues. Cue-evoked dopamine is influenced by multiple reward features such as magnitude, probability, and temporal predictability, which are often studied in isolation. However, in complex real-life environments, cue-evoked dopamine release likely reflects the combination of these features. In this study, we investigate how the combination of changes in temporal predictability and reward magnitude shape dopamine-encoded reward prediction errors. Using chronic fast-scan cyclic voltammetry (FSCV), we recorded dopamine release in the VMS during Pavlovian conditioning using male and female rats. We manipulated the temporal predictability of reward-predictive cues (presented for 5s before reward delivery) by using 30-second fixed versus variable inter-trial intervals (ITIs), while maintaining a constant reward rate between fixed and variable schedules. Our results reveal that rats can learn to accurately track 30-second fixed intervals, but not variable intervals. As expected, lower temporal predictability and larger reward magnitude both lead to greater cue-evoked VMS dopamine release. This effect emerges across learning in both male and female rats. However, when temporal predictability and reward magnitudes are both varied in the same task, dopamine release elicited by rare high-value cues is insensitive to temporal predictability. Presenting high-value cues in isolation, making cue identity and reward value predictable, rescues sensitivity to temporal predictability. Thus, when multiple reward-expectation features that contribute to dopamine reward prediction errors are combined, they do not necessarily interact in an additive or linear fashion. This work demonstrates that reward prediction errors encoded by dopamine release may be dominated by the features that provide the greatest reward expectation value.

P2-26 - Characterizing stimulated and behaviorally-evoked Neuropeptide Y release in the Nucleus Accumbens

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With the advent of genetically expressed sensors new opportunities have arisen to monitor previously unattainable analytes like neuropeptides. One such genetically expressed sensor, GRAB-NPY 1.0 has been developed for monitoring dynamic NPY release in the CNS. While previous studies on the function of NPY has been limited to exogenous application, transgene expression, or knock-out approaches combined with pharmacological manipulations, direct measurements in vivo have remained elusive. Indeed, previous studies measuring NPY with GRAB-NPY 1.0 have been limited to in vitro applications such as cell or cultured neurons preparations. Here we demonstrate NPY release in Nucleus Accumbens Core (NAcC), electrically and chemogenetically evoked from basolateral amygdala (BLA) stimulation. Release is thought to be activated by the stimulation of BLA glutamatergic efferents synapsing onto low-threshold spiking interneurons in the NAcC which are enriched in NPY. Here we demonstrate the presence of these NPY containing cells with RNA-FISH, and show that these cells do not co-localize with D1 or D2 containing cells of the NAcC. Preliminary data demonstrate that chemogenetic release (DREADD hM3-Dq expressed in the BLA) is dose dependent at the administered doses of 1, 5, and 10 mg/kg of CNO-administration i.p. Moreover, electrically evoked NPY release was tested at 5, 10, 20, 40, and 60 Hz stimulations at varying durations. NPY release appears to be able to support lower frequency stimulation up to 20 Hz stimulations, whereas signals at the higher frequencies (40 and 60 Hz) dropped in amplitude at 2s and longer stimulations. Ongoing and future studies are aimed at investigating NPY release during Pavlovian conditioning and to block electrically, chemogenetically, and behaviorally evoked release to test for sensor specificity.

P2-27 - Functionalized multi-modal arrays for real-time, site-specific neurochemical monitoring

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Mental health disorders are not driven by a single neurotransmitter, but instead involve complex interactions among multiple neurochemicals across interconnected brain regions. However, relatively little is known about how these neurochemicals fluctuate relative to one another during neuronal activation or pharmacological manipulation. Tools that enable simultaneous, site-specific measurement of multiple signals could help improve our understanding of brain function and support the development of new therapeutic strategies. We are developing a multi-modal sensing platform that combines amperometry, fast-scan cyclic voltammetry, and electrophysiology within a single electrode array. The platform integrates enzyme-modified biosensors for non-electroactive analytes, carbon fiber electrodes for FSCV-compatible measurements, and platinum-iridium recording sites for

amperometry or local field potential recordings. A complementary hardware and software package is being developed to synchronize and integrate data streams across multiple recording modalities. The sensor arrays are fabricated using a multi-barrel pulled capillary design and can be populated with platinum-iridium recording sites or carbon fiber electrodes. Platinum-iridium sites can be functionalized with enzyme coatings to detect analytes such as glucose, lactate, glutamate, and choline, or alternatively used for electrophysiological recordings. Functionalization of the probes is achieved by creating a 5-10 μm deep cavity at the tip of the electrode. Enzyme is immobilized within the cavity by entrapment in a chitosan membrane. A screening membrane is incorporated into each enzyme/chitosan matrix to prevent endogenous electroactive species (dopamine, ascorbic acid) from reaching the sensing surface. The platform is designed for flexible use in brain slices, anesthetized whole-brain preparations, and freely moving experiments. As part of this project, single-analyte 25 μm biosensors have also been developed, providing a flexible path toward real-time measurement of multiple neurochemical signals. Analyte targets include: glucose, lactate, glutamate, choline and oxygen. Together, these technologies support the long-term goal of simultaneous, site-specific neurochemical and electrophysiological monitoring in experimental models of brain function and disease.

P2-28 - Measuring neuronal activity in the medial orbitofrontal cortex during anxiety-like behavior in a mouse model for OCD

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Recent studies have linked mutations in the Bromodomain and WD repeat-containing protein 1 gene (Brwd1) to massively increased risk for obsessive-compulsive disorder (OCD). However, how deletion of Brwd1 alters brain activity and behavior, leading to increased OCD risk, is yet to be explored. We addressed this using Brwd1-heterozygous (Brwd1-het) mice. Because increased anxiety and excessive avoidance are core OCD traits, we asked whether Brwd1-het mice show an OCD-like phenotype. Abnormal activity in the orbitofrontal cortex (OFC) is associated with compulsive behavior and deficits in reversal learning, but its role in anxiety encoding remains underexplored. Previous literature has implicated other areas like the ventral hippocampus (vH) in “anxiety coding”: cells that respond to anxiogenic stimuli and are necessary for the performance of avoidance responses. Because the OFC is implicated in OCD and receives direct vH input, we asked whether it similarly contributes to “anxiety coding.” Thus, we compared behavior and single-cell medial OFC (mOFC) activity in Brwd1-het mice and their wild-type littermates (WT) during EPM exploration. We used endoscopic calcium imaging to record single-cell mOFC activity during behavior. To this end, we injected a virus expressing GCaMP7f in neurons and implanted a GRIN lens in the mOFC of Brwd1-het and WT mice. We used miniscopes to record single-cell activity while animals freely explored the EPM for 10 minutes. Behavior was recorded with an overhead camera and analyzed with custom Bonsai scripts to extract maze location. In addition, key events such as EPM arm entries and head-dips were manually scored using BORIS. We aligned neural activity to key behavioral events to characterize mOFC activity during specific anxiety-

related behaviors and approach-avoidance conflicts. Our preliminary results indicate that Brwd1-het mice show fewer entries into EPM open-arms than WT mice, suggesting increased anxiety consistent with OCD-like behavior. In WT mice, location-based analyses revealed neurons that respond to exploration of the different EPM compartments. However, we do not find a consistent preference in firing to open-arm entry across animals. Furthermore, we find subsets of cells that differentially fire depending on whether the animal is exploring or seeking safety, despite being in the same location. Similarly, a subset of cells shows diverging responses during head-dipping depending on the animal's future choice behavior: whether to continue exploring or to seek safety in the closed arms. The lack of clear preference for anxiogenic EPM compartments suggests that mOFC may play a role distinct from "anxiety coding". Instead, we hypothesize the mOFC to be key in the decision-making process that arbitrates approach-avoidance in the EPM. Additional animals will allow behavioral and neural comparisons between Brwd1-het and WT mice, providing insight into mechanisms that may increase OCD risk in humans.

