
# International Society for Serotonin Research

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# A Serotonin Symphony in Vienna
# Local Organizing Committee Welcomes You!

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ISSR 2025
A Serotonin Symphony
in Vienna

## ISSR President's Welcome

Dear colleagues,

As President of the International Society for Serotonin Research, it is a great pleasure to welcome you to the **21st ISSR Meeting**, held this year in the historic and culturally rich city of **Vienna, Austria**.

It has been **two years since our last in-person gathering in Cancun**, and while the pandemic years brought their share of disruption, they also underscored the value of scientific exchange and community – and this was for sure felt when we finally gathered in Mexico. I'm delighted that we can now reunite in person in central Europe to share our latest insights and celebrate the progress in 5-HT research.

The scientific program brings together **leading researchers from across the globe**, covering an impressive range of topics—from **molecular mechanisms and receptor pharmacology, to neural circuits, behavior, and therapeutic developments.** Each day will bring parallel symposia, followed by a vibrant mix of **plenary lectures, symposia, short talks, discussion rounds** and **poster presentation**s. It is also the premiere for a five-day meeting – a new road that needed to be taken since we received so many symposia applications of superb quality. We have 27 symposia – an all-time record – and more than 250 registered participants!

We are honored to welcome three exceptional Plenary speakers: **Anne Andrews, Susan Dymecki and Alan Frazer**, whose pioneering work continues to shape our field. We also feature **Jennifer Mitchell** and **David Nutt** in two **"Special Lectures"** on concurrent topics of serotonin and drug discovery. We're very pleased to feature **27 early career researchers** who will be supported by travel grants – and the program committee has selected **18 awardees** for short oral presentations, with all presenting posters that will remain on display throughout the meeting.

The program is complemented by several social events, starting with our **Welcome Reception at the Heurigen Restaurant "Fuhrgassl-Huber"**, offering a chance to reconnect with colleagues and meet new members of our growing community. The **Farewell Dinner at the Kunsthistorische Museum** will provide further opportunities for informal exchange in a relaxed setting – apart from the stunning architecture and the guided tour through the exhibitions.

We are deeply grateful to our **sponsors** for their generous support, which is essential to the success of our meetings. The theme of this year's event, **"A Serotonin Symphony in Vienna"**, reflects not only the central role of serotonin in neuroscience, but also the opportunity to gather in a city that has long been a hub of science, music, and culture. We encourage you to take some time to explore **Vienna's rich history, stunning architecture, and renowned cuisine** during your stay – the free evenings might provide you with this opportunity. However, more scientific oriented guided **"Lunch Tours"** will allow the participants therein already with a deep dive into the history of (serotonin) research in Vienna – when visiting the **"Freud Museum – Berggasse 19"**, the **"Fools' Tower"**, the **"Josephinum"** or simply strolling on the **"Walking Tour"** through the old General Hospital.

Thank you for joining us for what promises to be a stimulating and memorable meeting. I look forward to welcoming you to Vienna!

Warm regards,
John Neumaier

## Travel Award Recipients

**Isak Aarrestad**, University of California Davis, USA
**Maria Sancho Alonso**, University of Valencia, Spain
**Carla Arganaraz**, Instituto De Fisiología, Biología Molecular Y Neurociencias CONICET, Argentina
**Sara Asgharzadeh,** University of Ottawa, Canada
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**Beatrice Baumberger**, Imperial College London, UK
**Bettina Bohl**, Imperial College London, UK
**Emma Bonniwell**, Medical College of Wisconsin, USA
**Claire Deckers**, Temple University, USA
**Sixtine Fleury**, Dartmouth College, USA
**Rocio Foltran**, Instituto De Fisiología, Biología Molecular Y Neurociencias CONICET, Argentina
**Blake Fordyce**, University of North Carolina Chapel Hill, USA
**Maria Teresa Gallo**, University of Milan, Italy
**James Gattuso**, The Florey Institute, Australia
**Ralph Gradisch**, University of Zurich, Switzerland
**L. Sophie Gullino**, Vrije Universiteit Brussel, Belgium
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## Honorary Irvine Page Plenary Lecture

**Susan M. Dymecki, M.D./Ph.D.**
*Professor of Genetics*
*George Fabyan Professor of Genetics in the Field of Comparative Physiology*
*Harvard Medical School*

## Honorary Maurice Rapport Plenary Lecture

**Alan Frazer, Ph.D.**
*Professor/Research of Pharmacology*
*University of Texas Health San Antonio*

## Honorary Paul Vanhoutte Lecture

**Anne M. Andrews, Ph.D.**
*Professor of Psychiatry*
*University of California Los Angeles*

## Special Lecture

**Jennifer Mitchell, Ph.D.**
*Professor of Neurology*
*University of California San Francisco*

## Special Lecture

**David Nutt, Ph.D.**
*Edmond J. Safra Chair in Neuropsychopharmacology*
*Department of Brain Sciences*
*Imperial College London*

# Program Overview

| Room Codes | Great Lecture Hall | Seminar Room B1,B2 | Josephinum Great Hall | |
|---|---|---|---|---|
| MON \| July 07 | TUE \| July 08 | WED \| July 09 | THU \| July 10 | FRI \| July 11 |
| | Breakfast meeting for Travel Awardees<br>Josephinum | | | |
| | Parallel Symposia II<br>6 5 4 | Parallel Symposia IV<br>8 10 12 | Parallel Symposia VI<br>25 23 26 | Parallel Symposia VIII<br>21 22 24 |
| Registration | Coffee break | Coffee break | Coffee break | Coffee break |
| | Vanhoutte Lecture<br>**Andrews** Monitoring Serotonin Signaling: A Journey in Time and Space | Special Lecture I<br>**Nutt** 5-$HT_{2A}$ Receptor Agonists: From Human Neuroimaging to New Treatments of Brain Disorders | Rapport Lecture<br>**Frazer** Serotonin-You've Come a Long Way | Special Lecture 2<br>**Mitchell** The Long and Winding Road to FDA Approval of MDMA |
| | Lunch break | Lunch break<br>Lunch Discussions<br>Career Development and Training Opportunities | Lunch break<br>Lunch Discussions<br>ECNP Psychedelics Network | Lunch break |
| Opening Speech<br>Page Lecture<br>**Dymecki** Diversity and Unity-The Brain 5-HTergic Neuronal System | Poster Session I<br>Seminar Room A | Poster Session II<br>Seminar Room A | Young scientist's session | Parallel Symposia IX<br>14 20 |
| Coffee break | Coffee break | Coffee break | Coffee break | Coffee break |
| Parallel Symposia I<br>1 2 16 | Parallel Symposia III<br>15 9 ST I | Parallel Symposia V<br>13 19 ST II | Parallel Symposia VII<br>11 17 18 | Parallel Symposia X<br>3 27 7 |
| | | | Business Meeting | |
| Welcome Dinner \| Heurigen Restaurant "Fuhrgassl-Huber" Neustift am Walde 68 A-1190 Vienna | Dinner on own | Dinner on own | Evening Poster Session \| Wine & Cheese<br>Seminar A | Farewell Dinner \| Kunsthistorisches Museum Maria-Theresien-Platz, A-1010 Vienna |
| Room Codes | Great Lecture Hall | Seminar Room B1,B2 | Josephinum Great Hall | |

*Please wear your badge during all sessions and events.*

| Symposia | | | |
|---|---|---|---|
| 1 | *Serotonergic Sub-Circuits: Probing the Roles of Identified Serotonergic Populations in Behavior and Cognition*<br>Chair(s): Bénédicte Amilhon | 15 | *Sex and the Serotonin System: Reconciling Differential Susceptibility to Alcohol and Stress-Related Disorders*<br>Chair(s): Catherine Marcinkiewcz and Pingwen Xu |
| 2 | *Brain vs Gut Feelings": Serotonin as a Central and Peripheral Regulator of Autonomic Function, Mood, and Interoception*<br>Chair(s): Mark Ansorge and Michael Gershon | 16 | *Preclinical & Clinical Studies of the Mechanisms by which Psychedelics Engender Persistent Alleviation of Neuropsychiatric Illness*<br>Chair(s): Kevin Murnane |
| 3 | *Searching for Receptors Involved in the Antidepressant Effects of Psychedelics*<br>Chair(s): Carine Becamel and Joel Bockaert | 17 | *Diverse Stress Signaling Mechanisms in Dorsal Raphe*<br>Chair(s): John Neumaier |
| 4 | *Beyond Serotonin: The Multifaceted Metabolism of Tryptophan*<br>Chair(s): Stefano Comai and Ana Pocivavsek | 18 | *Serotoninergic Mechanisms of Psychedelic Therapeutics in Substance Use Disorders: Focus on Cognitive Flexibility*<br>Chair(s): Kathryn Cunningham and Stephanie Daws |
| 5 | *The Ascending Serotonin Neurons and Their Diverse Functions through the Serotonin Heteroreceptor Complexes*<br>Chair(s): Kjell Fuxe and Dasiel Borroto Escuela | 19 | *Lipidation within the Serotonergic Pathways: Implication for Depression and Anxiety*<br>Chair(s): Evgeni Ponimaskin and Mark Rasenick |
| 6 | *Psychedelics: A Stunning Antidepressant Effect*<br>Chair(s): Alain Gardier and Bruno Guiard | 20 | *Serotonin in Sudden Death*<br>Chair(s): Russell Ray |
| 7 | *Is Serotonin a Biomarker for Depression 2024?*<br>Chair(s): Parastoo Hashemi | 21 | *Early-Life Stress and 5-HT: Neurodevelopmental Mechanisms and Circuit Dynamics Driving Lifelong Behavioral Changes*<br>Chair(s): Derya Sargin |
| 8 | *Central 5-HT Signaling in Health and Disease*<br>Chair(s): Yanlin He and Pingwen Xu | 22 | *SSRIs in the Treatment of Depression: A Pharmacological Cul-de-Sac?*<br>Chair(s): Trevor Sharp and Philip Cowen |
| 9 | *Emerging Roles for Transporters in Serotonin Dysfunction in CNS Disorders*<br>Chair(s): Freja Herborg and Ulrik Gether | 23 | *Elevated MAOA and Treatment Resistance in Depression: New Twists on a Classic Hypothesis*<br>Chair(s): Jeffrey Meyer |
| 10 | *New Findings on Maternal Serotonergic Effects on Offspring Development*<br>Chair(s): Judith Homberg and Francesca Calabrese | 24 | *Targeting Presynaptic and Postsynaptic Serotonin Systems for Neuropsychiatric Treatment Strategies*<br>Chair(s): Harald Sitte |
| 11 | *Theme and Variations: Phenotypic Diversity and Dynamics of Serotonergic Neurons*<br>Chair(s): Skirmantas Janusonis and Benjamin Okaty | 25 | *Human in vivo Imaging of Genetic Variability within the Serotonin System*<br>Chair(s): Marie Spies and Elizabeth Bartlett |
| 12 | *Acute Clinical Psychological and Neurological Effects of Psychedelics*<br>Chair(s): Matthias Liechti | 26 | *Molecular Mechanism of Serotonin Transport by SERT, OCTs, and VMAT2*<br>Chair(s): Thomas Stockner |
| 13 | *Clinical Mechanisms for Persisting Positive Effects of Psychedelics*<br>Chair(s): Gitte Knudsen | 27 | *The 5-HT$_7$ Receptor as a Druggable Target*<br>Chair(s): Stephanie Watts and Finn Levy |
| 14 | *Serotonin, Its Detection and Function*<br>Chair(s): Yulong Li | | |

# Internet Access

| University Clinic of Dentistry | Josephinum |
|---|---|
| **[1] Connect your Device (e.g. Laptop) with the WiFi-Network "Gast-Wlan".**<br>**[2] Launch your web browser**<br>**[3] The login page will automatically appear. If not, enter any website.**<br>**[4] Enter Username and Password.**<br>**Username: V_1326**<br>**Password: 0h9f3**<br>**[5] You are now online!**<br>**[6] Use http://logon.now for re-login or status info**<br>**[7] Use http://ogoff.now to force a logoff.** | **Wifi-Network: "Josephinum"**<br>**No passcode required** |

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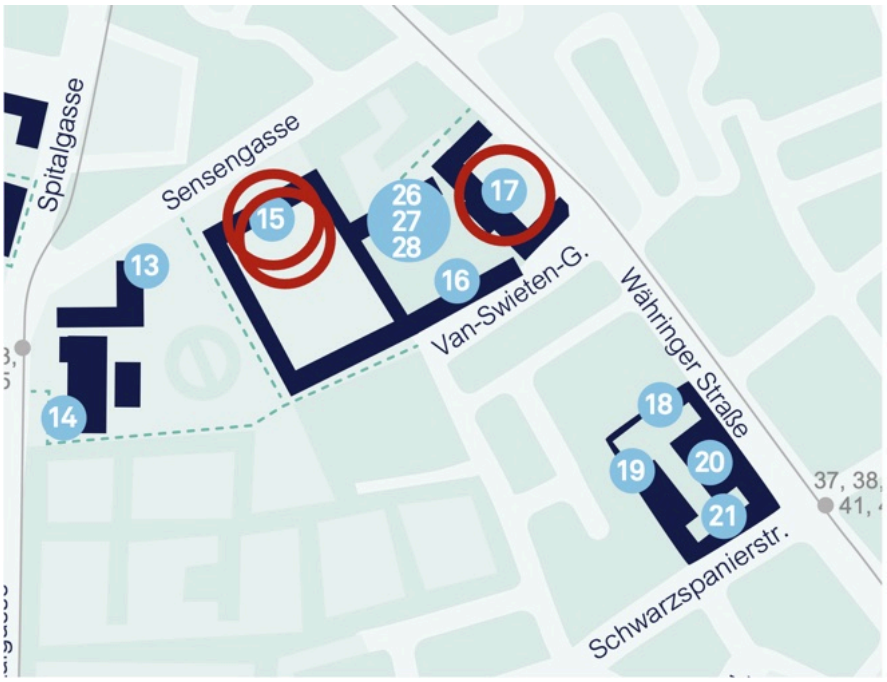
# Map of Venues




The Great lecture hall of the University Clinic of Dentistry and Seminar Hall A & B **(15)**
Josephinum, Währinger Strasse 25; Historical lecture hall and Museum for Lunch tour **(17)**
**REGISTRATION WILL BE IN THE ENTRY HALL OF THE UNIVERSITY CLINIC OF DENTISTRY (15)**

Here is how to walk most conveniently between the venues: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# Program Details

## Monday July 7th, 2025

| | |
|---|---|
| **10:00-16:00**<br>Entrance Hall | **Registration** |
| **14:15-14:45**<br>Great Lecture Hall | **Welcome Notes & Organisational Remarks**<br>*Michaela Fritz, Vice Rector for Research and Innovation, MedUni Vienna*<br>*John Neumaier, ISSR President*<br>*Harald H. Sitte & Matthäus Willeit, Chairs of the Local Organizing Committee* |
| **14:45-15:45**<br>Great Lecture Hall | **Page Lecture**<br>**Diversity and Unity - The Brain 5-HTergic Neuronal System**<br>*Susan Dymecki, Harvard Medical School*<br><br>*Chair & Introduction: John Neumaier* |

Serotonergic neurons, while unified by serotonin metabolism, diversify into numerous subtypes serving distinct biological processes. This diversity is the subject of Dymecki's Irving Page Plenary Lecture. Evidence of serotonergic neuron diversity was first observed decades ago through immunohistochemistry, electrophysiology, and neural tract-tracing-based studies, and has proven significant. The Dymecki Lab has sought to understand the extent of this heterogeneity—its generation developmentally, plasticity across the lifespan, and organizational logic—as revealed by applying molecular-genetic, genomic, and systems neuroscience approaches to genetically engineered rodent models. Findings suggest new ways to envisage the organization of these neurons and their functional classification, and provide new molecular tools to analyze specific serotonergic neuron subtypes. These data, together with rich findings in the field, support the idea that there are many different serotonergic neuronal subsystems, each influencing different physiological and behavioral processes. Further, the Dymecki lab applies these molecular findings and neuron classification models to postmortem human brain tissue. Together, this rodent and human-tissue work offers insights into which subtypes of serotonergic neurons and molecular signaling pathways may shape the clinical spectrum of brain disorders such as affective disorders, substance use disorders, sleep apnea, or the sudden infant death syndrome, to name a few examples.

| | |
|---|---|
| **15:45 - 16:15**<br>Catering Area | **Coffee - Tea - Break** |

**16:15 - 18:00**
Great Lecture Hall

## Parallel Symposia 1: Serotonergic Sub-Circuits: Probing the Roles of Identified Serotonergic Populations in Behavior and Cognition

*Chair: Bénédicte Amilhon*

Serotonergic neurons are diverse in their developmental origins, molecular footprint and electrophysiological properties. The complexity of serotonin neuron identity is paralleled by a modular organization of their input-outputs. An accumulation of work shows that anatomically defined serotonin neuron groups (B1-B9) serve distinct and sometimes opposite roles. Yet, increasing evidence suggests that the picture is even more complex, with traditional serotonin neuron groups hosting multiple sub-populations and circuits that support heterogenous properties and functions. Recent developments in genetic tools, viral vectors and optical strategies are allowing to further disentangle serotonergic populations and assess their roles in cognitive and behavioral functions. The speakers in this symposium use a variety of cutting-edge approaches to target identified serotonergic sub-circuits and elucidate their heterogenous roles in the mouse brain. Dr Bénédicte Amilhon addresses the roles of serotonin in the ventral hippocampus in relation to anxiety-like behavior. Using retrograde viral vectors to selectively target the raphe-ventral hippocampus serotonergic circuit, they uncover sex-specific roles of this circuit in modulating anxiety in female, but not male mice. Dr Christoph Anacker investigates the roles of serotonin in mediating long-term behavioral consequences of early life adversity. Using genetic models of altered serotonergic transmission and circuit studies, they show that altered serotonin release in the ventral dentate gyrus underlies sex differences in fear generalization after early life stress. Dr Nuno Dinis Alves studies the role of dorsal raphe-medial prefrontal cortex serotonergic circuits in cognitive flexibility. He will present novel data on the functional consequences of serotonergic input into the mPFC, including its adult plasticity. Dr Giacomo Maddaloni uncovers a novel role of developmentally defined mrEn1-Pet1 neurons in sleep and behavioral adaptation to variations in daylight duration. Their work notably reveals a new form of neurochemical plasticity, where reversible and branch selective expression of the vesicular glutamate transporter type 3 underlies synchronization to photoperiod change. Taken together, this symposium will highlight some of the most recent advances in probing the roles of identified serotonergic sub-circuits in cognition and behavior.

| | |
|---|---|
| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | A prospective code for value in the serotonin system<br>**Emerson Harkin**, Cooper Grossman, Jeremiah Cohen, Jean-Claude Béïque, Richard Naud |
| 16:45 - 17:10 | Serotonin's Role in Medial Prefrontal Cortex-mediated Cognitive Flexibility<br>**Nuno Dinis Alves**, PhD Ashlea Morgan, MSc Gregory Stevens, BSc Tamanna Yeasmin, MSc Alexandra Mackay, PhD Saige Power, PhD Derya Sargin, BSc Carla Hanna, BSc Arwa Adib, BSc Annette Ziolkowski-Blake, PhD Evelyn Lambe, PhD Mark Ansorge |
| 17:10 - 17:35 | Adaptation to seasonal photoperiods via dynamic serotonin-glutamate neurotransmitter segregation<br>Giacomo Maddaloni, YoonJeung Chang, Rebecca Senft, **Susan Dymecki** |
| 17:35 - 18:00 | Sex-specific modulation of anxiety by raphe-ventral hippocampus serotonergic circuits<br>**Bénédicte Amilhon** |

**16:15 - 18:00**

Seminar Room B1,B2

**Parallel Symposia 2: "Brain vs Gut Feelings": Serotonin as a Central and Peripheral Regulator of Autonomic Function, Mood, and Interoception**

*Chair: Mark Ansorge, Co-Chair: Michael Gershon*

The outstanding and comprehensive research on serotonin as a CNS- and gut-derived neurotransmitter has led to important discoveries regarding the roles of serotonin in central and enteric nervous system (CNS and ENS, respectively) development, mood, gastrointestinal motility, autonomic regulation, and pain. Many of these discoveries have segued into the clinical realm of patient care, where use of selective serotonin reuptake inhibitors (SSRIs) is often prescribed as a primary treatment for mood disorders and abdominal pain-related Disorders of gut-brain interaction (DGBI), the most common GI disorders internationally. Newer research, however, has increasingly revealed that serotonin and SSRIs have many more impacts, both inside and outside of the CNS, that need to be considered when utilizing interventions that systemically target serotonin signaling. In particular, gut-derived serotonin may have critical implications on mood and gut interoception. This symposium will help listeners to: (1) Understand the research outlining how serotonergic neurons within the CNS modulate diverse processes ranging from respiration and thermal balance to preference, avoidance, and coping behaviors; (2) Delineate the negative developmental impacts of in utero SSRI exposure on fetal neurodevelopment and long-term outcomes in regards to mood disorders and DGBI; (3) Appreciate, through a combination of basic and clinical research, how targeted serotonergic signaling within the gut may be a novel treatment for mood; (4) Learn how piezo-2 and enterochromaffin cells play important roles in gut interoception and pain.

| | |
|---|---|
| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | The medullary 5-HTergic neuron subtype called Tac1-Pet1 augments breathing during quiet wake and counters morphine-induced respiratory depression.<br>**Kathryn Lehigh**, Jordan Jones, M.D., Ph. D. Ryan Dosumu-Johnson, M.D., Ph.D. Susan Dymecki |
| 16:45 - 17:10 | Pathway-specific Roles for Serotonin and Their Developmental Malleability<br>**Mark Ansorge** |
| 17:10 - 17:35 | Intestinal SERT and SSRIs Play Important Roles in Gut-Brain Communication: Bench to Bedside Translation<br>**Kara Margolis** |
| 17:35 - 18:00 | Gut touch and the role of gut serotonin in the gut's intrinsic tactile sense<br>**Arthur Beyder** |

**16:15 - 18:00**

Josephinum
Great Hall

**Parallel Symposia 16: Preclinical and Clinical studies of the Mechanisms by which Psychedelics Engender Persistent Alleviation of Neuropsychiatric Illness**
*Chair: Kevin Murnane*

***Sponsorship by ASPET is gratefully acknowledged***

Psychedelics show promise in clinical trials for various neuropsychiatric conditions including depression, substance use disorder, and post-traumatic stress disorder, with remarkable persistence and effect sizes after only one or two treatments. These remarkable treatment responses are unlike therapies that have been developed previously, which typically require chronic daily administration to take effect and maintain efficacy. If such responses continue into larger trials and following market approval, this represents a transformative new paradigm for the treatment of neuropsychiatric illness. Further, this presents an opportunity to expand and shape human knowledge of the brain and behavior, by elucidating critical mechanisms in these persistent responses. Human studies of the mechanisms of action underlying the persistent therapeutic effects of psychedelics have been largely drawn from neuroimaging assays. In preclinical models, almost all efforts have been directed to analysis of synaptic plasticity. Specifically, increased synapse formation of excitatory neurons. In this panel, we will present exciting data that deepens knowledge on these mechanisms as well as identify and elucidate novel mechanisms by which psychedelics can induce persistent therapeutic effects. The first speaker, Dr. Kevin Murnane, will discuss and present aligned preclinical and clinical data on the mechanisms by which psychedelics can address compulsive methamphetamine use, as well as co-occurring affective disorders. The second speaker, Dr. Charles Nichols, will present preclinical data and discuss findings on the structural, functional, and pharmacological mechanisms by which psychedelics include persistent antidepressant-like effects. The third speaker, Dr. Peter Hendrics, will present clinical, psychological, and behavioral data on the mechanisms by which psychedelics can treat cocaine addiction and other neuropsychiatric illnesses. The fourth speaker, Dr. Wilder T. Doucette, will present preclinical data on the mechanisms by which psychedelics may induce metaplastic changes in brain circuitry relevant for neuropsychiatric illness. The panel will conclude with a joint effort to integrate, align, and inform these preclinical and clinical findings, as well as discussion of the areas that will advance the field moving forward.

| | |
|---|---|
| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | Preclinical and clinical studies of psychedelics for methamphetamine addiction<br>**Kevin Murnane**, Frances Vest, Bo Wood, Alexandru Dumitrescu |
| 16:45 - 17:10 | Psychedelics produce persistent antidepressant-like effects through functional plasticity rather than structural plasticity<br>**Charles Nichols** |
| 17:10 - 17:35 | Psilocybin in the Treatment of Cocaine Use Disorder<br>**Peter Hendricks** |
| 17:35 - 18:00 | Latent LSD induced metaplasticity is unmasked with medial frontal brain stimulation and captured by cortical-striatal network activity<br>Lucas Dwiel, **Wilder Doucette** |

18:30 - 22:00 **Welcome Dinner** by Invitation of the Mayor of the City of Vienna, Michael Ludwig, and the ISSR at the Heurigen Restaurant "*Fuhrgassl-Huber*", Neustift am Walde 68, 1190 Vienna (*find directions appended below*)

## Tuesday July 8th, 2025

| | |
|---|---|
| **08:00 - 11:00**<br>Entrance Hall | **Registration** |
| **07:30 - 08:30**<br>Josephinum Lesesaal | **Travel Grant Awardee Breakfast (*by invitation only*)** |
| **08:30 - 10:15**<br>Great Lecture Hall | **Parallel Symposia 6: Psychedelics: A Stunning Antidepressant Effect**<br>*Chair: Alain Gardier, Co-Chair: Bruno Guiard* |

Psychedelics are psychoactive substances that have been used in shamanic rituals for millennia, but are now attracting attention because of their potential in treating neuropsychiatric disorders, such as in patients with treatment-resistant depression. Psychedelics such as psilocybin found in 'magic mushrooms', lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-iodoamphetamine (DOI) and 5-methoxy-N,N-dimethyl-tryptamine(5-MeO-DMT), have already entered clinical trials with stunning results, i.e., a rapid antidepressant effect lasting for several weeks after a single administration (e.g., psilocybin). How these drugs engage molecular targets in the brain, and how this leads to their therapeutic and hallucinogenic effects not fully understood yet. Many psychedelics are chemically related to the neurotransmitter serotonin and bind to the 5-$HT_{2A}$ serotonin receptor. However, other G-protein-coupled serotonin receptors and the context of their administration might have crucial roles in the complex psychoactive and therapeutic effects of these drugs.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Psilocin, a stunning antidepressant effect in stress mice!<br>**Alain Gardier**, Makiath Adebo, Ons Laouej, Céline Defaix, Denis David, Erwan Poupon, Laurent Tritschler |
| 09:00 - 09:25 | LSD Treatment for Anxiety Disorders<br>**Stefano Comai, on behalf of Gabriella Gobbi** |
| 09:25 - 09:50 | The non-hallucinogenic serotonin 1B receptor is involved in the persisting behavioral effects and neural mechanisms of psilocybin in mice<br>**Sixtine Fleury** |
| 09:50 - 10:15 | Influence of the context of administration in the antidepressant-like response of the psychedelic 5-MeO-DMT<br>**Bruno Guiard**, Dr Romain Hacquet |

**08:30 - 10:15** **Parallel Symposia 5: The Ascending Serotonin Neurons and Their Diverse Functions through the Serotonin Heteroreceptor Complexes**
Seminar Room B1,B2
*Chair: Kjell Fuxe, Co-Chair: Dasiel Borroto Escuela*

The serotonin heteroreceptor complexes in the brain can operate via allosteric receptor-receptor interactions to participate in treatment of major depressive disorder (MDD) which will be presented (Dr. Borroto-Escuela). The discovery of the FGFR1-5-$HT_{1A}$R heterocomplexes in the dorsal hippocampus and the raphe bring together the serotonin hypothesis and the neurotrophic hypothesis of MDD. Disturbances in the molecular integration in serotonin heteroreceptor complexes can play a significant role in depression. It has also been found that oxytocin receptor forms heteroreceptor complexes with serotonin 5-$H_{2A}$R and 5-$HT_{2C}$R in the limbic system with potential relevance for social behavior and antidepressant treatment, which will be presented by Prof. Kjell Fuxe. They are found inter alia in the pyramidal cell layer of CA2 and CA3 in the dorsal hippocampus and the nucleus accumbens shell. Epidemiological data indicate a high rate of comorbidity between depression and substance use disorder (SUD). Prof. Frankowska found that administration of serotonin 5-$HT_{2C}$R agonists reduced cocaine reinforcement and protected against cocaine-seeking behavior in rats with a bilateral olfactory bulbectomy as well as in SHAM control rats undergoing cocaine self-administration. In contrast, acute administration of the 5-$HT_{2C}$R-preferring antidepressant mirtazapine did not alter cocaine reinforcement in either phenotype but reduced cocaine-seeking behavior. Neurochemical analyses revealed that cocaine reinforcement increased 5-$HT_{2C}$R levels in the ventral hippocampus, with a pre-existing depression-like phenotype enhancing this effect. In a last lecture, Véronique Sgambato, will discuss and present data on the role of the serotonergic system in impulse control disorders in Parkinson's disease. Impulse control disorders affect 14% to 33% of Parkinsonian patients treated with dopaminergic agonists and are notably underpinned by dysfunction of the dopaminergic system. There is no effective drug treatment to counteract these non-motor complications, apart from reducing dopa therapy, with the risk of withdrawal symptoms and worsening motor symptoms. However, non-dopaminergic mechanisms are thought to promote the genesis of these disorders. She investigated the potential involvement of the serotonin system in the pathophysiology of impulse control disorders, both in an animal model of Parkinson's disease and in parkinsonian subjects, using clinical, behavioral and imaging approaches. Preclinical and clinical results will be presented and discussed.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Brain serotonin heteroreceptor complexes in the Flinders Sensitive Line (FSL) rat model of depression and as targets for antidepressant treatment<br>**Dasiel Oscar Borroto-Escuela**, Marco Bartolini, Emmanuell Gonzalez-Cristo, Verty Ochoa-Torres, Adam Danielson, Javier Ruiz-Lasierra, Emilio Serra-Rojas, Francesca Frescura, Migdalis Hidalgo-Muniz, Orisley Franch de Armas, Kjell Fuxe |
| 09:00 - 09:25 | The role of the oxytocin receptor in the GPCR heteroreceptor complexes in the brain and Its relevance for brain integration.<br>**Kjell Fuxe**, Cristina Cuesta-Marti, Barbara Chruścicka-Smaga, Álvaro Enrique Cáceres-Quezada, Francesca Frescura, Sarah Beggiato, Luca Ferraro, Minerva Crespo-Ramírez, Angelica Maria Fierro-Huerta, Miguel Perez de la Mora, Harriët Schellekens, Dasiel Oscar Borroto-Escuela |
| 09:25 - 09:50 | Evaluation of 5-$HT_{2C}$ Receptor Drugs in a Preclinical Model of Depression and Cocaine Addiction Comorbidity<br>**Małgorzata Frankowska**, Dr Joanna Jastrzębska, Prof. Małgorzata Filip |
| 09:50 - 10:15 | One Molecule, Many Fates: Serotonin´s journey in search of neurochemical balance<br>**Angélica Fierro**, Matias Marambio, Juan Pablo Aguayo, Yuan Chang, Nicole Morales, Luis Dinamarca-Villarroel, Agustín Robles, Álvaro Cáceres, Gerald Zapata-Torres, Gonzalo E. Torres |

**08:30 - 10:15** **Parallel Symposia 4: Beyond Serotonin: The Multifaceted Metabolism of Tryptophan**
Josephinum Great Hall
*Chair: Stefano Comai, Co-Chair: Ana Pocivavsek*

Tryptophan metabolism encompasses an intricate network of biochemical pathways that extend far beyond the synthesis of serotonin. Indeed, as an essential amino acid, tryptophan serves as a precursor not only for serotonin but also for a diverse array of metabolic products that play crucial roles in physiological and pathological processes. The kynurenine pathway, for instance, leads to the production of several bioactive metabolites, including kynurenine, kynurenic acid, and quinolinic acid, each of which has significant implications for immune regulation, neuroprotection, and neurotoxicity. Our symposium aims to explore these multifaceted pathways, shedding light on the interconnected roles of tryptophan metabolites in health and disease. This exploration extends beyond serotonin synthesis and aims to generate substantive new ideas and foster collaborations around a rapidly expanding topic that is translationally relevant across different areas of medicine. In detail, Prof. Stone will provide an overview of the complexity of tryptophan metabolism along the different but interconnected pathways. Prof. Comai will present translational findings that demonstrate how the interplay between serotonin, melatonin, and kynurenine metabolites may underlie the associations among schizophrenia, metabolic disturbances, and cognitive impairment, potentially identifying new targets for treatment. Dr. Pocivavsek will discuss models that enhance tryptophan metabolism during development, with a special emphasis on the consequences of elevated kynurenine metabolites during the prenatal period on neurochemical and behavioral consequences later in life. Prof. Erhardt will discuss the significant finding of elevated cerebrospinal fluid (CSF) serotonin (5-HT) levels in patients experiencing their first-episode psychosis (FEP). Furthermore, Prof. Erhardt will delve into the comprehensive pathway analysis, which linked these metabolic alterations to inflammatory pathways, underscoring their crucial role in the pathogenesis and progression of psychosis.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Unveiling the complexity of tryptophan metabolism<br>**Trevor Stone** |
| 09:00 - 09:25 | Tryptophan Metabolism in During Neurodevelopment: Sleep, Cognition, and Mental Health<br>**Ana Pocivavsek** |
| 09:25 - 09:50 | The Role of Tryptophan Metabolism, Immune Activation, and Kynurenine Pathway Dysregulation in Psychotic Disorders<br>**Sophie Erhardt** |
| 09:50 - 10:15 | Bridging serotonin, melatonin, and kynurenines: insights into schizophrenia<br>Benedetta Barzon, Atea Shkodra, Sofia Nasini, Paola Fadda, Mirko Manchia, Antonella Bertazzo, Stefano Dall'Acqua, Alessio Squassina, Claudia Pisanu, Marta Bosia, **Stefano Comai** |

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**10:15 - 10:45** **Coffee - Tea - Break**

Catering Area

**Vanhoutte Lecture**

**10:45 - 12:00** **Monitoring Serotonin Signaling: A Journey in Time and Space**

Great Lecture Hall

*Anne Andrews, University of California Los Angeles*

*Chair & Introduction: John Neumaier*

We aim to understand how serotonin encodes information related to anxiety and stress. Throughout our research, we have developed and improved neuroanalytical methods to monitor serotonin levels in vivo with high temporal, spatial, and chemical resolution. Building on the pioneering work of Ralph Adams, we used electrochemical detection of serotonin to enhance fast microdialysis sampling combined with online HPLC. To directly sample the brain's extracellular signaling space, we developed rapid-pulse voltammetry paired with machine learning for multiplexed measurements of serotonin and dopamine. In addition to electrochemistry, we created electronic biosensors based on DNA aptamers for molecular recognition, coupled with field-effect transistors for electronic signal transduction. These sensors extend detection to non-electrochemically active neurotransmitters and hormones, allowing for implantable and wearable monitoring. Using high-resolution monitoring, we investigated the transgenerational impact of stress on anxiety-related behavior in adult mouse offspring and are working toward wearable multiplexed biomarker measurements for personalized insights into human stress responses.

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**12:00 - 14:00** **Lunch - Break (*opt. Lunch Tours - see Meeting points below*)**

Catering Area

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**14:00 - 15:45** **Poster Session I (Odd numbers)**

Seminar Room A

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**15:45 - 16:15** **Coffee - Tea - Break**

Catering Area

**16:15 - 18:00**
Great Lecture Hall

**Parallel Symposia 15: Sex and the Serotonin System: Reconciling Differential Susceptibility to Alcohol and Stress-Related Disorders**
*Chair: Catherine Marcinkiewcz, Co-Chair: Pingwen Xu*

Serotonin neurons in the dorsal raphe nucleus (DRN) are the main source of serotonergic input to the forebrain and orchestrate a wide variety of behavioral responses to stress, alcohol, and other drugs of abuse. Although they share many neurochemical and physiological properties in common, serotonin neurons are quite diverse and can be stratified into genetically and anatomically distinct subgroups that preferentially project to cortical or subcortical targets. Adding to this complexity, serotonin neurons express both types of nuclear estrogen receptors (ERα and Erβ) which, when activated, can modulate neuronal activity and expression of biosynthetic enzymes. It is therefore not surprising that the DRN serotonin system may be an important site of action for the expression of sex differences in stress-related disorders. The focus of this symposium will be on elucidating sex differences in stress-related disorders including PTSD and alcohol use disorder (AUD) through the lens of serotonin signaling across a variety of brain circuits. Dr. Xu will discuss new findings on the role of estrogens in modulating serotonergic neurons in the DRN in binge-like alcohol consumption. Dr. Marcinkiewcz will highlight the divergent effects of alcohol consumption on serotonin subsystems that modulate pain and social motivation in males and females. Dr. Burghardt will present evidence of a sex-dependent effect of serotonin on fear learning in the extended amygdala and its relationship to PTSD. Dr. Flanigan will focus on sex-specific effects of alcohol on social recognition behavior and the role of lateral habenula serotonin signaling in mediating these effects. The potential application of these collective results to precision medicine for stress-related disorders will be discussed.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | Estrogen Signaling in Dorsal Raphe 5-HT Neurons Regulates Binge-like Drinking in Mice<br>Valeria Torres Irizarry, Bing Feng, Qi Xu, Yanlin He, **Pingwen Xu** |
| 16:45 - 17:10 | Sex-dependent effects of chronic alcohol on serotonergic circuits in affective behavior<br>**Catherine Marcinkiewcz** |
| 17:10 - 17:35 | Serotonergic modulation of the BNST-CeA circuit promotes fear learning in female mice<br>Rebecca Ravenelle, Jinah Lee, Carolina Fernandes-Henriques, Jia Liu, Allyson Friedman, Ekaterina Likhtik, **Nesha Burghardt** |
| 17:35 - 18:00 | Abstinence from binge alcohol consumption produces sex-specific behavioral and physiological effects in the lateral habenula serotonin receptor $5HT_{2C}$ system.<br>**Meghan Flanigan** |

**16:15 - 18:00**
Seminar Room B1,B2

**Parallel Symposia 9: Emerging Roles for Transporters in Serotonin Dysfunction in CNS Disorders**
*Chair: Freja Herborg, Co-Chair: Ulrik Gether*

Serotonin is a crucial regulator of both basal and higher brain functions, and its dysfunction is linked to many CNS disorders, including depression, autism spectrum disorder, OCD, and anxiety. At serotonergic release sites, serotonin signals are tightly controlled by the serotonin transporter (SERT), a primary target of widely used antidepressants. Moreover, the extensive projections of serotonergic neurons underpin complex interactions with other neural circuits. However, the molecular and circuit mechanisms through which changes to serotonin signaling arise in diseased states and translate into altered serotonin-related emotional states, behaviors, and treatment responses remain poorly understood. In this session, the speakers will present recent work that offers new translational perspectives on serotonin dysfunction using exome sequencing, disease-associated patient-derived transporter mutants, animal disease models, and in vivo imaging of SERT in patients. Dr. Randy Blakely will open the session with new data on serotonergic plasticity in the context of genetic dopamine dysfunction. Following him, Dr. Freja Herborg will present new data on the identification and characterization of novel disruptive coding SERT variants found in patients with treatment-resistant chronic affective disorders. Next, Dr. Rupert Lanzenberger will offer novel insights from human SERT PET studies. Finally, Dr. Paola Brivio will introduce recent insights on Sex- and time-dependent effects of perinatal fluoxetine exposure on lifelong behaviors. Collectively, the session will highlight new transporter-related research on serotonin dysfunction and its neural, behavioral, and disease manifestations.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | She Made Me Do It: Behaviorally Penetrant Serotonergic Plasticity in the Context of Genetic Dopaminergic Dysfunction<br>**Randy Blakely** |
| 16:45 - 17:10 | Novel Insights from Hypomorphic SERT Variants in Patients with Affective Disorders<br>**Freja Herborg** |
| 17:10 - 17:35 | Serotonin Transporter Occupancy and Its Modulation: Neuroimaging Perspectives<br>**Rupert Lanzenberger** |
| 17:35 - 18:00 | Sex- and time-dependent effects of perinatal fluoxetine exposure on lifelong behaviors: insights into sensitive period dynamics<br>**Paola Brivio**, Maria Teresa Gallo, Anais Virenque, Alessia Golinelli, Fabio Fumagalli, Eero Castren, Francesca Calabrese |

**16:15 - 18:00**

Josephinum
Great Hall

**Short Talks I**
*Chair: Nicole Praschak-Rieder*

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:35 | Serotonin transporter abundance predicts the long-term SSRI treatment effect<br>**Matej Murgas** |
| 16:35 - 16:50 | The selectivity filter of serotonin transporter is comprised of extracellular loop 2 and 4<br>**Rong Zhu** |
| 16:50 - 17:15 | Evidence for low affinity of GABA at the vesicular monoamine transporter VMAT2<br>**Thomas Steinkellner** |
| 17:15 - 17:30 | Alterations of Cognitive Behaviours and Prefronto-Thalamic Circuits after Early-Life Exposure to Fluoxetine<br>**Nina Nitzan Soto** |
| 17:30 - 17:45 | Dynamic Duo: Serotonin Transporter and Organic Cation Transporter 3 Regulate Basolateral Amygdala Serotonin Clearance and Fear Memory Recall<br>**Lynette Daws** |
| 17:45 - 18:00 | SERT N-terminal domain encodes determinants of PKG/p38aMAPK activation<br>**Christina Fenollar Ferrer** |

**Dinner on own - enjoy your evening in Vienna** (*Dinner suggestions on conference webpage)*

*https://www.serotoninclub.org/2025-food-drink-recommendations*

*ISSR 2025*
*A Serotonin Symphony in Vienna*

ASPET 2026
Advancing Pharmacology. Building Community.
SUN, MAY 17–WED, MAY 20 | MINNEAPOLIS, MN

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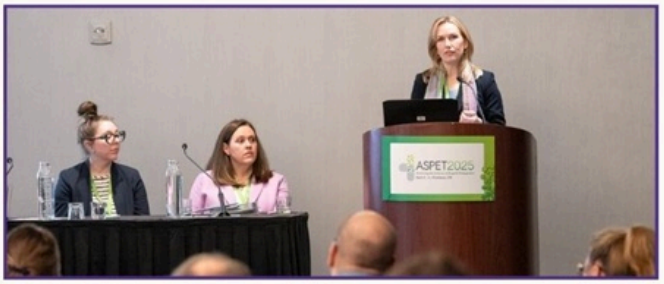

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## Wednesday July 9th, 2025

**08:00 - 11:00**
Entrance Hall

**Registration**

**08:30 - 10:15**
Great Lecture Hall

**Parallel Symposia 8: Central 5-HT Signaling in Health and Disease**
*Chair: Yanlin He, Co-Chair: Pingwen Xu*

The Symposium on serotonin (5-HT) and metabolic control is an academic gathering dedicated to exploring the complex role of 5-HT in regulating feeding behavior and energy balance. This interdisciplinary event brings together leading neuroscientists, biochemists, pharmacologists, and clinical researchers to discuss the latest findings and advancements in the field. Key topics of the symposium include: 1. Neurobiological Mechanisms: Presentations and discussions on how serotonin influences neural circuits involved in hunger and satiety. This includes the identification of specific serotonin receptors and pathways in the brain that modulate feeding behavior and energy balance. 2. Pharmacological Interventions: Examination of drugs targeting the serotonergic system to treat disorders related to feeding, such as obesity, anorexia, and bulimia. This involves understanding the therapeutic potential and side effects of such interventions. 3. Behavioral and Psychological Aspects: Analysis of how serotonin levels and receptor activities correlate with eating habits, mood, and disorders like anxiety and depression, which can impact feeding control. 4. Clinical and Translational Research: Updates on clinical trials and translational research aimed at developing new treatment strategies for metabolic disorders by modulating the serotonergic system. The symposium provides a platform for sharing cutting-edge research through keynote speeches, panel discussions, and poster presentations. Attendees have the opportunity to network, collaborate, and discuss future directions in serotonin research, with the ultimate goal of improving interventions for feeding-related health issues. This event is essential for anyone interested in the intersection of neuroscience, nutrition, and medicine, offering a comprehensive understanding of how serotonin impacts feeding behavior and the potential for novel therapeutic approaches.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Hypothalamic Serotonin Receptor Signaling and Energy Homeostasis<br>**Chen Liu** |
| 09:00 - 09:25 | 5-HT neurons and neurodevelopmental disorders<br>**Kensuke Futai**, Youngjae Ryu, Amy Cheung, Antonio Santana |
| 09:25 - 09:50 | Serotonin neurons in the dorsal raphe control food cravings during pregnancy in mice<br>**Yanlin He**, Pingwen Xu |
| 09:50 - 10:15 | Placental serotonin causes transcriptional and compositional changes in brain<br>**Roman Romanov** |

**08:30 - 10:15** Seminar Room B1,B2

**Parallel Symposia 10: New Findings on Maternal Serotonergic Effects on Offspring Development**

*Chair: Judith Homberg, Co-Chair: Francesca Calabrese*

While a dysfunctional serotonin (5-HT) system is one of the key pathophysiological mechanisms that underpin neuropsychiatric disorders, a clear understanding of its role remains incomplete. 5-HT is particularly well-known as a neurotransmitter, but also acts as a neurotrophic factor during neurodevelopment and a neurotransmitter during adulthood. This dual role of 5-HT asks for a deeper understanding of its contribution to the maturation and function of brain circuits and onset of neuropsychiatric disorders. In early-gestation, the developing forebrain relies on a placenta-derived source of 5-HT. As a result, 5-HT neurotrophic actions will be determined by maternal 5-HTergic factors rather than offspring 5-HTergic factors. In this symposium we will present the latest findings regarding maternal selective serotonin reuptake inhibitor (SSRI) exposure and maternal 5-HTergic genotype effects on offspring brain development and the behavioral consequences. Francesca Calabrese will introduce recent findings showing a sex- and time-dependent influence of perinatal exposure to fluoxetine on the development of cognitive deficits and anhedonia which are, furthermore, associated with symptom-specific molecular alterations in the brain and blood. Jocelien Olivier will present new findings on how maternal fluoxetine exposure alters placenta function and the gut microbiome in the mother and how this affects the expression of myelin-related genes and stress-related and social behavior in the offspring. Eelke Snoeren will discuss the effects of perinatal SSRI exposure on sociosexual behaviors displayed in a social context of a seminatural environment, and the effects of stressful life-events. Finally, Judith Homberg will switch to the effects of maternal genotype and demonstrate using rats how tryptophan-hydroxylase 1 genotype of the mother affects in a surprisingly profound manner behavior in cognition in the offspring. These presentations will together underline the importance of the maternal serotonergic status for offspring development and vulnerability to neuropsychiatric disorders, and in the future may shift attention from treatment to prevention of these disorders.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Molecular insights into long-term behavioral effects of perinatal serotonergic manipulation.<br>Maria Teresa Gallo, Paola Brivio, Arianna Palumbo, Fabio Fumagalli, **Francesca Calabrese** |
| 09:00 - 09:25 | Mom's gut feelings. Influences of SSRI treatment during pregnancy on maternal microbiome and offspring development<br>Anouschka Ramsteijn, Danielle Houwing, Mayerli Prado-Rivera, **Jocelien Olivier** |
| 09:25 - 09:50 | Perinatal SSRI exposure in rats and the effects on sociosexual behaviors in a seminatural environment<br>**Eelke Snoeren** |
| 09:50 - 10:15 | Beyond the own genes: Maternal serotonergic genotype shapes offspring's brain, cognition, and behaviour<br>**Judith Homberg**, Rogerio Castro, Jan Buitelaar |

**08:30 - 10:15** **Parallel Symposia 12: Acute Clinical Psychological and Neurological Effects of Psychedelics**
Josephinum Great Hall
*Chair: Matthias Liechti*

Psychedelics and MDMA produce powerful psychoactive effects via 5-$HT_{2A}$ receptor agonism and serotonin release, respectively. The psychoactive effects of psychedelics include alterations in visual and auditory perception, cognition of space, time and selfhood and intensification of mood. MDMA shares some of these effects, with more generally positive effects on mood and feelings of social connectedness, and weaker effects on perception and cognition. The fact that such remarkable psychoactive effects can be mediated by agonism in the serotonin system implicates that system as a widespread mediator of many aspects of conscious experience. Additionally, MDMA and psilocybin are both in phase 3 for the treatment of PTSD and treatment-resistant depression respectively and LSD is in phase 3 for GAD and their acute effects have been linked to clinical efficacy. Despite this, little is known about the neuropharmacological underpinnings of these remarkable acute effects. MDMA releases serotonin and oxytocin and has two pharmacologically distinct enantiomers and is clinically administered as the racemate. Prof. Matthias Liechti will describe the psychological acute effects of MDMA and its enantiomers compared to psychedelics. The psychedelic N,N-DMT has a very short plasma half-life allowing for better control of the duration of psychoactive effects. Dr. Severin Vogt will present data showing the acute psychological effects of N,N-DMT as a continuous infusion including participant-controlled self-titration. Dr. Chistopher Timmermann will present fMRI and EEG neuroimaging data from participants who received both ketamine and N,N-DMT to explain the neurological underpinnings of the psychoactive effects of DMT. Dr. Patrick Fisher will present fMRI data investigating the acute effects of psilocybin on functional brain entropy and hemodynamic effects in healthy volunteers to further nuance the neurological effects of psychedelic drugs in the human brain. Together, these sessions provide a thorough multi-model investigation of the acute effects of psychedelics in a clinical population across a range of drugs affecting the serotonin system.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Mechanism of action of acute subjective effects of psychedelics and MDMA<br>**Matthias Liechti** |
| 09:00 - 09:25 | Pharmacokinetics, acute subjective effects and tolerability of intravenous N,N-dimethyltryptamine<br>**Severin Benjamin Vogt**, Livio Erne, Matthias E. Liechti |
| 09:25 - 09:50 | Acute effects of MDMA, MDA and their prodrugs Lysine-MDMA and Lysine-MDA in healthy participants<br>**Isabelle Straumann** |
| 09:50 - 10:15 | Acute psilocybin effects on functional brain entropy and perfusion<br>**Patrick Fisher** |

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**10:15 - 10:45** **Coffee - Tea - Break**

Catering Area

**10:45 - 12:00**
Great Lecture Hall

**Special Lecture 1**
**5-$HT_{2A}$ Receptor Agonists: From Human Neuroimaging to New Treatments of Brain Disorders**
*David Nutt, Imperial College London, United Kingdom*

*Chair & Introduction: Matthäus Willeit*

***Sponsorship by Frontiers in Pharmacology is gratefully acknowledged***

The last decade has seen a remarkable resurgence of interest in psychedelic drugs such as psilocybin (from magic mushrooms) LSD and DMT (dimethyl tryptamine – the active ingredient of ayahuasca). This has been driven by the discovery that these psychedelics all act agonists of 5-HT2A receptors plus human imaging studies that reveal this action leads to profound alterations in brain measures of activity particularly in terms of increased entropy of EEG MEG and fMRI signals and reduced within-network, but increased between-network, connectivity. In addition they all can increase synaptic growth and brain plasticity. These findings not only explain the subjective nature of the psychedelic experience but also have implications for the treatment of internalising disorders such as depression, addiction, anorexia and OCD. Subsequent trials, particularly of psilocybin, in these disorders have revealed significant clinical benefits from even just a single administration. My talk will explore these brain mechanisms and clinical data and discuss the potential place of psychedelic medicine in the future of psychiatry.

**12:00 - 14:00**
Catering Area

**Lunch - Break (*opt. Lunch Tours - see Meeting points below; Discussion Round - Great Lecture Hall*)**

**14:00 - 15:45**
Seminar Room A

**Poster Session II (Even numbers)**

**15:45 - 16:15**
Catering Area

**Coffee - Tea - Break**

**16:15 - 18:00**
Great Lecture Hall

**Parallel Symposia 13: Clinical Mechanisms for Persisting Positive Effects of Psychedelics**
*Chair: Gitte Knudsen*

Psychedelic drugs have been shown to elicit significant and lasting positive psychological changes in various populations, including severe psychiatric patients and healthy volunteers. Remarkably, these positive changes have been observed following just a single psychoactive dose, yet no clinical research has fully uncovered the neurological mechanisms behind this effect. All psychedelic drugs act as agonists at the 5-HT2A receptor, and blocking this receptor inhibits the subjective effects of psychedelics. Therefore, understanding the mechanisms behind the lasting effects of psychedelics provides new insights into how the serotonin system influences mood and well-being. Several preclinical studies have demonstrated that psychedelics can increase markers of neuroplasticity, such as synapse formation and dendritic arborization. Additionally, modern advances in PET radiotracer technology allow for imaging synaptic density in the living human brain using [11C]UCB-J. Our first two speakers, Professors Gitte Moos Knudsen and Johan Lundberg, will present novel data on the lasting effects of psilocybin on synaptic density in healthy volunteers and major depression patients, respectively. These data provide clinical evidence for whether psilocybin produces pro-neuroplastic effects in the living human brain, and whether these are associated with positive clinical effects. Psilocybin has shown promise in phase 2a trials for treating addictive and compulsive disorders such as cigarette addiction, obsessive-compulsive disorder, and alcoholism. Dr. David Erritzøe will present data on the neurocognitive and clinical correlates of compulsivity to understand whether changes in compulsive behaviour underpin the lasting effects of psychedelics. Our final speaker, Prof. Tomas Palanicek, will explore the precision medicine component of psychedelic treatments, evaluating the effects of sex, previous exposure to psychedelics, and the setting of the session on the lasting effects of psychedelics. Together, these sessions represent the forefront of research into the mechanisms underlying the remarkable effects of psychedelics from neurological, psychological, and medical perspectives.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | Psilocybin's effect on human brain plasticity is contingent on setting<br>**Gitte Moos Knudsen** |
| 16:45 - 17:10 | Synaptic density as measured by [11C]-UCB-J positron emission tomography after psilocybin treatment<br>**Guusje Haver**, Hampus Yngwe, Pontus Plaven Sigray, Granville Matheson, Max Andersson, Mikael Tiger, Maria Beckman, Carl-Johan Ekman, Johan Lundberg |
| 17:10 - 17:35 | Detecting neuroplastic effects induced by ketamine in healthy human subjects: a multimodal approach<br>**Claudio Agnorelli** |
| 17:35 - 18:00 | The influence of sex, previous experience and setting on the phenomenology of psilocybin intoxication and its link to persistent effects in healthy volunteers<br>**Tomas Páleníček** |

**16:15 - 18:00**
Seminar Room B1,B2

## Parallel Symposia 19: Lipidation within the Serotonergic Pathways: Implication for Depression and Anxiety

*Chair: Mark Rasenick*

The last decades have led to an explosion of information and increased understanding of protein palmitoylation, a reversible post-translational modification (PTM) of proteins at cysteine residues. Methodical advances have uncovered a human palmitoylproteome containing several thousand proteins (app. 10% of all proteins encoded by the human genome). The functional importance of protein S-acylation in a variety of physiological settings has been uncovered and revealed significant changes in S-acylation patterns under pathological conditions. Dysregulation of the serotonin system has long been considered central to the etiology of depressive disorders, and the several serotonin receptors and transporters seem to play a key role in depressive neuropathology. Despite much effort, the molecular routes impairing the serotonin system in clinical depression and suicide remain largely enigmatic and highly controversial. Our objectives are to provide engaging speakers who will highlight the regulation and function of palmitoylation on proteins involved in serotonergic signaling with particular focus on depressive and anxiety disorders. We also hope to inspire attendees to consider how this PTM may be impacting the system they study. Topics to be covered include dynamic palmitoylation of heterotrimeric G proteins, the functional role of palmitoylation of serotonin transporter, the potential for anti-depressive drug development targeting S-acylation of serotonin receptors, and consequences of lipidation for receptor structure.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | G protein palmitoylation and antidepressant action<br>Jeffrey Schappi, **Mark Rasenick** |
| 16:45 - 17:10 | Exploring Cholesterol Sensitive Function of the Serotonin-1A Receptor: Excitements and Challenges<br>**Amitabha Chattopadhyay** |
| 17:10 - 17:35 | Regulation of Serotonin Transporter Kinetics and Trafficking by Palmitoylation: Implications for the Development and Treatment of Neurologic and Psychiatric Disorders<br>**James Foster**, Christopher Brown |
| 17:35 - 18:00 | Intentionally left blank due to cancellation |

**16:15 - 18:00**

Josephinum
Great Hall

**Short Talks II**
*Chair: Charles Nichols*

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:35 | Cross-species and mechanistic studies of maternal serotonin effects on offspring neurodevelopment<br>**Jeremy Veenstra-VanderWeele** |
| 16:35 - 16:50 | Socio-affective communication through ultrasonic vocalizations in Tph2-deficient rat pups: Communal nesting aggravates growth retardation despite ameliorating maternal affiliation deficits<br>**Markus Wohr** |
| 16:50 - 17:15 | Maternal high-fat diet promotes region and sex specific remodeling of serotonin circuits<br>**Michael Patton** |
| 17:15 - 17:30 | Multiplexed voltammetric serotonin measurements with carbon fiber multielectrode arrays<br>**Alexander Zestos** |
| 17:30 - 17:45 | Constitutive serotonin tone as a determinant of metabolic homeostasis: insights from selectively bred WZ-5HT rat sublines<br>**Jasminka Stefulj** |
| 17:45 - 18:00 | H3 Serotonylation Regulates Developmental Gene Expression in the mPFC Contributing to Behavioral Response to Early Life Adversity<br>**Ashley Cunningham** |

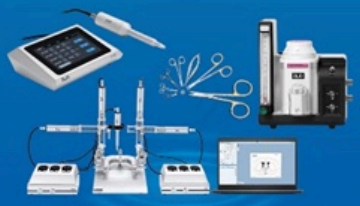
**Dinner on own - enjoy your evening in Vienna** (*Dinner suggestions on conference webpage)*

*https://www.serotoninclub.org/2025-food-drink-recommendations*

RWD 瑞沃德

Catalogue 2025

# Contributing our wisdom and effort to the improvement of the qualitty of life



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Pet healthcare facilities
20000+

Pharmaceutical companies
18000+

## Thursday July 10th, 2025

**08:00 - 11:00** Entrance Hall — **Registration**

**08:30 - 10:15** Great Lecture Hall — **Parallel Symposia 25: Human in vivo Imaging of Genetic Variability within the Serotonin System**
*Chair: Marie Spies, Co-Chair: Elizabeth Bartlett*

Human in vivo imaging with positron emission tomography and magnetic resonance imaging provides information on the density and distribution of serotonergic proteins as well as insight into brain structure and function, respectively. Imaging the impact of genetic and epigenetic factors on imaging outcomes informs on how genetic variability influences brain function and may reveal potential endophenotypes of psychiatric disease risk. Comprehensive understanding of this relationship deepens knowledge of psychiatric pathophysiology and may provide targets for risk management or treatment development. This symposium aims to showcase research elucidating the relationship between genetic and epigenetic factors and brain structure and function within the context of the serotonin system. Dr. Marie Spies (Medical University of Vienna, Austria) will present data on the impact of genetic and epigenetic changes within serotonin turnover genes on brain Monoamine Oxidase A levels, an index of cerebral serotonin degradation consistently shown to be altered in depression and a relevant drug target. Elizabeth A Bartlett (Columbia University, USA) will discuss imaging vulnerability for depression and suicidal behavior within the serotonin system. Silvia Bruzzone (Neurobiology Research Unit, Copenhagen, Denmark) will highlight modeling strategies for illustrating the impact of genetic and epigenetic factors on brain serotonin protein levels in the healthy and depressed state. In addition Dr Marin Jukic (Karolinska Institute, Stockholm, Sweden) will talk about neuroimaging of pharmacogenetic effects in the serotonin system.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Genetic and epigenetic regulation of serotonin turnover in seasonal affective disorder<br>**Marie Spies** |
| 09:00 - 09:25 | The Serotonin System in Depression and Suicidal Behavior: Novel Insights with PET Imaging<br>**Elizabeth Bartlett**, Francesca Zanderigo, J John Mann |
| 09:25 - 09:50 | Genetic and Epigenetic Contributions to Serotonin Neurotransmission in the Healthy and Depressed State<br>**Patrick Fisher** |
| 09:50 - 10:15 | Intentionally left blank due to cancellation |

**08:30 - 10:15** **Parallel Symposia 23: Elevated MAOA and Treatment Resistance in Depression: New Twists on a Classic Hypothesis**
Seminar Room B1,B2 *Chair: Jeffrey Meyer*

Treatment resistance in depression remains a significant problem, despite recently-approved fast-acting non-monoaminergic antidepressant (AD). Causes include the multifactorial nature of MDD and the limited utility of preclinical models for innovative drug development. Imaging studies aimed at identifying mechanisms of MDD and treatment resistance have reported (and replicated) elevated monoamine oxidase A (MAOA) levels in brain regions implicated in mood regulation, correlating with symptom severity, recurrence and postpartum or perimenopause periods of depression risk. MAOA inhibitors are available but seldom used due to broad side-effects. MAOA metabolizes monoamines, produces reactive oxygen species (ROS) and regulates mitochondrial activity, suggesting different pathways for promoting pathophysiology and AD resistance. Notably, no study has directly tested the causality of these putative links, nor has the MAOA pathology been modeled for drug discovery. This panel will first discuss a humanized mouse model overexpressing human MAOA in medial prefrontal cortex, with altered emotional behavior and serotonin neurotransmission as a model of treatment-resistant depression. Next, studies investigating elevated MAOA-induced altered mitochondrial activity and ROS production, and effects on antioxidants, will be presented. This will be followed by a human genetic study in a cohort of antidepressant-treated depressed patients, showing differential effect of rs979605(A>G) polymorphism, with worse improvement in female AA homozygotes compared to male A carriers after 6 months of treatment. Finally, results from a double-blind placebo-controlled study comparing a dietary supplement designed to counter effects of elevated MAOA to placebo on severity of postpartum blues, a strong risk factor for postpartum depression will be presented.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Modeling elevated MAO-A activity in mice: role in emotionality and antidepressant treatment response<br>**Jean-Philippe Guilloux**, Rodolphe Lebeau, Phuoc Quy Long Nguyen, Runhao Zhou, Jeffrey Meyer, Etienne Sibille, Toshifumi Tomoda |
| 09:00 - 09:25 | Elevated MAOA and altered bioenergetics underlying elevated emotionality in depression<br>**Toshifumi Tomoda**, Runhao Zhou, Rodolphe Lebeau, Akiko Sumitomo, Mounira Banasr, Rob Laister, Jeffrey Meyer, Jean-Philippe Guilloux, Etienne Sibille |
| 09:25 - 09:50 | Differential association of the MAOA rs979605(A>G) genetic polymorphism with clinical improvement in antidepressant-treated depressed males and females<br>**Kenneth Chappell**, Romain Colle, Jérôme Bouligand, Séverine Trabado, Bruno Fève, Laurent Becquemont, Emmanuelle Corruble, Céline Verstuyft |
| 09:50 - 10:15 | Randomized Double Blind Placebo Controlled Trial of a Dietary Supplement to Prevent Post Partum Blues With Six Month Follow Up of Depressive Symptoms<br>**Jeffrey Meyer**, ZhaoHui Wang, Apitharani Santhirakumar, Yekta Dowlati, Natalia Docteur, Asqa Shoaib, Jareeat Purnava, Yanqi Wang, Wei Wang, Ishrat Husain, Rashmi de Silva Wijeyeratne, Heba Reeyaz, Catalina Baena-Tan, Yuko Koshimori, Zahra Nasser, Valery Sit |

**08:30 - 10:15** **Parallel Symposia 26: Molecular Mechanism of Serotonin Transport by SERT, OCTs, and VMAT2**
Josephinum Great Hall
*Chair: Thomas Stockner*

An essential regulatory mechanism of serotonergic signalling and serotonin homeostasis is its cellular uptake by transporters. These include the neuronal serotonin transporter (SERT), the vesicular transporter VMAT2 that store serotonin in synaptic vesicles, and the promiscuous organic cation transporters OCT1-3 that show much broader expression patterns in the body. These transporters belong to three different families, differing in structure, driving forces, transport capacity, substrate selectivity, and transport velocity, but all carry out the physiologically essential transport of serotonin. In recent years the structures of all of these transporters have been elucidated, but nevertheless their transport cycle at an atomic level remained largely elusive as it involves a highly dynamical process which minimally includes steps such as substrate recognition, transporter occlusion and isomerization, substrate release and a return to the starting conformation. This symposium will present computational approaches that integrate experimental information from structure, biochemical and pharmacological characterization, site directed mutagenesis, ligand binding and electrophysiological measurements into extended simulations, enhanced sampling and free energy calculations to describe the mechanism and energetics of the transport cycle. We aim to provide in four talks a molecular picture of the transport mechanism and highlight the similarities and differences of these transporters. The transport is energized by the transmembrane gradient of sodium in SERT, by the transvesicular proton gradient in VMAT2, while OCTs solely depend on substrate concentration gradient. The talks will present data combining computational approaches with structural data and experiments to show the involved structures, highlight transporter dynamics and quantify associated energetic profiles to elucidate key interactions and driving forces orchestrating substrate transport. SERT is a high-affinity but slow transporter while the OCTs are low-affinity, but high-capacity transporter. SERT and VMAT2 are very specific transporters, while the OCTs are highly promiscuous. We will show data on the molecular origin of these differences and describe the properties that lead to substrate recognition.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Similarities and differences in the molecular mechanism, by which serotonin triggers transport in SERT and OCT3<br>**Thomas Stockner** |
| 09:00 - 09:25 | OCT1: another relevant serotonin transporter?<br>**Marleen Meyer-Tönnies** |
| 09:25 - 09:50 | Uncoupling the Gated-Pore Mechanism in the Human Serotonin Transporter – Novel Insights into the Conducting State<br>**Ralph Gradisch** |
| 09:50 - 10:15 | Allosteric modulation of serotonin and dopamine transporters: Insights from computations and experiments<br>**Ivet Bahar**, Hoang Nguyen, Mary Cheng, Ji Young Lee, Shaili Aggarwal, Ole Mortensen |

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**10:15 - 10:45** **Coffee - Tea - Break**

Catering Area

**10:45 - 12:00**
Great Lecture Hall

**Rapport Lecture**
**Serotonin-You've Come a Long Way**
*Alan Frazer, University of Texas Health San Antonio*

*Chair & Introduction: John Neumaier*

Research in the laboratory of Irvine Page at the Cleveland Clinic led to the identification of serotonin in 1948, with the first author of the publication of these findings being Maurice Rapport for whom this lecture is named. However, Vittorio Erspamer in Italy identified in 1947 an amine from enterochromaffin cells that he named enteramine. He studied it extensively in the early 1940's. When the structure of enteramine was elucidated in the mid 1950's, it was shown to be identical to that of serotonin, whose structure had been determined earlier. So the name serotonin stuck. Further research in 1954 by Betty Twarog in Page's lab found that serotonin was present in brain. This observation triggered much research into what functions serotonin played in brain and its possible involvement in psychiatric illnesses, particularly major depressive disorder. One outcome of such research was the demonstration in the mid 1970's that selective serotonin reuptake inhibitors (SSRIs) were effective antidepressants. Considerable research was also focused on the receptors through which serotonin acted. Building on such studies, my research focused on affinity states of the 5-HT1 receptor, behaviors associated with different types of serotonin receptors and the use of in vivo voltammetry to study the function of the serotonin transporter (SERT) in the brain. Key findings were that certain behaviors were elicited by activation of either 5-HT1 or 5-HT2 receptors and that repeated treatment with different classes of antidepressants had differential effects on such behaviors. We also found that it takes extensive destruction of serotonergic neurons to compromise their ability to clear extracellular serotonin. This may account for why there needs to be high occupancy (about 80%) or the SERT by different SSRIs to exert a therapeutic effect. Further, repeated treatment with SSRIs produces a time-dependent downregulation of the serotonin transporter which greatly compromises the ability of the SERT to remove serotonin.

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**12:00 - 14:00**
Catering Area

**Lunch - Break (*opt. Lunch Tours - see Meeting points below; Discussion Round - Great Lecture Hall*)**

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**14:00 - 15:45** **Young scientist's session**

Great Lecture Hall

*Chair: Lynette Daws*

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| 14:00 - 14:05 | Chair introduction |
| 14:05 - 14:20 | An immediate early gene and glutamate response is not necessary for the medicinal and neuroplasticity promoting effects of non-hallucinogenic 5-HT2A receptor agonists<br>**Isak Aarrestad** |
| 14:20 - 14:35 | Role of Epithelial 5HT4 Receptor in Gastrointestinal Motility and Visceral Pain<br>**Chalystha Yie Qin Lee** |
| 14:35 - 14:50 | Endogenous serotonin signaling in prefrontal cortex: frequency dependence, plasticity, and perturbation by chronic SSRI treatment<br>**Saige Power** |
| 14:50 - 15:05 | Serotonergic modulation of social cognition in mice<br>**Marco Niello** |
| 15:05 - 15:20 | Real-Time Analysis of Serotonin Dynamics in Human-Derived 3D Organoids and Spheroids Using Fast-Scan Cyclic Voltammetry<br>**Bettina Bohl** |
| 15:20 - 15:35 | Protocadherin-alphaC2 is required for fluoxetine-induced serotonin re-innervation and behavioral recovery after stroke<br>**Sara Asgharzadeh** |

**15:45 - 16:15** **Coffee - Tea - Break**

Catering Area

**16:15 - 18:00**
Great Lecture Hall

**Parallel Symposia 11: Theme and Variations: Phenotypic Diversity and Dynamics of Serotonergic Neurons**
*Chair: Skirmantas Janusonis, Co-Chair: Benjamin Okaty*

The developmental deployment of the brain's colossal meshwork of serotonergic axons depends on decisions made by each participating axon. These decisions include axon extensions in environments with and without directional cues, branching events, and responses to local neural activity. In addition, serotonergic neurons in the adult brain retain the expression of some developmentally active transcription factors and can regenerate their axons. Recent studies have revealed that, at any given time, serotonergic neurons express surprisingly diverse transcriptional programs. Some nodes in these gene networks are stable and developmentally hardwired, but some may reflect the neuron's unique history (including local signals received along the path of its axon as it traverses multiple brain regions). This raises intriguing questions about how the pluralism and dynamism of individual serotonergic neurons maintain regional serotonergic fiber densities, and how "diffuse" these projections really are. More broadly, studying these questions in serotonergic neurons may lead to a deeper understanding of the fundamental rules of brain self-organization that relies on deterministic and stochastic processes to achieve a remarkable degree of robustness. The symposium will discuss recent experimental evidence that demonstrates the diversity and dynamics of single-cell transcriptomes – in normal development and in the presence of environmental perturbations relevant to etiologies of mental disorders. The transcriptome profiles of small clusters of neurons will be related to their developmental projection maps which can now be reconstructed with state-of-the-art machine learning methods at the 3D whole-brain level. The first holotomographic time-lapse images of serotonergic growth cones will be presented, and several wiring modes available to a developing serotonergic axon will be discussed. The morphological diversity of serotonergic axons in soma-sized forebrain volumes will be demonstrated with Brainbow tagging, and some stochastic aspects of the serotonergic system will be noted. The speakers represent independent research groups that collectively have spearheaded these developments and will offer insights into current challenges and implications for serotonin neuroscience.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | Transcriptional and spatial dynamics of Dorsal Raphe serotonin neurons following SSRI administration<br>**Iskra Pollak Dorocic** |
| 16:45 - 17:10 | Transcriptomic Diversity and Dynamics of Serotonergic Neurons over Development and in Response to Stress.<br>**Benjamin Okaty** |
| 17:10 - 17:35 | Cartography of Forebrain Projecting Serotonin System<br>**Jing Ren** |
| 17:35 - 18:00 | Microglia and Serotonin 2B Receptors: Key Mediators of Fluoxetine-Induced Serotonergic System Plasticity and Remodeling<br>**Massimo Pasqualetti** |

**16:15 - 18:00**

Seminar Room B1,B2

**Parallel Symposia 17: Diverse Stress Signaling Mechanisms in Dorsal Raphe**
*Chair: John Neumaier*

Serotonin is an important integrator of physiological and behavioral responses to stress, and impaired functioning in the dorsal raphe nucleus (DRN) can contribute to maladaptive responses to stress. We will present data using diverse methodologies to elucidate the role of humoral and neuropeptide signals in regulating the activity of DRN serotonergic function in rodents. Dr. Christopher Lowry (University of Colorado Boulder) will present data showing the effects of chronic glucocorticoid administration on Tph2 mRNA and protein expression in the DRN and associated anxiety-like behavior, and the role of CRH1 and CRH2 receptors in these effects. He will compare the effects of chronic glucocorticoid administration with the effects of stress exposure. He will also compare CRH receptor priming in the extended amygdala (models of a chronic anxiety-like state) and direct application of CRH into the DRN. Dr. John Neumaier (University of Washington, Seattle) will discuss FKBP5, a key regulator of glucocorticoid receptor signaling, that is upregulated after stress in DRN serotonin neurons. He will also present data using optogenetics and GRAB-5-HT sensors in amygdala to address how FKBP5 impacts the capacity of 5-HT projection neurons to release serotonin, and how manipulating FKBP5 expression in these neurons alters their function. Dr. Nagalakshmi (Lakshmi) Balasubramanian (University of Iowa) investigates serotonergic (5-HT) circuits involved in stress and alcohol-related behaviors. She will present new data identifying a previously uncharacterized neural pathway that modulates anxiety and stress. This work reveals an interaction between CART peptide-expressing neurons in the Edinger-Westphal (EW) nucleus and 5-HT neurons in the dorsal raphe nucleus, in which EW CART peptides negatively regulate 5-HT activity via GABAergic interneurons. Dr. Lakshmi demonstrates that the CARTEW to DRN circuit acts as a critical driver of stress and anxiety-like behaviors in a sex-specific manner. Dr. Lynn Kirby (Temple University, Philadelphia) will present data to suggest that interactions between 5-HT and the stress neurohormone CRH within the DRN are involved in affective responses to both positive (heroin, alcohol, sucrose) and negative (footshock) reinforcers, influencing drug-taking behaviors. These findings include sex-differences in drug self-administration and new chemogenetic and fiber photometry data to elucidate the sex-dependent nature of 5-HT DRN signaling that may contribute to those effects.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | Chronic glucocorticoid intake alters basal Tph2 protein expression in anxiety-related midbrain serotonergic systems<br>**Christopher Lowry** |
| 16:45 - 17:10 | FKBP5, a potential integrator of adrenal steroids and serotonin<br>**John Neumaier** |
| 17:10 - 17:35 | CART Peptide Modulation of Serotonergic Activity: A Key Driver of Anxiety<br>**Nagalakshmi Balasubramanian** |
| 17:35 - 18:00 | Sex differences and affective motivation for drug self-administration: A role for corticotropin-releasing hormone (CRH)-5-HT circuits<br>**Lynn Kirby**, Claire Deckers, Bryan McElroy, Chen Li |

**16:15 - 18:00**

Josephinum
Great Hall

**Parallel Symposia 18: Serotoninergic Mechanisms of Psychedelic Therapeutics in Substance Use Disorders: Focus on Cognitive Flexibility**
*Chair: Kathryn Cunningham, Co-Chair: Stephanie Daws*

Substance use disorders (SUDs) continue to be an international public health threat. While treatments exist for certain SUDs, like opioid use disorder, treatment options are lacking for other types (i.e. cocaine use disorder). Existing treatments have also proven insufficient at reducing relapse in the long-term and thus, there is a need for novel treatment strategies. Relapse vulnerability is promoted by disruption of executive function and reduced cognitive flexibility, leading individuals attempting to abstain from drug use back to their old drug-seeking behavioral patterns. Recently, psychedelics have emerged as promising potential therapeutics for a number of neuropsychiatric disorders, including SUDs and other frequently comorbid disorders, like depression and anxiety. Serotonin (5-HT) systems contribute to the pathobiology of SUDs and several aspects of this system may be useful in increasing the precision of prevention, diagnosis and treatment of SUDs. The present panel will integrate knowledge across neurocircuitry, molecular and pharmacological mechanisms to inform the therapeutic potential of psychedelics for the treatment of SUDs. The present panel focuses on recent preclinical and clinical research directed to identify serotonergic mechanisms of psychedelics in persistent drug seeking, with a focus on the neural systems mediating these effects.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | The 5-$HT_{2A}$ receptor as a therapeutic target for opioid use disorder with comorbid alcohol use<br>**Jasper Heinsbroek**, Jamie Peters |
| 16:45 - 17:10 | 5-$HT_{2A}$R and 5-$HT_{2C}$R neurocircuitry in relapse vulnerability: Mechanisms and ligand discovery in cocaine use disorder<br>**Kathryn Cunningham**, Christina Merritt, Jia Zhou, Noelle Anastasio |
| 17:10 - 17:35 | Psilocybin-mediated inhibition of opioid-seeking phenotypes<br>**Stephanie Daws**, Gabriele Floris, Amy Stringer, Konrad Dabrowski, Mary Tresa Zanda |
| 17:35 - 18:00 | 5HT receptor activation and the glutamatergic interface of behavioral, neural and immunological responses to psychedelics<br>**Jan Ramaekers** |

**18:00 - 18:45** **Business Meeting (members only) - Great Lecture Hall**

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**19:00 - 22:00**

Catering Area
Seminar Room A

**Evening Poster Session - Wine & Cheese (+ grape juice etc.)**

## Friday July 11th, 2025

**08:30 - 10:15** Great Lecture Hall

**Parallel Symposia 21: Early-Life Stress and Serotonin: Neurodevelopmental Mechanisms and Circuit Dynamics Driving Lifelong Behavioral Changes**
*Chair: Derya Sargin*

Chronic childhood stress profoundly impacts brain development, heightening the risk for anxiety and mood disorders. However, the underlying neurobiological mechanisms remain elusive. Serotonin, one of the earliest emerging neurotransmitters, is crucial for brain development, modulating cellular proliferation, differentiation and the maturation of neural circuits. Dysregulation of serotonin during critical developmental periods increases the brain's vulnerability to stress, resulting in lifelong deficiencies in socioemotional and cognitive functions. Understanding how early-life adversities affect serotonin function and contribute to mood disorders is essential for developing effective treatment strategies. This symposium will provide evidence from developmental models that underscore the role of serotonin in socioemotional development across the lifespan. Dr. Catia Teixeira will discuss her research on the relation between prefrontal cortex activity during early life, specifically in the context of maternal separation stress, and cognitive performance on adulthood. She will highlight cellular (snRNAseq) and physiological consequences of early-life manipulations, with a focus on interneurons and serotonergic signaling. Dr. Mariano Soiza-Reilly will present findings on the maturation of the prefrontal cortex-to-raphe circuit during early postnatal life, examining how developmental alterations in this pathway affect adult emotional responses and increase stress vulnerability. Dr. Giulia Zanni will discuss the role of serotonin in the periaqueductal grey, investigating how this key neurotransmitter modulates unlearned fear responses in adulthood and after developmental interference in both rodents and humans. Dr. Derya Sargin will share her lab's latest research on how postnatal stress affects brain-wide connectivity and the efficacy of stimulation treatments for stress-induced anxiety. The symposium will feature data obtained through multidisciplinary approaches, including high-resolution microscopy, pharmacology, electrophysiological interrogation of synaptic circuits, and behavioral assessments. Together, these presentations will provide a comprehensive overview of how early-life stress impacts serotonin function and subsequent mental health outcomes, paving the way for potential therapeutic interventions.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Prefrontal cortex activity during early-life modulates cognitive performance in adulthood<br>**Catia Teixeira** |
| 09:00 - 09:25 | Postnatal Maturation of Prefrontal-to-Raphe 5-HT Neuron Circuit: Implications for Early-Life Stress Vulnerability and Psychiatric Disorders<br>**Mariano Soiza-Reilly** |
| 09:25 - 09:50 | Role of serotonin brain circuit in the developmental emergence of innate fear across species<br>**Giulia Zanni** |
| 09:50 - 10:15 | Serotonergic circuit activity in early life stress models<br>**Derya Sargin** |

**08:30 - 10:15** **Parallel Symposia 22: SSRIs in the Treatment of Depression: A Pharmacological Cul-de-Sac?**
Seminar Room B1,B2
*Chair: Trevor Sharp, Co-Chair: Philip Cowen*

Selective serotonin reuptake inhibitors (SSRIs) are currently the first-line pharmacological treatment of people with major depression, and safer and better tolerated than their tricyclic predecessors. However, SSRIs are not without their problems which include anxiety on initiation, sexual dysfunction, a problematic discontinuation syndrome as well overall modest efficacy, all of which show individual variability. This symposium will bring together experts in the field to provide an up-to-date account of the advantages of SSRIs, but also some of their challenges and insights into how these might be overcome. Philip Cowen (Oxford) will review obstacles associated with the clinical management of SSRI therapy, provide a comparison with other drug treatment options, and discuss the potential of 5-HT receptor-based strategies for treatment augmentation. Vibe Frokjaer (Copenhagen) will discuss some of the important downsides of SSRI therapy (including sexual dysfunction), recent advances in the search for biomarkers of treatment outcome, and molecular imaging insights into mechanisms of action. Jonathan Henssler (Berlin) will present the largest meta-analysis to date of the incidence of SSRI discontinuation symptoms in placebo-controlled trials. Finally, Trevor Sharp (Oxford) will review preclinical mechanisms of SSRI discontinuation and present new evidence of the involvement of a rebound activation of 5-HT neurons and how this might be prevented.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | SSRIs: A Pharmacological Cul-de-Sac?<br>**Philip Cowen** |
| 09:00 - 09:25 | Serotonin 4 receptor brain architecture in major depression, associations with sexual health, and antidepressant treatment outcomes<br>**Vibe G. Frokjaer** |
| 09:25 - 09:50 | Antidepressant Discontinuation Syndrome – Current Evidence on Incidence, Placebo Discontinuation, and Clinical Implications<br>**Jonathan Henssler** |
| 09:50 - 10:15 | Modelling the neurobiological effects of SSRI discontinuation<br>**Trevor Sharp**, Helen Collins, Sophie Gullino, David Bannerman |

**08:30 - 10:15** **Parallel Symposia 24: Targeting Presynaptic and Postsynaptic Serotonin Systems for Neuropsychiatric Treatment Strategies**
Josephinum Great Hall
*Chair: Harald H. Sitte*

In this symposium on serotonergic modulation by pre- and postsynaptic mechanisms, including the utilization of transporter-mediated efflux as well as receptor stimulation, we show how targeting the serotonergic system with both mechanisms may advance our therapeutic repertoire for neuropsychiatric disorders. Increasing extracellular serotonin (5-HT) levels in the brain has been shown to alleviate symptoms of depression and anxiety-related disorders, such as social phobias and PTSD. Recent preclinical and clinical studies have established the therapeutic potential of drugs inducing 5-HT release via the 5-HT transporter SERT. However, current 5-HT-releasing compounds in clinical trials pose risks of abuse and adverse side effects. Michael Baumann will talk about the mechanisms how MDMA (methylene-dioxi-methamphetamine, '*ecstasy*') and related drugs preferentially release 5-HT both ex-vivo and in-vivo, producing 5-HT-associated effects in preclinical behavioral models. He will also talk about new MDMA-like medications and their therapeutic potential. Francesco Papaleo will show how empathogens impact different social domains in mice by modulating cortical neurons. This is achieved by combining innovative socio-cognitive paradigms with in-vivo calcium imaging, allowing the observation of real-time neural activity. Additionally, the group uses circuit manipulations to understand the specific pathways involved and pharmacological challenges with empathogens to study their effects. John McCorvy studies serotonin G protein-coupled receptors involved in various psychoactive effects of drug action, in particular how the phenomenon known as "biased signaling" or "functional selectivity" can serve to provide mechanistic information on therapeutic efficacy versus side-effect profiles. McCorvy will show that psychedelic drugs as well as amphetamines such as MDMA analogs possess polypharmacology targeting multiple 5-HT-receptor subtypes and outline examples of biased agonists at these GPCRs that will inform their clinical efficacy or suspected side-effects. Michael Colwell will show how they utilize a selective serotonin releasing agent to directly increase synaptic serotonin in humans and examine its influence on key behavioral domains associated with serotonin function. Using computational techniques like reinforcement learning and drift diffusion modeling, they analyzed observed behaviors. Their reinforcement learning models revealed that increased synaptic serotonin specifically reduced sensitivity to outcomes in aversive, but not appetitive, contexts.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | Mining the new psychoactive substances library for MDMA-like therapeutic agents<br>**Michael Baumann** |
| 09:00 - 09:25 | Serotonergic modulation of cortical circuits in social cognition<br>**Francesco Papaleo**, Marco Niello |
| 09:25 - 09:50 | Polypharmacological and Biased Agonist Profiles of Psychedelics and MDMA Analogs<br>**John McCorvy**, Janelle Lanham, Natalie Cavalco, Allison Clark, Andrew Cao, John McKee, Hailey Bock, Marko Ivancich, Joseph Hennessey |
| 09:50 - 10:15 | Targeting presynaptic and postsynaptic serotonin systems for neuropsychiatric treatment strategies<br>**Michael Colwell** |

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**10:15 - 10:45** **Coffee - Tea - Break**

Catering Area

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| | **Special Lecture II** |
| **10:45 - 12:00** | **The Long and Winding Road to FDA Approval of MDMA** |
| Great Lecture Hall | *Jennifer Mitchell, University of California San Francisco* |
| | *Chair & Introduction: Noelle Anastasio* |

MDMA, in combination with therapy, has recently gained attention as a potential therapeutic for PTSD. This lecture will review the basic pharmacological and neurological actions of MDMA, summarize the phase 3 trials on safety and efficacy, and discuss the shortcomings that may have contributed to FDA disapproval of a New Drug Application (NDA) last summer. A brief discussion of possible next steps will follow.

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**12:00 - 14:00** **Lunch - Break (*opt. Lunch Tours - see Meeting points below*)**

Catering Area

**14:00 - 15:45**

Great Lecture Hall

## Parallel Symposia 14: Serotonin, Its Detection and Function

*Chair: Yulong Li*

Serotonin (5-HT), a pivotal monoamine neuromodulator, plays important roles in a wide range of physiological processes, such as emotion regulation, reward processing, appetite control, sleep-wake cycles, as well as learning and memory. Dysregulation of 5-HT transmission has been implicated in various brain disorders, including depression, anxiety, addiction, migraine and epilepsy. Consequently, the serotonergic system is a primary target for many psychotropic drugs, particularly serotonin reuptake inhibitors used in the treatment of depression. Therefore, understanding the complexities of serotonergic modulation is of utmost importance. Accurate detection of serotonin dynamics is a critical aspect in deciphering the serotonergic system. In combination with the monitoring and manipulation of serotonergic neurons, as well as exploring the role of 5-HT receptors, we can gain further insights into the serotonergic system. This symposium aims to combine the detection approaches and function studies of 5-HT. We will firstly showcase the recent advancements in fluorescent 5-HT sensors and their application for detecting spatiotemporal dynamics of 5-HT. Additionally, we will further delve into the significant roles of 5-HT modulation by two exemplary cases: (1) the intricate relationship between sex-specific expression of serotonin receptors and stress vulnerability; (2) the vital role of serotonin in the regulation of sleep. The chair and speakers, representing a balanced ratio of males and females, come from various countries, including China, Germany and the United States. By bringing together experts in the field, we aim to shed light on the detection and function of 5-HT, paving the way for future advancements and fostering a comprehensive understanding of the serotonergic system.