P2-29 - Electrochemical Detection of Neurotransmitters using Laser Induced Graphene from Parylene-N Microelectrodes (LIG-PNMEs)

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Fast Scan Cyclic Voltammetry (FSCV) is a well-established technique for neurotransmitter detection using carbon-based microelectrodes. As neurotransmitter concentrations in the brain are typically on the nM scale, highly sensitive electrodes are necessary to detect them during in vitro and in vivo studies. Prior work has developed a novel approach of using Parylene N and a near-IR femtosecond laser that induces 2-photon absorption to create Laser Induced Graphene from Parylene N Microelectrodes (LIG-PNMEs). This is a photothermal process that breaks sp² bonds in Parylene N and transforms it into porous graphene. While carbon-fiber microelectrodes (CFMEs) are traditionally used in FSCV for detecting monoamines, LIG-PNMEs are an alternative for improved neurotransmitter detection. Prior work showed that LIG-PNMEs had 1.5 times and 1.3 times higher sensitivity than CFMEs when detecting dopamine and serotonin. In this work, we examined the trapping effects LIG-PNMEs for the detection of dopamine, norepinephrine, epinephrine, DOPAC and Ascorbic Acid at a range of FSCV repetition frequencies. CFMEs have poor sensitivity for detecting the secondary oxidations products of neurotransmitters that undergo multiple oxidation steps due to their smooth surface, which allows analytes to diffuse rapidly from the electrode. For the same reason, CFMEs also exhibit poor reversibility, as reduction peaks are consistently smaller than oxidation peaks for reversible analytes. In contrast, LIG-PNMEs have rough surfaces that momentarily trap neurotransmitters, which increased their sensitivity for subsequent oxidations and improved their reversibility. The secondary oxidation products of these analytes are rarely seen on CFMEs, as they rapidly diffuse from the electrode after the first oxidation. CFMEs characteristically have a substantial drop in current at high frequencies, as analytes have less time to adsorb to the surface of the electrode. Currents at LIG-PNMEs were nearly frequency independent due to the analytes getting

trapped on the electrodes' surface. The catecholamines dopamine, norepinephrine, and epinephrine all undergo a primary oxidation at 0.6 V and a secondary cyclization product at 0.15 V. CFMEs had small currents for the secondary oxidation of the catecholamine, which became slightly pronounced at higher frequencies. LIG-PNMEs had larger secondary peaks of the catecholamines which increased significantly at higher frequencies. The improved temporal resolution of LIG-PNMEs make them optimal to use for in vitro and in vivo neurotransmitter detection.

P2-30 - Real-time dynamic molecular fingerprinting of neurotransmitters with machine learning and SERS optophysiology.

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Despite advances in electrophysiology and electrochemical sensing, simultaneously resolving the identity and spatiotemporal dynamics of multiple neurotransmitters at physiologically relevant concentrations remains beyond current methods. We introduce SERS Optophysiology, an integrated platform combining optogenetics with Surface-Enhanced Raman Spectroscopy (SERS) for multiplexed, near-real-time neurochemical detection. SERS exploits electromagnetic field enhancement at nanoscale gold surfaces to amplify molecular Raman signals by several orders of magnitude, enabling detection of molecular fingerprints at very low concentrations, characteristic of synaptic release. Our substrate, a gold nanoparticle-functionalised glass microfibre, geometrically analogous to an electrophysiology probe, enables sub-micron positioning adjacent to synapses in cell culture and brain tissue. Each neurochemical produces a unique vibrational spectrum, its molecular fingerprint, and our research so far has focussed on dopamine, glutamate, GABA and acetylcholine as the main neurotransmitters in Parkinson's disease models, though our selection is expanding to cover other key metabolites. Because SERS spectra from complex biological environments are high-dimensional and variable, we apply Random Forest classification to robustly identify and distinguish multiple neurotransmitters from overlapping spectral signatures simultaneously. Optogenetics and pharmacological models provide controlled stimulation of neuronal activity, and fast-scan cyclic voltammetry (FSCV) provides orthogonal electrochemical validation of our readouts. This approach extends foundational work by Lussier et al.¹ into a full SERS optogenetics-compatible platform where we can test live mouse brain tissue models. No existing technique simultaneously delivers molecular specificity, multiplexed detection, sub-second temporal resolution, and sub-micron spatial precision. We are currently validating this approach in neuronal cell models, both during optogenetic stimulation and under basal conditions, including cross-validation with fast-scan cyclic voltammetry (FSCV) to confirm reliability of the signals detected. Once fully validated, our SERS Optophysiology technique will soon be applied to mouse models of Parkinson's disease to study early neurotransmitter alterations and identify new therapeutic targets. References: 1 F. Lussier, T. Brulé, M.-J. Bourque, C. Ducrot, L.-É. Trudeau, J.-F. Masson, *Faraday Discuss.*, 2017, 205, 387.

P2-31 - Parasitic Capacitance Optimization for Reliable Multichannel FSCV Measurements Using Multiplexers

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Fast-scan cyclic voltammetry (FSCV) is widely used for real-time neurotransmitter detection. However, conventional systems are generally limited to single-subject or low-channel-count recordings. In this study, we developed a multiplexer (MUX)-based multichannel electrochemical measurement system capable of neurochemical monitoring across up to 16 channels. The system was designed to sequentially record neurochemical signals from a maximum of 16 channels using a single transimpedance amplifier and data acquisition device. Because MUX components can introduce parasitic elements that distort low-current electrochemical signals, we investigated the effects of parasitic capacitance and resistance through equivalent circuit simulations and dopamine detection experiments. The results showed that parasitic capacitance was the dominant factor affecting signal integrity, while on-resistance had little influence. In particular, capacitance values above 50 pF increased the measured current by more than 5%, providing a practical criterion for selecting MUX devices in FSCV systems. Based on this criterion, low-capacitance MUX devices were selected to construct an optimized system. Compared with a non-optimized MUX system, the optimized system reduced background current and signal noise to levels comparable to those of a standalone TIA system. The system also demonstrated high channel uniformity and long-term stability, with normalized root mean square error below 0.5% across 16 channels and Z-scores maintained within ± 1 over 100 hours. In dopamine detection experiments, the system successfully measured synchronized oxidation current responses from multiple electrodes after 1 μ M dopamine injection. These results demonstrate that the proposed MUX-based system enables reliable and scalable FSCV measurements across up to 16 channels. This study provides an engineering guideline for MUX selection in electrochemical measurement systems and may support efficient experimental designs for behavioral neuroscience and pharmacological studies.

P2-32 - Dual in vivo fiber photometry and electrophysiology to reveal striatal firing rate - dopamine relationships during learning.

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In vivo fiber photometry with genetically encoded biosensors and electrophysiology are two powerful techniques for investigating brain-behavior relationships. Performed simultaneously, these in vivo methods have the potential to answer previously un-addressable questions. For example, we are interested in understanding relationships between neuronal firing rates and the release of specific neuromodulators during learning. First, we developed a custom device to overcome the problems associated with dual tethered recordings, including movement restriction, cable tangling, and

degraded optical transmission. By including an automated assisted tracking system that commutates all signals electrically, we can perform simultaneous electrophysiology and photometry recordings in mice over multiple hours without any movement restriction or disruption in learning. Using this device, we simultaneously tracked extracellular dopamine and single-neuron firing in the ventral striatum (Nucleus Accumbens, NAc) while mice performed a reward- learning task. Specifically, two distinct cues signaled two different rates at which reward would be available for pressing a lever. Mice acquired this task, evidenced by their differential press rates during the two different cues, at the same rate as mice without dual recordings. We searched for trial-by-trial covariance between the dopamine signal and the firing of striatal neurons, focusing on key task events (cue-onsets, lever presses, and rewards). Striatal neurons were modulated around all key events. As expected, rapid dopamine transients occurred at cue-onset and reward delivery. We also observed some weak suppression around unrewarded lever presses. We also observed small negative transients around unrewarded lever presses. Moreover, we found robust trial-by-trial correlations between dopamine and striatal firing rates. Reliable correlations were observed in ~40% of neurons. Consistent with the known features of striatal neurons in the direct and indirect pathways, positive and negative correlations occurred at roughly equal frequency. Analyzing changes in the relationships between neuronal firing and extracellular dopamine as subjects adapt performance to task contingencies will provide novel insights into neural mechanisms of learning.