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| 14:00 - 14:05 | Chair introduction |
| 14:05 - 14:30 | Illuminating the Brain: New and Old Tools to Decipher Neuromodulatory Circuits<br>**Olivia Masseck** |
| 14:30 - 14:55 | Spying 5-HT dynamics by constructing multicolor GRAB sensors<br>**Yulong Li** |
| 14:55 - 15:20 | Sex-Specific Serotonin Receptor Expression Drives Stress Vulnerability in Adult Hippocampal Neural Stem Cells<br>**Juan Song** |
| 15:20 - 15:45 | Striatal Serotonin Release Signals Reward Value<br>**Katherine Nautiyal** |

**14:00 - 15:45**

Josephinum
Great Hall

**Parallel Symposia 20: Serotonin in Sudden Death**
*Chair: Russel Ray*

The central serotonin system plays key roles in arousal and cardiorespiratory homeostasis that place it at the center of multiple sudden death pathologies including sudden infant death syndrome/sudden unexpected infant death (SIDS/SUID) and sudden unexpected death in epilepsy (SUDEP). In this symposium speakers will cover the latest in clinical, basic, and translational research to understand the mechanistic roles of the serotonin system in sudden death pathophysiologies.

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| 14:00 - 14:05 | Chair introduction |
| 14:05 - 14:30 | The Serotonopathy of Sudden Infant Death Syndrome.<br>**Robin Haynes** |
| 14:30 - 14:55 | Sleep and Serotonin in Sudden Unexpected Death in Epilepsy<br>**Gordon Buchanan** |
| 14:55 - 15:20 | The Pendulum Between Breath and Death: Serotonin and Noradrenaline<br>**Savannah Lusk** |
| 15:20 - 15:45 | The Role of Htr1B in the Neonatal Autoresuscitation Reflex and its Implication to Sudden Infant Deaths<br>**Russell Ray** |

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**15:45 - 16:15** **Coffee - Tea - Break**

Catering Area

**16:15 - 18:00**
Great Lecture Hall

**Parallel Symposia 3: Searching for Receptors Involved in the Antidepressant Effects of Psychedelics**
*Chair: Carine Becamel, Co-Chair: Joel Bockaert*

Major depressive disorder is one of the leading causes of disability worldwide. It can affect up to 20% of people during the entire lifespan in developed countries, generating high social and economy burdens. A recent paradigm shift in the treatment of MDD was the discovery that serotonergic psychedelics, including Lysergic Acid Diethylamide (LSD) or psilocybin, induce rapid antidepressant effects even in patients resistant to conventional antidepressants such as serotonin reuptake inhibitors. Although there is a consensus that 5-HT2AR activation mediates the psychedelic effects of LSD, psilocybin and other hallucinogenic 5-HT2AR agonists in human and mice, its role in their antidepressant effects remains controversial. A related open question is whether psychedelic properties of 5-HT2AR agonists are necessary for mediating their antidepressant activity. New cryo-EM structures of the 5-HT2A receptor bound to both hallucinogenic/non-hallucinogenic agonists as well as G-protein and arrestin biased compounds revealing the molecular interactions responsible for selectivity and signaling bias will be presented by Ryan Gumpper. Carine Becamel will present genetic and pharmacological evidences showing that distinct mechanisms, dependent or not of the 5-HT2AR, contribute to the antidepressant-like effects of serotonergic psychedelics and that a psychedelic experience might not be required for the therapeutic response to 5-HT2AR agonists at least in a Chronic Despair model in mice. Similarly, in human, Lucie Berkovitch will present a dissociation between the psychedelic and therapeutic effects of psilocybin in resistant depression. Finally, Eero Castren will discuss his unexpected data showing that the target of psychedelics for treating depression are not 5-HT receptors but BDNF receptors. This is an entirely new concept.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | Decoding Psychedelics: Structural Insights into their Pharmacology and Receptor Interactions<br>**Ryan Gumpper** |
| 16:45 - 17:10 | Antidepressant-like effects of psychedelics in a chronic despair mouse model: is the 5-HT2A receptor the unique player?<br>**Carine Becamel** |
| 17:10 - 17:35 | 5-HT2A receptors role in psilocybin antidepressant effects in humans<br>**Lucie Berkovitch** |
| 17:35 - 18:00 | Interaction of antidepressants and psychedelic compounds with the BDNF receptor TrkB<br>**Anaïs Virenque**, Eero Castrén |

**08:30 - 10:15**
Seminar Room B1,B2

**Parallel Symposia 7: Is Serotonin a Biomarker for Depression 2024?**
*Chair: Parastoo Hashemi*

Serotonin has long been a molecule of interest in depression studies because of the monoamine hypothesis of depression. This theory posits that extracellular serotonin levels are lower during depression and this deficiency underpins the behavioural phenotypes associated with depression. The hypothesis has moved in and out of favour for decades, recently enjoying a resurgence because of clinical trials targeting depression with psychedelics (with high affinity for serotonin receptors). However there remains much controversy about the roles in serotonin in depression. In this symposium, given the state of the art in 2025, we ask is the serotonergic system altered during depression. And if so, can these alternations give us critical insights into the roles that this modulator may play in the pathology of depression?
Hashemi will talk about rapid serotonin measurements with FSCV in experimental and theoretical, computational models of depression and antidepressant action. Mayer will talk about a novel fluorescence-based method to characterize endogenous serotonin in vivo. Rabiner will follow with application of imaging methods to measure serotonin release depression patients. Finally, Hughes will talk from an industry perspective about the development of novel serotonin targeting agents as antidepressants. In sum, our symposium aims to re-explore serotonin as a marker of depression given important advances in technology over the last decade.

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| 08:30 - 08:35 | Chair introduction |
| 08:35 - 09:00 | An Ex Vivo System to Study how Inflammation Modulates Serotonin<br>**Parastoo Hashemi** |
| 09:00 - 09:25 | Leveraging a fluorescence-based approach to quantify extracellular neurotransmitter concentrations in vivo<br>**Felix Mayer**, Carl-Fredrik Bowin, Samuel Elliott Ovrom, Frederikke S. Petersen, Ulrik Gether |
| 09:25 - 09:50 | Evaluation of 5-HT release in the human brain in healthy volunteers and patients<br>**Ilan Rabiner** |
| 09:50 - 10:15 | Promising Translation of the PK/PD Relationship for the novel 5-HT2A receptor agonist, GM-2505<br>**Zoë Hughes** |

**16:15 - 18:00**

Josephinum
Great Hall

**Parallel Symposia 27: The 5-HT7 Receptor as a Druggable Target**
*Chair: Stephanie Watts, Co-Chair: Finn Levy*

The 5-$HT_7$ receptor, cloned now over 30 years ago, remains a receptor for which druggable solutions for treatment of disease have remained elusive. We gather experts from around the world who have brought focus to areas which implicate this receptor as either being causal of or potential ameliorator of disease. We hope this stimulates thought and progress in this receptor being a target in treatment of disease.

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| 16:15 - 16:20 | Chair introduction |
| 16:20 - 16:45 | State of the 5-$HT_7$ receptor: structure, signalling, pharmacology<br>**Finn Olav Levy** |
| 16:45 - 17:10 | The 5-$HT_7$ Receptor as a Target in Hypertension<br>**Stephanie Watts** |
| 17:10 - 17:35 | Targeting 5-$HT_7$ receptors as a therapeutic strategy for intestinal inflammation<br>**Jensine Grondin**, Yun Han Kwon, Benjamin Blass, Huaqing Wang, Suhrid Banskota, Kenneth Korzekwa, Min Ye, John C. Gordon, Dennis Colussi, Kevin M. Blattner, Daniel J. Canney, Waliul Khan |
| 17:35 - 18:00 | Biased Signaling at the 5-$HT_7$ Receptor: Novel Therapeutic Agents for Pain Modulation<br>**Séverine Morisset-lopez** |

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**19:00 - 23:00**

**Farewell Dinner | Kunsthistorisches Museum, Maria-Theresien-Platz, 1010 Vienna**
**(***directions are appended below***)**

| Poster Presentations | No. |
|---|---|
| **Alexander Zestos**<br><br>Multiplexed voltammetric serotonin measurements with carbon fiber multielectrode arrays | **P 1** |
| **Jeremy Veenstra-VanderWeele**<br><br>Cross-species and mechanistic studies of maternal serotonin effects on offspring neurodevelopment | **P 2** |
| **Michael Patton**<br><br>**Maternal high-fat diet promotes region and sex specific remodeling of serotonin circuits** | **P 3** |
| **Ashley Cunningham**<br><br>H3 Serotonylation Regulates Developmental Gene Expression in the mPFC Contributing to Behavioral Response to Early Life Adversity | **P 4** |
| **Rong Zhu**<br><br>The selectivity filter of serotonin transporter is comprised of extracellular loop 2 and 4 | **P 5** |
| **Markus Wöhr**<br><br>Socio-affective communication through ultrasonic vocalizations in Tph2-deficient rat pups: Communal nesting aggravates growth retardation despite ameliorating maternal affiliation deficits | **P 6** |
| **Matej Murgaš**<br><br>Serotonin transporter abundance predicts the long-term SSRI treatment effect | **P 7** |
| **Thomas Steinkellner**<br><br>Evidence for low affinity of GABA at the vesicular monoamine transporter VMAT2 | **P 8** |
| **Nina Nitzan Soto**<br><br>Alterations of Cognitive Behaviours and Prefronto-Thalamic Circuits after Early-Life Exposure to Fluoxetine | **P 9** |
| **Jasminka Štefulj**<br><br>Constitutive serotonin tone as a determinant of metabolic homeostasis: insights from selectively bred WZ-5HT rat sublines | **P 10** |

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| **Cristina Fenollar Ferrer**<br><br>SERT N-terminal domain encodes determinants of PKG/p38aMAPK activation | **P 11** |
| **Lynette Daws**<br><br>Dynamic Duo: Serotonin Transporter and Organic Cation Transporter 3 Regulate Basolateral Amygdala Serotonin Clearance and Fear Memory Recall | **P 12** |
| **Adriana Carrillo**<br><br>Psilocybin induces interneuron plasticity in a cell subtype specific manner | **P 13** |
| **Haley N Strong**<br><br>Serotonergic Modulation of Auditory Habituation: DiPT-Induced Plasticity in the Inferior Colliculus and Auditory Cortex | **P 14** |
| **Iwona Majkowska**<br><br>Investigation into the signalling pathways of CPL298 - a novel 5-HT7 receptor agonist demonstrating efficacy in preclinical models of neuropathic pain | **P 15** |
| **Mohammad Nazmul Islam**<br><br>Unlocking the potential of partial efficacy: a novel insight into monoamine neurotransmitter transporters | **P 16** |
| **John A. Rudd**<br><br>Action of Antidepressants to Induce Emesis and Alter Gastric Myoelectric Activity | **P 17** |
| **Marta Samina**<br><br>BEYOND a neurotransmitter: serotonin as a neuromodulator factor in the structural and behavioral development of the PFC | **P 18** |
| **Pablo Prieto Roca**<br><br>Morphological Analysis of Histamine Induced HumanDerived Serotonergic Neurons | **P 19** |
| **Sophie Marie Christine Skopec**<br><br>Is there a functional role of phosphorylation in organic cation transporter 3 (OCT3)? | **P 20** |
| **Pawel Zajdel**<br><br>Simultaneous blockade of 5-HT3 and 5-HT6 receptors produce antipsychotic and procognitive properties in animal models | **P 21** |

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| **Aliia Murtazina**<br><br>Serotonin signaling as a regulator of pelvic ganglion development in mice | **P 22** |
| **Maria Tkachenko**<br><br>Fluoxetine Exposure Impairs Oocyte Quality: Revealing the Critical Role of SERT-Mediated Serotonin Uptake in Mammalian Oogenesis | **P 23** |
| **Bo Wood**<br><br>Sex-Dependent Effects of 2,5-Methoxy-4-Iodoamphetamine (DOI) on Methamphetamine Self-Administration: Mechanistic Insights from Antagonism and PET Imaging Studies | **P 24** |
| **Frances Vest**<br><br>Naturalistic Psychedelic Use and Its Association with Drug Use Patterns, Response Inhibition, and Negative Affect in Methamphetamine Recovery | **P 25** |
| **Natalia Alenina**<br><br>DNAJC12, a novel regulator of serotonin synthesis | **P 26** |
| **Mariia Dorofeikova**<br><br>Effects of dopamine D2/D3 receptor agonist quinpirole and a monoamine oxidase inhibitor clorgyline on neonatal communication in tryptophan hydroxylase 2 knockout rats | **P 27** |
| **Brian Kangas**<br><br>Preclinical Studies Examining the Prohedonic Effects of Psychedelics | **P 28** |
| **Michael Bader**<br><br>Peripheral Serotonin Reduction Promotes Atherosclerosis Progression | **P 29** |
| **Janelle Lanham**<br><br>Investigating the Polypharmacological Signaling Profiles of Psychedelics | **P 30** |
| **Nina Kastner**<br><br>Prolintane and novel analogs induce serotonin transporter mediated efflux: A pharmacological characterization | **P 31** |
| **Ameya Kasture**<br><br>The role of somatodendritically-located serotonin transporter in phototactic behavior in Drosophila melanogaster | **P 32** |

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| **Oliver Kudlacek**<br><br>Local anesthetics in Cocaine: more than just a numb feeling? | **P 33** |
| **Nikita Shah**<br><br>Functional and Pathological Implications of a Highly Conserved N-terminal Arginine Residue in SLC6 Transporters | **P 34** |
| **Thomas Angenoorth**<br><br>Interactions of Quinone Derivates with Human Organic Cation Transporters 1-3 and Plasma Membrane Monoamine Transporter: Implications for Antimalaria Drug Pharmacokinetics | **P 35** |
| **Oliver Belleza**<br><br>A novel azobenzene paroxetine derivative and its interactions with biogenic amine transporters | **P 36** |
| **Chiara Sebastianelli-Schoditsch**<br><br>Homoamphetamines: Structural Optimization of Monoamine Transporter Function to Mitigate Abuse Liability and Enhance Therapeutic Safety | **P 37** |
| **Arne Hansen**<br><br>Serotonergic Modulation of Hippocampal Spatial Coding | **P 38** |
| **Yiwen Cui**<br><br>In vivo multiplex monitoring of neuromodulators with multispectral GRAB sensors | **P 39** |
| **Jinxia Wan**<br><br>Next generation of GRAB sensors for monitoring spatiotemporal serotonin dynamics in vivo | **P 40** |
| **Sanghwa Jeong**<br><br>Near-infrared Optical Nanoprobes for Dynamic Neurochemical Imaging | **P 41** |
| **Maja Peric**<br><br>Placental and Neonatal Serotonin (PlaNS) - A Prospective Birth Cohort Study in Zagreb, Croatia | **P 42** |
| **Mitsuko Kanamaru**<br><br>Optogenetic activation of serotonergic neurons in the brain affects periodic phenomena | **P 43** |

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| **Abigail Rogers**<br><br>Serotonergic neuron activity promotes oligodendrogenesis in the dorsal raphae | **P 44** |
| **Ana Rita Costa**<br><br>Neuronal Dynamics of Serotonin Pathways in the Regulation of Anxiety and Exploration-related behaviors | **P 45** |
| **Charlotta Henningson**<br><br>Spatio-molecular Organization of the Dorsal Raphe Nucleus and Transcriptional Effects of SSRI Treatment | **P 46** |
| **Ishanee Mazumder**<br><br>Investigating the 5-HT2A Receptor and BDNF-Mediated Effects of Serotonergic Psychedelics on Mitochondrial Dynamics in Rodent Cortical Neurons | **P 47** |
| **Yuji Odagaki**<br><br>Aripiprazole may be a partial agonist at 5-HT2A receptor coupled with G(alpha)q/11 proteins in rat cerebral cortical membranes | **P 48** |
| **Nikolett Arrasz**<br><br>Ga-Proteins as Novel Players in Mood Regulation: Exploring Serotonin Transporter Regulation and Serotonin Dynamics in C. elegans | **P 49** |
| **Emerson Harkin**<br><br>A prospective code for value in the serotonin system | **P 50** |
| **Joachim Neumann**<br><br>Mutated (D100A) 5-HT4-serotonin receptors in the mouse atrium | **P 51** |
| **Joachim Neumann**<br><br>Positive inotropic effects of felcisetrag in isolated human atrial preparations | **P 52** |
| **Felix Julian Morof**<br><br>The role of organic cation transporter 1 in determining serotonin levels in peripheral circulation | **P 53** |
| **Simone Mellert**<br><br>Sex-Specific Effects of Exercise on Serotonin Dynamics During Stress: Potential Role of GABAergic Inhibition in the Dorsal Raphe | **P 54** |

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| **Keith Henry**<br><br>Identification of the Amitriptyline Binding Site in the Human Serotonin Transporter by CryoEM and Computational Studies. | **P 55** |
| **Noelle Anastasio**<br><br>Serotonin:Glutamate Synergy Bridges High Impulsivity and Reward in Preclinical Studies | **P 56** |
| **Marco Niello**<br><br>Serotonergic modulation of social cognition in mice | **P 57** |
| **Roman Romanov**<br><br>Placental serotonin causes transcriptional and compositional changes in brain | **P 58** |
| **Isak Aarrestad**<br><br>An immediate early gene and glutamate response is not necessary for the medicinal and neuroplasticity promoting effects of non-hallucinogenic 5-HT2A receptor agonists | **P 59** |
| **Chalystha Yie Qin Lee**<br><br>Role of Epithelial 5HT4 Receptor in Gastrointestinal Motility and Visceral Pain | **P 60** |
| **Saige Power**<br><br>Endogenous serotonin signaling in prefrontal cortex: frequency dependence, plasticity, and perturbation by chronic SSRI treatment | **P 61** |
| **Ralph Gradisch**<br><br>Uncoupling the Gated-Pore Mechanism in the Human Serotonin Transporter – Novel Insights into the Conducting State | **P 62** |
| **Bettina Bohl**<br><br>Real-Time Analysis of Serotonin Dynamics in Human-Derived 3D Organoids and Spheroids Using Fast-Scan Cyclic Voltammetry | **P 63** |
| **Sara Asgharzadeh**<br><br>Protocadherin-alphaC2 is required for fluoxetine-induced serotonin re-innervation and behavioral recovery after stroke | **P 64** |
| **Sixtine Fleury**<br><br>The non-hallucinogenic serotonin 1B receptor is involved in the persisting behavioral effects and neural mechanisms of psilocybin in mice | **P 65** |

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| **Kathryn Lehigh**<br><br>The medullary 5-HTergic neuron subtype called Tac1-Pet1 augments breathing during quiet wake and counters morphine-induced respiratory depression | **P 66** |
| **Nagalakshmi Balasubrama**<br><br>CART Peptide Modulation of Serotonergic Activity: A Key Driver of Anxiety | **P 67** |
| **Isabelle Straumann**<br><br>Acute effects of MDMA, MDA and their prodrugs Lysine-MDMA and Lysine-MDA in healthy participants | **P 68** |
| **Emma Bonniwell**<br><br>Molecular Determinants of Serotonin 5-HT2C Receptor Non-canonical Signaling | **P 69** |
| **Claire Deckers**<br><br>Marked Sex Differences are Observed in Heroin Acquisition and Affective States in Rats, but Converge to Similar Levels of Footshock Stress-Induced Reinstatement | **P 70** |
| **Olivia Yang**<br><br>5-HT4 receptor activation reverses stress-induced dopamine system dysfunction | **P 71** |
| **Jacob Noeker**<br><br>Astrocytes respond to serotonin and regulate serotonin-induced synaptic transmission in the basolateral amygdala | **P 72** |
| **Blake Fordyce**<br><br>Investigation of 5-HT2A receptor localization using super resolution microscopy | **P 73** |
| **Maria Sancho Alonso**<br><br>Overexpression of α-synuclein in serotonin neurons alters the activity and connectivity profile of the mouse medial prefrontal cortex. Relation to anxiety disorders in PD | **P 74** |
| **Renata Sadretdinova**<br><br>Lateral hypothalamus promotes compulsive-like behavior through disinhibition of serotonin cells | **P 75** |
| **L. Sophie Gullino**<br><br>Biosensor evidence that glutamate co-released from 5-HT neurons modulates reward prediction error signals | **P 76** |

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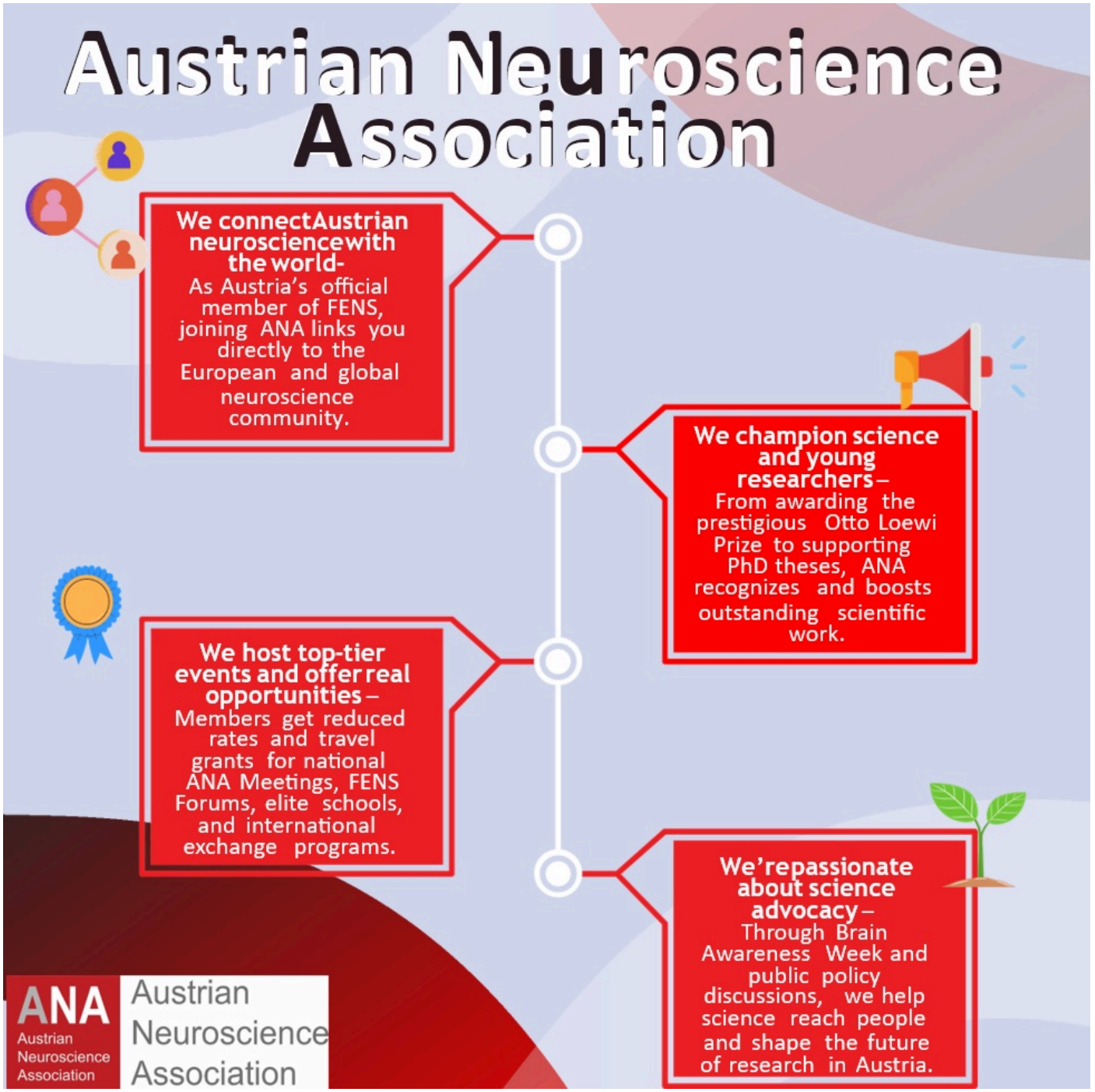
| **James Gattuso**<br><br>Acute but not Chronic Psilocybin Treatment Reduced Compulsive-like Behaviours in SAPAP3 Knockout Mice | **P 77** |
| **Rocio Beatriz Foltran**<br><br>Sex-dependent synergy of serotonin reduction with early life stress to produce adult depressive-like and anxiety phenotypes | **P 78** |
| **Jennyfer Payet**<br><br>Modulation of dorsal raphe nucleus connectivity and serotonergic signalling to the insular cortex in the prosocial effects of chronic fluoxetine | **P 79** |
| **Violette Richin**<br><br>Multimodal neuroimaging for PK/PD profile of NLX-204, a biased 5-HT1A receptor agonist | **P 80** |
| **Maria Teresa Gallo**<br><br>Perinatal fluoxetine exposure and lifelong behavioral alterations: shedding light on dynamics of sensitive periods | **P 81** |
| **Beatrice Baumberger**<br><br>Modeling the Neuroprotective Role of Estrogen and Progesterone in Brain Inflammation and Serotonin Regulation | **P 82** |
| **Carla Veronica Arganaraz**<br><br>Early-life stress alters development of prefrontal circuits modulating dorsal raphe serotonin neurons: Implications for maladaptive adult emotional behavior. | **P 83** |
| **Aurelija Ippolito**<br><br>Role of β-arrestin-2 in psychedelic drug-induced head-twitch responses and expression of plasticity-related genes in mice | **P 84** |
| **Lewis Yu**<br><br>The microbiome and serotonin immune interactions | **P 85** |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# We look forward to seeing you all at our next meeting in 2027!

# Symposia Abstracts

***Sorted by Symposia Numbers, Symposia Chair abstracts can be found above in the general program.***

## #1 Serotonergic Sub-Circuits: Probing the Roles of Identified Serotonergic Populations in Behavior and Cognition

**Bénédicte Amilhon (Chair)**
Harkin
A prospective code for value in the serotonin system
**Emerson Harkin**, Cooper Grossman, Jeremiah Cohen, Jean-Claude Béïque, Richard Naud
The in vivo responses of dorsal raphe nucleus serotonin neurons to emotionally salient stimuli are a puzzle. Existing theories centring on reward, surprise, salience and uncertainty individually account for some aspects of serotonergic activity but not others. Merging ideas from reinforcement learning theory with recent insights into the filtering properties of the dorsal raphe nucleus, here we find a unifying perspective in a prospective code for value. This biological code for near-future reward explains why serotonin neurons are activated by both rewards and punishments, and why these neurons are more strongly activated by surprising rewards but have no such surprise preference for punishments—observations that previous theories have failed to reconcile. Finally, our model quantitatively predicts in vivo population activity better than previous theories. By reconciling previous theories and establishing a precise connection with reinforcement learning, our work represents an important step towards understanding the role of serotonin in learning and behaviour.

Serotonin's Role in Medial Prefrontal Cortex-mediated Cognitive Flexibility
**Nuno Dinis Alves**, PhD Ashlea Morgan, MSc Gregory Stevens, BSc Tamanna Yeasmin, MSc Alexandra Mackay, PhD Saige Power, PhD Derya Sargin, BSc Carla Hanna, BSc Arwa Adib, BSc Annette Ziolkowski-Blake, PhD Evelyn Lambe, PhD Mark Ansorge
Cognitive flexibility, the ability to adapt patterns of behavior and thoughts to environmental or context changes, is a higher-order executive function strongly associated with Prefrontal Cortex (PFC)-related neuronal circuits. Within the PFC, specific regions mediate different forms of cognitive flexibility: the medial PFC (mPFC) is crucial for attentional set-shifting, while the orbital frontal cortex (OFC) is necessary for reversal learning. We found that specific serotonin release in the Prelimbic (PrL) region of the mPFC plays necessary and sufficient roles in cognitive flexibility through a mechanism dependent on 5-HT1A receptor activation. Using fiber photometry, we observed a pre-reward peak in serotonin neuronal activity of mPFC-projecting dorsal raphe (DR) neurons in the rule shift of the two-choice digging test, a mPFC-dependent attentional set-shifting task. The modulation of the activity of this pathway before reward alters performance, with terminal optogenetic stimulation improving and optogenetic inhibition impairing cognitive flexibility. Of note, we observed that optomodulation of this pathway does not impact anxiety-like behavior. Collectively, our data reveal a powerful and specific modulatory role of endogenous serotonin release from dorsal raphe-to-mPFC projecting neurons in cognitive flexibility.

Adaptation to seasonal photoperiods via dynamic serotonin-glutamate neurotransmitter segregation
**Giacomo Maddaloni**, YoonJeung Chang, Rebecca Senft, Susan Dymecki
Changes in daylight amount (photoperiod) drive pronounced alterations in physiology and behaviour. Adaptive responses to seasonal photoperiods are vital to all organisms – dysregulation is associated with disease, from affective disorders to metabolic syndromes. Circadian rhythm circuitry has been implicated yet little is known about the precise neural and cellular substrates that underlie phase synchronization to photoperiod change. Here we present a previously unknown brain circuit and novel system of axon branch-specific and reversible neurotransmitter deployment that together prove critical for behavioural and sleep adaptation to photoperiod change. We found that the recently defined neuron type called mrEn1-Pet1 located in the mouse brainstem Median Raphe Nucleus (MRN) segregates serotonin versusVGLUT3 (here proxy for the neurotransmitter glutamate) to different axonal branches innervating specific brain regions

involved in circadian rhythm and sleep/wake timing. Whether measured during the light or dark phase of the day this branch-specific neurotransmitter deployment in mrEn1-Pet1 neurons was indistinguishable; however, it strikingly reorganizes on photoperiod change. Specifically, axonal boutons but not cell soma show a shift in neurochemical phenotype upon change away from equinox light/dark conditions that reverses upon return to equinox. When we genetically disabled the deployment ofVGLUT3 in mrEn1-Pet1 neurons, we found that sleep/wake periods, voluntary activity, and clock gene expression failed to synchronize to the new photoperiod or were significantly delayed. Combining intersectional rabies virus tracing and projection-specific neuronal silencing in vivo, we delineated a Preoptic Area-to-mrEn1Pet1 connection responsible for decoding the photoperiodic inputs, driving the neurochemical shift and promoting behavioural synchronization. Our results reveal a previously unrecognized brain circuit along with a novel form of periodic, branch-specific neurotransmitter deployment that together regulate organismal adaptation to photoperiod changes.

Sex-specific modulation of anxiety by raphe-ventral hippocampus serotonergic circuits
**Bénédicte Amilhon**
Anxiety disorders are among the most prevalent mental disorders worldwide and affect women twice as often as men, yet the neural basis of the female brain's vulnerability to anxiety disorder is unclear. Raphe serotonergic neurons play a key role in the regulation of mood and anxiety and provide dense inputs to the ventral hippocampus (vHP). The vHP is also heavily involved in modulation of anxiety levels and show sex-specific involvement in a range of cognitive and emotional functions. We hypothesized that ventral hippocampal-projecting 5-HT neurons are instrumental in sex-specific control of anxiety levels. Using a combination of optogenetic tools and calcium sensors expressed specifically in raphe-vHP neurons, along with in vitro and in vivo electrophysiology, we show that the raphe-vHP pathway modulates behavior and hippocampus oscillatory activity differentially in male and female mice. Optogenetic activation of vHP-projecting 5-HT neurons elevated anxiety levels and stress-related behaviors exclusively in females. Increased anxiety in response to 5-HT release in the vHP was accompanied by changes in HP theta oscillation properties, again exclusively in females. Moreover, we identify sex differences in the electrophysiological properties of a subset of vHP-raphe 5-HT neurons located in the MnR. Together, our results provide novel mechanistic insight into the role of the raphe-vHP 5-HT pathway, with important implications for sex-related differences in anxiety and associated disorders.

## #2: Brain vs Gut Feelings": Serotonin as a Central and Peripheral Regulator of Autonomic Function, Mood, and Interoception

**Mark Ansorge (Chair), Michael Gershon (Co-Chair)**

The medullary 5-HTergic neuron subtype called Tac1-Pet1 augments breathing during quiet wake and counters morphine-induced respiratory depression.
**Ph.D. Kathryn Lehigh**, Jordan Jones, M.D., Ph. D. Ryan Dosumu-Johnson, M.D., Ph.D. Susan Dymecki
Brainstem serotonin (5-HT)-producing neurons modulate breathing, with distinct 5-HTergic neuron subtypes contributing to specific respiratory functions (Brust et al., 2014; Okaty et al., 2019). The Tac1-Pet1 neuron subtype, named by gene expression, distributes soma across raphe obscurus (ROb), raphe magnus (RMg), and lateral paragigantocellularis. Tac1-Pet1 neurons innervate brain and spinal cord regions involved in respiratory motor output (i.e., hypoglossal, phrenic, nucleus ambiguus) and nuclei critical for respiratory rhythm generation and modulation (i.e., preBotzinger, parabrachial). Tac1-Pet1 neurons are required for mounting a full respiratory response to hypercapnia (Hennessy et al., 2017). Until now, their sufficiency to drive breathing had not been assessed, though our axonal projection and functional data, as well as prior work on 5-HTergic ROb neurons, suggest that activating these neurons may augment breathing (Depuy et al., 2011; Pilowsky et al., 2014). We combined our mouse intersectional genetic platform with DREADD chemogenetics to activate Tac1-Pet1 neurons and measure respiration via awake whole-body plethysmography. We found that acute activation of Tac1-Pet1 neurons increases respiratory rate and minute ventilation – comparable to activating Pet1+ neurons en masse. Initial work using focal viral DREADD injections to infect ROb 5HTergic neurons has shown a similar increase in respiratory rate after

CNO-hM3Dq triggered neuron excitation, indicating brain specificity of the respiratory phenotype—in alignment with prior optostimulation of ROb 5HTergic neurons (Depuy et al., 2011). Given the sufficiency of Tac1-Pet1 neurons to increase minute ventilation, we are exploring the capability of Tac1-Pet1 neurons for augmenting breathing under conditions of drug-induced respiratory depression and assessing Tac1-Pet1 neuron modulation of motor output (upper airway tone and diaphragm EMG) and respiration across sleep-wake states. Preliminary findings demonstrate acute activation of Tac1-Pet1 neurons can counter opiate-induced disordered breathing.

Pathway-specific Roles for Serotonin and Their Developmental Malleability

**Mark Ansorge**

Serotonin is a key modulatory neurotransmitter regulating many brain functions such as mood, memory, and cognition. But before assuming its role in the mature brain, serotonin also modulates early brain development across phylogenetically diverse species. Increases in serotonin signaling during development lead to altered structure and function throughout the brain in adult mice. In fact, serotonin signaling is crucial to fetal brain development and plays a role in cell proliferation, neuronal differentiation, synaptogenesis, and neuronal migration. This developmental role has potential public health relevance because human fetal serotonin selective reuptake inhibitor (SSRI) exposure increases serotonin signaling during sensitive periods of human brain development. Currently approximately 6% of pregnant mothers take SSRIs. Here we report findings on serotonin's auto-inhibitory control over serotonergic projection development and their behavioral sequalae. Specifically, we review our findings on serotonergic projections to the hippocampus, medial prefrontal cortex, and the periaqueductal grey, with consequences to learning and memory, social bonding, cognitive flexibility, and innate fear.

Intestinal SERT and SSRIs Play Important Roles in Gut-Brain Communication: Bench to Bedside Translation

**Kara Margolis**

Disorders of gut-brain interaction (DGBI), such as irritable bowel syndrome (IBS), are the most common gastrointestinal problems internationally, affecting over 40% of the population. They are also highly linked to mood disorders; over 50% of people with a DGBI are also diagnosed with (mainly) anxiety or depression. Treatments for these co-occurring conditions are highly limited, largely due to a limited understanding of the pathophysiology of DGBI and also of their connections to mood. This talk will review the key serotonin-based links our lab has discovered, through clinical and preclinical studies, with regards to the connection between DGBI and anxiety. In particular, we will: focus on the links between in utero SSRI exposure and DGBI development in humans and in murine studies and; delineate the targeted actions of serotonin on the gut epithelium in the modulation of mood and GI pain.

Gut touch and the role of gut serotonin in the gut's intrinsic tactile sense

**Arthur Beyder**

Serotonin serves as a key signaling molecule in the gut-brain axis. Enterochromaffin cells (ECs) in the gastrointestinal (GI) tract lining are responsible for supplying the vast majority of peripheral serotonin, which not only regulates local motility and secretion but also communicates with the central nervous system, influencing both gastrointestinal function and behavior. The ECs in the GI tract lining respond to vast luminal stimuli, like nutrients, but also luminal forces through mechanosensitive ion channel Piezo2. In fact, Piezo2+ ECs are similar to Merkel cells, the skin's touch sensors. This led us to the concept of gut touch, a sensory modality intrinsic to the GI tract. In these ECs, Piezo2 mediates mechanotransduction, interacting with voltage-gated calcium and sodium channels to produce electrical excitability and sustained elevations in cytoplasmic calcium. This signaling cascade is crucial for serotonin release, which regulates motility and secretion through both intrinsic and extrinsic pathways. These gut touch mechanoreceptors endow the gut with a tactile sense, which it uses to assess the physical nature of luminal contents to improve digestion and waste management. Disruptions in these processes, including gut touch, may contribute to motility disorders and other GI-related conditions. This presentation will explore the mechanisms behind gut touch, focusing on serotonin's role and how disruptions in this system may lead to clinical disorders.

## #3 Searching for Receptors Involved in the Antidepressant Effects of Psychedelics

**Carine Becamel (Chair), Joel Bockaert (Co-Chair)**

Decoding Psychedelics: Structural Insights into their Pharmacology and Receptor Interactions
**Ryan Gumpper**
Psychedelics have recently garnered the interest of the psychiatric community for their promise to treat many intractable neuropsychiatric disorders, such as treatment-resistant depression, post-traumatic stress disorder, and anxiety, to name a few. These compounds exhibit many therapeutic and potentially serious side effects, so their complicated pharmacology and polypharmacology must be fully understood. Examining the molecular interactions across many receptors will reveal unique perspectives on their structure-activity relationships. This talk will explore the current state of the structural biology of psychedelic compounds and potential mechanisms that can be exploited in the development of safer, selective, and more effective psychedelic compounds.

Antidepressant-like effects of psychedelics in a chronic despair mouse model: is the 5-HT2A receptor the unique player?
**Carine Becamel**
Major depressive disorder is one of the most disabling psychiatric disorders in the world. First-line treatments such as selective serotonin reuptake inhibitors still have many limitations, including a resistance to treatment in 30% of patients and a delayed clinical benefit that is observed only after several weeks of treatment. Increasing clinical evidence indicates that the acute administration of psychedelic agonists of the serotonin 5-HT2A receptor (5-HT2AR), such as psilocybin, to patients with MDD induce fast antidepressant effects, which persist up to five weeks after the treatment. In our recent study, we investigated the effect of two psychedelics of different chemical families, DOI and psilocybin, and a non-hallucinogenic 5-HT2AR agonist, lisuride, in a chronic despair mouse model exhibiting a robust depressive-like phenotype. We showed that a single injection of each drug to wild type mice induces anxiolytic- and antidepressant-like effects lasting up to 15 days in wild-type mice. Notably, the antidepressant-like effects of DOI and lisuride were absent in 5-HT2A−/− mice, while psilocybin remained effective. Collectively, these findings indicate that 5-HT2AR agonists can produce antidepressant-like effects independently of their hallucinogenic properties through mechanisms involving or not the receptor. To go further and characterize changes in the synaptic phosphoproteome elicited upon 5-HT2A receptor stimulation we used quantitative phosphoproteomics. Our findings revealed that the administration of DOI in mice promotes the phosphorylation of synaptic proteins within a highly interconnected protein network, which includes the metabotropic glutamate (mGlu)5 (mGlu)5 receptor. Functional studies also revealed that neuroplasticity-promoting properties of psychedelics depend on a functional, reciprocal interplay between 5-HT2A and mGlu5 receptors.

5-HT2A receptors role in psilocybin antidepressant effects in humans
**Lucie Berkovitch**
Psilocybin, a compound found in "magic mushrooms." On top of its well-known acute subjective effects, the so-called psychedelic experience, psilocybin has shown promising results as a rapid-acting antidepressant. Indeed, clinical trials have demonstrated that psilocybin, administered in controlled settings, can significantly reduce symptoms of depression within hours, with effects lasting for weeks. The subjective experiences reported by participants, often described as mystical or spiritual, are thought to be integral to the therapeutic process.
Psilocybin main mechanism of action involves the activation of 5-HT2A serotonin receptors, which are densely expressed in the brain. 5-HT2A blockade leads to a suppression of its psychedelic effects. However, the role of 5-HT2A receptors activation and the associated psychedelic experience in psilocybin antidepressant effects remains unclear.
Several clinical trials have evidenced a correlation between the intensity of psychedelic experience and clinical response. In contrast, studies using animal models have shown that psilocybin can still exert antidepressant-like effects even when 5-HT2A receptors are blocked. The same result has been observed in a case-report where a patient incidentally taking a treatment antagonizing 5-HT2A receptors still exhibited a significant decrease of depressive symptoms after psilocybin intake, without experiencing any

acute subjective effects. This suggests that other mechanisms, such as the increased in neuroplasticity, could also contribute to its therapeutic benefits independently of 5-HT2A activation.
In this talk, we will present current evidence on the role of 5-HT2A receptors activation and psychedelic experience in psilocybin antidepressant effects and discuss how 5-HT2A receptors dependent and independent therapeutic effects can be disentangled and studied.

Interaction of antidepressants and psychedelic compounds with the BDNF receptor TrkB
**Anaïs Virenque**, Prof. Eero Castrén
Promotion of neuronal plasticity is considered critical for the action of all the drugs producing antidepressant effects. We have shown that many antidepressant drugs belonging to different chemical classes (including typical drugs SSRIs and tricyclics, but also ketamine) directly binding to TrkB, the receptor for brain-derived neurotrophic factor (BDNF) and allosterically increasing BDNF signaling. Furthermore, psychedelic compounds LSD and psilocin directly bind to TrkB with high affinity and thereby promote plasticity and antidepressant effects, but not the hallucinogenic effects. A point mutation within the predicted binding site within the transmembrane domain of TrkB prevents the binding as well as plasticity-promoting and antidepressant-like effects of antidepressants and psychedelic compounds. We are currently searching for other endogenous and synthetic molecules that interact with the antidepressant binding site within TrkB. Through promotion of BDNF signaling, antidepressants promote plasticity and thereby increase the sensitivity of neuronal networks to environmental influences, which suggests that active engagement of the patient together with drug-promoted plasticity is critical for mood recovery.