P2-33 - Optimization of Lactate Oxidase-Based Microelectrode Biosensors for Neurochemical Monitoring of Brain Energy Metabolism

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Lactate is increasingly recognized as a key neurometabolic molecule involved in brain energy metabolism, neuron-astrocyte metabolic coupling, and activity-dependent signaling. Reliable monitoring of lactate concentration dynamics in neural environments requires microbiosensors combining high sensitivity and selectivity, extended dynamic range, and reproducible enzyme immobilization. In this work, different lactate oxidase (LOx)-based immobilization strategies were evaluated on microelectrodes platforms with the aim of optimizing analytical performance for neurochemical monitoring applications. Several enzyme immobilization approaches were compared, including bovine serum albumin/glutaraldehyde (BSA/GA), chitosan and sol-gel matrices, with or without the addition of carbon nanotubes and metal nanoparticles. The analytical response of each configuration was assessed using the apparent Michaelis constant ($K_{M,app}$) and sensitivity as key performance indicators. In parallel, a design of experiments approach, including full factorial and Box-Behnken designs, was applied to evaluate the influence of LOx concentration, BSA concentration, and polyurethane diffusion-layer incorporation on sensor performance. Among the tested strategies, BSA/GA immobilization provided the most robust baseline platform, with high sensitivity and good reproducibility. Increasing LOx loading generally improved sensitivity, with LOx10.BSA1% and LOx10.BSA10% showing some of the highest responses. The introduction of polyurethane diffusion

layers markedly increased the apparent KM while decreasing sensitivity, confirming the role of polyurethane as a diffusion-controlling membrane. Full factorial analysis identified polyurethane thickness as the dominant factor modulating sensor behaviour, significantly increasing KM,app and significantly reducing sensitivity, whereas LOx and BSA concentrations showed positive but less statistically robust trends. Alternative immobilization strategies provided additional insight into sensor design. Carbon nanotubes and metallic nanoparticles did not consistently improve analytical performance under the tested conditions, suggesting that increased material complexity does not necessarily translate into improved biosensor response. Overall, the results demonstrate that lactate biosensor optimization is governed by a balance between enzyme availability and conformational stability, as well as substrate diffusion control, rather than by enzyme loading alone. The integration of empirical screening with experimental design enabled the rational selection of candidate architectures according to the intended application: highly sensitive sensors for low lactate concentrations or diffusion-controlled sensors for extended dynamic range. This work supports the development of optimized LOx-based microelectrode biosensors for real-time monitoring of lactate as a key molecule in neurometabolism.

P2-34 - Rapid fluorescence lifetime sensor development of LifeCamp enables transient and baseline absolute calcium measurements

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Genetically encoded calcium sensors (GECIs) have been instrumental for studying neuronal activity and intracellular signaling. GECIs are typically fluorescence-intensity sensors that change brightness upon calcium binding. Iterative improvements in GECIs have yielded indicators that report action potential-evoked calcium entry with high sensitivity and temporal resolution, enabling measurement of network activity across thousands of neurons. However, fluorescence intensity-based measurements generally cannot report baseline or absolute calcium levels and may confound neuromodulatory regulation of calcium handling with changes in action potential firing. Fluorescence lifetime sensors are insensitive to many artifacts that plague intensity-based measures and report absolute substrate levels, including those at rest. However, relatively few lifetime sensors for neuronal signals exist, and developing new sensors is typically difficult and low-yield. Here, we introduce a new rapid lifetime sensor development (RALISED) platform, which we use to build a new GCaMP8m-based high-speed lifetime calcium sensor, termed LifeCamp. We show that LifeCamp enables comparison of baseline calcium signals in cell culture, brain slices, and mice. In addition, we show that LifeCamp enables the detection of fast action potential-evoked calcium transients in single neurons from brain slices and in behaving mice. Using LifeCamp, we discovered calcium baseline changes associated with neuronal activity in brain slices and behaving mice, as well as slow average calcium changes in neuronal populations of freely moving mice. Altogether, this study highlights the utility of the RALISED method to rapidly develop new lifetime sensors and the application of the LifeCamp calcium lifetime sensor to study fast and slow calcium signaling.

P2-35 - Temporally Distinct Contributions of Ventral Pallidal Populations to Reward Seeking

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The ventral pallidum (VP) is a central node in the ventral basal ganglia that regulates motivated behavior and reward processing. Recent findings show complex cellular heterogeneity within this structure, and work from our lab and others implicate GABAergic VP (VP-GABA) neurons in reward seeking and glutamatergic VP (VP-Glu) neurons in aversion processing. The VP also contains fast-spiking neurons that produce parvalbumin (VP-PV), and regular spiking neurons that produce the opioid neuropeptide pro-enkephalin (VP-Penk). We investigated the contributions of these cell-types in the regulation of reward seeking under increasing-effort requirements using progressive ratio (PR) tests wherein we measured the maximum effort an animal is willing to exert (break point) to obtain a sucrose reward. Male and female mice (Vgat-Cre, Penk-Cre, PV-Cre, Vglut2-Cre; n = 8-10 per group) were surgically injected with AAVs carrying cell-specific chemogenetic (hM4D) or optogenetic (BiPOLES) actuators, and calcium (RCaMP) and/or glutamate and GABA sensors (iGlu-SnFR / iGABA-SnFR), and were implanted with optic fibers above the VP. Mice were trained to nose poke for sucrose (15 mg) on a fixed ratio schedule of reinforcement (FR1, 8d; FR5, 5d) followed by PR training and testing. Chemogenetic inhibition of VP-GABA neurons reduced break points ($p < 0.01$), and inhibition of VP-Glu neurons increased break points ($p < 0.05$) for sucrose. By contrast, chemogenetic inhibition of VP-Penk or VP-PV did not consistently alter break points. Optogenetic stimulation (630 nm, 40 Hz, 2s) of VP-GABA ($p < 0.01$), VP-Penk ($p < 0.01$) or VP-PV neurons during reward delivery or retrieval increased motivation for sucrose. By contrast, only optogenetic inhibition (450 nm, 2s) of VP-GABA neurons during reward delivery reduced motivation for sucrose ($p < 0.05$). Surprisingly, neither optogenetic stimulation, nor inhibition of VP-Glu neurons produced any effects. Photometry analyses revealed differential engagement of VP cell-types during reward seeking. VP-Glu neurons were most active during the presentation of reward-predictive stimuli, whereas VP-GABA neurons were most engaged during reward consumption. Concurrent neurotransmitter imaging showed that calcium activity within individual populations can be explained in part by contributions of glutamatergic and GABAergic signaling onto these cells. Our results reveal a complex temporal and cell-type specific regulation of motivation for rewards by different population of VP neurons. Specifically, the surprising divergence between continuous chemogenetic and temporally specific VP-Glu manipulations warrants further investigation. Ultimately, a better understanding of the cell-specific contributions of VP neurons to motivated behavior will be crucial for developing novel treatments for psychiatric disorders characterized by aberrant motivation such as major depressive disorder and substance use disorders.

P2-36 - Remotely-evoked Dopamine Release and Uptake in Whole, Live Zebrafish Brains

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Remotely-evoked Dopamine Release and Uptake in Whole, Live Zebrafish Brains Dineesha Chandrasiri and Michael A. Johnson Department of Chemistry and R.N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, 66047 USA In mammals, reward is encoded by dopamine release from the mesolimbic pathway, which projects from the ventral tegmental area to the nucleus accumbens. Our research team is interested in mechanisms that underlie the function of this pathway and how they are altered in Parkinson's disease and Alzheimer's disease. In this study, we conducted sub-second measurements of dopamine release and uptake with fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes in response to electrical stimulation in zebrafish whole brains ex vivo. This preparation is ideal because the whole brains of zebrafish remain viable after removal from the host. Thus, we have virtually unlimited access to all pathways present in the brain. The pathway that projects from the posterior tubercle (PT) to the dorsal nucleus of the ventral telencephalon (Vd) is analogous to the mesolimbic pathway in mammals. To confirm that our signal arose from dopamine release, we obtained measurements after the addition of GBR 12909, a dopamine transporter inhibitor, and desipramine, a norepinephrine transporter inhibitor. Furthermore, we assessed local and remote stimulation responses to perfusion with NMDA, an NMDA receptor agonist and MK-801, an NMDA receptor antagonist. This dual-site pharmacological approach allowed us to distinguish terminal-specific regulation from somatic NMDA-dependent control of the pathway that we are interested in.

P2-37 - Increased dopamine transmission in Disc1-L100P mice detected by [¹¹C]-PHNO PET imaging and amphetamine-induced locomotion

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Disc1-L100P mutant mice are an established animal model for schizophrenia, with abnormalities in pre-pulse inhibition, latent inhibition, cognition, brain development and cortical histology, and elevated levels of the D2 receptor-Disc1 complex, consistent with human studies of the disorder. Dopamine D2 receptors are the obligate target of all established antipsychotic medications used to treat schizophrenia. Here we extend the characterization of this mouse model by investigating dopamine transmission, which is increased in schizophrenia patients following acute amphetamine challenge. [¹¹C]-PHNO positron emission tomography (PET) imaging at baseline revealed significantly reduced striatal D2/D3 receptor availability in Disc1-L100P mice compared to wild-type littermate controls (BPND: 3.44 ± 0.312 SD (n=8) vs 3.92 ± 0.214 SD (n=7), $p = 0.0065$, $d = 1.802$). This result is consistent with elevated synaptic dopamine competing with tracer binding. Amphetamine (1 mg/kg

i.p.) also induced significantly greater locomotion in Disc1-L100P mutant mice vs. controls (amphetamine induced fold increase: Disc1-L100P = 1.34 ± 0.42 vs. WT = 0.93 ± 0.33 , $t(17) = 2.23$, $p = 0.040$, $d = 1.075$). These findings provide convergent neurochemical and functional evidence of increased striatal dopamine signaling in the Disc1-L100P mouse. This is another aspect of the Disc1-L100P mouse phenotype that is similar to schizophrenia, further strengthening the utility of this animal model.

P2-38 - Targeted metabolomics via microdialysis uncovers neurochemical differences between high and low responder rats during cocaine self-administration

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Substance use disorder (SUD) is a chronic, relapsing condition in which drug-seeking and drug-taking behavior persist despite negative consequences. Various human and animal studies have shown that the neurochemical mechanisms underlying the formation of a SUD primarily involve the mesolimbic dopamine system. However, the full scope of neurochemical adaptations that develop during the onset of drug use is not completely understood. Interestingly, only a subset of the population that experiments with a drug goes on to develop SUD. The neurochemistry associated with these differences in vulnerability is not well understood but may be a key component to furthering our knowledge of SUD. Animal models are critical for developing a better neurochemical understanding of addiction. The bred high responder (bHR) and bred low responder (bLR) rat model used in this work models vulnerability to SUD, as bHR have elevated rates of learning self-administration, amount of drug taking, and propensity to relapse compared to bLR. In this study, microdialysis samples were collected from the nucleus accumbens of 12 bHR and 12 bLR throughout a 15-day cocaine self-administration study to investigate baseline and longitudinal neurochemical differences between the two phenotypes. Each day, a baseline microdialysis fraction was collected prior to a 2-hour cocaine self-administration session, during which an additional fraction was collected. This study marks the first time that microdialysis samples have been collected throughout a cocaine self-administration study, allowing for unprecedented neurochemical monitoring while animals develop an addiction-like state. All samples were derivatized with benzoyl chloride and analyzed by a 3-minute liquid chromatography-mass spectrometry method to quantify 25 neurochemicals. The bHR rats acquired self-administration of cocaine on the first day of the experiment and maintained a stable number of infusions throughout, while the bLR largely did not self-administer. At baseline on day 1 of the experiment, six compounds were found to have significantly different concentrations between bHR and bLR; glutamate, histamine, and taurine were elevated in bHR, while levodopa, epinephrine, and normetanephrine were elevated in bLR. Dopamine, 3-MT, and norepinephrine had the greatest elevation due to cocaine use across the study. Basal dopamine concentrations were increased throughout the experiment for bHR but not bLR, a trend that was stronger for males than females.

These results reveal novel relationships between neurochemicals and SUD vulnerability, which lays the foundation for working toward treatment and prevention strategies.

P2-39 - Monitoring substance P dynamics in vitro and in vivo with a genetically encoded fluorescent sensor

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Substance P (SP) is a neuropeptide that regulates pain, inflammation, and emotional states via the neurokinin-1 receptor (NK1R). Dysregulated SP-NK1R signaling underlies various pathologies, but existing methods lack the spatiotemporal resolution to study its dynamics in living systems. We developed and validated a genetically encoded fluorescent indicator, NKLight1, that enables monitoring of SP release through sensor activation. Using this tool, we characterized SP signaling in vitro and in vivo. NKLight1 characterization in HEK293T cells and rat cortical neurons revealed wild type-comparable pharmacological properties while displaying abolished downstream signaling. The sensor enabled spatial and temporal characterization of photo-uncaged SP diffusion and detected endogenous SP release from primary mouse T cells upon antigen receptor stimulation. To characterize NKLight1 in vivo, we injected an AAV encoding NKLight1 and implanted an optic fiber coupled to a fluidic delivery device in the dorsomedial striatum (DMS) of mice, and observed increased NKLight1 signal in the DMS with infusion of exogenous substance P. We also demonstrated that NKLight can be used to measure endogenous SP dynamics in the DMS during an operant behavior paradigm; these dynamics were largely ablated with systemic administration of a NK1R antagonist, aprepitant. Collectively, NKLight1 offers a transformative platform for exploring neuropeptide dynamics in health and disease in various experimental contexts.

P3-41 - Effects of a muscarinic M4-PAM on striatal neurochemical dynamics

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The muscarinic acetylcholine receptor subtype 4 (M4) has been reported to influence dopamine-dependent behaviors, including measurement of psychostimulant-induced locomotion and antipsychotic-like responses in preclinical models. Owing to their integral role in striatal circuitry, M4 receptors have emerged as promising therapeutic target for a range of neuropsychiatric disorders. The M4 muscarinic acetylcholine receptor subtype is highly expressed in dopamine-rich brain regions that govern motor control, cognition, and neuropsychiatric disease processes. Within the striatum, M4 receptors are predominantly localized on GABAergic medium spiny neurons—particularly those expressing dopamine D1 receptors that form the direct striatonigral pathway. In addition, presynaptic M4 receptors are present on corticostriatal glutamatergic terminals, where they regulate glutamate release. Given the importance of integrating dopaminergic input in the striatum and prefrontal cortex, M4 receptor activation has a substantial impact on dopamine-mediated behaviors. Preclinical studies support this role, as global or cell-specific M4 receptor knockout mice exhibit dopamine hypersensitivity, including increased cocaine self-administration, elevated dopamine release, and enhanced locomotor responses to psychostimulants. We investigated the effects of an M4 positive allosteric modulator (PAM) on neurochemistry within the striatum. This was done firstly, by investigating the effect of a M4-PAM (0.1, 1.0, or 10 mg/kg) on amphetamine-induced dopamine increases in dorsal striatum using microdialysis. Secondly, also using microdialysis, we investigated the effect of an M4-PAM (10 mg/kg) on dopamine, serotonin, GABA, glutamate, and acetylcholine levels within three striatal compartments in freely behaving rats. M4-PAM pre-treatment did not change amphetamine-induced increases in dopamine at any of the doses tested. A dose of the M4-PAM known to reverse ~65% of amphetamine-induced hyperlocomotion did not change dopamine, serotonin, GABA, glutamate, or acetylcholine in any of the striatal compartments tested. These data show that the M4-PAM-induced pre-clinical effects observed on dopamine-mediated behaviors is unlikely to be caused by altered neurochemical dynamics such as changes in extracellular concentrations of dopamine, serotonin, GABA, glutamate, or acetylcholine. Although an M4-PAM-induced effect on dopamine has been broadly reported and discussed in the literature, based on the data presented here this potential mode of action seems unlikely, and therefore not driving the observed behavioral effects.