## #4 Beyond Serotonin: The Multifaceted Metabolism of Tryptophan

**Stefano Comai (Chair), Ana Pocivavsek (Co-Chair)**

Unveiling the complexity of tryptophan metabolism
**Trevor Stone**
Following the discovery of tryptophan in 1901, studies of its metabolism were largely concentrated on its conversion to nicotinamide adenine dinucleotide (NAD), via oxidation to kynurenine, quinolinic acid and nicotinamide (vitamin B3). The enzymes responsible were identified, together with other metabolites such as kynurenic acid, xanthurenic, anthranilic and quinaldic acids, all of which appeared to be biologically inactive. However, when 5-hydroxytryptamine (5-HT, serotonin) was discovered in the 1930s, with its range of receptor-mediated biological actions affecting synaptic transmission and smooth muscle contractility, it became the focus of interest for the next 50 years.
The tryptophan landscape changed dramatically when it was discovered that two metabolites of kynurenine also showed clear, receptor-mediated activity. Quinolinic acid excited neurons in the cerebral cortex by activating glutamate receptors sensitive to N-methyl-D-aspartate (NMDA). Subsequently, based on their structural similarities, kynurenic acid was found to block these receptors, in addition to other ionotropic receptors for kainate or AMPA. These actions led to the recognition of kynurenine metabolites as regulators of neuronal excitability, neurodevelopment and neurodegeneration.
At the same time that these neuromodulatory metabolites were being described (1981, 1982), it was observed that expression of the first enzyme in the kynurenine pathway (indoleamine-2,3-dioxygenase, IDO) was induced by inflammatory mediators, leading to the realisation twenty years later that this signalled a fundamental role of kynurenic acid in the immune system. Linking this information to that of neuromodulation has led to views of the kynurenine pathway as a key mediator of communication between the nervous and immune systems – the neuroimmune interface. The combined concepts have since been expanded further to the intestinal microbiome, where many bacteria synthesise tryptophan and produce indole derivatives affecting the mammalian host.
A more recently discovered mediator of tryptophan metabolism is interleukin-4-induced protein-1 (IL4i1), which metabolises tryptophan to indole pyruvate (and related compounds) which cyclises spontaneously to the glutamate antagonist kynurenic acid.
Finally, tryptophan metabolism is further complicated by interactions between the kynurenine and serotonin pathways, making it increasingly important to consider all these compounds for a more complete understanding of tryptophan biology.

Beyond Serotonin: The Multifaceted Metabolism of Tryptophan
**Stefano Comai**
Tryptophan metabolism encompasses an intricate network of biochemical pathways that extend far beyond the synthesis of serotonin. Indeed, as an essential amino acid, tryptophan serves as a precursor not only for serotonin but also for a diverse array of metabolic products that play crucial roles in physiological and pathological processes. The kynurenine pathway, for instance, leads to the production of several bioactive metabolites, including kynurenine, kynurenic acid, and quinolinic acid, each of which has significant implications for immune regulation, neuroprotection, and neurotoxicity.
Our symposium aims to explore these multifaceted pathways, shedding light on the interconnected roles of tryptophan metabolites in health and disease. This exploration extends beyond serotonin synthesis and aims to generate substantive new ideas and foster collaborations around a rapidly expanding topic that is translationally relevant across different areas of medicine. In detail, Prof. Stone will provide an overview of the complexity of tryptophan metabolism along the different but interconnected pathways. Prof. Comai will present translational findings that demonstrate how the interplay between serotonin, melatonin, and kynurenine metabolites may underlie the associations among schizophrenia, metabolic disturbances, and cognitive impairment, potentially identifying new targets for treatment. Dr. Pocivavsek will discuss models that enhance tryptophan metabolism during development, with a special emphasis on the consequences of elevated kynurenine metabolites during the prenatal period on neurochemical and behavioral consequences later in life. Prof. Erhardt will discuss the significant finding of elevated cerebrospinal fluid (CSF) serotonin (5-HT) levels in patients experiencing their first-episode psychosis (FEP). Furthermore, Prof. Erhardt will delve into the comprehensive pathway analysis, which linked these metabolic alterations to inflammatory pathways, underscoring their crucial role in the pathogenesis and progression of psychosis.

The Role of Tryptophan Metabolism, Immune Activation, and Kynurenine Pathway Dysregulation in Psychotic Disorders
**Sophie Erhardt**
Emerging evidence highlights the involvement of and connection between immune activation and tryptophan metabolism in the pathophysiology of psychotic disorders and cognitive dysfunctions. The kynurenine pathway (KP), the primary catabolic route of tryptophan degradation, plays a crucial role in modulating neuroactive metabolites, and enhanced central levels of kynurenic acid (KYNA) has been implicated in cognitive and psychiatric dysfunction. Increased immune activation, as reflected by elevated inflammatory markers, further supports the hypothesis of immune dysregulation in psychosis. In first-episode psychosis (FEP) patients, we have identified correlations between several immune markers and disease severity.
Metabolomic analyses of cerebrospinal fluid (CSF) and serum from the same FEP patients reveal significant alterations in tryptophan metabolism, including elevated serotonin (5-HT) levels in CSF. We have found that multiple kynurenine pathway enzymes, such as tryptophan 2,3-dioxygenase (TDO2), indoleamine 2,3-dioxygenases (IDO1/IDO2) and kynurenine aminotransferase (KAT) III, are induced by cytokines and plays a key role in regulating the production of kynurenine-derived metabolites, which subsequently influence neurotransmission. The identification of persistent metabolic and inflammatory changes in psychosis underscores the relevance of targeting the kynurenine pathway for potential therapeutic interventions.
Recent advancements in drug development have focused on KAT enzymes, particularly KAT II and KAT III, which are critical for the synthesis of KYNA. Recently, Kynexis reported promising results from a phase 1 clinical trial of a KAT II inhibitor, developed for the treatment of cognitive impairment associated with schizophrenia. Our own data demonstrates that immune activation specifically induces KAT III and increase KYNA production also in the absence of KAT II. Thus, blockade of additional KAT enzymes is necessary to normalize immune-induced KYNA concentration. It remains to be investigated how inhibition of KAT II and KAT III may influence serotonin synthesis and/or neurotransmission.

Tryptophan Metabolism in During Neurodevelopment: Sleep, Cognition, and Mental Health
**Ana Pocivavsek**
Recent findings highlight that tryptophan metabolism plays a key role in mental illness and neurodevelopment, involving serotonin and kynurenine pathway metabolites like kynurenic acid (KYNA).

Disruptions in this process, triggered by environmental risk factors (e.g., stress, infections, disrupted sleep, drug use), can impact postpartum health and fetal brain development. We investigate how dietary kynurenine affects sleep, arousal, and prenatal outcomes, hypothesizing that kynurenine pathway (KP) metabolism—linked to immune activation and high-fat diets—worsens sleep problems during pregnancy. KYNA, a cholinergic and glutamatergic modulator, is of particular interest. Female rats were fed chow laced with L-kynurenine sulfate (100 mg/day) from embryonic day (ED) 15 to ED 22 and a comparable eight consecutive days in nulliparous rats. EEG/EMG data classified vigilance states (wake, REM, NREM) using a deep neural network. In nulliparous females, kynurenine reduced REM sleep (-30%, $P<0.05$) and increased dark-phase NREM sleep (+33%, $P<0.05$). Pregnant dams showed increased dark-phase sleep (REM: +54%, $P<0.05$; NREM: +57%, $P<0.05$), but the kynurenine diet reduced REM sleep (-32%, $P<0.01$). Control dams exhibited lower cage activity, signaling rest needs, which the kynurenine diet disrupted. Offspring (PD28-56) of kynurenine-fed dams had reduced REM sleep and total sleep by PD56 ($P<0.05$). While control offspring showed robust sleep rebound post-deprivation, kynurenine-exposed offspring exhibited delayed and reduced rebound ($P<0.05$). Prepubertal EKyn females (PD28) showed elevated plasma kynurenine ($P<0.05$), KYNA ($P<0.01$), and cytokines (IL-10, IL-18; $P<0.01$), with no similar changes in males. Taken together, our data indicate that kynurenine diet impedes restorative sleep and supports our hypothesis that KYNA elevations negatively impact sleep quality, which may be particularly detrimental during pregnancy. Ongoing research continues to explore adolescence as a critical period for intervention, focusing on inhibiting kynurenine aminotransferase II (KAT II) as a mechanistic avenue to reduce KYNA and improve outcomes for individuals suffering from neurodevelopmental and psychiatric disorders.

## #5 The Ascending Serotonin Neurons and Their Diverse Functions through the Serotonin Heteroreceptor Complexes

**Kjell Fuxe (Chair), Dasiel Borroto Escuela (Co-Chair)**

Brain serotonin heteroreceptor complexes in the Flinders Sensitive Line (FSL) rat model of depression and as targets for antidepressant treatment

**Dasiel Oscar Borroto-Escuela**, PhD student Marco Bartolini, PhD student Emmanuell Gonzalez-Cristo, PhD student Verty Ochoa-Torres, MD student Adam Danielson, PhD student Javier Ruiz-Lasierra, PhD Student Emilio Serra-Rojas, PhD student Francesca Frescura, MD Migdalis Hidalgo-Muniz, MD Orisley Franch de Armas, Professor Kjell Fuxe

The concept of allosteric receptor–receptor interactions within G protein-coupled receptor (GPCR) heteroreceptor complexes provides a novel framework for understanding brain integration and neuropsychopharmacology. The observation of the GPCR heterodimer network (www.gpcr-hetnet.com) indicates that allosteric receptor-receptor interactions dramatically increase GPCR diversity and biased recognition, leading to enhanced specificity in signaling. Dysfunction of GPCR heteroreceptor complexes can lead to brain disease and offers new targets for drug development for central nervous system disorders, including major depressive disorder (MDD). This lecture presents findings from in situ proximity ligation assays conducted on the Flinders Sensitive Line (FSL) rat model of depression, focusing on the vulnerabilities of serotonin (5-HT) heteroreceptor complexes such as 5-HT1A-FGFR1, 5-HT1A-5-HT2A, 5-HT1A-GalR1-GalR2, and 5-HT1A-5-HT4R. Disturbances in these serotonin heterocomplexes within the raphe-hippocampal 5-HT system contribute to the pathophysiology of MDD, as evidenced by deficits in neuroplasticity and neurotransmission. For instance, dysfunction in 5-HT1A-FGFR1 complexes impairs neuroplasticity in the hippocampus and raphe nuclei, contributing to reduced neurogenesis and cortical network atrophy. Antidepressant-like effects of combined FGFR1 and 5-HT1A agonist treatments were diminished in FSL rats, potentially due to impaired uncoupling of 5-HT1A receptors from GIRK channels. Furthermore, astrocytic FGFR1-5-HT1A complexes in the hippocampus were identified, with pharmacological treatments enhancing structural plasticity and gamma oscillations, suggesting astroglial contributions to antidepressant mechanisms. Disturbances in the 5-HT1A-GalR1, GalR1-GalR2, and 5-HT1A-5-HT4R heteroreceptor complexes within the raphe-hippocampal 5-HT system were also found in FSL rats. In addition, inhibitory allosteric interactions within 5-HT1A-5-HT2A isoreceptor complexes, along with altered distribution and densities in FSL rats, were observed, suggesting a mechanism for reduced 5-HT1A signaling in depression. Taken together, these findings point to the significant role of serotonin

heteroreceptor complexes in the modulation of mood and their potential as therapeutic targets. These results highlight the importance of serotonin heteroreceptor complexes as key regulators of neuronal and astroglial network integration in depression and as novel targets for fast-acting antidepressants. Understanding their role in MDD offers critical insights into the development of precision therapeutics aimed at restoring disrupted receptor-receptor interactions.

The role of the oxytocin receptor in the GPCR heteroreceptor complexes in the brain and Its relevance for brain integration.

**Kjell Fuxe**, PhD Cristina Cuesta-Marti, PhD Barbara Chruścicka-Smaga, PhD student Álvaro Enrique Cáceres-Quezada, PhD student Francesca Frescura, PhD Sarah Beggiato, Professor Luca Ferraro, PhD Minerva Crespo-Ramírez, Professor Angelica Maria Fierro-Huerta, Professor Miguel Perez de la Mora, Professor Harriët Schellekens, PhD, MBA Dasiel Oscar Borroto-Escuela

Over the last decade, hypothalamic oxytocin axons have been shown to undergo extensive collateralization, leading to widespread innervation of the telencephalon and diencephalon. Similar to monoamines and neuropeptides, oxytocin primarily functions via volume transmission in the extracellular fluid, targeting regions such as the nucleus accumbens and caudate-putamen. A growing body of evidence highlights the existence of oxytocin receptor (OXTR) heteroreceptor complexes in the brain, including interactions with the ghrelin receptor (GHS-R1a). Ghrelin is a gut hormone (a 28-amino-acid peptide) that can cross the blood-brain barrier and reach the CNS, where it may target the GHS-R1a. There is evidence that the activated ghrelin receptor forms a complex with the OXTR, modulating its function through allosteric receptor-receptor interactions with implications for appetite regulation, anxiety, and depression. Our research revealed the existence of D2R-OXTR heteroreceptor complexes in the nucleus accumbens and dorsal striatum. These complexes exhibit enhanced receptor-receptor interactions, demonstrated by increased BRETmax values and validated by proximity ligation assay (PLA). Allosteric modulation by oxytocin increases D2 receptor (D2R) binding affinity, suggesting that these interactions underlie oxytocin's ability to enhance emotional and social behaviors through D2R signaling, especially in the nucleus accumbens. Such mechanisms are also relevant in the amygdala, where they may inform the development of anxiolytic drugs. Additionally, we have demonstrated the heteromerization of OXTR with serotonin receptors, including 5-HT2AR and 5-HT2CR, in limbic regions such as the hippocampus and nucleus accumbens. These findings, supported by in situ PLA and FRET analyses, show that activation of 5-HT2AR and 5-HT2CR protomers inhibits OXTR signaling, reducing its ability to facilitate social and cognitive processes. Evidence indicates that activation of the 5-HT2AR and 5-HT2CR protomers leads to inhibition of oxytocin protomer signaling, reducing its ability to enhance social and cognitive events. Thus, the known depressant actions of serotonin 5-HT2AR and 5-HT2CR can also be mediated by their reduction of oxytocin receptor-induced social events, in addition to their suppression of 5-HT1AR signaling, a well-known enhancer of antidepressant activity. Overall, these findings provide valuable insights into the interplay between oxytocin and serotonin systems, offering new perspectives for therapeutic strategies targeting cognitive, social, and mood disorders.

Evaluation of 5-HT2C Receptor Drugs in a Preclinical Model of Depression and Cocaine Addiction Comorbidity

**Małgorzata Frankowska**, Dr Joanna Jastrzębska, Prof. Małgorzata Filip

Drug addiction, also called substance use disorder (SUD), is a disease that affects a person's brain and behavior, leading to an inability to control the use of legal or illegal drugs or medicines. Epidemiological data indicate a high rate of comorbidity between depression and SUD. Administration of serotonin 2C (5-HT2C) receptor agonists reduced cocaine reinforcement and protected against cocaine-seeking behavior in rats with a bilateral olfactory bulbectomy (OBX; a model of depression) as well as in SHAM control rats undergoing cocaine self-administration. In contrast, acute administration of the 5-HT2C receptor-preferring antidepressant mirtazapine did not alter cocaine reinforcement in either phenotype but reduced cocaine-seeking behavior. Neurochemical analyses revealed that cocaine reinforcement increased 5-HT2C receptor levels in the ventral hippocampus, with a preexisting depression-like phenotype enhancing this effect. Ten days of cocaine abstinence reduced 5-HT2C expression in the dorsolateral striatum, while the coexistence of depression and SUD enhanced local receptor expression. The results support a key role for 5-HT2C in treating SUD and comorbid depression, highlighting the potential for further research into pharmacological strategies targeting these receptors.

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One Molecule, Many Fates: Serotonin´s journey in search of neurochemical balance
**Angélica Fierro**, Matias Marambio, Juan Pablo Aguayo, Yuan Chang, PhD Nicole Morales, PhD Luis Dinamarca-Villarroel, PhD Agustín Robles, Álvaro Cáceres, PhD Gerald Zapata-Torres, PhD Gonzalo E. Torres
Serotonin (5-HT) degradation by enzymes, signal integration through specific receptors, and uptake via the Serotonin Transporter (SERT) are distinct mechanisms that either generate a biological response or terminate the actions of this neurotransmitter. Dysregulation of 5-HT levels have been associated with various conditions, including attention deficits, depression, and Alzheimer's disease in mammals and feeding, reproduction and other actions in invertebrates.
To understand the interaction of 5-HT in each macromolecule, to elucidate the dynamic behavior and the key interactions of each protein-ligand complex a structural description is required. Thus, we carried out a systematic study in vertebrates and invertebrates using different molecular modeling methodologies to describe in enzymes, receptors and transporters the mechanisms involved when serotonin or another ligand interact.
After homology modeling to describe the architecture of macromolecules, docking studies to know main protein-ligand interactions, molecular dynamic simulations in all-atom and coarse grained method to describe a time-dependent structural and physicochemical evolution of each complex allows identified specific conformational changes associated at the mechanistic patterns of these membrane proteins interacting with serotonin and leads to define structural requirements to design new ligands with therapeutic or agrochemical use.
Acknowledgement: Fondecyt Grant 1161375, 1221030

## #6 Psychedelics: A Stunning Antidepressant Effect

**Alain Gardier (Chair), Bruno Guiard (Co-Chair)**

LSD Treatment for Anxiety Disorders
**Stefano Comai**, on behalf of Gabriella Gobbi

The non-hallucinogenic serotonin 1B receptor is necessary for the antidepressant effects of psilocybin in mice
Katherine Nautiyal, **Sixtine Fleury**
Serotonergic psychedelics are gaining traction as clinically effective therapies for a number of psychiatric disorders, including major depressive disorder. The persisting clinical effects of psychedelics, such as psilocybin, are most commonly attributed to activation of the serotonin 2A receptor (5-HT2A R) based on its role in the acute hallucinogenic effects of psychedelics. However, psilocin, the active metabolite of psilocybin, binds to many serotonin receptor subtypes, and its polypharmacology is potentially important for the long-lasting clinical effects. Our work tests the idea that the serotonin 1B receptor (5-HT1B R) is necessary for the anti-depressant effects of psilocybin in mice. As an inhibitory Gi-coupled receptor, 5-HT1B binds psilocin with high affinity, has been previously implicated in mediating neural plasticity, and is critical for the efficacy of other antidepressant therapies. Using chronic corticosterone and forced swim stress models of stress-induced depression, we established post-acute antidepressant-like effects of psilocybin in mice, including reductions in anxiety and anhedonia. Interestingly, these effects are absent in mice lacking 5-HT1BR binding using genetic or pharmacological loss-of-function models, suggesting that 5-HT1BRs are necessary for the behavioral responses to psilocybin in mice. We also measured the effects of 5-HT1BR on the neural activity changes induced by psilocybin. Quantification of whole-brain neural activity using c-fos labeling following psilocybin administration showed differential activation patterns depending on 5-HT1BR expression. In particular, 5-HT1BR expression influenced psilocybin-induced changes in c-fos expression in a number of limbic and cortical regions, including amygdala, basal ganglia, and prefrontal areas. Overall, our data shows that the non-hallucinogenic 5-HT1BR is necessary for the behavioral and neural effects of psilocybin in mice.

Influence of the context of administration in the antidepressant-like response of the psychedelic 5-MeO-DMT

**Bruno Guiard**, Dr Romain Hacquet

Evidence suggests that psychedelics have acute and long-lasting beneficial effects in the treatment of depression. Extra-pharmacological variables, known as “set and setting”, including individual dispositions (set) and the surrounding environment (setting) would influence the course of psychedelic experiences and subsequent well-being. This study investigated the anxiolytic/antidepressant-like activity of 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), examining how the context of administration influences the trajectory of behavioral effects. We also raised the possibility that 5-MeO-DMT reactivates a critical period of plasticity making the brain more susceptible to environmental reorganization of synaptic connectivity. In non-stressed control mice, a single high dose (5 or 10 mg/kg) of 5-MeO-DMT produced antidepressant-like effect 20 min after intraperitoneal administration, particularly in a post-synaptic 5-HT1A receptor-dependent manner. Interestingly, high doses retained antidepressant-like effects 24hr after administration, while a low dose (0.5 mg/kg) showed only delayed benefits in both control and corticosterone (CORT)-exposed mice, a model of depression. In these CORT mice, the behavioral activity of the low dose is enhanced when the drug is administered in a positive environment, but impaired in a negative setting. We then collected evidence that the antidepressant-like effects of 5-MeO-DMT correlates with a decrease in the percentage of Perineuronal Nets on hippocampal parvalbumin neurons when administered in a positive setting, while such a correlation was not unveiled in a negative context. Overall, our results underscore the importance of optimizing and standardizing the environmental conditions of psychedelic administration to enhance their efficacy in the treatment of depression and to limit adverse psychological events.

Psilocin, a stunning antidepressant effect in stress mice

**Alain Gardier**, PhD Student Makiath Adebo, Dr Celine Defaix, Pr Denis David, Pr Erwan Poupon, Dr Laurent Tritschler

Serotonergic psychedelics, 5-HT2A receptor (5-HT2AR) agonists, once banned, have recently displayed antidepressant (AD) potential in both humans and rodents. In 2019, the FDA granted "breakthrough therapy" status to psilocybin therapy in some clinical trials. Psychoplastogens are defined as a class of therapeutic compounds producing both rapid and sustained effects on neuroplasticity and AD behavior after a single administration. This classification includes both serotonergic psychedelics and ketamine, although their primary targets are distinct. Ketamine, a NMDA-R antagonist, induces neuroplasticity in glutamatergic pyramidal neurons, an effect potentially underlying its AD activity. In the lab, we found that a subanesthetic dose (R,S)-ketamine (10 mg/kg) increases extracellular serotonin (5-HText) levels in the medial prefrontal cortex (mPFC) associated with a sustained AD-like activity in BALB/cJ mice with anxiety phenotype. Cortical excitatory/inhibitory balance (Gluext/GABAext ratio) also increased t24h post-dose associated with an AD effects following either an intraperitoneal, intra-cortical or intranasal (R,S)-ketamine delivery. These data agree with the disinhibitory hypothesis explaining how NMDA-R blockade of inhibitory GABAergic interneurons increases excitatory synaptic drive in corticolimbic brain regions. By contrast, the mechanism by which 5-HT2AR activation leads to AD-like effects is still poorly defined. We hypothesize that ketamine and psilocin, the active metabolite of psilocybin, and a 5-HT2AR agonist may have similar effects on excitatory/inhibitory neurotransmission in the mPFC. Here, ketamine was used as a positive control to study neurochemical (mPFC microdialysis) and behavioral effects (FST, OF, SP, HTR) of a single dose of psilocin, at t24h post-dose in BALB/cJ mice. Synthesis of psilocin was performed in the CNRS BioCis lab. Preliminary results show that psilocin (0.25 mg/kg) increased swimming duration in the FST at t24h, but to a lesser extent compared to ketamine. A pretreatment with ketanserin, a 5-HT2AR antagonist (1 mg/kg) 30 min before psilocin (0.25 mg/kg) prevented neurochemical effects of psilocin in the mPFC. Further behavioral studies are currently performed to analyze the association of these neurochemical changes in the mPFC-DRN circuit with AD-like activity of psilocin

## #7 Is Serotonin a Biomarker for Depression 2024?

**Parastoo Hashemi (Chair)**

An Ex Vivo System to Study how Inflammation Modulates Serotonin

**Parastoo Hashemi**

In recent years, inflammation and depression have become essentially synonymous. Human patients with high levels of inflammation are less likely to respond to selective serotonin reuptake inhibitors and experimental therapies are underway to tackle depression at the level of inflammation. Inflammation affects neurotransmitters in the peripheral and central systems but due to complexity of both the immune system and the brain, it is very difficult to study these phenomena.

In this work, we create minimal, immune competent 3D human inducible pluripotent stem cell (iPSC)-derived serotonergic neurons. We perform an in-depth biochemical characterization and morphological analysis of these spheroids and investigate the effects of acute and chronic histamine (as a mimic for inflammation) and then LPS (direct inflammation) on serotonin dynamics and acute and chronic SSRI action. We put forth hypotheses for the interesting data from these studies. We thus present this exciting new method to study how inflammation modulates serotonin transmission in a human derived neuronal model.

Leveraging a fluorescence-based approach to quantify extracellular neurotransmitter concentrations in vivo

**Felix Mayer**, Carl-Fredrik Bowin, Samuel Elliott Ovrom, Frederikke S. Petersen, Ulrik Gether

The extracellular concentration of neurotransmitters (NM[EX]) dictates the response of adjacent and distal cells in neuronal networks. Basal (tonic) and phasic changes in NM[EX] shape fundamental processes in the brain, including the perception of reward, learning, sleep, social behaviors and memory formation. Consequently, abnormal, i.e. diminished or elevated, availability of NM[EX] has been implicated in numerous neuropsychiatric disorders. For example, ample evidence established a link between reduced extracellular serotonin and major depressive disorder. However, determining of NM[EX] remains challenging and often requires the use of sophisticated and costly equipment and/or may be limited by poor temporal and spatial resolution. Here, we propose a novel, fluorescence-based approach with excellent spatiotemporal resolution and generalizability to determine NM[EX] in freely moving animals. First, we expressed selected fluorescence-based sensors for NMs in primary neuronal cultures to identify the conditions that allow for determining the concentrations of exogenously applied NMs in a relative and quantitative manner. Subsequently, we applied this approach in vivo to determine basal as well as drug- and behavior-induced alterations in NM[EX], including serotonin, in various brain regions in freely moving mice. Notably, this novel approach enables scientists to investigate how tonic and phasic fluctuations in serotonin are disrupted in animal models for depression.

Evaluation of 5-HT release in the human brain in healthy volunteers and patients

**Eugenii Rabiner**

Experimental evidence consistent with the serotonin deficiency hypothesis of depression has been collected over the past 50 years. These data come from direct examination of 5-HT and its metabolites in the brains of preclinical models of depression and human post-mortem studies. Demonstration of mood lowering effects of pharmacological interventions that reduce brain 5-HT (such as dietary tryptophan depletion and tryptophan hydroxylase inhibitor p-chlorophenylalanine) as well as the finding that most classes of clinically effective antidepressants increase extracellular 5-HT provide further support to this hypothesis.

Direct evaluation of 5-HT release in the living human brain has not been feasible until recently. We have developed an experimental paradigm that utilises positron emission tomography with the 5-HT2 agonist radioligand [11C]Cimbi-36 to index 5-HT release in the human brain following a challenge with the monoamine releasing change, dex-amphetamine. Studies in healthy volunteers have demonstrated significant reductions in [11C]Cimbi-36 following the administration of 0.5 mg/kg of dex-amphetamine, consistent with the increased synaptic 5-HT blocking the binding of the radioligand. Follow-up studies in drug-free patients with depression have demonstrated significantly lower 5-HT release compared to healthy volunteers.

Our findings provide direct evidence for a reduced serotonin release capacity in patients with depression. Follow-up work is ongoing to replicate these findings in a larger cohort of depressed patients. In addition, these methods have been extended to examine 5-HT release capacity in patients with psychotic disorders and neurodegenerative conditions.

Promising Translation of the PK/PD Relationship for the novel 5-HT2A receptor agonist, GM-2505
**Zoë Hughes**
GM-2505 is a novel 5-HT2A agonist/5-HT releaser being developed for major depressive disorder (MDD). It was designed to have a half-life intermediate between DMT and psilocybin. We explored the translatability of the relationship between pharmacokinetic and pharmacodynamic data (PK/PD) for GM-2505 generated in rodents to data collected in healthy human volunteers.
In rats, GM-2505 (0.03-10 mg/kg) produced a dose-dependent increase in head twitches (peak @ 1 mg/kg). GM-2505 produced clear effects on EEG power measured in telemetered rats with skull screw electrodes. After administration of GM-2505 (1-3 mg/kg) low frequency EEG power was decreased, and after the 3 mg/kg dose, gamma power was increased. Antidepressant efficacy of GM-2505 (0.3 and 1 mg/kg) was seen in the chronic mild stress paradigm (CMS) in WKY rats. GM-2505 reversed stress-induced deficits in sucrose intake, anxiety and cognitive impairment ≥ 24h after a single dose. In vivo target engagement was demonstrated by dosing rats with GM-2505 (1-10 mg/kg) and [$^{11}$C]Cimbi-36 and showing dose-dependent displacement of radiotracer binding in frontal cortex.
A Phase 1 trial in healthy adult participants evaluated the safety, PK and PD effects of single ascending doses of GM-2505 (0.34-20 mg). GM-2505 was safe and well tolerated at the doses tested and achieved dose proportional increases in exposure with a median t½ of 45 min. The TEAEs were consistent with a psychedelic drug including altered states of consciousness and perception. Transient increases in blood pressure were observed at ≥10 mg. GM-2505 produced dose-dependent subjective effects (5D-ASC and MEQ-30) and reduced low-frequency EEG power (especially alpha 8-13Hz) at lower doses and increased gamma power at higher doses.
The clinical PK and PD effects of GM-2505 were consistent with an intermediate duration 5-HT2A agonist. There was a strong relationship between plasma exposure, subjective effects and EEG. Clinical plasma exposures associated with robust PD effects were well aligned with exposure causing peak head twitch in rats. Doses of GM-2505 with efficacy in CMS achieved ≥50% 5-HT2A receptor occupancy consistent with the published clinical PET study with psilocybin (Madsen et al., Neuropsychopharmacology (2019) 44:1328–1334). Robust translatability of preclinical PD effects provides confidence in the prospective translation of preclinical antidepressant effects of GM-2505 to patients with MDD.

## #8: Central 5-HT Signaling in Health and Disease

**Yanlin He (Chair), Pingwen Xu (Co-Chair)**

Hypothalamic Serotonin Receptor Signaling and Energy Homeostasis
**Chen Liu**
The central serotonin (5-hydroxytryptamine, or 5-HT) system has been a key target of weight-loss medications since the 1960s. Compounds that increase brain 5-HT levels are known to reduce food intake and body weight. For example, the anorectic effect of d-fenfluramine—the active ingredient in the once-popular diet pill Fen-Phen—is partly mediated through the activation of 5-HT2C receptors.
More recently, we identified triptans, a class of drugs commonly prescribed for migraines, as possessing anorexigenic properties. Our findings reveal that the hypophagic effect of triptans depends on endogenous 5-HT1B receptors and is absent in mice lacking these receptors.
Through a combination of genomic, genetic, and behavioral analyses, our ongoing research elucidates the neural circuit mechanisms by which serotonin receptor signaling in the hypothalamus regulates food intake and body weight.

5-HT neurons and neurodevelopmental disorders
**Kensuke Futai**, Ph.D. Youngjae Ryu, M.D./Ph.D. Amy Cheung, Mr. Antonio Santana
Serotonergic (5-HT) neurons in the raphe nuclei extend axonal projections throughout the brain and regulate many essential brain functions, including emotion, learning and memory, social, reward, sleep, and appetite. Volume transmission is the major mode for 5-HT transmission but mechanisms underlying 5-HT signaling are still largely unknown. Abnormal brain 5-HT levels and function have been implicated in autism spectrum disorder (ASD). Neurexin (Nrxn1-3) genes encode presynaptic cell adhesion molecules important

for regulating synaptic neurotransmitter release, notably glutamatergic and GABAergic transmission. Mutations in Nrxn genes are associated with neurodevelopmental disorders including ASD. However, the role of Nrxn genes in the 5-HT system is poorly understood. We have generated the mouse models with Nrxn genes disrupted specifically in 5-HT neurons to study how Nrxns affect 5-HT system development and social behaviors. We recently reported that loss of Nrxns in 5-HT neurons impairs 5-HT release and sociability, highlighting functional roles for Nrxns in 5-HT neurotransmission and executing complex cognitive behaviors. In this session, I will present our further progress on the roles of Nrxns in 5-HT system development and social behaviors.

Serotonin neurons in the dorsal raphe control food cravings during pregnancy in mice
**Yanlin He**, Dr. Pingwen Xu
During pregnancy, physiological changes can significantly alter food intake behaviors. However, the underlying neural mechanisms remain unclear. Our previous studies have identified 5-hydroxytryptamine (5-HT) neurons in the dorsal raphe nuclei (DRN) as key regulators of feeding behaviors such as binge eating and anorexia nervosa in rodents. In this study, we investigate the role of DRN 5-HT neurons in regulating food-craving behavior in pregnant mice. We observed that DRN 5-HT neuronal firing activity is impaired during pregnancy through increased small conductance calcium-activated potassium type 3 (SK3) ion channel activity. Genetic knockout of the SK3 ion channel from DRN 5-HT neurons abolishes the increases in DRN 5-HT neuronal firing activity caused by pregnancy. It reduces food-craving behavior in pregnant mice compared to virgin mice. Moreover, activation of DRN 5-HT to the ventral tegmental area (5-HT DRN->VTA) projections inhibits food-craving behavior in pregnant mice compared to control littermates. These findings provide novel insights into the role of 5-HT signaling in modulating food cravings during pregnancy and highlight potential targets for managing pregnancy-associated appetite dysregulation.

Placental serotonin causes transcriptional and compositional changes in brain
**Roman Romanov**
Here we argue that placental serotonin (5-HT) can work as a transgenerational non-genetic factor of fetal neurodevelopment. Our results show that in pregnant rodents stress causes the physiological increase of 5-HT levels that lead to changing the balance of hormones and behavioral patterns of coping strategies in the adulthood of their offspring. However, the effects of increased serotonin levels on the developing brain remain unexplored.
To address the hypothesis that an increase in serotonin levels during the gestational period leads to enduring alterations in the transcriptional and compositional profiles of brain cells we focused on the hypothalamus since exactly this brain region is involved in regulating hormone secretion, metabolism, and homeostasis. Moreover, the hypothalamus plays a crucial role in coordinating adaptive responses to environmental cues including social behavior. As a model to increase the serotonin, we used pregnant rats fed with 5-HT precursor during a period of intensive neurogenesis (E11 – E14). After analysis of scRNA-seq datasets obtained from control and experimental animals at different stages (E20, P28), we revealed trajectory shifts leading to the compositional changes including specific populations of neurons and glia. The single-cell analysis of cis-regulatory elements confirmed that serotonin precursor administration causes massive rearrangement of epigenetic profiles and tuning of developmental programs. Particularly, our investigation encompassed the identification of gene programs associated with WNT signaling and shift in neurogenesis-gliogenesis balance.
Our data suggest that placental serotonin non-genetically directs brain development highlighting its relevance in the context of neurodevelopmental disorders and long-term health outcomes in offspring.

## #9: Emerging Roles for Transporters in Serotonin Dysfunction in CNS Disorders

**Freja Herborg (Chair), Ulrik Gether (Co-Chair)**

She Made Me Do It: Behaviorally Penetrant Serotonergic Plasticity in the Context of Genetic Dopaminergic Dysfunction
**Randy Blakely**
In a number of brain regions critical for cognition, mood, motor function and reward, raphe serotonergic projections overlap with dopaminergic projections arising from the ventral tegmental area or substantia nigra. Decades of research demonstrate a functional interdependence of these systems in terms of signaling, plasticity and behavioral modulation. Prior research has identified rare, functional human coding variation in both serotonin (5-HT) and dopamine (DA) transporters (SERT, DAT) associated with neurobehavioral disorders whose etiology and/or treatment implicate both neurotransmitters. These findings raise the question as to whether genetic changes impacting one system result in compensatory changes in the other, with the alterations in both driving a more complete spectrum of disrupted behavioral traits and justify the use of mixed action pharmacology in treatments. In a published study (Stewart et al, 2019), we established that mice with a disease-associated DAT Val559 mutation fail to exhibit locomotor activation following cocaine administration, although hyperactivity can be readily demonstrated with amphetamine or methylphenidate. Genetic, pharmacological and behavioral studies revealed that the lack of locomotor response to cocaine arises from serotonin-dependent locomotor suppression that emerges in the context of developmental DAT Val559 expression. In work to be presented, I will discuss our new findings that, as with early-life DA neuron lesion-induced animals, expression of DAT Val559 during development results in the sprouting of serotonergic axons in a sex- and region-specific manner that can explain cocaine-induced locomotor suppression as well as other behavioral phenotypes arising in the context of DAT Val559 expression. These findings offer a cogent example of an “adaptive pathology” that can drive the complex phenotypes of neurobehavioral disorders and illuminate the basis for mixed-action therapeutic strategies.

Novel Insights from Hypomorphic SERT Variants in Patients with Affective Disorders
**Freja Herborg**
Affective disorders are a leading cause of disability worldwide. The serotonergic system has been heavily implicated in the complex etiology and serves as a therapeutic target. The serotonin transporter (SERT) is a key regulator of serotonin neurotransmission, yet the role of rare genetic variants in psychiatric disease remains poorly understood. In this talk, I will present the identification and functional characterization of two novel disease-associated SERT variants found in a cohort of patients with treatment-resistant chronic affective disorders and a history of electroconvulsive therapy. Both variants were significantly enriched in the patient cohort compared to control populations. Functional analyses revealed that the mutations exert distinct perturbations to SERT function and a converging partial loss-of-function molecular phenotype. Furthermore, we explore the ability of noribogaine to rescue the surface trafficking of the mutants through pharmacochaperoning and peak into preliminary in vivo data. These findings expand the genetic landscape of mood disorders by implicating coding loss-of-function SERT variants and resulting serotonergic disturbances as risk factors for chronic, treatment-resistant affective disorders.

Serotonin Transporter Occupancy and Its Modulation: Neuroimaging Perspectives
**Rupert Lanzenberger**
Positron emission tomography (PET) with highly selective and highly specific radioligands such as DASB enables voxel-wise quantification of the serotonin transporter across the entire brain with a spatial resolution of a few millimeters. Until now, dynamic measurements of up to 90 minutes were required, making it necessary to perform two separate measurements to assess both the effects of antidepressant drugs on the serotonin transporter availability and the transporter occupancy by medication.
Here, we present new methods that allow the quantification of occupancy dynamics within a single PET measurement in the context of SSRI challenge studies. Such methods will gain importance with the advent of new highly sensitive whole-body PET systems, which enable significantly longer or later measurement times, much higher temporal resolution in the range of seconds, and, most importantly, valid measurements in cortical regions.

Different psychiatric patient groups are compared, and the latest findings on SERT biomarkers for predicting treatment success with antidepressants such as SSRIs and ketamine are presented.

Sex- and time-dependent effects of perinatal fluoxetine exposure on lifelong behaviors: insights into sensitive period dynamics

**Paola Brivio**, Dr. Maria Teresa Gallo, Dr. Anais Virenque, Dr. Alessia Golinelli, Prof. Fabio Fumagalli, Prof. Eero Castren, Prof. Francesca Calabrese

Serotonin (5-HT) is a crucial modulator of brain development, and disruptions in serotonergic signaling during perinatal periods can significantly affect neural function, potentially contributing to the onset of neuropsychiatric disorders later in life.

In the first stages of life brain plasticity is heightened during specific windows of development, the so-called “sensitive periods” and the maturation of GABAergic interneurons is crucial for the correct maintenance of the sensitive periods. Indeed, alterations in this interneuron subpopulation have been linked to neuropsychiatric disorders.

To investigate the relationship between 5-HT dysregulation and the pathophysiology of these pathological conditions, we exposed male and female Wistar rats to fluoxetine (FLX), during two key developmental windows: gestation (prenatal-FLX) and lactation (postnatal-FLX). In particular, the drug was administered to the dams at a dose of 15 mg/kg/day via drinking water. Following a behavioral battery at post-natal day (PND) 35 and 70, we observed that prenatal-FLX led to anhedonic-like behavior in adult male rats whereas postnatal-FLX caused cognitive deficits in adult female rats, whereas no symptoms were manifested during adolescence.

Hence, the aim of this study was to investigate whether sensitive periods dynamic in prefrontal cortex and dorsal hippocampus was modulated by perinatal FLX administration.

In particular, we evaluated the expression of parvalbumin-positive (PV+) interneurons and the formation of perineuronal nets (PNNs), specialized extracellular matrix which regulate neuronal plasticity and stabilize synaptic connections during brain development in adolescent rats. Moreover, we investigated the expression of trigger and brakes genes, targets that mediates sensitive periods dynamics during the lifespan at PND 21, 35 and 70.

Our results revealed that the period of FLX exposure shapes PV+ interneuron expression and PNNs formation, as well as altered the opening and closure of sensitive periods in the brain regions considered in a sex-specific manner, suggesting that FLX exposure during critical developmental windows alters the dynamics of these stages characterized by elevated plasticity.

This work provides valuable insights into the molecular and behavioral consequences of serotonergic dysregulation, highlighting its role in shaping neurodevelopmental trajectories.

## #10: New Findings on Maternal Serotonergic Effects on Offspring Development

**Judith Homberg (Chair), Francesca Calabrese (Co-Chair)**

Molecular insights into long-term behavioral effects of perinatal serotonergic manipulation.
Dr Maria Teresa Gallo, Dr Paola Brivio, Arianna Palumbo, Prof Fabio Fumagalli, **Francesca Calabrese**
Perturbation of serotonin (5-HT), especially when happens during perinatal stages leads to alterations of brain functioning and the onset of neuropsychiatric disorders later in life.
To investigate the complex interplay between 5-HT and the pathophysiology of these diseases we manipulated the serotonergic system by exposing male and female rats to fluoxetine (FLX) (15mg/Kg/day in drinking water) during gestation (prenatal-FLX) or breastfeeding (postnatal-FLX).
Interestingly, we observed sex- and timing-dependent behavioral alterations that became manifest in adulthood but are not present in adolescence. Indeed, adult male rats developed an anhedonic-like phenotype when exposed to prenatal-FLX, whereas females were more sensitive to postnatal manipulation showing cognitive deficits. At the molecular level, we looked for potential peripheral markers providing indications on windows of vulnerability that could suggest what is happening in the brain. In particular, we analyzed the expression of genes involved in different processes: synaptic plasticity, hypothalamic–pituitary–adrenal axis, immediate early genes, early responsive genes, autophagy-related genes, mitochondrial activity-related genes, and proinflammatory cytokines. We focused on brain regions involved in psychiatric conditions, such as the prefrontal cortex and the dorsal and ventral subregions of the hippocampus. Lastly, since we are looking for peripheral biomarkers, we conducted the same molecular analysis in the blood.
Interestingly, we identified, peripherally and centrally, biological functions altered by perinatal serotonin modulation regardless of the timing of FLX exposure and sex, while other pathways were specific for the pathological-like phenotypes.
Focusing on the experimental groups that developed pathological-like behaviors, we observed specific common modulations in blood and brain. In particular, our results support the potential relationship between the anhedonic-like phenotype and alterations regarding growth factors, response to steroid hormone, apelin, and NF-kappa B signaling pathways. On the other hand, cognitive impairment (females postnatal-FLX) seems to be associated with variations in autophagic processes, NF-kappa B, NOD-like receptor and mTOR signaling pathways, apoptosis, and thermogenesis. Moreover, we found alterations in this group in the response to starvation, which could be related to the lower body weight.
In conclusion, these results provide new insights into potential biomarkers associated with specific behavioral phenotypes that may be useful for broadening knowledge about psychiatric conditions.

Mom’s gut feelings. Influences of SSRI treatment during pregnancy on maternal microbiome and offspring development
Dr. Anouschka Ramsteijn, Dr. Danielle Houwing, MSc Mayerli Prado-Rivera, **Jocelien Olivier**
Serotonin plays a crucial role in early vertebrate development, yet the regulatory mechanisms governing serotonergic-dependent neurodevelopment remain poorly understood. These mechanisms are vital, as they influence a wide range of biological processes. Maternal environmental factors can impact the embryo through placental interactions, and approximately 90% of the body's serotonin is synthesized in the gut. The gut microbiota regulates serotonergic transmission and is a key component of the bidirectional communication network between the central nervous system and the gastrointestinal tract.
Given the increasing use of selective serotonin reuptake inhibitors (SSRIs) during pregnancy, we investigated how maternal microbiota adapt during pregnancy and lactation in response to SSRI treatment and how these changes influence social behavior in offspring. Additionally, we examined gene expression alterations in the prefrontal cortex (PFC) and amygdala of offspring from SSRI-treated mothers. Our findings indicate that myelin-related genes are significantly altered at the juvenile stage. Currently, we are assessing myelin-related gene expression across different developmental stages in the PFC, amygdala, and hippocampus following SSRI exposure.
The observed microbiome shifts, behavioral outcomes, and myelin-related gene alterations highlight the critical role of maternal serotonin levels in shaping developmental processes in offspring.