P3-42 - Fiber photometry reveals reduced dopamine signaling in diet-induced obesity

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The increasing prevalence of obesity is a pressing public health challenge. Regular physical activity is a cost-effective strategy to support weight management and improve metabolic health. However, many individuals with obesity struggle to adhere to exercise programs. Dopamine transmission from ventral tegmental area (VTA) neurons to the nucleus accumbens (NAc) is known to play a critical role in action initiation, movement vigor, and reinforcement learning. We propose that diet-induced obesity impairs dopamine signaling within the nucleus accumbens, thereby limiting engagement in behaviors that support weight loss. To test this hypothesis, we investigated obesity-related alterations in tonic and phasic dopamine signaling in C57BL/6 mice. In the first experiment, mice were fed either a standard chow diet or a high-fat diet (HFD) for six weeks, resulting in significant weight gain in the HFD group. Mice received a unilateral injection of the genetically encoded dopamine sensor GRABDA into the nucleus accumbens, along with implantation of an optical fiber to enable fiber photometry recordings of dopamine signaling in freely moving animals. Fiber photometry analyses revealed that HFD-fed mice exhibited fewer spontaneous dopamine transients and reduced low-frequency signal power, indicating impaired tonic dopamine signaling in the NAc, likely contributing to impaired action initiation and motor vigor. In a second experiment, we longitudinally assessed phasic dopamine signaling during the development of obesity using two behavioral paradigms: (1) a two-choice bandit task, a reward-based decision-making task in which mice had to poke the correct port (left or right) to receive a chocolate pellet; and (2) a Pavlovian conditioning task, in which an auditory cue predicted chocolate pellet availability. Our results showed that HFD-fed mice exhibited significantly attenuated dopamine responses to both reward delivery in the bandit task and reward-predictive cues in the Pavlovian task. These findings indicate impaired phasic dopamine signaling, which may contribute to deficits in action initiation and reinforcement learning. Overall, our fiber photometry recordings reveal reduced tonic and phasic dopamine signaling in diet-induced obesity. These impairments may contribute to the reduced engagement in physical activity and lifestyle interventions commonly observed in individuals with obesity.

P3-43 - Fast and sensitive analysis of dopamine, serotonin and other neurotransmitters in microdialysis samples with high time resolution, using the ALEXYS™ Neurotransmitter Analyzer

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Neurotransmitter analysis in microdialysis samples requires the reliable quantification of picomolar concentrations in small volume fractions ($\leq 5 \mu\text{L}$), posing significant analytical challenges. The

ALEXYS™ Neurotransmitter Analyzer, based on the DECADE Elite and SenCell (electrochemical detector and flow cell), has been designed to meet these demands, and delivers superior sensitivity and selectivity for fast, reproducible trace analysis of a wide range of neurotransmitters and metabolites. It comprises of several integrated system solutions, which have been developed for trace analysis of neurotransmitters in microdialysis samples, both online using a sampling valve and offline using an autosampler. System innovations include parallel and serial detection schemes with multiple flow cells, dual and triple loop injection valves for minimal sample consumption, and optimized UHPLC separations using sub-2 μm particle columns. With these innovations a neuroscientist is able to extract more information using a minimum amount of sample, ultimately saving time, resources, and test animals. Several examples of optimized assays for the analysis of monoamines and metabolites, acetylcholine, GABA, glutamate, will be shown to demonstrate the performance of the ALEXYS™ neurotransmitter analyzer. The analyzer enables fast and sensitive detection of acetylcholine, GABA, glutamate, monoamines, amino acids, and their metabolites, with detection limits as low as 0.15 fmol for monoamines (30 pM, 5 μL), 3 fmol for acetylcholine (0.3 nM, 10 μL), and 15 fmol for GABA (10 nM, 1.5 μL). Basal levels of neurotransmitters can be quantified accurately. For online in vivo microdialysis, a dual-loop sampling valve (Fig. 1) allows online collection and simultaneous analysis of 1.5 μL fractions, achieving temporal resolutions below 2 minutes with detection limits of 100 pmol/L for both dopamine and serotonin. By raising the separation temperature from 35 °C to 60 °C, analysis times are reduced to less than 1 minute, allowing an even higher temporal resolution of in vivo experiments (Fig. 2).

P3-44 - Translating FSCV to human model systems: real-time serotonin sensing in 3D iPSC-derived spheroids

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Major depressive disorder remains mechanistically elusive, in part due to the lack of tools to monitor neurotransmission in human systems with high temporal resolution. While fast-scan cyclic voltammetry (FSCV) has transformed measurements of monoamines in animal models, its application to human-derived systems has remained limited. Here, we establish a translational electrochemical platform by integrating FSCV with induced pluripotent stem cell (iPSC)-derived 3D serotonergic spheroids to enable real-time monitoring of human serotonin dynamics. We optimized FSCV for use in complex 3D stem cell-derived cultures, including electrode geometry, stimulation paradigms, and analysis workflows, enabling stable, label-free detection of serotonin with sub-second resolution and nanomolar sensitivity. This approach allows direct quantification of both spontaneous and evoked release events, as well as reuptake kinetics, providing a functional readout of serotonergic signalling that is not accessible through conventional molecular assays. Using this platform, we demonstrate its capacity to resolve pharmacological mechanisms, as selective serotonin reuptake inhibitors (SSRIs) produced distinct signatures in release amplitude and clearance kinetics, reflecting differences in transporter engagement. Furthermore, exposure to histamine, a key mediator of stress and

inflammation, modulated serotonin availability and blunted SSRI responses, highlighting the sensitivity of FSCV to detect dynamic changes in neurotransmitter regulation under pathophysiological conditions. To extend the system toward more physiologically relevant models, we incorporated iPSC-derived microglia to generate immune-competent spheroids. In this multicellular context, electrochemical recordings revealed altered serotonin clearance dynamics and inflammation-dependent modulation of release and reuptake, demonstrating the ability of FSCV to interrogate neuroimmune interactions in real time. Together, this work expands the application of FSCV from animal models to human stem cell-derived systems, establishing a versatile platform for monitoring neurotransmitter dynamics in complex in vitro environments. By enabling direct, real-time measurement of serotonin in human-derived tissues, this approach provides a powerful bridge between electrochemical sensing technology and translational neuroscience, with implications for drug discovery, disease modeling, and precision neuropsychopharmacology.

P3-45 - A spatially and transcriptionally defined mu-opioid receptor-enriched MSN population mediates opioid reward learning

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Opioid use disorder (OUD) remains a public health crisis, yet the specific neuronal populations mediating opioid reward are incompletely understood. Opioids reinforce behavior primarily through activation of mu opioid receptors (μ ORs), encoded by *Oprm1*, within mesolimbic reward circuitry including the nucleus accumbens (NAc). However, *Oprm1* expression is unevenly distributed across NAc medium spiny neurons (MSNs), and the functional contributions of these μ OR-enriched MSN populations remains unclear. To identify and characterize *Oprm1*-expressing MSN populations, we performed single-nucleus RNA sequencing (snRNA-seq) of the rat NAc and integrated these data with high-resolution 10x Genomics Xenium spatial transcriptomics across multiple anterior-posterior coordinates of the mouse NAc. Cell identities were assigned using canonical markers and validated with Allen Institute's MapMyCells tool. These approaches identified a transcriptionally distinct MSN subtype marked by *Chst9* (*Chst9*-MSNs) that exhibits exceptionally high *Oprm1* expression. snRNA-seq analysis revealed that *Chst9*-MSNs comprise ~7% of NAc cells yet contain over one-third of total *Oprm1* transcripts. Spatial transcriptomic analysis identified 22 molecularly distinct spatial domains within the NAc, including a domain highly enriched for *Chst9*-MSNs across several anterior-posterior planes, where they form spatially organized streaks and clusters along the medial and ventral borders of the NAc shell. Cross-species comparisons demonstrated conservation of this population across mouse, rat, nonhuman primate, and human datasets. To enable selective targeting of this population, we generated a *Chst9*-Cre-tdTomato transgenic rat line. Whole-cell patch clamp recordings demonstrated that *Chst9*-MSNs are robustly inhibited by the μ OR agonist DAMGO, an effect reversed by naloxone. Importantly, Cre-dependent deletion of *Oprm1* in *Chst9*-MSNs abolishes fentanyl conditioned place preference in female rats. Together, these findings identify *Chst9*-MSNs as a

spatially and transcriptionally defined μ OR-enriched MSN population that is both directly responsive to opioids and necessary for opioid reward learning. This work defines a cell-type-specific substrate of opioid reinforcement and highlights Chst9-MSNs as a potential novel target for OUD treatment.

P3-46 - Striatal lactate shapes dopamine release through cholinergic interneurons

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Striatal dopamine (DA) signalling is fundamental for action selection, goal-directed behaviour, and reinforcement learning, and is profoundly disrupted in Parkinson's disease. The release of DA from its extensively branched striatal axonal arbors can be regulated locally by mechanisms that govern axonal excitability, action potential propagation, and release probability. A range of striatal neuromodulators and receptors directly and potently modulate striatal DA release in this manner, such as acetylcholine (ACh) released from striatal cholinergic interneurons (ChIs) acting at nicotinic receptors (nAChRs). In parallel, accumulating evidence indicates that dopaminergic activity is associated with activity-dependent elevations in striatal extracellular lactate. Although the astrocyte-neuron lactate shuttle identifies lactate as a major energetic substrate supporting neuronal activity, whether lactate regulates striatal dopamine release remains unknown. Here, using a combination of fast-scan cyclic voltammetry (FSCV), G-protein-coupled receptor activation-based (GRAB) imaging, and patch-clamp electrophysiology in acute mouse striatal slices, we investigated the role of lactate in supporting striatal DA transmission. Using FSCV, we show that lactate is sufficient to sustain evoked dopamine release in absence of glucose, demonstrating that striatal dopaminergic circuits can efficiently use lactate as an energetic substrate. Using GRAB-DA imaging, we further demonstrate that pharmacological blockade of monocarboxylate transporters (MCTs) with 4-CIN strongly reduces DA release, revealing that endogenous lactate trafficking is required to maintain normal dopaminergic transmission. Mechanistically, blocking β 2-containing nicotinic acetylcholine receptors largely occluded the effect of 4-CIN on dopamine release suggesting a critical involvement of ChIs, key modulators of dopamine release within the striatum. Consistently, GRAB-ACh imaging revealed that MCT blockade with 4-CIN also reduces acetylcholine release. At the cellular level, patch-clamp recordings of ChIs further showed that 4-CIN decreases the spontaneous firing activity. Together, these findings uncover a previously unrecognized metabolic interaction between lactate, cholinergic interneurons, and dopaminergic axons, positioning lactate not merely as a metabolic substrate, but as a dynamic regulator of striatal function. Ongoing work aims to investigate the cellular and circuit mechanisms governing striatal lactate dynamics using a new generation of genetically encoded lactate sensors. These mechanisms may represent an important metabolic component of striatal dysfunction in Parkinson's disease and could provide novel therapeutic targets for restoring dopaminergic signalling.

P3-47 - Investigating the representation of outcome controllability across valences in dopamine and serotonin signaling in the ventromedial striatum

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Controllability, the degree to which actions can influence outcomes, is central to adaptive decision making and stress resilience. In humans, impaired perception of control is implicated in psychiatric disorders such as depression, obsessive-compulsive disorder, and pathological gambling, suggesting that altered controllability perception has transdiagnostic relevance and may contribute to maladaptive decision making and pathological behavioral patterns. Animal studies have shown that experience with controllable or uncontrollable aversive stimuli can alter later avoidance or escape responses. However, most of this work has focused on aversive controllability alone and often using between-subject designs. It therefore remains unclear whether controllability is represented as a general action-outcome feature across appetitive and aversive contexts, or whether its neural representation depends on outcome valence. The serotonergic and dopaminergic systems are implicated in functions that relate to outcome controllability. Serotonergic signaling is implicated in behavioral inhibition, aversive-outcome controllability, and stress sensitivity. Dopaminergic signaling is involved in reinforcement learning, value encoding, and motivated behavior across valences. The ventromedial striatum (VMS) is particularly interesting for studying controllability across appetitive and aversive outcomes, as it receives both dopaminergic and serotonergic input and integrates information about value, valence, and action. In this study, we developed a behavioral paradigm to compare controllable and uncontrollable conditions for both appetitive and aversive outcomes within the same animal and experimental session. This design includes four trial types: appetitive controllable, appetitive uncontrollable, aversive controllable, and aversive uncontrollable. By comparing these conditions within the same animal and experimental session, the task reduces between-subject and across-session variability, enabling a more direct assessment. Our behavioral results suggest that rats learned the task structure and adjusted their behavior according to trial type. This behavioral paradigm will be combined with fiber photometry recordings to simultaneously monitor VMS serotonin and dopamine using a dual sensor approach already established in our group.

P3-48 - Real-time monitoring of dopamine dynamics in the *Drosophila* mushroom body in vivo using fast-scan cyclic voltammetry

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Drosophila melanogaster is an excellent genetic model for studying the fundamental principles of neural signaling, as its neurotransmitter systems are highly homologous to those of mammals. In the *Drosophila* brain, the mushroom body acts as a central hub for processing sensory information related to associative learning. Dopaminergic neurons in this region are critical for evaluating

appetitive and aversive stimuli. To understand the regulatory dynamics of these signals, it is essential to monitor real-time dopamine release in response to physiological stimuli. Nonetheless, real-time measurement of neurotransmitters in *Drosophila* remains a significant challenge due to its microscale brain size. Because of the surgical difficulties associated with in vivo access, most previous electrochemical studies have been conducted using dissected, isolated brains. While useful, these ex vivo preparations lack the intact sensory pathways required to study dopamine fluctuations during natural behavior. Consequently, it remains unclear how dopamine release in the mushroom body responds to natural physiological stimuli compared to artificial pharmacological stimulation. The purpose of this study is to characterize real-time dopamine dynamics in the mushroom body of adult flies in vivo using Fast-scan cyclic voltammetry (FSCV). FSCV is an electrochemical technique that provides high temporal resolution for monitoring rapid changes in extracellular neurotransmitter levels. In this work, we developed an in vivo recording setup by preparing a head cuticle window to access the brain while keeping the fly alive. We performed measurements using carbon-fiber microelectrodes to detect sub-second dopamine transients. The setup was validated by detecting dopamine release evoked by exogenous acetylcholine application. We are currently using this approach to compare endogenous dopamine transients elicited by natural sensory inputs, specifically appetitive stimuli (sugar solution) and aversive stimuli (electric shock). This in vivo methodology will provide a substantial advance in comprehending how neural circuits process sensory information and regulate reward-based behaviors in real time.