Perinatal SSRI exposure in rats and the effects on sociosexual behaviors in a seminatural environment

**Eelke Snoeren**
Serotonin plays an important role in sociosexual behavior, but little is known about the influence of serotonin during early development on behavioral functioning in adulthood. During early development, serotonin acts as neurotrophic factor, while it functions as a modulatory neurotransmitter in adulthood. The occurrence of serotonin release, could thus have different effects on behavioral outcomes, depending on the developmental period in which serotonin is released. In our research, we study the behavioral effects of perinatal selective serotonin reuptake inhibitors (SSRI) exposure on adult rats in a seminatural environment in which rats live in groups and can freely express their full repertoire of behavior. Pregnant mothers received oral gavage of 10 mg/kg fluoxetine or vehicle daily, from gestational day 0 until postnatal day 21. Fluoxetine can cross the placenta, and thus increase serotonin levels and affect the development of the fetus and newborn. Subsequently, at adulthood, the offspring were tested in groups of 8 rats (4 females and 4 males) in the seminatural environment during a period of 8 days in which they were also exposed to stressful life events in the form of white-noise. We analyzed several types of behavior on different days to unravel the effects of perinatal SSRI exposure on e.g. social, prosocial, sexual, and stress-coping behaviors. In this presentation, I will give an overview of our findings.
Financed by Helse Nord: PFP1295-15

Beyond the own genes: Maternal serotonergic genotype shapes offspring's brain, cognition, and behaviour
**Judith Homberg**, Msc. Rogerio Castro, Prof.dr. Jan Buitelaar
Serotonin (5-HT), crucial for neurodevelopment, is synthetized by tryptophan hydroxylase (TPH). While the TPH2 isoform produces 5-HT centrally, TPH1 is expressed in peripheral organs. Despite its peripheral distribution, TPH1 gene alterations have been linked to vulnerability to stress and psychiatric disorders. Using TPH1 knockout (TPH1-/-) rats, we evaluated the impact of the lack of peripheral 5-HT on cognition and behavior. We confirmed that TPH1-/- rats exhibit less anxiety-like behavior and greater accuracy in an operant cognitive task. Importantly, this phenotype was associated with immune system, 5-HTergic metabolism and gut microbiota alterations. However, beyond the own genes, also maternal genotype influences neurodevelopment. Before the embryo is able to synthetize 5-HT itself, the mother provides 5-HT via the placenta. Compromised expression of TPH1 reduces 5-HT levels in the maternal peripheral tissues, resulting in altered transport of 5-HT via the placenta to the embryo. By testing TPH1+/- rats with distinctive maternal genotypes (TPH1-/- or TPH1+/+), we analyzed the implications of the maternal genotype on the offspring's phenotype. Our data revealed that offspring of TPH1-/- mothers presented several developmental and physiological abnormalities, including motor and sensory development retardation and increased body weight. Offspring of TPH1-/- mothers also showed cognitive impairments, and males, but not females, presented attention-deficits and increased anxious-like behavior, aggression, and impulsivity. These results indicate that, beyond the own genes, the maternal 5-HTergic genotype has a significant impact on the offspring's brain, cognition, and behavior. Further studies are ongoing to investigate the role of the gut microbiome in the effects of maternal TPH1 genotype and to elucidate the translational applicability of these findings to human health.

## #11: Theme and Variations: Phenotypic Diversity and Dynamics of Serotonergic Neurons

**Skirmantas Janusonis (Chair), Benjamin Okaty (Co-Chair)**

Transcriptional and spatial dynamics of Dorsal Raphe serotonin neurons following SSRI administration
**Iskra Pollak Dorocic**
The Dorsal Raphe Nucleus contains the majority of the brain's serotonin neurons, yet the molecular diversity and spatial organization of these cells remains incompletely characterized. Using spatial transcriptomics, a spatially resolved, unbiased RNA-sequencing method that preserves tissue architecture, we analyzed mouse brain sections containing the Dorsal Raphe Nucleus and adjacent midbrain structures. Our analysis revealed six distinct serotonergic subpopulations, each with unique molecular signatures and precise spatial distributions within the Dorsal Raphe. These subpopulations showed differential expression of neuropeptides, receptors, and signaling molecules, suggesting functional specialization. To explore how this heterogeneity responds to pharmacological perturbation, we examined the effects of fluoxetine, a selective serotonin reuptake inhibitor (SSRI) commonly used in depression treatment. Both acute and chronic SSRI administration induced distinct transcriptional changes across serotonergic subpopulations, affecting key pathways including Ras, MAPK and cAMP signaling, as well as axonal guidance pathways. Notably, we observed treatment-dependent opposing transcriptional changes in neuropeptides, particularly Thyrotropin-releasing hormone (Trh) and Prodynorphin (Pdyn), which showed distinct spatial localization patterns. Our transcriptomic and complementary in situ hybridization analyses demonstrate that the Dorsal Raphe Nucleus contains molecularly and spatially diverse serotonin neuron subpopulations with differential responses to pharmacological manipulation, suggesting phenotypic differences of molecular serotonin subtypes.

Transcriptomic Diversity and Dynamics of Serotonergic Neurons over Development and in Response to Stress.
**Benjamin Okaty**
The capacity to synthesize and release the monoamine neurotransmitter serotonin (5-Hydroxytryptamine; 5-HT) in the mature central nervous system is restricted to a relatively small subpopulation of brainstem neurons that express the transcription factor Fev, also known as Pet1. Our lab and others have shown that mature Pet1-expressing neurons can be classified into distinct molecularly-defined neuron subtypes that are phenotypically and functionally heterogeneous, displaying diverse neurochemical co-transmitter and receptor phenotypes, innervation profiles, electrophysiological properties, and anatomical distributions of cell bodies. How and when the mature molecular organization of Pet1 neuron subtypes emerges over the course of development is poorly understood. To address these questions, we applied single cell RNA sequencing (scRNAseq) to purified Pet1 neurons harvested from transgenic mice (in which neurons with a history of Pet1 expression are fluorescently labeled) at progressive stages of postnatal development – postnatal day 0 (P0), P5, P15, and P30. Integrating this data with our previously published scRNAseq studies of rhombomerically fate-mapped and anatomically microdissected Pet1 neurons collected from P30-P90 mice (Okaty, et al. 2015; Okaty, et al. 2020), we were able to link developmental single cell transcriptomes to rhombomere of origin, brainstem anatomy, and adult-defined Pet1 neuron subtype. We identified thousands of differentially expressed genes, with the top two principal components correlating with developmental gene variance followed by neuron subtype-specific gene variance, respectively. Despite displaying dramatic developmental transcriptomic dynamics, we were able to reliably classify Pet1 neurons harvested from as early as P0 mice into adult-defined Pet1 neuron subtypes on the basis of stably expressed marker genes, suggesting that the mature subtype organization of the Pet1 system is specified embryonically. Our Pet1 neuron subtype-specific developmental analyses revealed that different Pet1 neuron subtypes mature at different rates, including with respect to expression of genes related to neurotransmitter phenotype, such as Tph2 and Gad2, encoding biosynthetic enzymes for 5-HT and GABA, respectively. Only a minority of Pet1 neuron subtypes expressed mature levels of Tph2 transcript at P0, while the majority showed dramatic up-regulation of Tph2 over postnatal development. Currently, we are exploring how early life stressors – such as maternal separation and postnatal fluoxetine exposure (P2-P15) – impact the molecular maturation of serotonergic neurons.

Cartography of Forebrain Projecting Serotonin System

**Jing Ren**

Dysregulated serotonin transmission has been implicated in neurodevelopmental processes and is strongly associated with the onset of mental health disorders such as anxiety and depression. The developmental trajectory of the serotonin system closely aligns with critical periods during which these phenotypes emerge. Although serotonin projections are present in most forebrain regions at birth, the maturation of the serotonin system involves prolonged axonal innervation that continues through postnatal development and adolescence, ultimately achieving its mature adult morphology. Previous studies have highlighted that this innervation process occurs in a regionally and temporally specific manner, suggesting tightly regulated developmental mechanisms. However, a comprehensive, brain-wide understanding of the spatial and temporal dynamics of postnatal serotonin innervation remains lacking.
In this study, we employed advanced tissue clearing and light-sheet imaging techniques to generate a high-resolution, 3D whole-brain map of serotonin axonal innervation at multiple postnatal developmental stages. By integrating this imaging approach with our recently developed machine-learning pipeline, we systematically quantified and characterized the spatial and temporal dynamics of serotonin innervation across the entire brain. These findings provide critical insights into the postnatal development of the serotonin system and establish a foundational resource for understanding how disruptions in serotonin wiring may contribute to neurodevelopmental disorders

Microglia and Serotonin 2B Receptors: Key Mediators of Fluoxetine-Induced Serotonergic System Plasticity and Remodeling

**Massimo Pasqualetti**

Serotonin is a neurotransmitter crucial for the functioning of the central nervous system where it plays key roles not only in the regulation of physiological processes such as mood, sleep, and appetite but also in brain development, contributing to the maturation and plasticity of neural networks. Dysregulated serotonergic signaling is linked to neuropsychiatric disorders including depression and anxiety which are often treated with drugs that modulate the serotonergic system, such as the selective serotonin reuptake inhibitor (SSRI) fluoxetine. However, the therapeutic effects of SSRIs typically emerge only after weeks of treatment, suggesting that the clinical benefits are primarily associated not with the immediate increase in serotonin levels but with the more protracted processes of neural and synaptic remodeling.
Previous work from our laboratory has shown that the architecture of the serotonergic system in brain regions critically involved in mood regulation (such as the hippocampus and medial prefrontal cortex) exhibits high plasticity in response to fluoxetine-induced alterations of serotonergic homeostasis. Notably, this plasticity varies depending on whether treatment occurs during adulthood or early life stages. These findings highlight an intriguing, tight link between the fluoxetine-induced morphological changes of serotonergic axons and its antidepressant effects on behavior.
We sought to identify mediators of this serotonergic fiber plasticity by investigating whether microglia, via serotonin 2B receptor (HTR2B) signaling, might play a role. Early-life and adult Tph2GFP knock-in heterozygous mice were treated chronically with either the selective HTR2B agonist BW723C86 to mimic fluoxetine's effects or fluoxetine combined with the selective HTR2B antagonist RS127445. Three-dimensional (3D), high-resolution analyses suggest that fluoxetine-induced modulation of HTR2B signaling indeed results in plastic changes in the morphology and density of serotonergic fibers.
To gain new insights into the dynamics and flexibility of serotonergic axons under different experimental conditions, in a parallel approach we are using 3D-holotomography (a refractive index-based imaging) to track axons with submicrometer resolution in ex vivo explants. This ongoing study visualizes fast (on the order of seconds) movements of intracellular organelles, the dynamics of growth cones, and the extensions of axons. These data will contribute to the understanding of how much the local environment and gradients shape the diverse morphologies and paths of individual serotonergic axons.

## #12: Acute Clinical Psychological and Neurological Effects of Psychedelics

**Matthias Liechti (Chair)**

Mechanism of action of acute subjective effects of psychedelics and MDMA

**Matthias Liechti**

Psychedelics (psilocybin, LSD, DMT) and MDMA are used in research and therapeutically in patients and are investigated in phase 2-3 clinical trials to develop them into medications. Information on the clinical pharmacology and acute effects of these substances is presented to aid in the design and conduct of future trials. The presentation will describe acute effects, dose-response, concentration-effect relationship and the neuropharmacological mechanisms involved in the acute subjective effects of psychedelics and MDMA and its enantiomers.

Pharmacokinetics, acute subjective effects and tolerability of intravenous N,N-dimethyltryptamine
**Severin Benjamin Vogt**, Livio Erne, Matthias E. Liechti
N,N-dimethyltryptamine (DMT) is unique among classical serotonergic psychedelics because of its short-lasting effects when administered intravenously. In recent years, various administration regimes have been investigated, including intravenous bolus doses, continuous infusions lasting 30 to 120 minutes, and combined regimens of an initial bolus dose followed by a continuous infusion. Dose-response effects have been characterized for bolus doses ranging from 5 to 25 mg and continuous infusion rates of 0.6 to 2.4 mg/min. This presentation will focus on the pharmacokinetics, acute subjective effects, and tolerability of intravenous DMT, based on data from modern clinical trials in humans. Additionally, the feasibility of self-guided dose-titration with DMT will be discussed. DMT exhibits dose-proportional pharmacokinetics and induces dose-dependent subjective effects. Intravenous bolus doses produce very rapidly increasing and often overwhelming acute subjective effects, which are short-lived. In contrast, continuous infusions result in more gradually increasing, intense and well-tolerated effects that reach a plateau after 20–30 minutes. Self-guided dose-titration allows for a flexible and very well-tolerated administration of DMT, with subjective effects adapting within 5–10 minutes after a dose adjustment. The short duration of DMT's effects allows for rapid adaptation, enabling highly flexible administration and dosing strategies that could be tailored to the specific needs of patients.

Acute effects of MDMA, MDA and their prodrugs Lysine-MDMA and Lysine-MDA in healthy participants
**Isabelle Straumann**
3,4-Methylenedioxymethamphetamine (MDMA) is used recreationally, in research, and in MDMA-assisted psychotherapy. The effects of its psychoactive metabolite 3,4-methylenedioxyamphetamine (MDA), have never been directly compared to the effects of MDMA in humans. Prodrugs like Lysine-MDMA and Lysine-MDA were developed to enhance tolerability and reduce abuse potential.
We compared acute responses to MDMA (100 mg), MDA (93.9 mg), Lysine-MDMA (171.7 mg), Lysine-MDA (165.6 mg), and placebo dosed equimolarly and in a counterbalanced order. Outcome measures included acute subjective and autonomic effects, and pharmacokinetics.
MDA and MDMA induced effects of comparable intensity. MDA induced more subjective stimulant-like effects, more negative "bad drug" effects and tended to produce slightly more fear and visual changes. The effect duration (mean ± SEM) of MDA was 6.6 ± 0.7 hours and longer compared to the effect duration of MDMA of 3.7 ± 0.4 hours. Lysine-MDA did not induce different effects than MDA other than a slightly later effect onset and a longer time to maximal effect. The plasma elimination half-life (geometric mean ± SEM) of MDMA and MDA was 7.3 ± 0.7 and 8.4 ± 0.4 hours, respectively. When Lysine-MDMA was given, no MDMA could be measured in the blood samples and no subjective or autonomic effects occurred.
MDMA and MDA produce similar acute subjective and autonomic effects. MDA produced more stimulant-type effects and acted longer than MDMA. Lysine-MDA represents a functional slow-release prodrug form of MDA. Lysine-MDMA did not release MDMA due to the tertiary amine structure and is therefore not a functional prodrug of MDMA.

Acute psilocybin effects on functional brain entropy and perfusion
**Patrick Fisher**
Classic psychedelic drugs, e.g., psilocybin, induce profound alterations in consciousness primarily via activation of the serotonin 2A receptor (5-HT2AR). Psychedelic drugs show promising therapeutic effects, i.e., fast-acting and persistent clinical benefits in patients with, e.g., depression, anxiety, and substance abuse. Critical to advancing their safe and effective therapeutic use is resolving brain mechanisms associated with acute and persistent effects. We evaluated acute psilocybin effects on brain function, functional connectivity, and neurophysiological measures. Twenty-eight healthy individuals completed a study wherein they were scanned before and multiple times after psilocybin administration (0.2-0.3 mg/kg,

p.o.). We examined acute effects on emotional face processing and resting-state connectivity with BOLD fMRI, cerebral blood flow (CBF) with pseudo-continuous arterial spin labeling, and intra-carotid artery diameter (ICA-diameter) with time-of-flight angiography; some sequences were acquired >100 times across participants. Accompanying scan acquisitions, participants rated the subjective drug intensity (SDI, scale: 0-10) and we quantified plasma psilocin levels (PPL). Psilocybin significantly attenuated amygdala reactivity to emotional faces in a drug- and intensity-dependent manner. During psilocybin sessions, PPL and SDI were correlated with disruption of within- and between-network functional connectivity. We observed pronounced effects of psilocybin administration on some but not all measures of brain entropy considered. Global cerebral blood flow (CBF) measured with pcASL was reduced ~11% at peak PPL and SDI. Regional effects were strongest in the parietal cortex where CBF was decreased 15%. Psilocybin significantly reduced ICA-diameter (10%), evidencing acute arterial constriction. Taken together, these findings highlight broad acute psilocybin effects on neural signaling and neurophysiology. Observed constriction of the ICA alludes to neurophysiological effects not reported previously and deserving of consideration in future studies. These findings will be considered in the context of the growing psychedelic brain imaging literature, prominent models of psychedelic effects on brain function, and highlighting remaining knowledge gaps for future study.

## #13: Clinical Mechanisms for Persisting Positive Effects of Psychedelics

**Gitte Knudsen (Chair)**

Psilocybin's effect on human brain plasticity is contingent on setting

**Gitte Moos Knudsen**

The therapeutic potential of psilocybin for treatment of mental and neurological conditions has garnered significant attention. A pivotal question pertains to the mechanism through which a single dose of psilocybin can elicit enduring positive changes. In the present study, we investigate in healthy humans the effects of a single psychedelic dose of psilocybin on cerebral Synaptic Vesicle glycoprotein 2A (SV2A), as a measure of neuroplasticity, and the effect of the environment in which the psychedelic session is taking place.

Fifteen healthy volunteers were given peroral psilocybin (0.3 mg/kg) and subsequently underwent a psychedelic session while either lying on a bed in a peaceful room with sitters (N=10) or while being scanned an MR-scanner (N=5). In a single-arm, open-label design, we measured cerebral SV2A binding with [11C]UCB-J positron emission tomography before and one week after administering psilocybin. The volume of distribution (VT) was the SV2A outcome measure and change in SV2A binding was assessed with one-tailed paired t-tests. Effects of the setting on subjective effects and on SV2A binding were evaluated using linear mixed effects models and latent variable models.

The scores for positive subjective effects during the intervention and at three months follow-up were significantly affected by the environment where the session took place, with the MR-scanner environment being significantly less associated with positive acute and lasting effects. While there compared to baseline was no overall significant change in VT's in hippocampal or frontal cortex one week after psilocybin, the setting during the session mattered. The change in VT is significantly negatively affected by being in an MR-scanner compared to an intervention room ($p < 0.001$). Further, an increase in SV2A is positively associated with mystical-type experiences during the session.

We conclude that the subjective effects during the psychedelic session as well as enduring effects at 3-months are contingent of the setting of the psychedelic session. Further, a reinforcing environment is associated with a higher increase in cerebral synaptic density after a psychedelic dose of psilocybin.

Synaptic density as measured by [11C]-UCB-J positron emission tomography after psilocybin treatment

**Guusje Haver**, Hampus Yngwe, Dr Pontus Plaven Sigray, Dr Granville Matheson, Max Andersson, Dr Mikael Tiger, Dr Maria Beckman, Dr Carl-Johan Ekman, Adjunct professor Johan Lundberg

Introduction: [11C]UCB-J ((4R)-1-[(3-[11C]methyl-4-pyridyl)methyl]-4-(3,4,5-trifluorophenyl)pyrrolidin-2-one) is a radiotracer that binds to Synaptic Vesicle Protein 2A (SV2A), a ubiquitously expressed vesicle protein. Since its development, many papers have been published in the fields of neurology and psychiatry, to investigate differences in synaptic density in various diseases, such as epilepsy, Alzheimer's and depression. Since psychedelics are shown to have neuroplastic effects in animals , measuring synaptic density in vivo in man in psychedelics research is of particular interest.

Previously, test-retest statistics have been reported for healthy controls and patients with Alzheimers, but not in psychiatry. Here we present the first test-retest statistics in patients with Major Depressive Disorder (MDD), as well as preliminary results from depressed patients treated with psilocybin. We hypothesized that psilocybin could increase synaptic density in several brain regions to cause its long-lasting antidepressant effects.

Methods: 34 patients with MDD were enrolled in a double blind, randomized controlled trial where they received a dose of 25mg of psilocybin as active treatment, or 100mg of niacin as a control together with psychological support. Niacin produces a flushing reaction but has no proposed effects on synaptic density or depressive symptoms.

From the 17 niacin-treated patients, 28 pre- and post-dosing scans were used to investigate test-retest reliability of [11C]-UCB-J. The other scans were discarded because of excessive motion. From the treatment group, all 34 scans were used. During the scan, arterial blood was sampled, which allows us to use full kinetic modelling. We extract tissue time-activity curves (TAC's) in several regions of interest, which will be kinetically modelled to calculate the Volume of distribution (Vt). From the niacin group we calculate test-retest statics, and from the entire cohort we test the hypothesis if psilocybin has an effect on synaptic density as measured by Vt.

Detecting neuroplastic effects induced by ketamine in healthy human subjects: a multimodal approach
**Claudio Agnorelli**
Introduction: Ketamine, is an anesthetic that also has utility in the treatment of depression and substance misuse. However ,its mechanisms of action, particularly regarding its neurophysiological and therapeutic effects in humans are not well understood. In clinical studies, psychedelic-like doses of ketamine produce lasting mood improvements after a single or few administrations. Extensive animal research has demonstrated that ketamine enhances neuroplasticity and reopens developmental windows for learning, both of which might explain its clinical effects . However, examining these mechanisms in humans has proven challenging.
Materials and Methods: We investigated ketamine's neuroplastic effects in healthy human subjects using integrated Positron Emission Tomography (PET)/Magnetic Resonance Imaging (MRI). Eleven participants underwent two PET/MRI scans before and 1-8 days after a single dose of ketamine known to elicit psychedelic effects (1 mg/kg). [11C]-UCBJ PET was used to quantify pre-synaptic terminal density/plasticity , while changes in glutamate concentration were assessed via 1H-MRS, and intrinsic brain activity, and functional connectivity plus graph-theoretic metrics via resting-state fMRI.
Results: While group-level analyses post-ketamine showed only trend-level increases in the [11C]-UCBJ signal , we observed significantly elevated Anterior Cingulate Cortex (ACC) glutamate levels. Functional connectivity analyses revealed decreased within-network integrity, particularly in high-order networks like the default mode network (DMN), alongside increased low-to-high-order network integration. Our multimodal analysis showed that increased synaptic plasticity correlated with reduced intrinsic brain activity in DMN regions and decreased influence of the posterior cingulate cortex (PCC) in global network dynamics.
Discussion: These results suggest that the reorganization of brain functional architecture induced by ketamine during the altered state of consciousness persists in the days following administration and is linked to enhanced neuroplasticity. By linking molecular and network-level changes, our results point to the PCC as a central hub where ketamine may reshape brain hierarchies in the long term, providing new directions for understanding its therapeutic mechanisms and developing targeted treatments.
Funding: General Electric (GE)

The influence of sex, previous experience and setting on the phenomenology of psilocybin intoxication and its link to persistent effects in healthy volunteers
**Tomas Páleníček** T. Klučková, M. Nikolič, F. Tylš, V. Vikotrin, Vejmola, Č. M. Viktorinová, A. Bravermanová, R. Androvičová, V. Andashko, J. Korčák, P. Zach, M. Hájková, M. Kuchař, M. Balíková, M. Brunovský, J. Horáček J
Background: Recent studies intensively explore psilocybin's antidepressant potential, but variables like prior experience, repeated use, setting, and sex remain underexplored. This study examines acute and long-term effects of psilocybin in healthy individuals.

Methods: A double-blind, placebo-controlled, cross-over study included 40 healthy participants (20 females, mean age 38). Each received two doses of psilocybin (0.26 mg/kg) at least 56 days apart (mean 354) in two study arms (EEG and fMRI). Nearly half had prior psychedelic experience. Acute effects were measured using the Altered States of Consciousness Scale (ASC) and a visual analogue scale (VAS) for emotional valence. The Persisting Effects Questionnaire (PEQ) assessed long-term effects.

Results: All results were independent of observed variables. Acute effects were moderate on the ASC, with VAS ratings showing mostly pleasant or fluctuating experiences and only one unpleasant session. All experiences resolved in a positive or neutral state by the session's end. Psilocybin produced lasting positive effects across all PEQ domains, with negligible negative effects. Oceanic Boundlessness and Visual Restructuralization correlated with positive outcomes, while Dread of Ego Dissolution, typically associated with fear, did not predict negative effects. The nature of the acute experience (pleasant or mixed) was not linked to the direction or intensity of long-term outcomes. Peak experiences ending in a positive mood were strongly associated with favorable long-term effects.

Conclusion: Repeated psilocybin administration in healthy individuals induces positive, lasting effects, with challenging experiences in controlled settings not causing adverse outcomes. These findings support

psilocybin's psychological safety and its repeated use in clinical trials.

Clinical trial registration: EudraCT 2012-004579-37

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## #14: Serotonin, Its Detection and Function

**Yulong Li (Chair)**

Illuminating the Brain: New and Old Tools to Decipher Neuromodulatory Circuits
**Olivia Masseck**
Understanding how neuromodulatory circuits generate complex behavior is one of the major goals of Neuroscience. Neurotransmitter and Neuromodulators are crucial for information flow between neurons and understanding their dynamics is the key to unravel their role in behavior.
Our lab recently developed a new family of genetically encoded serotonin (5-HT) sensors (sDarken) on the basis of the native 5-HT1A receptor and circularly permuted GFP (Kubitschke et al. 2022). sDarken 5-HT sensors are bright in the unbound state and diminish their fluorescence upon binding of 5-HT. Sensor variants with different affinities for serotonin were engineered to increase the versatility in imaging of serotonin dynamics. As demonstrated here, the designed sensors show excellent membrane expression, have high specificity and a superior signal-to-noise ratio, detect the endogenous release of serotonin and are suitable for in vivo imaging. However, to overcome current limitations in intensity based fluorescent measurements, we employ lifetime imaging as a new tool for the readout of serotonin.
In addition a new red-shifted genetically encoded calcium indicator (PinkyCaMP) based on mScarlet will be presented. PinkyCaMP surpasses existing redshifted calcium sensors in brightness, photostability and compatibility with optogenetics. PinkyCaMP is well tolerated from neurons and shows no toxicity or aggregation neither in culture nor in vivo.

Spying 5-HT dynamics by constructing multicolor GRAB sensors
**Yulong Li**
The serotonergic system is pivotal in regulating various physiological and pathological processes and remains a crucial therapeutic target for numerous psychiatric disorders. Recent developments in genetically encoded GFP-based serotonin (5-HT) sensors have provided valuable insights into serotonergic neurotransmission, yet their sensitivity and spectral profiles limit their ability to dissect 5-HT signals within complex conditions effectively. To address these constraints, we optimized green fluorescent G-protein-coupled receptor (GPCR)-activation-based 5-HT (GRAB5-HT) sensors and introduced a novel red fluorescent variant. These sensors demonstrate exceptional cell surface trafficking, specificity, sensitivity, and spatiotemporal resolution, rendering them adept for in vivo monitoring of 5-HT dynamics. Leveraging these advancements, the subcortical 5-HT release was recorded in freely moving mice using fiber photometry. Interestingly, we observed both uniform and gradient 5-HT release patterns within the mouse dorsal cortex under varying circumstances with mesoscopic imaging. Additionally, employing dual-color imaging, we observed seizure-induced waves of 5-HT release across the cortex, following with calcium and endocannabinoid waves. In essence, these innovative 5-HT sensors offer unprecedented opportunities to delve into the complexities of serotonergic neurotransmission in both physiological and pathological conditions.

Sex-Specific Serotonin Receptor Expression Drives Stress Vulnerability in Adult Hippocampal Neural Stem Cells

**Juan Song**
Women are more vulnerable to stress and at higher risk for mood disorders, yet the underlying mechanisms remain unclear. The serotonin (5HT) system plays a key role in stress response and mood regulation, with adult hippocampal neurogenesis serving as a critical substrate for these effects. Here we found that female mice express functional 5HT1A receptors (5HT1ARs) in hippocampal NSCs, and deleting these receptors selectively reduces the NSC pool in females. This loss is driven by a shift in serotonergic signaling, where 5HT1AR deletion leads to 5HT-induced depolarization via upregulated 5HT7 receptors (5HT7Rs). Furthermore, repeated restraint stress (RRS) impairs the maintenance of NSCs in females through a 5HT1AR-dependent mechanism. In contrast, male NSCs naturally express 5HT7Rs, which are downregulated by stress, preventing NSC depletion. These findings suggest that sex-specific serotonin receptor expression and their differential responses to stress may contribute to sex differences in stress vulnerability.

Striatal Serotonin Release Signals Reward Value
**Katherine Nautiyal**
Serotonin (5-HT) modulates diverse behavioral phenotypes, many of which have reward-related components, though the mechanisms by which 5-HT encodes and regulates these phenotypes remain unclear. Past studies which have measured and manipulated dorsal raphe nucleus (DRN) neuron activity illustrate the complexity in timing and location of 5-HT modulation of reward-related behaviors. Taking advantage of a genetically encoded fluorescent biosensor (GRAB-5-HT), we focused on measuring 5-HT release in reward-related forebrain regions in mice. Our results show that 5-HT release in the dorsomedial striatum (DMS) increases in anticipation of reward consumption. However, in the medial nucleus accumbens shell (mNAc), 5-HT levels decrease below baseline levels following reward consumption, with a sharp offset of inhibition at the termination of consumption. Both regions show encoding of extrinsic reward value as well as internal satiety state (both over and undervaluing rewards). Our studies also compared 5-HT release in the context of volitional vs avolitional rewards, cued vs unpredicted rewards, and aversive stimuli. Interestingly, tracing studies suggest that a significant portion of DRN cells projecting to the DMS also send collaterals to the mNAc, raising the possibility that the differential release patterns are due to terminal regulation of 5-HT release. Overall, our characterization of 5-HT release using the GRAB-5-HT sensor uncovers reward-related patterns of 5-HT release in the striatum which we are now probing to determine their functional relevance in the regulation of reward-related behavior.

## #15: Sex and the Serotonin System: Reconciling Differential Susceptibility to Alcohol and Stress-Related Disorders

**Catherine Marcinkiewcz (Chair), Pingwen Xu (Co-Chair)**

Estrogen Signaling in Dorsal Raphe 5-HT Neurons Regulates Binge-like Drinking in Mice
BS Valeria Torres Irizarry, PhD Bing Feng, BS Qi Xu, PhD Yanlin He, **Pingwen Xu**
Estrogens promote binge alcohol drinking and contribute to sex differences in alcohol use disorder. However, the mechanisms are largely unknown. This study aims to test if estrogens act on 5-hydroxytryptamine neurons in the dorsal raphe nucleus (5-HTDRN) to promote binge drinking. We found that female mice drank more alcohol than male mice in chronic drinking in the dark (DID) tests. This sex difference was associated with distinct alterations in mRNA expression of estrogen receptor α (ERα) and 5-HT-related genes in the DRN, suggesting a potential role of estrogen/ERs/5-HT signaling. In supporting this view, 5-HTDRN neurons from naïve male mice had lower baseline firing activity but higher sensitivity to alcohol-induced excitation compared to 5-HTDRN neurons from naïve female mice. Notably, this higher sensitivity was blunted by 17β-estradiol treatment in males, indicating an estrogen-dependent mechanism. We further showed that both ERα and ERβ are expressed in 5-HTDRN neurons, whereas ERα agonist depolarizes and ERβ agonist hyperpolarizes 5-HTDRN neurons. Notably, both treatments blocked the stimulatory effects of alcohol on 5-HTDRN neurons in males, even though they have antagonistic effects on the activity dynamics. These results suggest that ERs' inhibitory effects on ethanol-induced burst firing of

5-HTDRN neurons may contribute to higher levels of binge drinking in females. Consistently, chemogenetic activation of ERα- or ERβ-expressing neurons in the DRN reduced binge alcohol drinking. These results support a model in which estrogens act on ERα/β to prevent alcohol-induced activation of 5-HTDRN neurons, which in return leads to higher binge alcohol drinking.

Sex-dependent effects of chronic alcohol on serotonergic circuits in affective behavior
**Catherine Marcinkiewcz**
Drugs that enhance serotonin (5-HT) transmission in the brain are often used to treat co-morbid anxiety and depression in individuals with AUD, but emerging evidence suggests that serotonin may worsen negative outcomes associated with alcohol abstinence. Prior work from our group suggests that chronic alcohol promotes hyperexcitability of 5-HT neurons in the dorsal raphe nucleus (DRN), which are known to play important roles in affective behavior. The goal of the present study was to elucidate the neural circuits underlying affective disturbances after chronic intermittent ethanol (CIE) in a mouse model.
Our results indicate that CIE induced social deficits in male, but not female, C57BL/6J mice. 5-HT hyperexcitability was also limited to males. Using an unbiased 3D imaging technique (iDISCO), we then showed that chemogenetic activation of DRN 5-HT neurons in ethanol-naïve mice enhanced c-Fos expression in the nucleus accumbens (NAcc), while CIE increased 5-HT transients in the NAcc during social interation in males only. These results suggest that excessive 5-HT signaling in the NAcc may drive CIE-induced social deficits in males. This was confirmed by showing that chemogenetic manipulation of the 5-HT DRN-->Nacc pathway selectively modulates social behavior in male mice exposed to CIE.
We then showed that 5-HT2c receptor-expressing dynorphin neurons in the NAcc could mediate this process. Specifically, pro-dynorphin and 5-HT2c mRNA expression was upregulated in the NAcc of CIE mice and humans with AUD. Ex vivo electrophysiology showed a 5-HT2c-dependent increase in excitability of NAcc dynorphin neurons in CIE mice. Importantly, genetic ablation of pro-dynorphin or 5-HT2c receptors in dynorphin neurons, or chemogenetic inhibition of dynorphin neurons in the NAcc, could rescue social deficits in CIE mice. Finally, chemogenetic activation of NAcc dynorphin neurons inhibited local dopamine release during social interaction.
Taken together, our results indicate that CIE activates NAcc dynorphin neurons via 5-HT2c receptors, which in turn inhibits dopamine release during social interaction and may reduce the motivation to engage in social behavior. These findings suggest that caution may be advised when prescribing selective serotonin reuptake inhibitors (SSRIs) to patients with AUD, and that targeted therapies that modulate 5-HT receptors in specific neuronal circuits may be more efficacious.

Serotonergic modulation of the BNST-CeA circuit promotes fear learning in female mice
PhD Rebecca Ravenelle, B.A. Jinah Lee, PhD Carolina Fernandes-Henriques, PhD Jia Liu, PhD Allyson Friedman, PhD Ekaterina Likhtik, **Nesha Burghardt**
Post-traumatic stress disorder is characterized by intense fear memory formation and is diagnosed more often in women than men, implicating a sex difference in the underlying circuits. Using auditory fear conditioning as a model of fear learning in mice, we show that there is a sex difference in the effects of increasing extracellular levels of serotonin on fear memory recall and communication in the extended amygdala. We found that female mice showed higher sensitivity to the effects of pharmacologically increasing serotonin during acquisition, which enhanced tone recall in both sexes. Optogenetically stimulating serotonin terminals in the anterior dorsal bed nucleus of the stria terminalis (adBNST) of TpH2-ChR2-YFP-Bac mice during fear conditioning enhanced tone recall in females but not males. This sex-specific effect was accompanied by an increase in c-Fos expression in the adBNST and central nucleus of the amygdala (CeA) and was blocked by an intra-BNST infusion of a 5-HT2C receptor antagonist. Our in vivo electrophysiology experiments revealed that increasing serotonin in the adBNST during learning also enhanced adBNST-CeA high gamma (90-140Hz) synchrony and the directionality of adBNST-to-CeA communication in high gamma during fear memory recall in females only. Chemogenetic inhibition of adBNST output to the CeA blocked serotonin-enhanced recall in females, demonstrating a necessary role of the CeA in downstream circuits. Finally, we show that in the absence of serotonin stimulation, fear conditioning increased endogenous levels of serotonin in the adBNST to the same extent in males and females, with no sex differences in freezing during tone recall. Together, our findings indicate that even though projections from the adBNST to the CeA are not usually required for tone recall, serotonin in females is uniquely positioned to recruit the adBNST-CeA circuit and increase fear memory only when

serotonin is enhanced beyond endogenous levels. Our findings have implications for individuals taking medications that increase serotonin and for those whose endogenous serotonin levels are high.

Abstinence from binge alcohol consumption produces sex-specific behavioral and physiological effects in the lateral habenula serotonin receptor 5HT2c system.
**Meghan Flanigan**
Diagnoses of Alcohol use disorder (AUD) is rapidly growing in women, but potential sex differences in the pathophysiology of AUD have not been thoroughly explored. Here, we demonstrate that binge alcohol produces sex-specific behavioral and only partially physiological effects on serotonin signaling in the lateral habenula (LHb). Male and female adult mice were permitted to freely drink alcohol in their home cage on a binge-like schedule called Drinking in the Dark (DiD). After three weeks of DiD and one week of abstinence, female mice displayed deficits in social recognition behavior, while male mice displayed enhanced acoustic startle behavior. We then used fiber photometry to record calcium and serotonin signals from LHb neurons expressing the serotonin 5HT2c receptor in DiD animals during exposure to social or startle-related stimuli. Remarkably, DiD males showed enhanced calcium responses to startle stimuli while DiD females showed a trend towards both increased calcium and 5HT responses to social stimuli. Chemogenetic inhibition of these neurons reversed DiD-induced social deficits in females and startle potentiation in males. Through a series of patch-clamp electrophysiology experiments in LHb-5HT2c cells, we found that abstinence from DiD is associated with increased resting membrane potential, increased tonic firing, reduced bursting, and reduced 5HT modulation of bursting. These adaptations were all dependent on serotonin-independent activation of the 5HT2c receptor in the LHb, suggesting a possible DiD-induced regulation of constitutive 5HT2c activity in the LHb in females. In males, abstinence from DiD is associated with increased evoked excitability, increased tonic firing, reduced bursting, and reduced 5HT modulation of bursting. Similarly to females, 5HT-independent activity of LHb-5HT2c neurons mediated these effects. Hence, abstinence from DiD promotes similar physiological adaptations in the LHb in males and females that constrain neuronal activity to tonic rather than burst firing and reduce sensitivity to 5HT. However, the behavioral outcomes of these physiological adaptations diverge between males and females. The results of this study may have important implications for our understanding of AUD and promote the development of sex-informed treatment approaches.

## #16: Preclinical and Clinical studies of the Mechanisms by which Psychedelics Engender Persistent Alleviation of Neuropsychiatric Illness

**Kevin Murnane (Chair)**

Preclinical and clinical studies of psychedelics for methamphetamine addiction
**Kevin Murnane**, Frances Vest, Bo Wood, Alexandru Dumitrescu
Background: Methamphetamine is a highly addictive psychomotor stimulant, for which there are no Food and Drug administration approved pharmacotherapies. Studies with psychedelics have suggested that they may be useful for methamphetamine addiction. However, to the best of our knowledge, no previous study has reported the effects of psychedelics in gold-standard animal models or in individuals suffering from methamphetamine addiction. The aim of this research is to investigate the effects of multiple psychedelics on the use of methamphetamine, as well as additional vulnerability factors to relapse, in preclinical models and individuals in recent abstinence from methamphetamine use.
Methods: In this study, we determined whether two structurally different psychedelics could attenuate the rewarding effects of methamphetamine using conditioned place preference and intravenous self-administration procedures, two well established animal models. In parallel, we examined the impact of self-reported use of psychedelics on use of methamphetamine in recently abstinent individuals in a residential treatment center that meet criteria for a stimulant use disorder. Furthermore, the impact of self-reported use of psychedelics on negative affect and behavioral control, two established vulnerability factors to relapse, was also examined.
Results: Multiple psychedelics attenuated methamphetamine-induced place conditioning. Likewise, they attenuated methamphetamine self-administration as doses that did not impact food-maintained behavior. Interestingly, individuals recently abstinent from methamphetamine who report a history of psychedelic use

exhibited better cognitive flexibility and an increased ability to inhibit unwanted responses. Moreover, self-reported use of psychedelics was associated with lower levels of stress, depression, and anxiety on validated instruments.
Conclusions: Together, the data presented here demonstrate that compounds from two distinct structural classes of psychedelics attenuate the rewarding and reinforcing effects of methamphetamine. In concordance, stratifying individuals in recent abstinence from methamphetamine use demonstrates a phenotype that is likely to be protective against relapse in individuals who report a history of psychedelic use. These findings warrant deep mechanistic studies in animal models as well as prospective and controlled clinical trials of the potential of psychedelics for methamphetamine addiction.

Psychedelics produce persistent antidepressant-like effects through functional plasticity rather than structural plasticity
**Charles Nichols**
Activation of serotonin 2A (5-HT2A) receptors is thought to underly the long-lasting antidepressant effects of psychedelics such as psilocybin, but beyond that the molecular and cellular mechanisms involved are not well understood. Recent preclinical studies using mice have primarily examined relatively short time points after drug administration, which does not address the long-lasting effects of psilocybin in humans (i.e. several months or more). We seek to address how a single administration of a psychedelic can affect neural physiology and behaviors that can last several months or longer. We utilized a rat experimental system to demonstrate both psilocybin and the selective 5-HT2A receptor agonist 25CN-NBOH reduce immobility in the forced swim test without a decrease in effect size for at least three months after a single administration of drug. Our results with 25CN-NBOH suggest 5-HT2A receptor activation is sufficient to produce long-lasting behavioral changes. We also investigated functional cellular plasticity in neurons from the medial prefrontal cortex (mPFC) of these animals, particularly targeting the infralimbic region, ~ 100 days after the single dose of drug using brain slice electrophysiology. Functional plasticity was evident for both drugs, and layer V excitatory pyramidal neurons from both treatment groups demonstrated significant changes in resting membrane potential, firing rates and synaptic excitation. Recorded neurons were examined by microscopy for synaptic density and spine classification, which found no differences between control and drug treated. Further, gene expression studies for several presynaptic and postsynaptic markers in the mPFC indicated no differences in gene expression between groups. Together, our results indicate a single treatment with a psychedelic, acting through 5-HT2A receptors, is sufficient to elicit very long-lasting behavioral and cellular changes through enduring function plasticity rather than structural plasticity.

Psilocybin in the Treatment of Cocaine Use DIsorder
**Peter Hendricks**
Background: Annual cocaine-related deaths are estimated at 28,000 in the US, an almost 75% increase since 2019. Most interventions for cocaine use disorder (CUD) result in modest success rates, and there are no approved pharmacotherapies for CUD. Prior research suggests that psilocybin may have broad anti-addictive properties, with recent clinical trials suggesting safety and efficacy of psilocybin in the treatment of alcohol use disorder and tobacco user disorder. Methods: This clinical trial randomized 40 individuals with CUD to receive either 25 mg/70 kg psilocybin (n = 20) or 100 mg diphenhydramine (n = 20). Both groups received an 8-session manualized cognitive behavioral therapy (CBT) intervention for CUD. Drug administration sessions occurred after the 4th CBT session. Biochemically verified percentage of abstinent days, complete abstinence rates, and time to first lapse through 180 days after end-of-treatment were compared between groups using intent-to-treat analysis. Results: No unexpected or serious adverse events were attributed to psilocybin or diphenhydramine. Percentage of abstinent days, complete abstinence rates, and time to first lapse were significantly greater among those randomized to psilocybin, with large effect sizes. Conclusions: One psilocybin session, compared to diphenhydramine, with manualized CBT, significantly and substantially increased long term cocaine abstinence. These results indicate the promise of psilocybin in the treatment of CUD.