P3-49 - Green and Red GRAB Sensors for Real-Time Monitoring of Endocannabinoid Dynamics

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Endocannabinoids (eCBs), including Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are key retrograde neuromodulators involved in diverse physiological processes. However, their distinct in vivo dynamics remain poorly understood due to the lack of tools with sufficient spatiotemporal resolution and molecular specificity. We previously developed a non-selective green GRAB sensor, eCB2.0, which detects both AEA and 2-AG with robust fluorescence changes ($\sim 300\% \Delta F/F_0$), enabling visualization of overall eCB dynamics in living animals. To distinguish between these two major eCBs, we now report the development of two selective green sensors: gAEA2.0, specific for AEA, and g2-AG2.0, selective for 2-AG. When expressing in vivo, we monitored both AEA and 2-AG signals during foot shock in the NAc core and BLA, whereas sucrose reward evoked time-locked 2-AG—but not AEA—signals in the VTA. To enable simultaneous monitoring of AEA and 2-AG in behaving animals, we further engineered red-shifted versions, rGRABAEA and rGRAB2-AG, compatible with multiplexed imaging alongside their green counterparts. We further investigated their dynamics in the NAc shell during administration of different psychoactive drugs, including nicotine and $\Delta 9$ -tetrahydrocannabinol ($\Delta 9$ -THC). Remarkably, nicotine evoked endogenous AEA signals, whereas $\Delta 9$ -THC elicited only 2-AG

responses. In summary, these selective and spectrally-distinct eCB sensors provide powerful tools for real-time, dual-color monitoring of AEA and 2-AG in vivo, and open new avenues to dissect their individual contributions to brain function and behavior.

P3-50 - Cost shapes striatal dopamine value signals during sequentially revealed cost-benefit offers in rats

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Heightened sensitivity to the costs of reward pursuit is a core feature of many dopamine-related disorders, yet how striatal dopamine represents cost and shapes reward valuation during choice remains unclear. A major challenge is that phasic striatal dopamine is influenced by both expected reward and action initiation, making it difficult in typical cost-benefit tasks to separate value-related signals from movement-related changes. In addition, most work has focused on single striatal regions, so it remains unclear how different subregions differentially contribute to cost-benefit integration. To address these, we used fibre photometry in rats performing a novel cost-benefit decision task designed to isolate valuation from action initiation and examined how anticipated cost and reward is reflected in cue- and action-related dopamine signals in nucleus accumbens (NAc) and dorsomedial striatum (DMS). Male and female Lister Hooded rats (n=21) were presented with cost-benefit offers while holding in an initiation port, with cost (near versus far reward port, steady v flashing port light) and reward size (zero, small, medium or large; 4 auditory cues) revealed sequentially during the offer period. Rats could accept the offer by holding until the offer period ended and pay the action cost to obtain the reward, or exit early and forgo it. Hold completion rates demonstrated a significant cost × reward-size interaction. Using the dLight1.3b dopamine sensor and fibre photometry, we recorded dopamine release from NAc and DMS on alternating days. Results demonstrate that anticipated cost modulates dopamine release across distinct valuation and movement epochs, and that the impact of cost on these dopamine signals differs between NAc and DMS.

P3-52 - A decrease in phasic dopamine precedes the rapid satiation of sodium appetite

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Body fluid homeostasis in the face of constant deviations (e.g., due to fluid loss and food/water intake) is a critical problem for most animals. A range of physiological mechanisms – both autonomic and endocrine – can help water and solutes to be conserved, but eventually water and salt need to be acquired via motivated behaviour. In fact, deviations in fluid balance induce powerful drive states that

dramatically change the affective value of fluids of different tonicity, making thirst and sodium appetite key models for studying the relationship between motivated behaviour and homeostasis. Mesolimbic dopamine has long been linked to motivated behaviours and is thought to play a key role in assigning value to stimuli based on biological needs. Sodium deprivation or experimentally-induced depletion radically changes appetitive behaviour directed at, and the value of, sodium. We have previously shown that, in sodium deplete rats, intraoral infusions of hypertonic sodium result in robust dopamine release in nucleus accumbens. Such responses are not seen when animals are sodium replete. A key remaining question is what exactly happens during restoration of sodium balance and how is dopamine related to the transition between the value of sodium in deplete versus replete states. We have addressed this by adjusting infusion parameters (sodium concentration and infusion duration). Using DeepLabCut to track postural changes and SimBA to classify observed behaviours, rats exhibited a remarkably rapid and sharp transition in appetitive behaviour across a single session. The behavioural transition point was determined for each rat. By simultaneously measuring dopamine release in the lateral nucleus accumbens shell (with fibre photometry recordings of GRAB-DA fluorescence), we show that dopamine responses also decrease rapidly during the session. Realignment of trials to each rat's behavioural transition point resulted in an even steeper decrease in dopamine about the transition. Cross-correlation of the signals shows that changes in dopamine actually precede decreases in appetitive behaviour by just a few trials. Together, these data support a model in which phasic dopamine responses to ingested sodium cause a rapid updating of appetitive behaviour.

P3-53 - Surface Functionalization of Carbon Nanotube Yarn Electrodes for Enhanced Dopamine Adsorption Kinetics and Tonic Dopamine Detection

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Dopamine is a major neurotransmitter involved in reward, motor control, and neurological function, and measuring its basal concentration is important for understanding brain neurochemical states. Carbon nanotube yarn (CNTY) electrodes are promising for dopamine sensing because their porous fiber structure provides a large electroactive surface area. However, pristine CNTY is hydrophobic, which limits electrolyte penetration into the internal pore network and restricts effective dopamine adsorption. Here, we introduce an electrochemical functionalization method that modifies the CNTY surface with oxygen-containing functional groups to improve dopamine adsorption kinetics and enable reliable tonic dopamine detection. CNTY microelectrodes were fabricated from twisted fibers drawn from vertically aligned CNT forests, sealed in glass capillaries, and beveled into cone-shaped tips with a diameter of approximately 30 μm . Electrochemical functionalization was performed in Tris buffer by applying a triangular waveform from -0.4 V to $+1.8\text{ V}$ at 400 V/s . Cyclic voltammetry and electrochemical impedance spectroscopy showed that functionalization increased capacitive background current and reduced impedance over a broad frequency range. The phase shift observed

in the Bode plot further indicated improved ion access into the porous CNTY structure and reduced interfacial resistance. To examine how surface modification affected dopamine adsorption and desorption, flow-injection data were analyzed using an FSCV-based kinetic model. Functionalized CNTY electrodes showed an increased adsorption rate constant and a decreased desorption rate constant, resulting in a substantially higher equilibrium constant. These results indicate that oxygenated surface sites promote stronger and more stable dopamine binding. In addition, improved electrolyte penetration stabilized the electrical double layer during rapid voltage switching, reducing capacitive charging variability in multiple cyclic square wave voltammetry. As a result, background differences between consecutive scans approached zero, allowing tonic dopamine faradaic signals to be more clearly extracted. Overall, controlled electrochemical functionalization of CNTY electrodes enhances dopamine adsorption kinetics while stabilizing charge behavior within the porous electrode structure. This combined improvement provides a practical electrochemical interface for tonic dopamine sensing and may support future in vivo studies of basal neurotransmitter dynamics in neurological and psychiatric disorders.

P3-54 - Development of a multi-analyte neurochemical monitoring unit

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No conflict of interest. Traumatic Brain Injury (TBI) is the leading cause of death and disability in adults under 35 and is recognised by the World Health Assembly as a major yet under-recognised global health problem [1]. A critical challenge in TBI care is the lack of real-time monitoring of brain neurochemistry. My research addresses this by developing a continuous neurochemical monitoring unit coupled with cerebral microdialysis to provide an objective molecular window into the injured brain. Current neurocritical care enables metabolic monitoring, but real-time assessment of the brain's excitatory-inhibitory balance remains inaccessible. In severe TBI, the dynamic interplay between excitatory glutamate and inhibitory GABA is central to secondary injury mechanisms including excitotoxicity and spreading depolarisations. My work focuses on developing highly sensitive microfluidic-amperometric biosensors capable of simultaneous glutamate and GABA detection in real time. We developed a multi-component sensing platform. First, amperometric glutamate biosensors were fabricated with an average sensitivity of 2.0 pA/μM, improved from ~1 pA/μM using previous methods [2], with best-sensor performance reaching 4.0 pA/μM. Second, a GABA biosensor using a dual-enzyme cascade (GABA-AT and GlutOx) was developed to convert GABA into detectable H₂O₂. This achieved an average sensitivity of 0.4 pA/μM and best-sensor sensitivity of 0.6 pA/μM, substantially improved from 0.045 pA/μM. A key challenge is that the GABA sensor also detects endogenous extracellular glutamate (GLUT(ECF)). Accurate GABA quantification therefore requires simultaneous measurement of background glutamate and real-time signal subtraction: $\text{GABA Signal} = \text{Total Signal} - \text{GLUT(ECF) Signal}$. To enable this, we designed and validated a novel microfluidic platform allowing two biosensors to sample the analyte stream at the same flow point rather than in series. This system produced simultaneous responses within 15 ms and achieved a three-fold

improvement in T90 response time compared with the previous serial configuration. This validated platform enables the real-time signal subtraction required for monitoring glutamate/GABA dynamics in TBI. It provides a pathway toward a deployable bedside monitoring system for neurocritical care and establishes a foundational technology for broader ICU applications. By offering high-time-resolution neurochemical monitoring currently unavailable to clinicians, this work could improve understanding of secondary injury and guide future therapeutic interventions. Following laboratory and animal validation, the system will progress toward proof-of-concept clinical studies with collaborators in major trauma centres in London and Copenhagen. References: 1.Khattak et al. Brain Sci. 2026. doi:10.3390/brainsci16020134.2.Samper et al. Lab Chip. 2019. doi:10.1039/c9lc00044e.