Latent LSD induced metaplasticity is unmasked with medial frontal brain stimulation and captured by cortical-striatal network activity
PhD Lucas Dwiel, **Wilder Doucette**

Background: Psychedelic drugs have resurged in neuroscience and psychiatry with promising success in psychedelic-assisted therapy for the treatment of anxiety, depression, and addiction. At the cellular level, psychedelic drugs elicit neuroplastic processes 24 hours after administration, priming neural circuits for change. The acute effects of psychedelic drugs are well characterized with functional imaging and neural oscillations showing an increase in the entropy of spontaneous cortical activity.
Hypotheses: We hypothesized that cortical-striatal oscillations recorded in rats would confirm the effects of psychedelic drugs. We also hypothesized that brain stimulation delivered 24 hours after LSD administration would lead to different and more persistent effects than brain stimulation alone and that these differences would relate to mTOR pathway activation.
Methods: We recorded local field potential (LFP) oscillations from rats following lysergic acid diethylamide (LSD) or saline administration and determined if exposure to these treatments altered the effect of brain stimulation targeting the medial frontal cortex 24 hours later.
Results: We confirmed acutely decreased low frequency power across the brain when rats are given LSD. We also demonstrated these altered states return to baseline after 24 hours. Brain stimulation applied in the previously reported window of heightened neuroplasticity produced distinct shifts in brain state compared to brain stimulation applied 24 hours after saline. Further, the changes in brain state induced by brain stimulation after LSD lasted longer than those induced by brain stimulation alone. Lastly, we found that mTOR activation (i.e., pS6 levels) was elevated in rats given LSD and brain stimulation compared to either intervention alone within the medial frontal cortex.
Conclusions: Despite the acute effects of LSD disappearing after 24 hours at the level of cortical-striatal LFPs, there are still latent effects that interact with brain stimulation to create distinct changes in brain activity compared to brain stimulation alone, and these changes last longer than those induced by brain stimulation alone. Further, the interaction between LSD and brain stimulation may be mediated, in part, through mTOR pathway activation. Our proof-of-concept findings are the first to suggest that psychedelic drugs could work in combination with brain stimulation to achieve enhanced effects on brain activity.

## #17: Diverse Stress Signaling Mechanisms in Dorsal Raphe

**John Neumaier (Chair)**

Chronic glucocorticoid intake alters basal Tph2 protein expression in anxiety-related midbrain serotonergic systems
**Christopher Lowry**
Stress-related psychiatric disorders, such as anxiety and mood disorders, are often correlated with disruption of hypothalamic-pituitary-adrenal (HPA) axis function and dysfunction of brain serotonergic systems. It is, however, unclear what role glucocorticoids (GCs), the primary effector stress hormones of the HPA axis, without the experience of external stressors per se, play in the etiology of these disorders. Previous research demonstrated that chronic GC intake via the drinking water dose-dependently disrupts the diurnal expression pattern of tph2, the gene encoding the rate-limiting enzyme for brain serotonin synthesis (tryptophan hydroxylase 2, Tph2), by increasing tph2 mRNA expression during the rats' inactive light-phase. Here, we used western blot techniques to test the hypothesis that chronic GC treatment increases basal Tph2 protein expression. Adrenal-intact, adult male rats were treated with 100 µg/ml corticosterone (CORT) or vehicle (0.45% 2-hydroxypropyl-β-cyclodextrin) via the drinking water for 21 days, and their emotional behavior was assessed in the elevated plus-maze (EPM) and forced swim tests (FST) after 2 weeks. In concordance with previous studies, chronically GC-treated rats displayed more anxiety-like behavior on the EPM, and in tendency less proactive stress-coping behavior in the FST. In GC-treated rats, light-phase Tph2 protein expression was elevated specifically in anxiety-related subdivisions of the DR, namely the dorsal part (DRD). Dark-phase Tph2 expression in the DR remained unaltered by GC treatment. Our results suggest that anxiety-related brain serotonergic systems in the DR are particularly sensitive to GC-induced alterations of Tph2 protein expression, with anxiety-related DR subdivisions being disrupted during the inactive light-phase.

FKBP5, a potential integrator of adrenal steroids and serotonin
**John Neumaier**

The serotonin (5-HT) system and the hypothalamic-pituitary-adrenal (HPA) axis are two critical components of the body's stress response system. Both systems are implicated in the vulnerability and resilience to stress exposure and disorders including PTSD.
Previously we found that repeated forced swim stress increased FKBP5 expression in 5-HT neurons. This was initially detected using RiboTag-Seq in mice and was confirmed by RTqPCR, RNAscope, and immunohistochemistry. FKBP5 is a chaperone protein that provides negative feedback on glucocorticoid receptor signaling and may also regulate other receptor systems. There is limited research to date on how glucocorticoid signaling impacts serotonin neurons function. Our hypothesis is that FKBP5 interferes with 5-HT neuron function, perhaps by impairing with the capacity of 5-HT neurons to release serotonin, rendering the serotonin neurons resistant to circulating glucocorticoids.
Our recent experiments demonstrate that other stressors also induce increased FKBP5 in dorsal raphe nucleus using a "two hit" model of stress exposure in early life and young adulthood. We combined subcutaneous injection of vehicle or lipopolysaccharide (LPS) into PND 14 male and female mice with delivery of three blast wave exposures, a well-validated model of mild traumatic brain injury (mTBI). We found that both males and females had dramatically increased FKBP5 mRNA in dorsal raphe after blast, and there was a modest synergistic increase of LPS prior to mTBI. Several peripheral cytokines also responded with a synergistic increase to these treatments.
Next, we are examining the effect of 5-HT neuron-specific overexpression or knockout of FKBP5 on fear learning and memory. Using Cre-dependent AAV vectors injected into Pet1-Cre mice, we can either increase or decrease FKBP5 in DRN 5-HT neurons. Our data so far shows that experimentally increased FKBP5 exacerbates contextual and cued freezing while reduced FKBP5 reduces freezing. We plan a more complex fear paradigm that will examine fear recall, extinction, and retrieval. We hypothesize that FKBP5 interferes with the capacity to synthesize and release serotonin, and we plan to present data on the effect of increasing or decreasing FKBP5 in DRN 5-HT neurons on real-time 5-HT release using a GRAB-5-HT fluorescent biosensor expressed in basolateral amygdala.

CART Peptide Modulation of Serotonergic Activity: A Key Driver of Anxiety
**Nagalakshmi Balasubramanian**
Serotonin (5-HT) plays a critical role in stress signaling, both by regulating the physiological stress response and by contributing to the development of stress-related disorders such as anxiety and depression. Neuropeptides can modulate 5-HT release either directly or indirectly. Our recent work identified projections of the small neuropeptide CART (cocaine- and amphetamine-regulated transcript) to the dorsal raphe nucleus (DRN), a major source of forebrain-projecting serotonergic neurons. Despite limited research on the role of CART in stress signaling within the brainstem, some studies have reported reduced levels of CART in the Edinger-Westphal nucleus (EWcp) of individuals with major depressive disorder.
To investigate the role of CART in DRN-5HT signaling, we employed a combination of conventional and advanced techniques. Our initial findings revealed that central administration of CART peptide led to reduced c-Fos expression in ventral 5HT-DRN neurons. Furthermore, direct infusion of CART into the DRN elicited anxiogenic behaviors in male C57BL/6J mice. This behavioral phenotype was accompanied by a reduction in 5HT-DRN neuronal activity and serotonin release, as measured by in vivo fiber photometry. Using circuit-tracing approaches, we identified CART inputs to the DRN from several subcortical regions, with the centrally projecting Edinger-Westphal nucleus (EWcp) showing heightened activation following acute restraint stress. Targeted chemogenetic activation of DRN-projecting CART neurons in the EWcp replicated the anxiogenic effects of intra-DRN CART infusion in males, but not in females, indicating a sex-specific role for this pathway. In vivo fiber photometry and c-Fos experiment further revealed that the chemogenetic activation of the CART EWcp→DRN circuit inhibited DRN-5HT neurons through the recruitment of GABAergic interneurons.
In summary, our findings identify the CART EWcp→DRN circuit as a key regulator of anxiety-like behavior through feedforward inhibition of 5HT neurons in a sex-dependent manner. This pathway represents a novel and promising therapeutic target for the treatment of depression.

Sex differences and affective motivation for drug self-administration: A role for corticotropin-releasing hormone (CRH)-5-HT circuits
**Lynn Kirby**, Claire Deckers, Bryan McElroy, Chen Li

The serotonergic dorsal raphe nucleus (DRN) is positioned at the intersection of stress and reward circuits. Interactions between 5-HT and the stress neurohormone CRH within the DRN impact affective responses to both positive and negative reinforcers that can influence drug-taking behaviors. Recent work in our laboratory in rats has uncovered sex-differences in 5-HT DRN neuronal excitability, alcohol and opioid self-administration as well as the ultrasonic vocalizations (USVs) which signal positive (50 kHz) and negative (22 kHz) affective state that accompany them. Females exposed to an adolescent social isolation stress show hypoexcitability of 5-HT DRN neurons in adulthood, self-administer more alcohol and have a more compulsive (punishment-resistant) drinking phenotype compared to males. Females also self-administer more heroin than males, display subtle differences in stress-induced heroin reinstatement patterns compared to males and show elevated GABAergic spontaneous inhibitory postsynaptic currents in 5-HT DRN neurons following stress-induced reinstatement. During heroin self-administration, males display more anticipatory 50 kHz USVs prior to availability of heroin, potentially signaling sex-specific affective motivation for the drug. Chemogenetic activation of 5-HT DRN neurons in Tph2-iCre rats suppresses alcohol and sucrose self-administration but elevates heroin self-administration, though these effects may reflect a leftward shift in the dose-response curve across all drug classes, indicating a reduction in reward value. Punished responding for both alcohol and heroin is elevated by chemogenetic activation of DRN 5-HT neurons, indicating a more compulsive phenotype. Ongoing studies are using in vivo fiber photometry in Tph2-iCre rats to determine how 5-HT neurons encode natural and drug rewards compared to aversive footshock and their combination in punished drug self-administration models. Initial findings indicate valence-dependent, bi-directional encoding of 5-HT DRN neurons to rewards vs punishers and the cues that predict them. Future studies will also employ Crh-Cre rats for chemogenetic and fiber photometry studies to monitor activity in CRF afferents to the DRN during drug self-administration and to determine the causal relationship between CRH-5-HT circuits, affective and drug-taking behaviors. Both strategies will also be used to elucidate the sex-dependent nature of 5-HT DRN signaling that contribute to sex-differences in affective motivation for drug self-administration.

## #18: Serotoninergic Mechanisms of Psychedelic Therapeutics in Substance Use Disorders: Focus on Cognitive Flexibility

**Kathryn Cunningham (Chair), Stephanie Daws (Co-Chair)**

The 5-HT2A receptor as a therapeutic target for opioid use disorder with comorbid alcohol use
**Jasper Heinsbroek**
There is currently huge interest in the possibility that psychedelic drugs might aid fast and lasting remission from various neuropsychiatric disorders. Both classical psychedelics and modern non-hallucinogenic psychedelic-like compounds enhance neuronal structural plasticity through action at the 5-HT2A receptor, but the specific neuronal ensembles and cell types responsible for their therapeutic effects are unknown. We recently showed that the psychedelic (±)-DOI and the non-hallucinogenic, ibogaine-derivative tabernanthalog (TBG) effectively reduced motivation for heroin in rats with a history of comorbid heroin and alcohol self-administration. Blocking the 5-HT2A receptor eliminated these therapeutic effects. We will discuss possible neural mechanisms that might underpin these anti-addiction effects.

5-HT2AR and 5-HT2CR neurocircuitry in relapse vulnerability: Mechanisms and ligand discovery in cocaine use disorder
**Kathryn Cunningham**, Dr. Christina Merritt, Dr. Jia Zhou, Dr. Noelle Anastasio
Dysregulated function of serotonin 5-HT2A receptor (5-HT2AR) and 5-HT2CR in meso-corticolimbic circuitry is implicated in the neural mechanisms underlying vulnerability to relapse in cocaine use disorder (CUD). While there is evidence that 5-HT2AR and 5-HT2CR ligands have the potential as CUD therapeutics, the ~80% homology of their orthosteric binding pockets challenges the synthesis of subtype-selective ligands. Employing fragment-based, rational drug design approaches, we have crystalized strategies to explore 5-HT2AR and 5-HT2CR function with small molecule allosteric modulators. A fragment-based, rational drug design approaches were employed to craft a novel molecule series which was evaluated in 5-HT-induced signaling assays in h5-HT2CR, h5-HT2AR and h5-HT2BR cells in vitro. We identified molecules with selectivity as 5-HT2AR, 5-HT2CR or dual 5-HT2AR/5-HT2CR allosteric

modulators with on-target properties, acceptable pharmacokinetics and brain penetration parameters and negligible displacement of orthosteric sites of ~50 GPCRs and transporters. In silico analyses suggest binding to less conserved, extracellular sites vs. the orthosteric 5-HT site. Efficacy profiles of PAMs were characterized in preclinical models, and the findings are consistent with more constricted 5-HT2R agonist-like actions vs. a full 5-HT2R-specific agonists. Optimization of 5-HT2CR, 5-HT2AR and dual 5-HT2CR/5-HT2AR PAMs will provide novel pharmacological tools to promote greater understanding of the roles of these receptors in CUD and new prospects to develop selective, novel candidate medications with distinct profiles at these closely related receptors.

Psilocybin-mediated inhibition of opioid-seeking phenotypes
**Stephanie Daws**, Gabriele Floris, Amy Stringer, Konrad Dabrowski, Mary Tresa Zanda
Psychedelic drugs such as psilocybin have received increased attention for their potential to treat neuropsychiatric disorders, including substance use disorder (SUD). Using a rat model of heroin self-administration, we have evaluated the efficacy of psilocybin for reducing opioid seeking behaviors. Our data demonstrate that psilocybin administration does not impact heroin self-administration but significantly reduces heroin relapse following forced abstinence. We have begun to delineate the potential signaling mechanisms through which psilocybin may be accomplishing inhibition of heroin seeking using RNA sequencing methods. We will present data that highlights psilocybin-mediated modulation of inflammatory signaling as a potential therapeutic avenue for reduction of heroin seeking.

5HT receptor activation and the glutamatergic interface of behavioral, neural and immunological responses to psychedelics
**Jan Ramaekers**
Models of therapeutic effects of psychedelics should preferably integrate cognitive, neural, neuroplastic and immunological responses to explain improvements in psychopathologies. Evidence suggests that long-term therapeutic effects of psychedelics may result from changes in behavioral (decreased rumination, enhanced cognitive flexibility), central (downregulation of the hippocampal-prefrontal cortex axis, normalization of glutamate concentrations, increased neuroplasticity) and peripheral processes (downregulation of inflammatory markers). While each individual process can independently produce a therapeutic response, the interconnection of these processes implies they don't operate in isolation. Rather these changes collectively interact to trigger a sustained therapeutic response. Within this framework, the glutamatergic system emerges as the main interface orchestrating these interconnected processes.

## #19: Lipidation within the Serotonergic Pathways: Implication for Depression and Anxiety

**Mark Rasenick (Chair)**

G protein palmitoylation and antidepressant action
Dr Jeffrey Schappi, **Mark Rasenick**
Lipid rafts are cholesterol-rich, cytoskeletal associated structures providing a rigid island within the more mobile phospholipid regions of the membrane. Previous studies have revealed that the G protein, Gsα, inhabits both raft and non-raft domains, but GPCR/Gsα-driven activation of adenylyl cyclase is more facile in non-raft domains. Gsα is anchored to membranes through Gβγ subunits, which are prenylated, and palmitoylation of Gsα also plays a role in lipid raft and plasma membrane attachment.
We have developed a cellular paradigm for antidepressant action. Treatment of neural or glial cells for three days (or rodents for three weeks) results in a redistribution of Gsα from raft to non-raft membrane fractions, where it enjoys a more facile interaction with adenylyl cyclase. Rapid acting antidepressants show an accelerated action, requiring only 15 minutes treatment.
Treatment with antidepressants depalmitoylates Gsα and treatment of cells with depalmitoylation inhibitor, palmostatin. blocks the antidepressant-induced depalmitoylation of Gsα and also blocks the antidepressant-induced, Gsα-mediated activation of adenylyl cyclase. Expression of mutationally-altered GFP-Gsα that either cannot be palmitoylated or canot be depa,mitoylated shows no response to antidepressant treatment. This is in sharp contrast to GFP-Gsα, which is translocated from lipid rafts by antidepressants.

Thus, it appears that one action of antidepressants may be to depalmitoylate Gsα. The universality of this finding and its relevance for the treatment of depression, awaits further experimentation.

Exploring Cholesterol Sensitive Function of the Serotonin-1A Receptor: Excitements and Challenges
**Amitabha Chattopadhyay**
G protein-coupled receptors (GPCRs) are cellular nanomachines that represent the largest group of integral membrane proteins in the human proteome involved in signal transduction across membranes. The serotonin-1A receptor is an important neurotransmitter receptor of the GPCR superfamily. It is implicated in the generation and modulation of various cognitive, behavioral and developmental functions and serves as an important drug target. Pioneering work from our group demonstrated that membrane cholesterol is necessary for ligand binding, G-protein coupling and signaling of serotonin-1A receptors. In our recent work, we explored the molecular basis of cholesterol sensitivity exhibited by the serotonin-1A receptor by generating site-specific mutants of key residues in CRAC motifs in transmembrane helices (TM) 2 and 5 of the receptor supported by all-atom MD simulations. Notably, we showed that a lysine residue (K101) in one of the CRAC motifs is crucial for sensing altered membrane cholesterol levels (Kumar et al. (2021) Science Advances 7: eabh2922 (recommended in Faculty Opinions (F1000Prime)). These results constitute one of the first reports comprehensively demonstrating that cholesterol sensitivity could be knocked out by a single point mutation at a cholesterol binding site. Our observations are further supported from all-atom molecular dynamics simulations which reveal a tightly bound cholesterol molecule between TM1 and TM2 by establishing polar contacts with K101 that leads to stabilization of extracellular loop 1. I will end my talk by presenting our recent exciting observations on the role of cholesterol in endocytosis (spatiotemporal regulation) and trafficking of the serotonin-1A receptor and their implications in pathophysiology and therapeutics.

Regulation of Serotonin Transporter Kinetics and Trafficking by Palmitoylation: Implications for the Development and Treatment of Neurologic and Psychiatric Disorders
**James Foster**, PhD Christopher Brown
Serotonin (5-HT) is an important signaling molecule that modulates several physiologic functions including mood, learning, memory, sleep, cognition and gastric motility. The serotonin transporter (SERT) plays a critical role in the spatial and temporal control of extracellular 5-HT concentrations in serotonergic signaling, with dysregulation of this process being implicated in the pathogenesis of major-depressive, obsessive-compulsive, and autism-spectrum disorders. Moreover, SERT is a primary target for selective serotonin and serotonin-norepinephrine reuptake inhibitors (SSRIs and SNRIs), therapeutic drugs employed to treat these disorders. Human SERT has recently been identified as a palmitoylated protein, and using the irreversible palmitoyl acyl-transferase inhibitor 2-bromopalmitate (2BP), we have shown that acute inhibition of SERT palmitoylation results in decreased transport capacity (Vmax) without changes in Km or surface expression, while chronic inhibition or elevated concentrations of 2BP results in loss of cell surface and total cellular SERT, suggesting that palmitoylation is important in regulating SERT trafficking and maintenance of SERT protein levels through biogenic or anti-degradative processes. Using an overexpression approach with nine palmitoyl acyltransferases (DHHCs), we have identified five DHHCs that increased SERT palmitoylation that was accompanied by increased SERT cell surface and total cellular expression and increased 5-HT uptake. Preliminary data suggests that different DHHCs have differential impacts on SERT function, mediating trafficking-dependent enhancement of transport capacity and/or an increase in Vmax independent of SERT surface density. In addition, we have found that 3h exposure to the SSRI escitalopram results in decreased SERT palmitoylation, decreased cell surface expression, and decreased 5-HT uptake capacity that persists after thorough washout of the drug. Together, these results reveal palmitoylation is a major regulatory mechanism for SERT kinetics and trafficking that may participate in the development and treatment of neurologic and psychiatric disorders.

## #20: Serotonin in Sudden Death

**Russel Ray (Chair)**

The Serotonopathy of Sudden Infant Death Syndrome.

**Robin Haynes**
Sudden infant death syndrome (SIDS) remains the leading cause of post-neonatal mortality in the U.S.– despite implementation of safe sleep practices. While SIDS is a heterogeneous disorder, mounting evidence support abnormalities in the serotonergic system in some SIDS infants. Brainstem abnormalities in serotonin (5-HT) have been found in key regions of the medulla that participate in the maintenance of cardiorespiratory homeostasis in sleep, including processes involved in chemoreception, arousal, and autoresuscitation. Among these abnormalities are age- and region- specific alterations in 5-HT receptors 5-HT1A and 5-HT2A/C, decreases in 5-HT levels, and abnormalities in 5-HT cell number and morphology. Abnormalities in 5-HT in SIDS extend beyond the brain into the periphery with increased serum, plasma, and platelet 5-HT in a subset of SIDS infants. Together these data support that a subset of SIDS is a global serotonopathy representing biological vulnerability that puts an infant at risk for SIDS.

Sleep and Serotonin in Sudden Unexpected Death in Epilepsy
**Gordon Buchanan**
Epilepsy is a very common neurological disorder affecting more than 70 million people worldwide. About one-third of patients with epilepsy will not gain control of their seizures with currently available medical therapies. These patients are at highest risk for dying from sudden unexpected death in epilepsy (SUDEP), the leading cause of death in patients with refractory epilepsy. Mechanisms for SUDEP are poorly understood but seizure associated respiratory, cardiac and arousal deficits have been implicated. Various deficiencies in serotonin signaling have also been implicated. SUDEP also happens more commonly during the night, perhaps during sleep. Serotonin is involved in modulating many of these factors associated with SUDEP. Here we will discuss potential roles for serotonin in the various etiologies for SUDEP.

The Pendulum Between Breath and Death: Serotonin and Noradrenaline
**Savannah Lusk**
Sudden Infant Death Syndrome (SIDS) remains a leading cause of infant mortality worldwide, often linked to abnormalities in the serotonergic (5-HT) and noradrenergic (NA) systems of the respiratory network. This study employs innovative conditionally expressed inhibitory and excitatory DREADD models to understand the contributions and interactions of these two neurotransmitter systems in SIDS pathology. By using cre- and FLPo- drivers, we created models that allow singular excitation or inhibition of either the entire 5-HT or NA systems to enable concurrent excitation or inhibition of both systems in the same animal.
The failure of the neonate autoresuscitation reflex is hypothesized to be a common endpoint in many SIDS cases. This reflex was assayed in neonate mice through repeated bouts of exposure to an anoxic gas to induce bradycardia and apnea, followed by the restoration of room air to observe successful or failed autoresuscitation. We assessed the mice using a new closed-loop robotic neonate cardiorespiratory assessment platform, with outcomes analyzed by our comprehensive respiratory analysis platform, Breathe Easy.
We found four groups with decreased survival: NA activation, NA activation and 5-HT activation, NA activation and 5-HT inhibition, and, interestingly, 5-HT activation. Preliminary results suggest a cardiovascular mechanism of autoresuscitation failure independent of respiratory outcomes. At baseline, the four groups with decreased survival showed increased heart rates, while controls and groups with no change in survival either stayed the same or decreased. Ventilatory frequency, tidal volume, and ventilation increased in the three groups with NA activation but decreased in all other groups, including those with 5-HT activation. Additionally, there appeared to be a unique relationship between heart rates during early versus late recovery in the groups with decreased survival.
These results reveal a previously uncharacterized dynamic functional interplay between the 5-HT and NA systems in protective respiratory reflexes, setting the stage for a more comprehensive understanding of SIDS neuropathology.

The Role of Htr1B in the Neonatal Autoresuscitation Reflex and its Implication to Sudden Infant Deaths
**Russell Ray**
Aim: Serotonin receptor binding is decreased in subsets of SIDS cases and in vivo modeling has the importance of the serotonergic system in cardiorespiratory function. Our study aims to elucidate the molecular mechanisms by which serotonin modulates the life-saving cardiorespiratory autoresuscitation reflex.

Methods: Utilizing an automated cardiorespiratory phenotyping pipeline developed in the lab, we tested serotonin receptor 1B (Htr1B) function globally and in specific neuronal subpopulations. Following phenotypic assessment, we tested pharmacological interventions with the goal of rescuing failed cardiorespiratory reflexes.
Results: Global loss of Htr1B decreased survival to anoxic challenges in a sex-specific manner ($p<0.05$), perturbed heart rate and respiratory rate recovery following the initial anoxic challenge ($p<0.05$), and delayed gasping following the initial challenge ($p<0.05$). Importantly, the loss of Htr1B from Pet1-cre defined (largely serotoninergic) neurons did not result in measurable differences. However, loss of Htr1B in Engrailed 1-expressing neurons (rhombomere 1 and midbrain) led to a vulnerability, specifically in male autoresuscitation. We hypothesized that caffeine, a commonly used therapeutic in the NICU, could rescue these vulnerabilities. Administration of caffeine at 10mg/kg prior to cardiorespiratory challenge in Htr1B-/- rescued survival, heart rate recovery, respiratory rate recovery, and gasping capabilities ($p<0.05$).
Conclusions: We highlight that serotonergic signaling for the autoresuscitation reflex is modulated by Htr1B. Additionally, we highlight the complexity of the regulatory machinery in serotonin levels at the synapse and provide evidence in support of broader application of caffeine in at-risk infants.

## #21: Early-Life Stress and Serotonin: Neurodevelopmental Mechanisms and Circuit Dynamics Driving Lifelong Behavioral Changes

**Derya Sargin (Chair)**

Prefrontal cortex activity during early-life modulates cognitive performance in adulthood
**Catia Teixeira**
Caregiver behavior is arguably one of the most important environmental factors regulating brain function during early-life. Previously, we have reported that the presence or absence of rat dams from the nest altered prefrontal cortex (PFC) local field potential power. In this study we aimed at understanding whether prefrontal activity during the early postnatal period in mice affected cognitive performance in the adult. We show that inhibiting the PFC of mouse pups leads to cognitive deficits in the adult similar to those seen following maternal separation. We also show that activating the PFC during maternal separation can prevent these behavioral deficits. To test the effects of maternal separation on transcriptional profile of the PFC, we performed single-nucleus RNA-sequencing. Maternal separation led to differential gene expression almost exclusively in inhibitory neurons. In addition, we found changes in GABAergic and serotonergic pathways in these interneurons. Interestingly, both maternal separation and early-life PFC inhibition led to changes in physiological responses in prefrontal activity to GABAergic and serotonergic antagonists that were similar to the responses of more immature brains. Prefrontal activation during maternal separation prevented these changes. These data emphasize a crucial role of PFC activity and development that manifests in adult behavior.

Postnatal Maturation of Prefrontal-to-Raphe 5-HT Neuron Circuit: Implications for Early-Life Stress Vulnerability and Psychiatric Disorders
**Mariano Soiza-Reilly**
We investigate in mice the maturation of the PFC-to-raphe circuit during early postnatal life, analyzing whether developmental alterations on this pathway could affect adult emotional responses to stress and mood control. We apply a multidisciplinary approach that includes high-resolution microscopy, pharmacology and electrophysiological interrogation of synaptic circuits, together with behavioral assessments. Our study shows that either early-life stress or the excess in 5-HT levels has a similar impact on prefrontal circuit's maturation accompanied by detrimental consequences on adult emotional behavior.

Role of serotonin brain circuit in the developmental emergence of innate fear across species
**Giulia Zanni**
Fear is the human emotion that is elicited in threatful experiences. Maladaptive fear patterns are common in anxiety disorders. A risk factor for anxiety is increased serotonin (5-HT) during early life, i.e. due to early life trauma or serotonin reuptake inhibitors (SSRIs) use in pregnancy. In rodent models postnatal day 2 to 11 (P2-11) fluoxetine treatment causes enduring changes in 5-HT function and decreased innervation of several brain structures. We found that perinatal SSRIs enhance innate fear-related responses and fear

brain circuit activation that are conserved across species. SSRI-administered mice showed increased defense responses to a predator odor that were associated with stronger fMRI-based fear circuit activation (amygdala, PAG, thalamus) when compared to saline controls. Similarly, human adolescents from the ABCD study exposed to SSRIs in utero showed greater activation of fear brain structures (amygdala, putamen, thalamus) than unexposed adolescents.
Fear responses are orchestrated by the activation of stimulus-specific neural circuits that converge in the periaqueductal gray (PAG). This structure enables active coping strategies critical for survival in threatful environments. The PAG receives 5-HT projections that control local activity of GABAergic interneurons signaling during fear assessment. Using projection-specific optogenetics (Pet1Cre::Ai32 or Ai39), we found that 5-HT projection to the PAG is necessary for fear behavior.
It remains unknown if increased developmental 5-HT signaling alters adult 5HT dlPAG circuit function to increase innate fear.
We employed 1) chemogenetic (Pet1Cre::Hm3d, CNO, 2 mg/kg) and pharmacologic (fluoxetine, 10 mg/kg) approaches to increase 5-HT signaling during P2-11 in mice, 2) we used fiber photometry (FP) to measure 5-HT (iSeroSnFR) input onto GABAergic (JRGeco1a) output in VgatCre mice injected with fluoxetine or saline during P2-11 in mice.
Both chemogenetic and pharmacologic increases of 5-HT signaling during P2-11, lead to heightened innate fear in adults. Using FP recording we found that during threat assessment, both 5-HT levels and GABAergic activity increase, establishing a direct relationship of 5-HT in controlling GABA signaling during fear in naïve mice. In contrast, in P2-11 fluoxetine injected mice we found that 5-HT levels and GABAergic activity are both altered.
Overall, increased 5-HT levels in early life enhances innate fear responses and fear brain circuit activation that are conserved across species.

Serotonergic circuit activity in early life stress models
**Derya Sargin**
Early life stress (ELS) is associated with disrupted brain connectivity and increased risk for emotional and social deficits later in life. The correlation between functional changes in brain circuits and subsequent alterations in specific behaviors is unknown. In rodent studies, ELS-induced alterations in memory and cognition have been associated with structural changes in brain regions that receive strong serotonin inputs. Using established mouse models of chronic developmental stress, my lab's recent findings revealed that ELS disrupts the connectivity of the serotonin system which could have implications for the treatment of affective disorders that arise from early life adversities. Here, I will present our work on established mouse models of chronic developmental stress, focusing on the critical pathways that modulate serotonin responses and underlie ELS-induced long-lasting behavioral impairments. Understanding the mechanisms of how ELS disrupts brain-wide connectivity of the serotonin system, we aim to develop treatments acting at the level of circuits.

## #22: SSRIs in the Treatment of Depression: A Pharmacological Cul-de-Sac?

**Trevor Sharp (Chair), Philip Cowen (Co-Chair)**

SSRIs: A Pharmacological Cul-de-Sac?
**Philip Cowen**
The widespread adoption of selective serotonin reuptake inhibitors (SSRIs) as first line pharmacological treatments in the management of clinical depression, transformed the landscape of drug therapy for this condition. SSRIs are safer and better tolerated than the tricyclic antidepressants (TCAs) which they replaced. However, they have limitations which may have placed a ceiling on expectations of first-line pharmacological treatment. Notable problems with SSRIs include induction of anxiety on treatment initiation, delayed onset of significant therapeutic effect, sexual dysfunction, sleep disturbance and overall modest efficacy, particularly against symptoms of anhedonia. This talk will describe the development of SSRIs from TCAs and the pharmacological properties, for example, blockade of 5-HT2A/2C receptors, that might account for the superior efficacy of TCAs in some people with depression.

Serotonin 4 receptor brain architecture in major depression, associations with sexual health, and antidepressant treatment outcomes

**Vibe G. Frokjaer**

In this talk I will cover findings from a recent clinical trial documenting serotonergic brain architecture changes in patients with moderate to severe Major Depresssive Disorder, in particular the involvement of the serotonin 4 receptor in depression and antidepressant treatment mechanisms. This work offers molecular brain imaging informed insights into mechanisms of actions of SSRIs including those relevant for core symptoms of anhedonia and potential side effects related to sexual dysfunction. Also I will present and discuss sexual health data across standard antidepressant treatment with SSRis and highlight potential sex differences. Last we will discuss perspectives for refining future antidepressant treatment regimes in the light of the presented results.

Antidepressant Discontinuation Syndrome – Current Evidence on Incidence, Placebo Discontinuation, and Clinical Implications

**Jonathan Henssler**

Antidepressant discontinuation symptoms are becoming an increasingly important aspect of clinical practice, yet their incidence remains largely unknown and subject to sometimes heated debate. An accurate estimate of the incidence of antidepressant discontinuation symptoms is urgently needed to properly inform both patients and clinicians during treatment discontinuation, while also providing valuable information to researchers studying antidepressant treatments.

This presentation will cover findings from a recent meta-analysis—the largest to date—on the incidence of antidepressant discontinuation symptoms. It is also the first to systematically assess discontinuation symptoms in patients discontinuing placebo, thus providing insight into nocebo effects, symptom specificity, and the proportion of symptoms that may be attributed to pharmacological withdrawal in the strict sense.

The presentation will offer a nuanced view of the current evidence base, address critiques of the meta-analysis, and explore the clinical implications of these findings.

Modelling the neurobiological effects of SSRI discontinuation

**Trevor Sharp**, Helen Collins, Sophie Gullino, David Bannerman

Selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs) are currently first-line pharmacological treatments for major depressive disorder and anxiety-related disorders. However, abrupt cessation SSRI treatment can produce a debilitating discontinuation syndrome, typified by symptoms such as anxiety and sleep disruption. Recent clinical reports estimate that half of patients experience the discontinuation syndrome, and more than 40% report that their symptoms are severe. Currently the mechanism underpinning SSRI discontinuation syndrome is unknown. In a combination of behavioural, neurochemical and immunocytochemical experiments we have explored the effect of SSRI discontinuation in mice. We observed that mice discontinued from two weeks treatment with paroxetine showed a short-lasting increase in anxiety-like behaviour as well as evidence of fragmented sleep. In in vivo microdialysis and ex vivo neurochemistry experiments, paroxetine discontinued mice demonstrated evidence of increased 5-HT neuronal activity over and above baseline levels. These findings were supported by immunocytochemical measurements of c-Fos/TPH2 doubled-labelled neurons in the midbrain raphe. Mismatched timing of the anxiety and 5-HT neuron changes suggested that the latter effect may not itself be sufficient to cause anxiety. Overall, our data indicate that SSRI discontinuation can be modelled in mice. Moreover, our findings support the idea that SSRI discontinuation is associated with a rebound activation of 5-HT neurons. This effect is reminiscent of neurotransmitter changes associated with other psychotropic drug withdrawal states, suggesting a common unifying neurochemical mechanism.

## #23: Elevated MAOA and Treatment Resistance in Depression: New Twists on a Classic Hypothesis

**Jeffrey Meyer (Chair)**

Modeling elevated MAO-A activity in mice: role in emotionality and antidepressant treatment response

**Jean-Philippe Guilloux**, Rodolphe Lebeau, Phuoc Quy Long Nguyen, Runhao Zhou, Jeffrey Meyer, Etienne Sibille, Toshifumi Tomoda

A pathological feature of major depressive disorders (MDD) that may underlie decreased monoamines, depressive symptoms, and variable response to antidepressants (AD), is elevated levels of monoamine oxidase A (MAOA). Replicated clinical studies revealed an increase in MAOA levels across the brain of MDD patients compared to healthy controls. Whether elevated MAOA is a causal link to the MDD-vulnerability, neurochemical changes and AD treatment resistance remains to be tested. To answer this question, we established an experimentally manipulable system in mice, based on the introduction of human MAOA (hMAOA) gene into several brain regions and cell types (CamkII+ or GFAP+ cells).
Adeno-associated viral (AAV) vectors allowing neuronal or glial hMAOA expression were injected in the PFC of 8-weeks old mice. Increase in MAOA expression/activity was confirmed using immunohistochemical, transcriptomic and proteomic experiments. Effects of increase MAOA on emotional behavior were assessed and neurochemical effects of increased hMAOA and response to AD (fluoxetine, ketamine, moclobemide) treatment were tested using in vivo microdialysis in the PFC and vHipp.
Selective CamkII+-AAVhMAOA injection in the PFC increase MAOA mRNA/protein levels, leading to increased MAOA activity, without affecting endogenous MAOA and MAOB expression. Emotional behaviour analyses revealed that elevated hMAOA in CamkII+ neurons do not affect anxious-like behavior but induced an anhedonic effect. In vivo microdialysis experiments showed that elevated hMAOA activity leads to a 40% decrease in basal [5-HT]ext levels without affecting the 5-HT transporter activity and prevents the fluoxetine-induced increase in [5-HT]ext levels in the PFC compared to controls. Interestingly, hMAOA increase in GFAP+-cells in the PFC results in anxious-like behavior, but with no effect on anhedonia. In glial cells, elevated hMAOA activity prevents the fluoxetine-induced increase in [5-HT]ext levels in the PFC compared to controls, as observed when hMAOA was elevated in CamkII+ neurons.
Overall, our results confirmed that increased hMAOA activity results in lower 5-HT neurotransmission associated with changes in emotional behavior. Whether these regio-selective neurochemical changes can lead to greater susceptibility to stress remains to be tested. Additionally, while our study focused on the effects of MAOA overexpression in the PFC, other brain regions involved in mood regulation will be tested to decipher the regional selectivity of these effects.

Elevated MAOA and altered bioenergetics underlying elevated emotionality in depression
**Toshifumi Tomoda**, B.S. Runhao Zhou, Ph.D. Rodolphe Lebeau, Ph.D. Akiko Sumitomo, Ph.D. Mounira Banasr, Ph.D. Rob Laister, M.D., Ph.D. Jeffrey Meyer, Ph.D. Jean-Philippe Guilloux, Ph.D. Etienne Sibille
Treatment for major depressive disorder (MDD) using conventional antidepressants (ADs) is only effective in approximately 50% of patients with limited efficacies. Elevated expression of monoamine oxidase A (MAOA) in corticolimbic brain areas has been consistently reported in MDD via positron emission tomography (PET) studies. MAOA is an outer mitochondrial membrane protein that catalyzes the oxidative deamination of monoamines (e.g., serotonin and norepinephrine), while producing hydrogen peroxide and electrons to fuel bioenergetic machinery. High MAOA is therefore thought to contribute to low brain serotonin levels and hence depressed mood, or resistance to conventional monoaminergic AD treatment. However, the causal link of elevated MAOA to the pathobiology of MDD and/or treatment resistance has not been tested, and the mechanisms underlying these psychopathological conditions remain to be studied.
To test this causality, we have developed a humanized model of elevated MAOA in mice, in which human MAOA gene is selectively expressed in relevant neuronal or glial cell types within corticolimbic areas, and studied the impacts on emotional behavior, neurochemistry and cellular metabolism, with particular emphasis on mitochondrial bioenergetic pathways.
Increasing MAOA in pyramidal neurons in the medial prefrontal cortex (mPFC) resulted in anhedonia-like behaviors in mice, while elevated MAOA in astrocytes in PFC resulted in anxiety behaviors, with concurrent decrease (~40%) in serotonin levels. We also investigated the effects of elevated MAOA in primary neurons/astrocytes on mitochondrial bioenergetics, metabolites and associated proteomic changes. Elevated MAOA led to significant increase in reactive oxygen species (ROS) production, and reduction in mitochondrial membrane potential, oxygen consumption rate (OCR) and ATP production differentially in neurons vs. astrocytes. These bioenergetic changes were at least partially rescued by antioxidant treatment but not by the therapeutic dose of serotonin supplementation.
Together the results demonstrated that elevated MAOA in distinct cell types differentially caused impaired neurochemical profiles, emotionality behaviors, and mitochondrial bioenergetic functions, and further suggested a novel mechanism for neuron/glia interplay that regulates emotional behavior. Further studies will assess the impact of elevated MAOA in mediating resistance to conventional and novel AD treatment

regimen, using the mitochondrial bioenergetic assay system as a platform to test the efficacy at mechanistic levels.

Differential association of the MAOA rs979605(A>G) genetic polymorphism with clinical improvement in antidepressant-treated depressed males and females

**Kenneth Chappell**, Romain Colle, Jérôme Bouligand, Séverine Trabado, Bruno Fève, Laurent Becquemont, Emmanuelle Corruble, Céline Verstuyft

Major depressive disorder (MDD) is the leading cause of disability worldwide. First-line treatments, antidepressant drugs (ATD), target the levels of monoamine neurotransmitters like serotonin (5-HT). However, only half of ATD-treated patients respond sufficiently. Monoamine oxidase (MAO), which metabolizes 5-HT to 5-hydroxy indoleacetic acid (5-HIAA), is a candidate biomarker of treatment response. Genetic variants in the X-chromosome-linked MAO-encoding genes, MAOA and MAOB, have been associated with clinical improvement following ATD treatment in depressed patients. Our aim was to analyze the association of MAOA and MAOB genetic variants with clinical improvement and the plasma 5-HIAA/5-HT ratio in 6-month ATD-treated depressed patients.

Clinical (n=378) and metabolite (n=148) data were obtained at baseline and 1 (M1), 3 (M3), and 6 months (M6) after beginning ATD treatment in patients of METADAP, a multicentric, prospective, and naturalistic cohort. Mixed-effects models were used to assess the association of genetic variants with the Hamilton Depression Rating Scale (HDRS) score, response and remission rates, and the plasma 5-HIAA/5-HT ratio. The sex × variant interaction was included to help control for factors linked to X-chromosome inactivation (XCI).

The MAOA rs979605(A>G) and MAOB rs1799836(T>C) polymorphisms were analyzed. The sex × rs979605 interaction was significantly associated with the HDRS score (P=0.011), suggesting a potential difference in the A allele's association with the HDRS score between males (coef=-2.04) and females (coef=0.70). At M6, A allele-carrying males had a lower marginal mean (±standard error) HDRS score (n=24, 10.9±1.61) compared to AA homozygous females (n=14, 18.1±1.87; P=0.0067). The MAOB rs1799836 polymorphism was significantly associated with the plasma 5-HIAA/5-HT ratio (P=0.018). Overall, CC/C females/males had a lower ratio (n=44, 2.18±0.28) compared to TT/T females/males (n=60, 2.79±0.27; P=0.047). Forty MAOA polymorphisms were in strong linkage disequilibrium with rs979605. According to Genotype-Tissue Expression data, rs979605, 35 of its linked variants, and rs1799836 were associated (i.e., P<0.05) with MAOA expression in the hippocampus, hypothalamus, and/or basal ganglia.

The differential association of the MAOA rs979605(A>G) A allele with higher and lower HDRS scores in females and males, respectively, suggest it may be a potential biomarker of interest for ATD treatment response. More studies of these two variants are needed.

Randomized Double Blind Placebo Controlled Trial of a Dietary Supplement to Prevent Post Partum Blues With Six Month Follow Up of Depressive Symptoms

**Jeffrey Meyer**, ZhaoHui Wang, Apitharani Santhirakumar, Dr. Yekta Dowlati, Natalia Docteur, Asqa Shoaib, Jareeat Purnava, Yanqi Wang, Dr. Wei Wang, Dr. Ishrat Husain, Rashmi de Silva Wijeyeratne, Heba Reeyaz, Catalina Baena-Tan, Dr. Yuko Koshimori, Zahra Nasser, Valery Sit

Background: Postpartum blues (PPB) is a frequent syndrome of sad mood, crying spells, anxiety, restlessness, reduced appetite, and irritability, typically peaking day 5 postpartum. When severe, it greatly increases risk for later postpartum depression. This trial compared a dietary supplement to placebo on PPB severity. The supplement was designed to counter downstream effects of elevated monoamine oxidase A level, implicated in causing PPB.

Methods: Participants recruited by advertisement from the Toronto region completed procedures at CAMH, Canada and/or participants' homes. Oral supplement or identical appearing relatively inert placebo were administered in randomised, double-blind fashion. Supplement was blueberry juice and extract given four times between nighttime day 3 and morning day 5 postpartum; tryptophan 2g nighttime day 4 postpartum, and tyrosine 10g morning day 5 postpartum. On day 5, depressed mood induction procedure (MIP) and postpartum blues were assessed. All data is presented. (NCT03296956 closed, clinicaltrials.gov).

Results: Between January 2019 and December 2022, participants took supplement (n=51) or placebo (n=52). There was no significant effect on primary outcome MIP on visual analogue scale for depressed mood (mean difference=-0·39mm, 95% CI: -6·42 to 5·65mm). COVID-19 waves associated with crying frequency on the Center for Epidemiologic Scale (CES-D) the week before supplement intake, likely

influencing MIP and postpartum blues. Stein Maternity Blues scores, exploratory PPB measure, was lower in the active group (effect size 0·62; median, interquartile range (IQR): active 2·00 (IQR 1, 4); placebo 4·00 (IQR 1·75, 6); regression with general linear model, supplement effect, β coefficient = -1·50 (95% CI -2·60, -0·40), p=0·008; effect of CES-D crying category before supplement, p=0·03 to 0·00000023). Twenty-six and 40 different adverse events occurred within 25% and 42% of supplement and placebo cases respectively (Chi-Square, p=0·06). Exploratory six month follow up of total CES-D scores favored supplement condition (time*treatment interaction, mixed effects model, p=0·05; alternatively piecewise regression, p=0·01).
Conclusions: The primary outcome was negative for effect on depressed mood induction. However the supplement moderately reduced PPB and severity of depressive symptoms 6 months later.
(see Meyer et al. eClinicalMedicine, 2024)

## #24: Targeting Presynaptic and Postsynaptic Serotonin Systems for Neuropsychiatric Treatment Strategies

**Harald Sitte (Chair)**

Mining the new psychoactive substances library for MDMA-like therapeutic agents
**Michael Baumann**
3,4-Methylenedioxymethamphetamine (MDMA) is a monoamine-releasing compound that has shown promise as a medication adjunct for treating post-traumatic stress disorder. However, MDMA administration is associated with certain risks, such as abuse liability and cardiovascular stimulation. The therapeutic actions of MDMA are thought to involve transporter-mediated release of 5-hydroxytryptamine (5-HT), whereas rewarding and cardiovascular actions involve release of dopamine and norepinephrine, respectively. Current research efforts are aimed at developing MDMA-like compounds with better efficacy and safety profiles. To this end, we assayed a vast library of new psychoactive substances for in vitro activity at transporters for 5-HT (SERT), dopamine (DAT), and norepinephrine (NET) in rat brain synaptosomes. Amphetamine, indane, cathinone, and benzofuran derivatives were provided by academic and pharmaceutical collaborators. Our experiments identified a number of novel compounds which exhibit high potency and efficacy for release at SERT, with lower potency and/or efficacy for release at DAT and NET. Collectively, our findings demonstrate the feasibility of designing monoamine-releasing agents with optimized activity at SERT relative to DAT and NET. Ongoing studies are characterizing the in vivo effects of candidate lead compounds using drug discrimination and biotelemetry methods in laboratory rodents.

Serotonergic modulation of cortical circuits in social cognition
**Francesco Papaleo**, Marco Niello
Social cognition is impaired in numerous neuropsychiatric disorders. Serotonin (5HT) is classically associated with the processing of social information and serotonergic neurons project to the medial prefrontal cortex (mPFC), a fundamental hub of socio-cognitive functions. However, the role of 5HT dynamic in social cognition, the related circuits as well as the impact of pharmacological manipulation are still unexplored. Here, to address this topic, we combined recently developed socio-cognitive tasks for mice with circuit manipulations, fiber photometry, and in vivo pharmacology. We observed that manipulating the 5HT system with serotonin releasing agents impacted different aspects of social cognition in mice going beyond commonly assessed “prosocial” effects. Consistently, 5HT manipulation impacts mPFC local circuits classically engaged in socio-cognitive processes. Our findings underscore the pivotal role of the serotonergic system in modulating distinct domains of social cognition, providing a foundation for innovative therapeutic approaches to neuropsychiatric disorders.

Polypharmacological and Biased Agonist Profiles of Psychedelics and MDMA Analogs
**John McCorvy**, Janelle Lanham, Natalie Cavalco, Allison Clark, Andrew Cao, John McKee, Hailey Bock, Marko Ivancich, Joseph Hennessey
Classical psychedelics, such as LSD and psilocybin, and entactogens like MDMA show rapid-acting and durable effects in a plethoral of neuropsychiatric disorders and are extraordinarily useful as neuropsychopharmacological agents in serotonin research. However, traditional psychedelics like LSD and psilocin/psilocybin act at serotonin receptors in a post- and pre-synaptic mechanisms, but MDMA is thought

to primarily act at the serotonin transporter (SERT) in a presynaptic mechanism. Given that the 5-HT2A receptor appears to a critical receptor to mediate psychedelic effects, many of other 12 serotonin G protein-coupled receptors (GPCRs) that are also expressed in brain have not been fully interrogated for additional neuropsychopharmacological effects that may contribute toward antidepressant, prosocial and/or therapeutic properties. Therefore, using a panel of psychedelic and MDMA analogs, we employ G protein dissociation BRET platforms and second messenger signaling assays to form an extensive map of structure-activity relationships (SAR) for each chemical scaffold (tryptamine, phenethylamine, amphetamine etc) revealing chemical determinants for agonist activity across the serotonin GPCRome. Our results reveal that very few psychedelic or MDMA analogs have little selectivity for the 5-HT2A receptor and reveal off-target activity at 5-HT2B and 5-HT1B/1D/1e/1F receptors, which may be responsible for cardiotoxicity and prosocial/antimigraine effects, respectively. Importantly, we show that psychedelics produce robust and efficacious Gq/11 signaling efficacy at the 5-HT2A receptor, unlike “non-psychedelic” agents, which show partial 5-HT2A Gq/11 agonist efficacy. Finally, we show key SARs for psychedelic and MDMA analogs that appear to avoid 5-HT2B cardiotoxic effects and can be re-engineered toward pathway-selectivity and/or biased agonism. These results establish a framework for rational and structure-based design of novel serotonin agents that can be fine-tuned for rapid-acting and durable neuropsychopharmacological and therapeutic properties.

Targeting presynaptic and postsynaptic serotonin systems for neuropsychiatric treatment strategies
**Michael Colwell**

## #25: Human in vivo Imaging of Genetic Variability within the Serotonin System

**Marie Spies (Chair), Elizabeth Bartlett (Co-Chair)**

Genetic and epigenetic regulation of serotonin turnover in seasonal affective disorder
**Marie Spies**
Genetic variants within - and epigenetic regulation of - serotonin turnover genes may impact upon risk for affective disorders. Though the impact of singular genetic or epigenetic factors is likely limited and their effects complex, changes within serotonin turnover may serve as a link between genetic risk and clinical phenomenology. Data from three studies shining light on genetic and epigenetic regulation of serotonin turnover are presented.
The impact of genetic variants within the tryptophan hydroxylase 2 (TPH2) and monoamine oxidase A (MAOA) genes, the main enzymes in cerebral serotonin (5-HT) turnover, on global MAO-A distribution volume (VT) assessed using [11C]harmine positron emission tomography (PET) was tested in 21 patients with seasonal affective disorder (SAD) and 30 healthy controls (HC). Increased brain MAO-A levels are an oft replicated PET finding in depression. An effect of rs1386494 genotype on brain MAO-A VT (ca. 25% difference between genotypes) was observed. The role of rs1386494 on TPH2 levels or function is unclear. Theoretically, TPH2 function may affect MAO-A levels via their common product/substrate, serotonin. (Spies M, Murgas M et al., 2023).
The impact of MAOA promoter / exon I / intron I region DNA methylation on brain MAO-A VT was tested in a largely overlapping sample. We did not observe a significant effect, even after correction for diagnosis, season, MAOA VNTR genotype and sex, though an effect of season on methylation was detected in women, with higher levels in spring and summer. The latter is in accordance with extensive evidence for seasonal effects on the serotonin system and suggests these may be facilitated via methylation (Handschuh PA, … Spies M et al., 2023).
To further assess the impact of environmental factors on epigenetic regulation of serotonin turnover, an overlapping sample was used to test for effects of daily sunshine hours gleaned from regional meteorological measurements on serotonin transporter gene (SLC6A4) promoter methylation, correcting for 5-HTTLPR, age and sex. Higher sunshine hours were associated with lower methylation, though only across the entire group, not within diagnoses. This suggests a general phenomenon rather than pathophysiologically relevant process (Handschuh PA et al., 2023).
Together these findings highlight the impact of genetic and epigenetic regulation on serotonin turnover.

The Serotonin System in Depression and Suicidal Behavior: Novel Insights with PET Imaging
**Elizabeth Bartlett**, Dr Francesca Zanderigo, Dr J John Mann
This talk highlights recent in vivo positron emission tomography (PET) analyses of brain serotonin transporter (5-HTT) and 1A receptor (5-HT1A) binding to better understand depression and suicidal behavior.
Analysis 1: Twenty-five unmedicated participants with major depression (MDD), including 13 suicide attempters, underwent same-day [11C]DASB (for 5-HTT) and [11C]CUMI-101 (for 5-HT1A) PET scans to quantify binding potential (BPND) using the NRU 5-HT Atlas. Ecological momentary assessment (EMA) captured daily stress over one week proximal to PET. Positive correlations between EMA-measured stress and 5-HT1A BPND were found in 9 of 10 brain regions in attempters, while an inverse correlation was found in attempters with 5-HTT BPND. Serotonin system stress-responsivity among attempters may indicate a serotonergic phenotype that contributes to vulnerability for suicidal behavior, which may be targeted to boost resiliency to stress.
Analysis 2: Sixty participants with MDD and 31 healthy volunteers (HVs) underwent [11C]DASB scans to quantify BPP. [11C]DASB binding along a tract of serotonergic axons was automatically delineated on the PET scans and revealed significantly lower 5-HTT binding in MDD vs. HVs, particularly near the tract's origin, close to the raphe nuclei, which dissipated as the distance along the tract from the origin increased. Future studies are needed to determine the source of dysregulated 5-HTT binding in proximal axon segments, which could inform targeted antidepressant treatment, including neuromodulation, and may extend to other serotonin-related disorders.
Analysis 3: Radiomics and machine learning were applied to [11C]WAY100635 (5-HT1A) PET data to develop classifiers distinguishing MDD from HVs. Radiomics quantifies multi-scale textural and shape information in the images through extraction of voxel interrelationships but has rarely been applied in psychiatry. Radiomics was applied to 5-HT1A BPF brainstem voxel-wise maps (46 participants with MDD, 30 HVs), extracting 107 unique features from each image. Machine learning models were constructed using an 80/20 training/testing split and 10, 5-fold cross-validations. The optimal model was applied to the testing set, yielding 80% accuracy, 66.7% sensitivity, and 88.9% specificity to classify MDD vs. HVs, demonstrating radiomics' potential in developing PET-based biomarkers in psychiatry.
These findings underscore the promise of serotonin PET imaging, particularly using radiomics, to identify biomarkers for depression and suicidal behavior, with implications for targeted psychiatric treatments.

Genetic and Epigenetic Contributions to Serotonin Neurotransmission in the Healthy and Depressed State
**Patrick Fisher**
Alterations in serotonin neurotransmission have been linked with depression, which has a strong genetic component. Nonetheless, little is known about the genetic contribution to in-vivo levels of proteins that are key to serotonin neurotransmission, such as the serotonin transporter (5-HTT). In addition, environmental factors (e.g. early life stress) also critically contribute to depression risk. Epigenetic variation of serotonin genes (e.g. serotonin transporter [SLC6A4] and tryptophan hydroxylase 2 [TPH2]), which can dynamically regulate gene expression in response to the environment, has been linked to both early life stress and depression, representing a potential gene-by-environment risk marker. Importantly, epigenetic mechanisms (e.g. DNA methylation) are tissue specific. Most research linking SLC6A4 or TPH2 methylation is based on measurements from peripheral tissues, such as blood or saliva. Positron emission tomography (PET) studies combined with genetic and epigenetic markers offers a unique opportunity to resolve effects on the in-vivo brain serotonergic architecture.
Using PET to estimate brain 5-HTT and 5-HT4 levels, we evaluated whether five genetic variants within regulatory regions of genes central to serotonin neurotransmission (HT2AR, HT1AR, SLC6A4, MAOA, BDNF) were: 1) associated with and 2) predicted brain 5-HTT levels in healthy adults (N=140). Next, we evaluated whether SLC6A4 or TPH2 DNA methylation was linked with early life stress measures, depressive symptoms, and brain 5-HTT (N=140) and 5-HT4 (N=112) levels in healthy participants and with 5-HT4 in individuals with depression (N=90).
An MAOA variant putatively associated with lower MAOA activity was associated with higher 5-HTT levels. However, genetic information was not sufficient to predict brain 5-HTT levels. We did not observe evidence for a link with SLC6A4 or TPH2 methylation and measures of early stress, depressive symptoms, nor 5-HTT or 5-HT4, neither in healthy participants nor in patients with depression. Our findings suggest that informative genetic markers of in-vivo serotonin neurotransmission lie elsewhere and that epigenetic

variation within serotonin-relevant genes measured peripherally may provide a limited representation of in-vivo serotonergic neurotransmission in adults. Future studies are needed to more fully elucidate the genetic and epigenetic effects on the human serotonergic system.

Effectiveness and safety of escitalopram treatment personalized based on therapeutic drug monitoring of drug plasma concentration: A prospective cohort study

**Marin Jukic**

This is the first prospective study aiming to quantify the effectiveness and safety of escitalopram monotherapy initiation where therapeutic drug monitoring (TDM) was used to achieve the therapeutic reference range (TRR) of plasma concentration. PsyCise-E (NCT05210140) was a hospital-based study conducted in Belgrade, Serbia, involving 92 outpatients with a baseline Hamilton Rating Scale for Depression (HAM-D) score higher than 13. The primary endpoint was the relative reduction in HAM-D score from baseline to week eight, with dose personalization based on TDM four weeks after treatment initiation. Patients were categorized into groups: (1) unadjusted (they achieved TRR at 10 mg/day), (2) adjusted (their dose was adjusted to achieve TRR) and (3) inadequate (they did not reach TRR). Safety was assessed by the occurrence of adverse drug reactions (ADRs) and QTc interval prolongation. Most patients required a dose escalation beyond 10 mg/day (71/92), and most patients achieved TRR after eight weeks (79/92). The 55% (95% CI: 47-64) reduction in HAM-D scores did not correlate with escitalopram plasma concentrations and did not differ between groups; however, response and remission rates were significantly higher in patients who achieved TRR by week four. The incidence of ADRs (47/92) increased by 3.2% (0.1-6.3) per ng/ml escitalopram, with no significant differences between the groups. QTc prolongation of 5.5 ms (1.8-9.3) did not correlate with plasma concentration and did not differ between groups. While TDM-guided dosing likely only marginally improved escitalopram effectiveness, it increased treatment safety as TDM-guided dose escalation did not lead to ADRs or QTc prolongation.

## #26: Molecular Mechanism of Serotonin Transport by SERT, OCTs, and VMAT2

**Thomas Stockner (Chair)**

Similarities and differences in the molecular mechanism, by which serotonin triggers transport in SERT and OCT3.

**Thomas Stockner**

SERT and OCT3 both transport serotonin and both transporters are expressed in the brain. They strongly differ in the Km and in the maximal transport capacity. In this talk I will elaborate on the molecular mechanism of substrate transport from the perspective of the transporters, highlighting similarities and key differences, but also from the perspective of transporter substrates. Which are the key interactions leading to substrate transport, how is selectivity achieved? An analysis of the energetics at the atomic level underlying the transport process sheds light on an important cornerstone of substrate translocation, which is one that is notoriously difficult to investigate.

OCT1: another relevant serotonin transporter?

**Marleen Meyer-Tönnies**

The membrane transporter OCT1 (SLC22A1) is almost exclusively expressed in human hepatocytes. OCT1 mediates the hepatic uptake of a variety of structurally diverse substances, including drugs such as sumatriptan, fenoterol, and morphine, as well as endogenous compounds like thiamine (vitamin B1). Notably, OCT1 exhibits high genetic variability, with five common genetic variants leading to a reduction or complete loss of transport function in up to 9% of Europeans. Carriers of loss-of-function variants have higher systemic concentrations of sumatriptan, fenoterol, and morphine. The exact transport mechanism of OCT1 is not fully understood. However, based on the recently available cryo-EM structures of OCT1, several key amino acids have been proposed as essential for OCT1 transport.
Serotonin is known to be a substrate of mouse organic cation transporters. In the mouse brain, OCT2 has been reported to mediate serotonin reuptake alongside SERT, with knockout mice exhibiting behavioral phenotypes and altered responses to SSRIs. In the periphery, serotonin is bioinactivated in hepatocytes by monoamine oxidase, but the role of OCT1 in this process remains unknown.

In my talk, I will discuss the potential role of OCT1 in the homeostasis of peripheral serotonin in humans. Additionally, I will present examples of how we use species differences to identify structural determinants of OCT1 transport mechanisms.

Uncoupling the Gated-Pore Mechanism in the Human Serotonin Transporter – Novel Insights into the Conducting State
**Ralph Gradisch**
The presynaptically located serotonin transporter (SERT) utilizes the sodium gradient as a driving force for the reuptake of serotonin (5-HT), hence fine-tuning serotonergic signalling. Importantly, SERT is pharmacologically targeted for the treatment of neuropsychiatric diseases. High-resolution structures of SLC6 members, including SERT, revealed their conserved three-dimensional architecture and highlighted the structural elements enabling the alternating access mechanism. The transport cycle is initiated upon binding of 5-HT and requires structural rearrangements to transition from the outward-open state to the occluded and, subsequently, the inward-open state. Movements of the dynamic bundle domain towards and away from the rigid scaffold domain regulate the gates above and below the central substrate binding site (S1) to facilitate either extracellular or intracellular accessibility to the S1. The "gated pore" and "rocking bundle" mechanisms ensure that the S1 can only be accessed from one side of the membrane, but never from both simultaneously. This feature discriminates channels from transporters. However, not all features of SERT can be explained by these models. Electrophysiological recordings revealed excessive ion flux in the presence of substrate, surpassing the proposed transporter stoichiometry. Although much smaller than channel-mediated currents, this high-conducting state is reminiscent of a ligand-gated channel. This study demonstrates that a single point mutation above the S1 is sufficient to render a transporter a ligand-gated pore. By integrating molecular dynamics simulations with experimental approaches, we unravelled SERT's sealing mechanism. Besides adding another layer of complexity to the alternating access model, this study highlights the extremely fine line separating transporters and channels.

Allosteric modulation of serotonin and dopamine transporters: Insights from computations and experiments
**Ivet Bahar**, Hoang Nguyen, Mary Cheng, Ji Young Lee, Shaili Aggarwal, Ole Mortensen
Advances in the structural characterization of dopamine and serotonin transporters have opened the way for structure-based modeling and simulations, which, together with experimental data, now provide mechanistic understanding of their transport function and interactions. We will review recent progress in the elucidation of the structural dynamics of these monoamine transporters (MATs), their conformational landscape and transitions, and their allosteric regulation mechanisms. We will present a comparative study of the structural dynamics and ligand-binding properties of two MATs, dopamine transporter (DAT) and serotonin transporter (SERT), with focus on the allosteric modulation of their transport function. Allosteric regulation is achieved by drugs or substrates that consistently bind a secondary site S2. Our analysis of a dataset comprising 50 structures resolved for DAT and SERT, in the presence of various ligands/drugs, identifies key residues such as E494, P561, and F556 in hSERT that consistently coordinate small molecule allosteric modulators. Further analysis reveals how DAT and SERT's the first principal mode (PC1) of structural changes underlies the transition between outward- and inward-facing states as well as their gating; whereas PC2 supports the rearrangements of TM helices near the S2 site. Finally, the examination of cross-correlations between hSERT structural elements points to coupled motions between TM6a residues F335 and G338 and TM10 E493-E494-T497, which supports the allosteric communication between S1 and S2.
These results support the design of allosteric modulators for better control of specific interactions and cellular pathways, rather than simply inhibiting the transporter at its orthosteric site.

## #27: The 5-HT7 Receptor as a Druggable Target

**Stephanie Watts (Chair), Finn Levy (Co-Chair)**

State of the 5-HT7 receptor: structure, signalling, pharmacology
**Finn Olav Levy**
The 5-HT7 receptor is one of the three Gs-coupled 5-HT receptors. It is detectable in several tissues, but more prominent in the nervous system and gastrointestinal tract, and is involved in regulation of mood,

cognition, digestion, and vascular tone. It exists in three splice variants in humans. In addition to coupling to the G protein Gs, it has also been shown to couple to G12. All splice variants display significant constitutive activity in heterologous expression systems, i.e. they activate Gs in the absence of ligand, an activity that can be blocked to different extent by various antagonists which show varying degree of inverse agonist activity. The structure of the 5-HT7 receptor has been solved in an active complex with Gs. In addition, the 5-HT7 receptor displays some properties which are not yet fully explained. When expressed in cells, it seems to prevent other receptors from activating adenylyl cyclase. One possible reason for this is that the receptor appears preasscociated with Gs, i.e. associated with Gs in the absence of ligand. This has been shown with several imaging methods, such as FRAP (fluorescence recovery after photobleaching), FRET (fluorescence/Förster resonance energy transfer) and single molecule imaging. Although the significance of this in vivo is unknown, a possible effect is to maintain the dynamic range of agonist-induced signalling.

The 5-HT7 Receptor as a Target in Hypertension

**Stephanie Watts**

Hypertension is the number 1 modifiable factor for lowering risk of cardiovascular disease. While first and second line therapies exist, the management of hypertension faces the challenge of three new R's: regression of blood pressure control; resistance (uncontrolled with 3 meds); and refractoriness (uncontrolled with 5 meds). This sobering problem is the impetus for new discoveries that can help transform the treatment of a disease that literally leads to death. We have spent years understanding how serotonin (5-hydroxytryptamine, 5-HT) reduces blood pressure; serotonergics are absent from current therapies. We share mechanisms of how this hypertension is effected, at least in the rat. Venous relaxation and arteriole relaxation is mediated by the 5-HT7 receptor, validated by use of a 5-HT7 receptor knockout lab we created. Additionally, the activated 5-HT7 receptor suppresses 5-HT2A receptor mediated contraction. The discovery of the ability of the 5-HT7 receptor to be biased in signalling provided an additional avenue to consider relative to therapeutic development. We used the recently described β-arrestin selective 5-HT7 receptor agonist Serodolin to test the hypothesis that 5-HT7 activation does not cause vascular relaxation or hypotension via the β -arrestin pathway. Isolated abdominal aorta (no functional 5-HT7) and vena cava (functional 5-HT7) from male Sprague Dawley rats were used in isometric contractility studies. Serodolin (1 nM – 10 μM) did not change baseline tone of isolated tissues and did not relax the endothelin-1 (ET-1)-contracted vena cava or aorta. In the aorta, Serodolin acted as a 5-HT2A receptor antagonist, In the vena cava, Serodolin acted as a 5-HT7 receptor antagonist, shifting concentration response curve to 5-HT left and upward (% of10 μM NE contraction; Veh = 3.2±1.7; Ser (10 nM) = 58±11; $p < 0.05$) and blocking relaxation of the contracted tissue to the 5-HT1A/7 agonist 5-carboxamidotryptamine. In anesthetized rats, though 5-HT caused concentration-dependent depressor responses, Serodolin caused an insignificant small depressor responses at all three infusion rates. Moreover, Serodolin blocked the 5-HT-induced fall in blood pressure. Thus, activation of the Gs pathway of the 5-HT7 receptor may prove beneficial in two ways: activating a relaxant receptor and promoting restraint of the contractile 5-HT2A receptor.

Targeting 5-HT7 receptors as a therapeutic strategy for intestinal inflammation

**Jensine Grondin**, Yun Han Kwon, Benjamin Blass, Huaqing Wang, Suhrid Banskota, Kenneth Korzekwa, Min Ye, John C. Gordon, Dennis Colussi, Kevin M. Blattner, Daniel J. Canney, Waliul Khan

Inflammatory bowel diseases (IBD) are serious chronic inflammatory conditions of the human bowel. In recent years, progress has been made in developing improved therapeutic strategies for IBD. However, these therapies have several disadvantages, including the potential for severe side effects, toxicity, and loss of efficacy over time. In addition, existing therapeutics only provide symptomatic relief, and a cure for IBD remains elusive. Thus, developing better treatment strategies for IBD, potentially by identifying new therapeutic targets, is of great importance. In IBD patients and across various animal models of colitis, alterations in serotonin (5-hydroxytryptamine; 5-HT) signalling has been observed. Of the 14 known receptor subtypes, 5-HT receptor type 7 (5-HT7) is one of the most recently discovered and is also present within the intestinal tract. Previously, we have reported that blocking 5-HT signalling with either a selective 5-HT7 receptor antagonist (SB-269970) or genetic ablation ameliorated intestinal inflammation in murine colitis. More recently, we developed novel antagonists targeting 5-HT7 receptors with high selectivity. When utilized in vivo, these antagonists significantly alleviated the severity of intestinal inflammation across various models of experimental colitis. These findings suggest that inhibition of 5-HT7 receptor signalling by

these novel antagonists may serve as an alternative mode of treatment to alleviate intestinal inflammatory conditions, including IBD.

Biased Signaling at the 5-HT7 Receptor: Novel Therapeutic Agents for Pain Modulation

**Séverine Morisset-lopez**

Transmembrane signaling through G protein-coupled receptors (GPCRs), originally described as requiring coupling to intracellular G-proteins, can also occur through G-protein-independent pathways via β-arrestin recruitment. Biased ligands, by stabilizing specific bioactive conformations of GPCRs, enable selective activation of these distinct signaling pathways. This concept of functional selectivity has emerged as a promising avenue for developing new therapeutic molecules, offering the potential for enhanced efficacy and fewer side effects by precisely modulating GPCR activity.

Over the past decade, the 5-HT7 receptor (5-HT7R) has gained attention as a therapeutic target for neuropsychiatric disorders, sleep and circadian rhythm disturbances, and pathological pain. We identified a small-molecule compound, Serodolin, which binds to 5-HT7R with nanomolar affinity. Our findings indicate that Serodolin exhibits biased activity: it acts as an antagonist/inverse agonist on Gs-mediated signaling while promoting ERK activation through a β-arrestin-dependent mechanism that requires c-SRC activation.

Furthermore, Serodolin significantly reduces hyperalgesia and pain perception in response to inflammatory, thermal, and mechanical stimuli. This antinociceptive effect was absent in 5-HT7R knockout mice and was completely blocked by SB269-970, a selective 5-HT7R antagonist, confirming the specificity of Serodolin's action. While 5-HT7R activation is traditionally linked to Gs-dependent adenylyl cyclase stimulation, our study, using a β-arrestin-biased agonist, uncovers an alternative molecular signaling mechanism.These findings highlight the therapeutic potential of 5-HT7R modulation in pain management.

# Short Oral Talks, Poster, and Travel Awardee Abstracts

**P1**

Multiplexed voltammetric serotonin measurements with carbon fiber multielectrode arrays

**Zestos A**, Alyamni N

Fast scan cyclic voltammetry (FSCV) is an electrochemical technique that measures several biomolecules via the oxidation at the electrode surface. Carbon fiber microelectrodes (CFMEs) have been used to measure neurotransmitters with FSCV due to their ability to adsorb cationic monoamine neurotransmitters such as serotonin. Carbon fiber multielectrode arrays has risen to target multiple brain regions simultaneously. We characterized a novel carbon fiber multielectrode array (MEA), a 4-channel electrode, and found it comparable to the single channel CFME in sensitivity and selectivity. The MEA, along with a multichannel potentiostat, had the additional capability of multiplexing neurotransmitter measurements in vitro by multi-waveform application. We utilized the multielectrode array and four-channel potentiostat to measure selective serotonin reuptake inhibitor (SSRI) escitalopram stimulated levels of serotonin in the caudate putamen in coronal mouse brain slices ex vivo. This yielded a sensitivity of 3.33 nA/μM with a limit of detection 100 nM. Here, we utilize the MEA to detect neurotransmitters in the brain using electrical stimulation and SSRI induced serotonin release. We measured endogenous levels of serotonin and other monoamine neurochemicals such as dopamine and norepinephrine in the ventral tegmental area, caudate putamen, raphe nuclei, and substantia nigra. Measuring neurotransmitters will aide in understanding complex brain heterogeneity, the dynamic neurochemical environment, complex behaviors, and the effect of neurological disorders and drugs on the brain. We will measure the effects of SSRI anti-depressants and psychostimulants such as cocaine and amphetamine on serotonin transporter function and the extracellular increase in neurochemical levels.

**P2**

Cross-species and mechanistic studies of maternal serotonin effects on offspring neurodevelopment

Siegel R, Haft J, Yueh H, Kee N, Gomez W, Lee K, Ahn A, Rochwarger Y, Pineda I, Irfan A, Shuffrey L, Anderson G, Simpson N, Underwood M, **Veenstra-VanderWeele J**, O'Reilly Sparks K

We previously reported associations between maternal serotonin (5-HT) and offspring neurodevelopment in autism (2018; 2024). Here, we sought to further examine this relationship and potential mechanisms across species. In humans, we leveraged cord blood and placental samples from a South African birth cohort. Remarkably, cord blood and placental 5-HT were not correlated, suggesting post-delivery placenta levels are unlikely to represent fetal 5-HT exposure. We found a strong negative association between being born preterm and infant cord blood 5-HT ($p<0.00001$), and a positive association between gestational age and cord blood 5-HT in term babies ($p=0.01$). We further found a positive association between Mullen Early Learning Composite Score and cord blood 5-HT levels ($p=0.02$). In pregnant mice, injection of [3H]5-HT (i.v.) at E13.5-15.5 led to measurable [3H] counts in placenta and fetal brains ($p<0.001$). Increasing the amount of [3H]5-HT resulted in a non-linear increase in fetal brain counts ($p=0.03$). Pretreatment with the SSRI escitalopram (20 mg/kg, i.v.) resulted in a 5-fold increase in fetal brain counts ($p<0.00001$); whereas inhibition of the low affinity serotonin transporter, OCT3, had no effect ($p=0.98$). Knock-in mice with an overactive serotonin transporter showed increased maternal blood counts ($p<0.05$) and had offspring with decreased fetal brain counts ($p=0.01$), despite no significant differences in placenta counts. Preliminary data indicate that increasing maternal 5-HT levels may lead to increased pup vocalizations. These findings suggest that the maternal 5-HT system, likely including serotonin transport from the mother to the embryo, contributes to fetal brain 5-HT levels during an important neurodevelopmental window.

**P3**

Maternal high-fat diet promotes region and sex specific remodeling of serotonin circuits

**Patton M**, Bilbo S

Serotonin neurons are among the earliest to develop in the fetal brain, and proper circuit formation depends on maintaining optimal serotonin levels. Maternal nutrition is closely linked to fetal brain development. Our laboratory previously showed that a maternal diet high in saturated fat (mHFD) reduces fetal brain serotonin levels in male, but not female, offspring by altering microglial function in the dorsal raphe nucleus (DRN). Building on these findings, we now demonstrate that mHFD differentially affects the development of specific serotonin circuits: microglia underprune serotonin fibers in the nucleus accumbens (NAc) but not in the orbitofrontal cortex (OFC). We propose that heterogeneity in microglial serotonin receptor expression may underlie why some serotonin networks are vulnerable to diet-induced changes, while others remain resilient.

**P4**

H3 Serotonylation Regulates Developmental Gene Expression in the mPFC Contributing to Behavioral Response to Early Life Adversity

**Cunningham A**, Chan J, Brindley E, Holt L, Estill M, Shen L, Nestler E, Maze I

The serotonergic (5HTergic) system and its homeostasis in early life are essential for establishing proper brain architecture, and early life stress (ELS) can disrupt this process, increasing lifetime risk for affective disorders. Beyond its receptor-mediated roles, 5HT can covalently bind to histone H3 tails, forming the novel histone modification H3 serotonylation (H3 Ser.). While H3 Ser. regulates neuroplasticity in vitro, its role during postnatal development and how ELS impacts this modification in vivo remains unclear. Here, we used region-specific transcriptomic profiling to examine normal and ELS-induced transcriptional changes in the dorsal raphe nucleus (DRN) and their impact on serotonergic projections to the medial prefrontal cortex (mPFC). We observed reduced 5HTergic innervation and serotonin abundance in the mPFC, a region central to stress response and behavioral regulation. Using FANS-coupled CUT&RUN and RNA-sequencing in male and female mice, we characterized H3 Ser. enrichment across development and under ELS. Developmental and ELS-induced differences in H3 Ser. were sex-specific and cell-type dependent, with pronounced effects in glia. In glia, ELS increased H3 Ser. differential loci by over 150-fold in adolescence—the most dramatic shift across all conditions. Bioinformatics identified oligodendrocytes as particularly impacted. ELS increased H3 Ser. signal in oligodendrocyte-lineage cells and oligodendrocyte precursor cell numbers, which may impact synaptic pruning in the mPFC. Importantly, these circuit, transcriptional, and epigenetic changes were associated with impairments in cognitive flexibility during adolescence. Together, these findings reveal how ELS disrupts serotonergic signaling in the DRN and its projections, contributing to PFC-dependent behavioral alterations through dysregulated H3 serotonylation.

**P5**

The selectivity filter of serotonin transporter is comprised of extracellular loop 2 and 4

**Zhu R**, Sandtner W, Holy M, Kudlacek O, Ebner A, Gruber H, Freissmuth M, Newman A, Sitte H, Hinterdorfer P

Serotonin (SERT) and dopamine (DAT) transporters regulate neurotransmission by reabsorbing released serotonin and dopamine into presynaptic neurons. These transporters are key drug targets for treating

disorders like depression, ADHD, and addiction. Drug selectivity is crucial—SERT inhibitors treat depression, while DAT inhibitors alleviate ADHD symptoms. Traditionally, selectivity was attributed to dissociation rates (koff), but recent studies suggest that association rates (kon) also play a role (ref.1,2,3), implying a selectivity filter in the transporters' entry pathway. To investigate this, we used atomic force microscopy (AFM) to measure desipramine's interaction forces with SERT and DAT at the molecular level. Since desipramine contains a secondary amine, we developed a novel method to conjugate it to AFM tips using a bromoacetate reaction. Force measurements on CHOK1 cells expressing fluorescently tagged SERT or DAT confirmed binding specificity, as free desipramine prevented interaction. By fitting unbinding forces to Bell-Evans' theory, we determined koff, while kon was obtained by varying AFM dwell times on the cell surface. Our results showed that while koff was similar for both transporters, kon for SERT was six times higher than for DAT, indicating that desipramine's selectivity is driven primarily by its association rate. Blocking extracellular loops 2 and 4 reduced kon for SERT but not DAT, with negligible effects on koff. This suggests these loops contribute to a selectivity filter that facilitates desipramine entry to SERT's primary binding site, providing new insights into transporter-targeting drug design.

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**P6**

Socio-affective communication through ultrasonic vocalizations in Tph2-deficient rat pups: Communal nesting aggravates growth retardation despite ameliorating maternal affiliation deficits

Wang T, Homberg J, Boreggio L, Samina M, Castro R, Kolk S, Alenina N, Bader M, Dai J, **Wöhr M**

A lack of serotonin (5-hydroxytryptamine, 5-HT) in the brain due to deficiency of the rate-limiting enzyme in 5-HT synthesis, tryptophan hydroxylase 2 (TPH2), was recently reported to result in impaired maternal affiliation across species, including mice, rats, and monkeys. In rodents, this was reflected in a lack of preference for maternal odors and reduced levels of isolation-induced ultrasonic vocalizations (USV), possibly contributing to a severe growth retardation phenotype. Here, we tested whether growth retardation, maternal affiliation deficits, and/or impairments in socio-affective communication caused by Tph2 deficiency can be rescued through early social enrichment in rats. To this aim, we compared male and female Tph2-/- knockout and Tph2+/- heterozygous rat pups to Tph2+/+ wildtype littermate controls, with litters being randomly assigned to standard nesting (SN; one mother with her litter) or communal nesting (CN; two mothers with their two litters). Although CN ameliorated the maternal affiliation deficit caused by Tph2 deficiency, CN did not rescue the socio-affective communication deficits and aggravated growth retardation. Deficits in socio-affective communication were reflected by reduced emission of isolation-induced USV, associated with changes in acoustic features, clustering of subtypes, and temporal organization. To close the communicative loop between mother and pup, we assessed maternal preference and showed that mothers display a preference for Tph2+/+ controls over Tph2-/- pups, particularly under CN conditions. Together, this indicates that CN aggravates growth retardation despite ameliorating maternal affiliation deficits in Tph2-deficient rat pups, possibly due to reduced and acoustically altered isolation-induced USV, hindering efficient socio-affective communication between mother and pup.

**P7**

Serotonin transporter abundance predicts the long-term SSRI treatment effect

**Matej Murgaš**

Major depressive disorder (MDD) is a highly prevalent psychiatric condition, with selective serotonin reuptake inhibitors (SSRIs) serving as a first-line pharmacological treatment. This study investigates the relationship between serotonin transporter (SERT) levels and SSRI treatment response. Using a [11C]DASB PET scan, we measured the volume of distribution (VT) of SERT in 30 unmedicated MDD patients (16 female), all of whom had been drug-free for at least 3 months. Following the scan, participants commenced a 6-week course of 10mg escitalopram. Symptom severity was evaluated using the Montgomery Åsberg Depression Rating Scale (MADRS) at baseline, week 2, and week 6. We examined the association between the percentage change in MADRS and VT in the anterior cingulate cortex, insula, amygdala, hippocampus, putamen, thalamus, striatum, midbrain, dorsal raphe nuclei (DRN), and medial raphe nuclei. A general linear model, adjusted for age, revealed an association ($p_{corr}<0.05$) between the change in MADRS after 6 weeks and VS of all regions except the insula and ACC. However, only the DRN ($F=10.36$; $p_{corr}<0.05$) remained statistically significant after correction for multiple tests. Post-hoc analysis indicated a positive correlation between VS and change in MADRS ($r_{DRN}=0.52$). No association between SERT distribution and the change in MADRS after 2 weeks was observed. Consistent with previous findings, the therapeutic effects of SSRIs generally manifest only after an extended treatment period. The observed relationship between pretreatment SERT levels and alterations in symptom severity may serve as a predictor of treatment outcome, potentially enhancing therapeutic efficacy.

**P8**

Evidence for low affinity of GABA at the vesicular monoamine transporter VMAT2

**Steinkellner T**, Srinivasan S, Limani F, La Batide-Alanore S, Hnasko T

Monoamine (dopamine, DA; serotonin, 5-HT; norepinephrine, NE) neurons comprise a heterogeneous population of cells. For instance, some DA and 5-HT neurons express vesicular glutamate transporters allowing these cells to co-release monoamines and glutamate. Additionally, GABA may be co-released from DA neurons. However, most DA neurons do not express the canonical machinery to synthesize GABA or the vesicular GABA transporter VGAT. Instead, GABA seems to be taken up into DA neurons by a plasmalemmal GABA transporter (GAT1) and stored in synaptic vesicles via the vesicular monoamine transporter VMAT2. Yet, it remains unclear whether GABA indeed interacts with VMAT2, or whether another transmitter could be responsible for the observed inhibitory effects attributed to GABA. Here, we used radiotracer flux measurements in VMAT2 expressing HEK-293 cells and synaptic vesicles from rodents to determine whether GABA qualifies as substrate at VMAT2. We found that GABA reduced uptake of VMAT2 substrates (5-HT and DA) in mouse synaptic vesicle preparations from striatum and cerebellum at millimolar concentrations but had no effect in VMAT2-expressing cells. Interestingly, while the closely related amino acid glycine did not affect substrate uptake at VMAT2 in mouse synaptic vesicles, the amino sulfonic acid taurine reduced uptake similar to GABA. Lastly, we discovered that 20-60 % of monoamine neurons in the substantia nigra, raphe nuclei and locus coeruleus express VMAT2 and GAT1 suggesting that many of them could be capable of co-releasing monoamines and GABA. Together, our findings suggest that GABA is a low-affinity substrate at VMAT2 with potential implications for monoamine physiology.

**P9**

Alterations of Cognitive Behaviours and Prefronto-Thalamic Circuits after Early-Life Exposure to Fluoxetine

**Soto N**, De Stasi A, Keshishian L, Gaspar P, Aguirre A, Karalis N, Bacci A

Depression research traditionally focused on emotional symptoms, but cognitive and neurovegetative impairments are also key features. The prefrontal cortex (PFC) is central to cognitive control and emotional regulation, and its dysfunction is implicated in depression pathogenesis. In the context of cognitive control, the PFC forms reciprocal interactions with the mediodorsal nucleus of the thalamus (MD), among other regions. During early postnatal development, a subset of deep-layer PFC pyramidal neurons (PNs) transiently express the serotonin transporter (SERT), making them particularly sensitive to environmental influences as selective-serotonin-reuptake-inhibitors like fluoxetine (FLX). Perinatal fluoxetine treatment (PNFLX) in rodents results in PFC hypo-excitability, altered SERT+ PN firing, and impaired adult emotional behaviours. Given the dense bidirectional connectivity between SERT+ PNs and MD-neurons, we used anatomical labelling, in-vivo and in-vitro electrophysiology, and cognitive tests to dissect the effects of PNFLX on the prefronto-thalamic loop and cognitive functions. We found that PNFLX leads to cognitive deficits, including novelty aversion and sex-specific impairments in learning and attentional flexibility, tasks reliant on intact prefronto-thalamic connectivity. Optogenetic mapping of reciprocal connections between the PFC and MD revealed that PNFLX reduced corticothalamic inputs from SERT+ PNs onto MD-neurons. Conversely, MD recruitment of L2/3-PNs was increased by PNFLX. Ongoing high-density electrophysiology can further elucidate network-level changes. Our findings suggest that altered serotonin signalling during early development disrupts prefronto-thalamic circuits, producing cognitive symptoms associated with neuropsychiatric disorders. This highlights the importance of developmental timing and circuit-specific effects in the cognitive pathology of depression, and underscores the PFC-MD pathway as a target for intervention.

**P10**

Constitutive serotonin tone as a determinant of metabolic homeostasis: insights from selectively bred WZ-5HT rat sublines

**Štefulj J**, Kesić M, Baković P, Čičin-Šain L

Serotonin (5-hydroxytryptamine, 5HT) is an important regulator of metabolic homeostasis, with its central and peripheral actions often exerting opposing effects. Using selectively bred Wistar-Zagreb 5HT (WZ-5HT) rat sublines with constitutionally high (high-5HT) or low (low-5HT) whole-body 5HT tone, we investigated how endogenous serotonin activity modulates energy balance, adiposity, glucose metabolism, thermogenesis, and diet response. On standard chow, high-5HT animals exhibited higher body weight, greater white adipose tissue and worse glucose/insulin tolerance compared to low-5HT anaimals, accompanied by modest transcriptional shifts favoring orexigenic signaling in the hypothalamus and lipogenic pathways in white adipose tissue. In addition, the high-5HT animals showed reduced brown adipose tissue (BAT) activity and thermogenic gene expression both under control conditions and in response to cold exposure or β3-adrenergic stimulation. Surprisingly, these high-5HT rats demonstrated relative metabolic resilience when challenged with a high-fat diet, while the low-5HT counterparts were more susceptible to diet-induced obesity and insulin resistance. Our results indicate that constitutionally higher serotonin tone promotes positive energy balance and adiposity under normal conditions, but may confer protection against metabolic deterioration in an obesogenic environment. These findings provide new insights into the bidirectional role of 5HT in the regulation of metabolic plasticity and suggest a potential contribution of 5HT to the phenomenon of metabolically healthy obesity.