P3-55 - Glycation-induced Dopaminergic Dysfunction in Parkinson's Disease Model Zebrafish

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by impaired motor function mainly due to the loss of dopaminergic neurons in the substantia nigra. Emerging evidences suggest that metabolic dysfunction, particularly glycation associated with diabetes, may worsen PD pathology. Methylglyoxal (MGO), a reactive carbonyl species and a byproduct of glycolysis, acts as a precursor in the formation of advanced glycation end-products (AGEs) that are found in the substantia nigra neurons of the PD brain. When glycated, α -synuclein, the hallmark aggregates in PD, may alter neurotransmitter function and receptor sensitivity, increasing neurotoxicity. Mitochondrial dysfunction and oxidative stress are well known contributors to dopaminergic dysfunction in the PD brain. However, the mechanisms underlying dopaminergic impairments due to glycation in the PD brain are not well understood. Therefore, it is crucial to explore the effects of glycation inhibitors on neurotransmitter dysregulation. In this work, we developed an acute glycation-induced PD model in *Danio rerio* (zebrafish) to investigate possible mechanistic link between glycation and dopamine signaling impairments. Adult zebrafish were treated with rotenone via the immersion method for 30 consecutive days to mimic the mitochondrial dysfunction characteristic to PD. Individual fish then received intraperitoneal injections of MGO to induce acute glycation. We then assessed dopamine release and uptake properties with fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes. Preliminary FSCV data suggested that MGO resulted in further impairments in dopaminergic signaling in rotenone-treated zebrafish indicating a synergistic effect of metabolic and oxidative stress on neurotransmission. To address these impairments, we are evaluating the anti-glycating effect of carnosine, a naturally occurring dipeptide known for its ability to scavenge reactive carbonyl species. While preliminary brain perfusion experiments suggested a decline in dopamine reuptake, these findings remain under investigation to differentiate physiological effects from potential electrochemical artifacts. To further investigate the link between metabolic state and neurotransmission, enzyme-based lactate biosensors developed by Pinnacle Technology Inc were validated for real-time measurements. These tools will enable simultaneous monitoring of glucose

dynamics alongside dopamine signaling, providing a platform to study neurochemical-metabolic coupling under relevant disease conditions. Overall, this approach serves as a novel bioanalytical platform for analyzing glycation-induced neurotransmitter dysfunction and investigating potential therapeutics with glycation inhibiting characteristics.

P3-56 - Baseline Firing Rate and mPFC Stimulation Frequency Jointly Shape Phasic Dopamine Release in the Nucleus Accumbens

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Dopamine release in the nucleus accumbens is central to reward processing, motivation, and goal-directed behavior, yet the mechanisms linking cortical input and intrinsic dopaminergic tone remain poorly understood. We combined fast-scan cyclic voltammetry (FSCV) in rats with a data-driven mathematical model to examine how medial prefrontal cortex (mPFC) stimulation frequency and the baseline firing rate (BFR) of dopaminergic neurons interact to control evoked dopamine dynamics. Electrical stimulation of the mPFC evoked robust phasic DA release in the NAc. Concurrent electrophysiological recordings were acquired simultaneously with FSCV to monitor local field potentials and multi-unit activity in the mPFC and NAc during stimulation trains. For stimulation trains longer than 5 s, 10–20 Hz frequencies produced significantly larger peak dopamine concentrations than 60 Hz, revealing a clear frequency-by-duration interaction. In contrast, brief (≤ 5 s) trains were most effective at 60 Hz. These frequency-dependent effects were absent during direct medial forebrain bundle stimulation, indicating that mPFC regulation operates through circuit-specific mechanisms. A modified model incorporating BFR (1–8 Hz) and D2 autoreceptor inhibition quantitatively reproduced the observed variability. Higher BFR elevated tonic DA levels while attenuating the peak phasic response and reducing stimulus-evoked overshoot. BFR further interacted with reuptake kinetics (V_{max} , K_m) and D2 autoreceptor binding rates (k_{on} , k_{off}), demonstrating that the pre-stimulation state of the dopaminergic system strongly modulates its responsiveness to incoming cortical drive. These results show that both the frequency of mPFC input and the instantaneous BFR of DA neurons are critical determinants of NAc DA release. Accounting for BFR and frequency tuning may improve the design of deep brain stimulation protocols for disorders involving dopaminergic dysregulation, such as depression, addiction, and Parkinson's disease.

P3-57 - Tachykinin control of striatal neuromodulator release during reward learning

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Substance P (SP) is an abundant striatal neuropeptide released primarily from D1-expressing medium spiny neurons in response to salient stimuli. Despite its abundance, its role in regulating

neuromodulatory signaling in the striatum during reward learning remains poorly understood. SP has been shown to modulate cholinergic interneuron (CIN) excitability through its action on the neurokinin 1 receptor (NK1R), which is enriched in striatal CINs, positioning it to influence both acetylcholine (ACh) signaling and downstream dopamine (DA) dynamics within striatal circuits. Because coordinated interactions between ACh and DA are critical for reinforcement learning, value encoding, and action selection, SP may serve as an important regulator of striatal neuromodulatory balance. Disruption of these signaling processes has been strongly implicated in neuropsychiatric disorders including substance use disorder, depression, and schizophrenia. Here, we investigate how SP regulates coordinated dopaminergic–cholinergic signaling during reward-associated behavior. Previous work has shown that SP can modulate CIN activity in vitro, but whether SP regulates coordinated DA and ACh signaling in vivo during learning has not been established. Using RNAscope, we confirmed that the cognate receptor for SP, neurokinin 1 receptor (NK1R), is highly expressed in striatal CINs, positioning SP to directly influence cholinergic signaling and indirectly shape dopaminergic transmission. To determine how SP regulates these neuromodulatory systems during behavior, we used dual-color in vivo fiber photometry with the genetically encoded sensors gACh3.0 and rDA3m to simultaneously monitor ACh and DA dynamics in the dorsomedial striatum and nucleus accumbens during reward learning tasks. Simultaneous photometric recordings revealed coordinated cue-evoked ACh and DA signals during reward learning that evolved across training. Importantly, global SP knockout (Tac1 KO) mice exhibited markedly diminished cue-evoked ACh release alongside enhanced DA release both during the cue and reward consumption in Pavlovian and operant reward tasks. Behavioral analyses further demonstrated impaired reversal learning in Tac1 KO mice, linking disrupted neuromodulator coordination to deficits in reward learning and action selection. Together, these findings identify SP as a critical regulator of coordinated DA-ACh signaling in the striatum and establish multiplexed neurotransmitter photometry as a powerful approach for dissecting interactions between neuromodulatory systems in vivo. More broadly, this work provides a mechanistic framework for understanding how peptidergic signaling shapes striatal circuit function and suggests that disruption of SP-dependent neuromodulatory balance may contribute to maladaptive learning processes relevant to addiction and other neuropsychiatric disorders.