**P11**

SERT N-terminal domain encodes determinants of PKG/p38aMAPK activation

**Fenollar Ferrer C**, Quinlan M, Meinke C, Lüthi D, Hofmaier T, Schütz G, Stockner T, Sitte H, Blakely R

The serotonin (5-HT) transporter (SERT) terminates 5-HT signaling between neurons. SERT transport rates can be modulated by Ser/Thr directed protein kinases as well as lipid interactions. PKG activation increases intrinsic 5-HT transport rate accompanied by Thr276 phosphorylation, residue required for SERT activation. A single mutation in the N-terminus of SERT, Gly56Ala, is sufficient to induce 5-HT transport rate acceleration via a PKG/p38MAPK dependent mechanism. Interestingly, the N-terminus is also necessary for pCA-induced 5-HT efflux, showcasing the N-terminus as a key player in SERT regulatory processes as well as conformations linked to 5-HT efflux. However, the mechanisms by which this small 80 residue tail contributes to SERT activation and 5-HT efflux are still unknown, mainly due to the lack of structural information of this domain. Here we report our results using in vitro biochemical studies in association with computational techniques to gain insight into the roles of the SERT N-terminus in the mechanisms by which a) SERT achieves rate acceleration and b) pCA-induced 5-HT efflux arises. We examined Val11, Thr53, Ser54, Glu58 and Ala59 as N-terminal residues whose mutation to their human SERT identities restores PKG/p38MAPK sensitivity on an otherwise PKG/p38MAPK insensitive mouse 129S6 strain. The PKG/p38MAPK activation-determining residues are located along with Gly56 within a binding site that may also be linked to lipid binding. This interconnection of PKG/p38MAPK and lipid regulatory pathways is further supported by their structural co-localization with Thr276, highlighting the key role of the N-terminus in SERT activation.

**P12**

Dynamic Duo: Serotonin Transporter and Organic Cation Transporter 3 Regulate Basolateral Amygdala Serotonin Clearance and Fear Memory Recall

Shin S, Honan L, Owens W, Horton R, Toney G, **Daws L**

Dysregulation of serotonin (5-HT) neurotransmission in basolateral amygdala (BLA), a key integration hub for processing emotional stimuli, is crucial for aberrant fear memory. The high-affinity, low-capacity 5-HT transporter (SERT), the target of selective 5-HT reuptake inhibitors (SSRIs) commonly used to treat post-traumatic stress disorder (PTSD), is richly expressed in BLA. However, SSRIs often provide suboptimal therapeutic benefit. Organic cation transporter 3 (OCT3), a low-affinity, high-capacity 5-HT transporter is also richly expressed in BLA. However, its contributions to BLA 5-HT clearance and fear memory are unknown. Here, we tested the hypothesis that SERT and OCT3 on 5-HT neurons are important both for BLA 5-HT clearance and for fear memory formation and recall. Using in vivo high-speed chronoamperometry, we found that 5-HT clearance in BLA was slowed at low but not high extracellular concentrations and that the SSRI fluvoxamine was without effect in mice with SERT knockout compared to controls. In OCT3 knockout mice, 5-HT clearance was slowed both at low and high extracellular concentrations, suggesting OCT3 contributes more to 5-HT uptake than previously appreciated. Behaviorally, fear learning was unaffected by SERT or OCT3 knockout on 5-HT neurons. In OCT3 knockout mice, however, freezing was reduced during context recall 4 days later. Preliminary results suggest SERT knockout mice display reduced freezing in cued recall. Findings suggest that SERT and OCT3 each play an active role in fear memory recall, opening the possibility that pharmacotherapeutics targeting OCT3 and SERT concurrently may have greater therapeutic response than SSRIs alone.

**P13**

Psilocybin induces interneuron plasticity in a cell subtype specific manner

**Carrillo A**, Strong H, Hines D, Hines R

Psilocybin has been shown to be an effective therapeutic in disorders of mental health, yet its mechanism of action remains to be fully understood. Psilocybin and other psychedelics have been demonstrated to produce a critical period-like state characterized by increases in excitatory pyramidal cell dendritic spine density. Rapid increases in dendritic spine density, unaccompanied by uncontrolled activity is suggestive of the presence of a homeostatic regulatory mechanism. GABAergic interneurons of several classes modulate cortical signaling by regulating pyramidal cell firing rates, but changes to interneuron morphology and connectivity have not been explored following psilocybin treatment. We find that psilocybin most potently increases disinhibitory Vasoactive Intestinal Polypeptide (VIP) interneuron dendrite arborization in comparison to other interneuron subclasses. The effect on arborization is primarily concentrated on lower order dendrites of the VIP cells. In contrast, cells of basket and chandelier morphology show reductions in arborization following psilocybin. We corroborate these results by examining VIP puncta, and find an increase in the total number of VIP positive clusters. Parvalbumin (PV) positive cluster densities decrease following psilocybin treatment, suppression that is consistent with the effects on basket and chandelier cell morphology. In contrast to PV, cholecystokinin (CCK) positive cluster densities increase, suggesting that this subtype of basket cell is facilitated following psilocybin treatment. With the growing list of disorders implicating subtypes of interneurons in pathophysiology, this understanding may lead to new applications for psychedelics and related compounds.

**P14**

Serotonergic Modulation of Auditory Habituation: DiPT-Induced Plasticity in the Inferior Colliculus and Auditory Cortex

**Strong H**, Bothwell M, Carrillo A, Hines R, Hines D

Psychedelics, through their modulation of serotonergic systems, have garnered interest for their therapeutic potential in neuropsychiatric disorders. While much research has focused on their effects on mood and cognition, less is known about their influence on sensory processing. The understudied psychedelic N,N-Diisopropyltryptamine (DiPT) uniquely alters auditory perception, providing a window into serotonergic modulation of auditory circuits. Using mouse models, we examined the effects of DiPT on auditory habituation and plasticity within auditory nuclei. Electroencephalography revealed significant alterations in auditory event-related potentials (ERPs), including prolonged latencies and amplitude shifts indicative of impaired habituation. Histological analysis of the inferior colliculus (IC) and auditory cortex using Golgi-Cox staining showed increased spine density, particularly in neurons involved in high-frequency sound processing. These findings suggest that DiPT enhances synaptic plasticity in auditory pathways, potentially disrupting habituation mechanisms. Ongoing studies are exploring synaptic remodeling mechanisms mediated by neuronal and non-neuronal cells. These findings provide insight into how psychedelics influence auditory processing and may inform therapeutic approaches for disorders characterized by sensory and habituation deficits, such as autism spectrum disorder and schizophrenia.

**P15**

Investigation into the signalling pathways of CPL298 - a novel 5-HT7 receptor agonist demonstrating efficacy in preclinical models of neuropathic pain

**Majkowska I**, Skieterska K, Kokhanovska S, Skowrońska K, Krzemień J, Jędrzejewska N, Żero P, Janusz A, Górka M, Szybiński M, Moszczyński-Pętkowski R, Gurba-Bryśkiewicz L, Smuga D, Dubiel K, Kurowska K, Kordylewski S, Lenda T, Satała G, Hogendorf A, Bojarski A, Wieczorek M, Matłoka M

Neuropathic pain is a chronic and often debilitating condition resulting from nerve damage, caused by injury, diabetes or chemotherapy-induced neurotoxicity. Current treatments include anticonvulsants, antidepressants and opioids, which offer limited pain relief and can have significant side effects. As a result, there is a growing need for more effective therapy for neuropathic pain to improve patient outcomes and quality of life. 5-HT7R, a G-protein coupled receptor for serotonin, has been implicated in modulation of pain. It is expressed in regions related to nociceptive pathways in central and peripheral nervous system. Activation of 5-HT7R has been shown to have antinociceptive effects in animal models, making it a promising target for new therapies. Here, we present CPL298, a novel and specific agonist for 5-HT7R. We previously have demonstrated that CPL298 reduces both mechanical and thermal hypersensitivity in several animal pain models. To determine signalling pathways activated by CPL298, HEK293 cells overexpressing 5-HT7R were treated with agonists with or without inhibitors, including a PKA inhibitor and an inhibitor of the β-arrestin/β2-adaptin interaction. Our results show that CPL298 is a potent 5-HT7R agonist that activates Gs protein, leading to an increase in cAMP and PKA dependent phosphorylation of ERK1/2 and CREB. Data from pharmacokinetic study in mice show that CPL298 has limited penetration into brain and spinal cord, indicating peripheral action – possibly targeting dorsal root ganglia and primary sensory neurons. The obtained results provide insights into the mechanism and site of action of CPL298, enhancing our understanding of the drug's antinociceptive effect.

**P16**

Unlocking the potential of partial efficacy: a novel insight into monoamine neurotransmitter transporters

**Islam M**, Niello M, Gajdošová K, Kooti F, Sitte H

Partial efficacy refers to a ligand's ability to induce submaximal response in the protein of interest, where precision and balance replace the blunt force of classical therapeutic strategies. While partial efficacy and its benefits at G protein-coupled receptors has been extensively characterized, it is still a puzzling phenomenon at monoamine neurotransmitter transporters (MATs) that encompass, among other monoamines, the transporter for serotonin (SERT). MATs are crucial membrane proteins that balance synaptic neurotransmission by removing previously released neurotransmitter molecules from the synaptic cleft back into the releasing neuron. Their activities make them critical molecular determinants in the treatment of neuropsychiatric disorders. However, fine-tuning the activity of these homeostatic machines remain a challenge. Current classical MATs modulators often lead to poor therapeutic responses, with inhibitors leading to delayed onset of therapeutic action and releasers causing unrestrained neurotransmitter release. Thus, it is imperative to understand partial efficacy at MATs to unlock a promising strategy for fine-tuning synaptic neurotransmission in a controlled manner. In the current study, we have systematically probed several established chemical compounds in MATs-expressing HEK293 cells by combining radiotracer flux assays (uptake and release) and patch-clamp electrophysiology in the whole-cell mode. Among the compounds tested at SERT, three out of seven robustly demonstrated partial efficacy. These compounds induce 5-HT release via SERT at a lower magnitude compared to the fully efficacious releasing agents. Our findings provide structure-activity relationships of partial efficacy at SERT and suggest that drug-induced neurotransmitter release could be pharmacologically fine-tuned with enhanced precision and safety.

**P17**

Action of Antidepressants to Induce Emesis and Alter Gastric Myoelectric Activity

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Strategies to treat depression and other disorders include the use of drugs to elevate extracellular levels of serotonin, noradrenaline and/or dopamine. The increases in central monoamine and/or catecholamine levels are thought to induce secondary neurochemical/receptor changes necessary for a positive clinical outcome. However, side effects of nausea and emesis are thought to involve the gastrointestinal tract and abdominal vagus. Ferrets and Suncus murinus (house musk shrew) were dosed orally with fluoxetine (10-100 mg/kg), duloxetine (30-100 mg/kg), venlafaxine (30-100 mg/kg), or vehicle (mulgofen, 10 ml/kg) and observed for 24 h. Fluoxetine was slightly more potent than duloxetine to induce emesis in both the ferret and Suncus murinus; venlafaxine was not consistently emetic. Fluoxetine did not modify food intake, but duloxetine and venlafaxine reduced food consumption in the ferret, but not Suncus murinus. The effect of drugs on Suncus murinus gastrointestinal slow wave activity was assayed using tissue sections on a microelectrode array platform. Fluoxetine (10 μM), duloxetine (10 μM), and venlafaxine (10 μM), reduced the frequency of slow wave activity in the duodenum; in the ileum only fluoxetine (10 μM) and duloxetine (10 μM) were inhibitory. Duloxetine (10 μM) and venlafaxine (10 μM) enhanced slow wave activity in the colon, but fluoxetine (10 μM) was inhibitory. The relative potencies of the antidepressants in the behavioural studies indicates that serotonin may contribute to emesis more than dopamine and/or noradrenaline. The action to modify upper and lower gastrointestinal slow wave activity may contribute to emesis and effects on food consumption.

**P18**

BEYOND a neurotransmitter: serotonin as a neuromodulator factor in the structural and behavioral development of the PFC

**Samina M**, Castro R, Lodder T, Merkelijn T, Hanswijk S, Garcia L, Witteveen J, Migliarini S, Pasqualetti M, Alenina N, Bader M, Homberg J, Kolk S

The prefrontal cortex (PFC), responsible for higher cognitive tasks, is often impaired in serotonin (5-HT)-related neuropsychiatric disorders and often finds its origins during development. Proper cortical development relies on intrinsic (transcription factors, local placental-derived 5-HT) and extrinsic (instructing 5-HT and catecholaminergic fibers) events. Recently, our group showed a functional interplay between the 5-HT and catecholaminergic prefrontal projection systems during development. Surprisingly, lack of the high-affinity 5-HT transporter (5-HTT), resulting in high 5-HT levels, affected not only 5-HT- but also TH+ projections towards the PFC. In 5-HTT-/- rats, the number and distribution of reelin-releasing Cajal-Retzius cells were affected in early postnatal stages, as was cortical layer identity. Further, we showed that 5-HTT is also expressed in the cortex during embryonic development even before any neurotransmitter system reaches the PFC. Therefore, we hypothesize that there is an intrinsic and extrinsic effect of 5-HT levels during PFC development, which can affect reelin release, cortical layering, and PFC-mediated cognitive functioning. To test this, we assessed how lifelong high (5-HTT-/-) and low (TPH2-/-) 5-HT levels affect prefrontal innervation and PFC microstructure during prenatal stages. Preliminary data showed altered dopaminergic transporter (DAT) expression and disrupted 5-HT and TH+ innervation in 5-HTT-/- embryos. Concurrently, a longitudinal behavioral assessment in TPH2-/- rats revealed deficits in motor and sensory reflex development early postnatally, physiological abnormalities, and impaired exploratory and anxiety-related behaviors in late adolescence and adulthood. Taken together, we aim to dissect the role of 5-HT in PFC development, its impact on postnatal maturation, and consequently on PFC-steered behavior.

**P19**

Morphological Analysis of Histamine Induced HumanDerived Serotonergic Neurons

**Pablo Prieto Roca**, Bettina Bohl, and Parastoo Hashemi

Depression is a common mental health disorder that affects over 300 million people worldwide [1]. Despite its prevalence, the pathophysiology of depression remains fragmented across various theories [2]. Recent research suggests that histamine may play a crucial role in synergizing the inflammation and monoamine theories [3]. Inflammation-induced histamine has been implicated in neurotransmission by modulating extracellular serotonin levels [4]. However, the exact role of this interplay in the pathophysiology of depression remains unclear. Emerging evidence suggests that stress-induced mental illnesses, mediated by neuroinflammation, result in alterations in the morphology of the serotonergic system [5]. Interestingly, this co-modulation of serotonin (5-HT) and histamine is also evident in a mouse model of stress-induced depression, highlighting histamine's potential role in brain remodeling [6]. We hypothesized that histamine may change the morphology of serotonergic neurons, and that these changes may play an important role in how plasticity is involved in depression. The state of the art for neuronal morphology analysis has not been extended to iPSC-derived neurons. In this study, we present an efficient, open-source computational tool for Sholl analysis of neuronal iPSC cultures. With this tool, we demonstrate that histamine induces distinct morphological changes in neuronal cultures, particularly reducing proximal neuritic branching while promoting distal neuritic growth. This result adds to the knowledge of how inflammation may affect plasticity. Furthermore, we anticipate that this work will lay a robust foundation for general and rapid analyses of neuronal morphology.

**P20**

Is there a functional role of phosphorylation in organic cation transporter 3 (OCT3)?

**Skopec S**, Angenoorth T, Yang J, Sitte H

The organic cation transporter 3 (OCT3) is a transmembrane protein involved in the transport of various endogenous substances such as serotonin and other monoamines - as well as xenobiotics across cellular membranes. The aim of this study is to investigate the effects of phosphorylation on OCT3 functions with in vivo phosphorylation sites and responsible protein kinases and phosphatases. Additionally, the study seeks to understand the pharmacodynamic effects of OCT3 genetic variants on its phosphorylation-mediated functions and the involvement of phosphorylation in pharmacokinetics of tyrosine kinase inhibitors (TKIs). The utilized techniques include site-directed mutagenesis, radiotracer flux assays, mass spectrometry (MS) and confocal laser scanning microscopy. Initial experiments identified the phosphorylation at Ser3 and Ser537 on OCT3, from which dephosphomimetic mutants were generated. Both showed no significant difference in substrate uptake compared to the wildtype transporter, suggesting that these phosphorylation sites are not crucial for substrate influx via OCT3. MS identified low molecular weight protein tyrosine phosphatase (LMW-PTP) in the OCT3 complex (as an interacting protein of OCT3) and its functional implication was examined with LMW-PTP inhibitors. They did not significantly affect the uptake function of OCT3. We will continue to study the functional involvement of pS3/pS537 and interacting protein kinases/phosphatases as well as the effects of OCT3 phosphorylation on the so-called phosphotyrosine switch mechanism of TKIs and possibly different transport inhibition properties of TKIs for OCT1, 2 and 3, which will provide novel mechanistic insights into drug-drug interactions and their side effects.

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**P21**

Simultaneous blockade of 5-HT3 and 5-HT6 receptors produce antipsychotic and procognitive properties in animal models

**Zajdel P**, Malikowska-Racia N, Gołębiowska J, Mekki L, Becamel C, Cyrano E, Grychowska K, Lamaty F, Nikiforuk A, Marin P, Popik P

The majority of novel antipsychotics are characterized by multitarget profile of action and preferentially block dopamine D2 receptors and/or serotonergic 5-HT2A/5-HT1A receptors. They partly alleviate psychotic symptoms but fail to treat the negative symptoms and cognitive deficits. An analysis of receptor profile of clozapine, the only medication used in the drug-resistant schizophrenia, revealed that in addition to antagonism of 5-HT2A receptor, it behaves as antagonist at 5-HT3 and 5-HT6 receptors. These observations prompted us to develop a compound FPPQ, the first-in-class dual 5-HT3/5-HT6 receptor antagonist. FPPQ displays balanced low-nanomolar affinity at both receptors, shows selectivity over 87 targets and decent brain penetration. We showed that FPPQ (1, 3 mg/kg, po) reversed PCP-induced hyperactivity and produced pro-cognitive effect in PCP-induced short-term memory model. Herein we report that FPPQ produced antipsychotic-like properties in the prepulse inhibition (PPI) test (3, 6 mg/kg, ip) in rats (a reliable tool displaying high predictive validity for the “positive” symptoms), evoked no catalepsy (1–6 mg/kg, ip) in rats and produced procognitive effects (2.5 mg/kg, ip) in the novel object recognition (NOR) task in Disc1-L100P mutant mice, a genetic model of schizophrenia. Overall, present data suggest that simultaneous blockade of 5-HT3 and 5-HT6 receptors provide a novel therapeutic approach to alleviate positive symptoms of schizophrenia and cognitive comorbidities. Further studies would focus on evaluating effects on FPPQ on the negative symptoms of schizophrenia to highlight a strategy of dual 5-HT3/5-HT6 receptor antagonists in antipsychotic drug discovery.

Acknowledgements

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**P22**

Serotonin signaling as a regulator of pelvic ganglion development in mice

**Murtazina A**, Galimullina R, Erickson A, Principi F, Adameyko I

The parasympathetic system, which antagonizes the sympathetic stress response, forms during embryogenesis from nerve-associated multipotent glial progenitor cells. However, the transcriptional mechanisms of parasympathetic ganglia formation, and diversity, remain unclear. The goal of our study is to identify critical cell states leading to parasympathetic ganglia during embryogenesis. To achieve this, we performed single-cell transcriptomic analysis of micro-dissected mouse parasympathetic ganglia on embryonic days E13.5 and E18.5. In particular, we hypothesized that serotonin signaling might play a role in parasympathetic neuronal differentiation, because we previously discovered a transitionary serotonin-responsive cell state called "bridge cells" which are necessary for adrenal gland development from Schwann cell precursors. Analyzing the pelvic ganglion, we indeed observed Htr3a-positive "bridge cells" at E13.5. Further, we found expression of serotonin in the pelvic ganglia at multiple embryonic stages. To understand the role of serotonin in the development of the pelvic ganglion, we injected 5-HTP into pregnant females at different stages of development. We found that if the injection occurred before or just at the beginning of pelvic ganglion formation (E11.5 and E12.5) it resulted in a reduction of pelvic ganglion size by almost half at E13.5, indicating a direct requirement for serotonin signaling in the pelvic ganglia development. Conversely, later-stage (E13.5 and E14.5) 5-HTP injection instead increased pelvic ganglia size. Thus, serotonin signaling may control parasympathetic ganglia formation by acting as an early regulator of cell fate decisions in Schwann cell precursor bridge state, but also promote later growth of the

pelvic ganglia by a separate mechanism.

**P23**

Fluoxetine Exposure Impairs Oocyte Quality: Revealing the Critical Role of SERT-Mediated Serotonin Uptake in Mammalian Oogenesis

**Tkachenko M**, Nikishin D

Serotonin is known to be a regulator of oocyte maturation in a large number of animal species. In maturing mammalian oocytes, the source of serotonin is selective membrane transporter of serotonin (SERT) activity. Previously we demonstrated that selective serotonin reuptake inhibitors (SSRIs) reduce the amount of serotonin in mouse blood, ovarian tissue and oocytes. In this work, we investigated the effects of reduced peripheral serotonin on preovulatory and postovulatory stages of oogenesis. To reduce the amount of serotonin in blood serum and oocytes, female mice were treated with fluoxetine dissolved in drinking water (0,13 mg/mL) for 10 days. After superovulation protocol we analyzed the oocytes matured under conditions of decreased serotonin levels. Quantitative analysis of postovulatory (MII) oocytes obtained during superovulation protocol revealed reduced number of ovulated oocytes per mouse in the experimental group. Morphological analysis based on the size of perivitelline space, the shape of the oocyte and first polar body, and form of the meiotic spindle revealed no significant differences between the fluoxetine-treated and control groups. The labeling of reactive oxygen species (ROS) revealed increased oxidative stress in oocytes related to the reduced mitochondrial activity. To assess the potential effects of fluoxetine on earlier stages of oogenesis, we analyzed the maturation of GV-oocytes obtained from the ovary after gonadotropin stimulation. The analysis of GV-oocyte chromatin staining with DAPI in the fluoxetine-treated group compared to the control group revealed a reduction in the proportion of SN (surrounded nucleolus) GV-oocytes which are considered to have better developmental potential. Moreover, quantitative analysis of in vitro matured (IVM) oocytes revealed a decrease in the proportion of oocytes developed to the stage of MII in the fluoxetine-treated group and an associated increase in the proportion of degenerated oocytes. Quantitative PCR analysis of these oocytes showed upregulation of cytoplasmic immaturity marker Zar1 in fluoxetine-exposed MII oocytes compared to controls. These results collectively indicate that serotonin, via SERT-mediated uptake, plays a critical role in ensuring proper oocyte maturation and developmental competence.

**P24**

Sex-Dependent Effects of 2,5-Methoxy-4-Iodoamphetamine (DOI) on Methamphetamine Self-Administration: Mechanistic Insights from Antagonism and PET Imaging Studies

**Wood B**, Patisual P, Christopher C, Barnes M, Lokitz S, Blough B, Murnane K

Methamphetamine (METH) is a highly addictive psychostimulant with no FDA-approved treatments, despite its significant public health impact. Psychedelics 5-HT2A agonists, are gaining clinical attention, yet their potential to attenuate METH reinforcement remains underexplored. This study investigated the effects of the synthetic psychedelic 2,5-methoxy-4-iodoamphetamine (DOI) on METH self-administration and 5-HT2A receptor involvement using the antagonist M100907. Male and female Sprague-Dawley rats were trained to self-administer METH on a fixed ratio 4 schedule of reinforcement. Following stable responding, DOI (0.1 or 0.32 mg/kg) was administered 15 minutes before sessions to assess dose-dependent effects on METH intake. The peak DOI dose was tested across varying METH doses (0.03 and 0.06 mg/kg/infusion) to evaluate shifts in the dose-response curve. To confirm 5-HT2A involvement, M100907 (0.1 mg/kg) was administered prior to the peak DOI dose. Food-maintained rats served as controls to assess behavioral specificity. PET imaging assessed in vivo 5-HT2A receptor occupancy (DOI: 0.1, 0.32, 1 mg/kg). DOI

significantly reduced METH intake in males but not females, with effects blocked by M100907. DOI more potently suppressed METH self-administration compared to food controls in males. At the peak dose (0.32 mg/kg), DOI achieved ~80% 5-HT2A receptor occupancy in both sexes, despite behavioral differences. These findings suggest 5-HT2A agonists, like DOI, could reduce METH intake, with sex-specific mechanisms warranting further study. Future research will explore the efficacy of psilocybin and other psychedelics to better understand their therapeutic potential in stimulant use disorder.

**P25**

Naturalistic Psychedelic Use and Its Association with Drug Use Patterns, Response Inhibition, and Negative Affect in Methamphetamine Recovery

**Vest F**, Barnett B, Wood B, Dumitrescu A, Cannon C, Bierbaum K, Patterson J, Murnane K

Background: Recent shifts in substance use disorder (SUD) treatment frameworks recognize that complete abstinence is not the only meaningful outcome, with the FDA now accepting changes in substance use patterns as clinically significant. Given serotonin's role in cognition and emotion regulation, serotonergic psychedelics may influence cognitive and affective factors relevant to drug use changes. This study examines whether a history of naturalistic psychedelic use is associated with reduced methamphetamine intake, prolonged abstinence, improved response inhibition, and lower negative affect.

Methods: Participants (N=37) were recruited from in-patient addiction treatment facilities and stratified based on lifetime psychedelic use (N=19 with history, N=18 without). Naturalistic psychedelic use was defined as non-clinical use of classical psychedelics. Participants reported their longest period of methamphetamine abstinence and estimated annual methamphetamine intake (g/year). Response inhibition was assessed using the Stroop Color & Word Task (SCWT), and negative affect was measured using the Depression Anxiety Stress Scale (DASS).

Results: Individuals with history of psychedelic use reported longer abstinence durations and lower annual methamphetamine intake. They exhibited faster, more accurate SCWT performance, indicating better response inhibition, and scored lower on the DASS, suggesting reduced negative affect.

Conclusions: Naturalistic psychedelic use may be associated with prolonged abstinence, reduced drug intake, enhanced cognitive control, and lower negative affect in individuals recovering from methamphetamine addiction. Future research should investigate underlying mechanisms and consider psychedelic use as a potential confound in addiction studies.

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**P26**

DNAJC12, a novel regulator of serotonin synthesis

Cao Y, Popp O, Milani N, Qadri F, Kühn R, Mertins P, Bader M, **Alenina N**

Tryptophan hydroxylases TPH1 and TPH2 are enzymes responsible for serotonin synthesis in the periphery and the central nervous system, respectively. Both enzymes belong to the aromatic amino acid hydroxylase (AAAH) family, which also includes phenylalanine hydroxylase (PAH), responsible for degrading phenylalanine, and tyrosine hydroxylase (TH), which produces dopamine. Here, we demonstrate that the co-chaperone DNAJC12 is specifically expressed in serotonergic neurons in the brain and is downregulated

in mice lacking TPH2, and thus central serotonin. DNAJC12 has previously been shown to regulate the stability of PAH, and mutations in its gene cause hyperphenylalaninemia and neurological symptoms in patients. We show that DNAJC12 also binds to and stabilizes TPH1 and TPH2 in transfected cells by preventing their proteasome-mediated degradation. To elucidate the role of DNAJC12 in regulating serotonin synthesis in vivo, we generated DNAJC12-deficient mice. These mice exhibit reduced levels and activity of PAH, TPH2, and TPH1 in the liver, brain, and pineal gland, respectively, and develop hyperphenylalaninemia along with central and peripheral serotonin deficiency. These findings highlight a pivotal role for DNAJC12 in the regulation of AAAH enzymes, and thereby in neurotransmitter synthesis and phenylalanine homeostasis, providing a mechanistic explanation for the complex neurological symptoms observed in patients carrying DNAJC12 mutations.

**P27**

Effects of dopamine D2/D3 receptor agonist quinpirole and a monoamine oxidase inhibitor clorgyline on neonatal communication in tryptophan hydroxylase 2 knockout rats

**Dorofeikova M**, Boreggio L, Wang T, Wöhr M, Bader M, Alenina N

Depletion of serotonin signaling in the brain by ablation of tryptophan hydroxylase 2 (TPH2) in rodents has been shown to lead to early postnatal growth retardation, among other delays in the expression of key developmental milestones, including a deficiency in neonatal communication, which may underlie growth retardation. Since the dopaminergic system is known to affect pup ultrasonic vocalizations (USVs) and may be mediating the serotonin effects, we used the monoamine oxidase inhibitor clorgyline, that reduces the breakdown of serotonin, norepinephrine, and dopamine and the more specific D2/D3 receptor agonist quinpirole to explore their effects on weight gain and neonatal communication in Tph2-deficient (TPH2-/-) rats. Clorgyline (10 mg/kg), quinpirole (1 mg/kg) or vehicle were administered from postnatal day (PND) 2 until PND 14. USVs were recorded on PND 4, 8 and 12 and analyzed using DeepSqueak. Tph2-/- rat pups had significant weight and communication deficits compared to Tph2+/+ animals. Clorgyline significantly increased weight gain in Tph2-/- rat pups and their USV frequency range. In a second set of experiments, Tph2-/- rat pups and their heterozygous littermates were administered quinpirole. Tph2-/- rat pups demonstrated significantly impaired communication, and quinpirole treatment was able to rescue deficits in the number of USVs and their frequency range towards PND 12. Tph2-/- rats gained significantly less weight than their Tph2+/- cage mates. The results of this study support the involvement of the dopaminergic system in the effects of serotonin on neonatal communication but not on weight gain.

**P28**

Preclinical Studies Examining the Prohedonic Effects of Psychedelics

**Kangas B**

There has been increased research into the medicinal potential of psychedelics, such as the 5-HT2A receptor agonist psilocybin, for a host of neuropsychiatric disorders. Anhedonia, defined as the loss of pleasure from previously rewarding stimuli, is a prominent and often debilitating phenotype in transdiagnostic clinical populations. The current studies evaluated the effects of psilocybin and key positive and negative control drugs in the rodent version of the Probabilistic Reward Task (PRT), a touchscreen-based assay that was reverse-translated from an RDoC-recommended task to objectively quantify anhedonic phenotypes in patient populations. Across groups of male rats (n=12/experiment), the effects of psilocybin (0.1-1.0 mg/kg), with or without pretreatment of the 5-HT2A antagonist volinanserin (0.03-0.3 mg/kg), the NMDA antagonist ketamine (10 mg/kg), the psychedelic selective 5-HT2A agonist (±)-DOI (0.3-3.0 mg/kg), the non-psychedelic 5-HT2A agonist lisuride (0.1-1.0 mg/kg), and the prototypical

SSRI fluoxetine (0.3-3.0 mg/kg) were studied for their ability to produce prohedonic phenotypes in the PRT. Psilocybin produced persistent dose-dependent increases in reward responsivity. Pretreatment with volinanserin blocked this effect, consistent with 5-HT2A mediation. Ketamine and (±)-DOI treatment produced similar effects acutely, although DOI's effects were no longer present 24 hours post dose. Lisuride failed to produce prohedonic phenotypes. Likewise, fluoxetine did not affect PRT performance, which is consistent with the inability of SSRIs to produce prohedonic effects in humans. Overall, the present findings demonstrate that psilocybin has rapid and enduring prohedonic efficacy in a translational rodent task, and that agonism of the 5-HT2A receptor is necessary, but not sufficient, for these effects.

**P29**

Peripheral Serotonin Reduction Promotes Atherosclerosis Progression

Alenina N, Parma L, Barbic S, Dorofeikova M, **Bader M**, Duchene J

Atherosclerosis is a chronic inflammatory disease characterized by plaque formation within arterial walls and is the primary cause of cardiovascular diseases (CVDs), the leading cause of death worldwide. Depression, another major global health burden, is two to three times more prevalent in CVD patients than in the general population and is considered a CVD risk factor. Serotonin acts as an autacoid in the periphery and as a neurotransmitter in the central nervous system. It is synthesized by two tryptophan hydroxylase enzymes: TPH1 in the gut and TPH2 in the brain. Its levels are regulated by uptake through the serotonin transporter (SERT), which controls both circulating and central serotonin. SERT is also the primary target of selective serotonin reuptake inhibitors (SSRIs), a widely used class of antidepressants. To investigate the role of serotonin in atherosclerosis progression, we used aged Apoe-/-Sert-/- and Apoe-/-Tph1-/- mice as models of long-term SERT inhibition and peripheral serotonin depletion, respectively. Both models developed significantly larger atherosclerotic plaques than Apoe-/- mice, suggesting that peripheral serotonin loss exacerbates disease progression. In addition, Apoe-/-Sert-/- and Apoe-/-Tph1-/- mice exhibited elevated cholesterol levels, indicating metabolic dysregulation. Of note, female Apoe-/-Sert-/- mice had fewer circulating monocytes, especially the protective nonclassical subsets, and reduced CD44 expression, pointing to impaired monocyte adhesion. In summary, long-term SERT inhibition may accelerate atherosclerosis through both metabolic and immune pathways, raising concerns about prolonged SSRI use in individuals at high cardiovascular risk.

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**P30**

Investigating the Polypharmacological Signaling Profiles of Psychedelics

**Lanham J**, Cavalco N, Bock H, Ivanich M, Bonniwell E, Hennessey J, McCorvy J

Psychedelics of several chemical classes have known affinity for G protein-coupled receptors (GPCRs) other than 5-HT2A, an important mediator of psychedelic effects. The polypharmacological profile examining other serotonin subtype signaling has not been thoroughly investigated. We used a BRET-based G protein biosensor platform capable of directly detecting G protein dissociation activity, avoiding common assay artifacts such as non-specificity and assay amplification due to receptor reserve. Our results show that tryptamines and ergoline psychedelics are the most promiscuous chemical series, with almost pan-5-HT receptor agonism. Activity of phenylethylamines, such as DOI, are more restricted toward the 5-HT2 subtypes. Other phenethylamines, such as 2C-B, shows potent agonist activity at 5-HT1B/1D/1e. Mescaline shows weak activity at 5-HT2 receptors, but unexpectedly shows similar potency at 5-HT1 targets. Comparing the most potent psychedelic chemical classes, ergolines and N-benzyls, we reveal that

LSD potently activates sympatholytic adrenergic (ADR) GPCR subtypes, ADRα2, unlike 25I-NBOMe, which is highly selective for 5-HT2 receptors. All psychedelics tested activated 5-HT2B to some degree, which is implicated in causing cardiac valvulopathy. Our results suggest that the psychopharmacology and possibly the therapeutic effects of psychedelics may involve several 5-HT receptors. These results shed light on safety and toxicity concerns with certain psychedelics.

**P31**

Prolintane and novel analogs induce serotonin transporter mediated efflux: A pharmacological characterization

**Kastner N**, Islam M, Dybek M, Roth E, Heisinger S, Holy M, Jäntsch K, Baumann M, Brandt S, Wallach J, Sitte H, Kudlacek O

Prolintane is a synthetic central nervous system stimulant that exerts its effects primarily through the inhibition of dopamine and norepinephrine reuptake. Originally introduced as a therapeutic agent for attention deficit hyperactivity disorder (ADHD), its clinical application was halted due to concerns over its potential for abuse and addiction. Despite this, current pharmacotherapy for ADHD, including amphetamines and methylphenidate, continues to present similar risks, underscoring the importance of identifying novel compounds with improved safety profiles. This study explores the structure-activity relationship of a series of ring-substituted prolintane analogs synthesized via a modified one-pot Mannich Barbier reaction and investigates their pharmacological properties. Functional assays using radiotracer flux in stably expressing HEK293 cells revealed that the introduction of methyl groups at ortho, meta, or para positions of the aromatic ring increased potency at the serotonin transporter (SERT) while simultaneously decreasing activity at the norepinephrine transporter (NET). Similarly, fluorine substituted analogs displayed increased SERT interaction and lowered SERT/dopamine transporter (DAT) ratios. Furthermore, all tested analogs elicited SERT-mediated inward currents and promoted reverse transport/efflux. These findings indicate that prolintane analogs act not only as DAT and NET inhibitors but also as SERT substrates and releasers, classifying them as “hybrid compounds”. The enhanced SERT activity combined with serotonin-induced efflux is associated with anti-addictive effects and supports further in vivo evaluation to evaluate therapeutic potential. This work illustrates the impact of targeted structural changes on prolintane's pharmacological profile and provides a basis for further investigation.

**P32**

The role of somatodendritically-located serotonin transporter in phototactic behavior in Drosophila melanogaster

**Kasture A**, Dittrich R, Ottendorfer T, Hummel T, Sucic S

The serotonin transporter (SERT), responsible for the uptake of extracellular serotonin, is expressed in both somatodendritic and presynaptic compartments. Previously, we demonstrated that mutating the conserved R607 residue of the ER export motif (R607/I608) located in the carboxyl tail of SERT, prevents its recruitment by the COPII component SEC24C. This, in turn, confines SERT to the somatodendritic compartment of raphe neurons. In Drosophila melanogaster, the corresponding R599A mutation similarly caused somatodendritic enrichment of the functional transporter in serotonergic neurons. Notably, both somatodendritically and presynaptically expressed SERT contribute distinctly to olfactory responses in flies. Given the critical role of serotonin in modulating phototactic responses, we investigated the role of compartmentalized SERT using a novel phototactic assay combined with mechanical stress. Flies displayed consistent phototactic performance over a three-day period. We found that mechanical stress reduces phototactic performance in fruit flies, which can be restored by citalopram, a selective serotonin

reuptake inhibitor. Using CRISPR-Cas9, we generated a SERT knockout (KO) and observed that mechanical stress no longer affected phototactic performance in the absence of SERT. Expression of the mutant dSERT R599A in a sensitized background using the GAL4-UAS system led to decreased phototactic behavior, which remained unaltered following mechanical stress treatment. Our findings highlight the essential role of serotonin in phototactic behavior and emphasize that presynaptic enrichment of SERT is crucial for an apt phototactic response.

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**P33**

Local anesthetics in Cocaine: more than just a numb feeling?

**Kudlacek O**, Holy M, Bicher J, Kooti F, Senning N, Karden A, Ludwig I, Luf A, Stockner T, Sitte H

Cocaine remains one of the most frequently used illicit drugs. Due to the lack of regulation on the black market, quality and purity of cocaine vary greatly. A high percentage of cocaine from the street market is mixed with other substances (adulterants) to increase the profit. Some of these additives mimic cocaine's effects, while others are included for less clear reasons. Local anesthetics are frequently detected as cocaine adulterants. Since Cocaine originally used as a local anesthetic, it is interesting whether other local anesthetics also share psychoactive effects with cocaine. We investigated Procaine, Lidocaine and Benzocaine for their potency on neurotransmitter uptake via the SLC6 transporters SERT, DAT and NET, and the SLC22 transporters OCT1, OCT2 and OCT3. Procaine and Benzocaine displayed some inhibitory property (although less than cocaine) on DAT and NET, but affected SERT to a much lesser extent. Lidocaine as a member of the amide family of local anesthetics had no effect on the uptake via SLC6 transporters. The organic cation transporters (OCTs) displayed a different pattern of susceptibility. Procaine blocked uptake in a range similar to cocaine, lidocaine was less effective and benzocaine didn't inhibit any transport via the OCTs. Although the inhibitory potency on OCTs might be relevant for effects and side effects of procaine and lidocaine, a relevant cocaine-like action on SERT, DAT and NET seems unlikely. Therfore mimicking the numb feeling on the tongue, when testing cocaine, is the most likely explanation why local anesthetics are used as adulterants.

**P34**

Functional and Pathological Implications of a Highly Conserved N-terminal Arginine Residue in SLC6 Transporters

**Shah N**, Kasture A, Sucic S

Background: Neurotransmitter transporters, such as γ-aminobutyric acid (GABA) transporter 1 (GAT-1), serotonin transporter (SERT) and dopamine transporter (DAT), are crucial for regulating neurotransmission by taking up substrates from synaptic cleft into neurons and astrocytes. Mutations in the N-terminal R79ETWGKK motif of SERT disrupt the conformational cycle between inward- and outward-facing states. Similarly, mutations at the R44 locus in human GAT-1 (homologous to SERT-R79) are linked to epilepsy. In this study, we investigate the molecular mechanisms underlying these variants, using in vitro (HEK293 cells) and in vivo (Drosophila melanogaster) models.

Methods: The R44 mutations were created by site-directed mutagenesis and functionally examined by radiotracer GABA uptake assays. Their cellular localisation and expression were studied by confocal microscopy and Endo H-deglycosylation and dominant negative phenotype tested on the wild-type

transporter. Transgenic flies were generated using phi-integrase system.

Results: The mutations resulted in loss of GABA uptake activity, with mutant proteins either residing intracellularly or expressed at the plasma membrane with reduced stability. Conversely, wild-type GAT-1 was correctly targeted to the cell surface. Several small molecules rescued mutant transporter expression, stability and function. Consistent with the in vitro findings, expression of R44 mutant proteins in Drosophila mirrored these results.

Conclusion: Our findings highlight the importance of the N-terminus in maintaining the integrity and function of SLC6 transporters. Furthermore, targeting key molecular regulators along the trafficking pathway can restore transporter expression and function, providing a promising strategy for developing effective therapies for SLC6A1-related disorders.

This work is supported by Austrian Science Fund (project P36574-B27)

**P35**

Interactions of Quinone Derivates with Human Organic Cation Transporters 1-3 and Plasma Membrane Monoamine Transporter: Implications for Antimalaria Drug Pharmacokinetics

**Angenoorth T**, Baralic E, Stancovic S, Holy M, Yang J, Sitte H, Maier J

Interactions between quinone derivatives and human OCT1-3 (SLC22A1-3) and PMAT (SLC29A4) may influence antimalarial drug pharmacokinetics, as many prescribed drugs interact with these transporters. This study examined the inhibitory and substrate properties of 17 quinone derivatives on hOCT1-3 and PMAT using radiotracer-based uptake inhibition assays and HPLC/LC-MS-based uptake assays in HEK293 cells stably expressing these transporters. All quinones moderately to strongly inhibited hOCT1, with IC50 values from 2.14 to 37.17 µM. hOCT2 was similarly inhibited, except by dihydroartemisinin. Most compounds also inhibited $MPP^+$ uptake via hOCT3. In contrast, PMAT showed moderate to no inhibition. Regarding substrate properties, most quinones were not transported by hOCT1-3 or PMAT. However, some uptake was observed: primaquine was taken up by all four transporters; cinchonidin by hOCT2, hOCT3, and PMAT; and mepacrine by hOCT2. These findings suggest that quinone-transporter interactions may affect drug disposition and therapeutic efficacy in malaria treatment and could play a role in drug-drug interactions or side effects. Further research is needed to confirm the involvement of these transporters in antimalarial drug action. Overall, this study provides valuable insight into transporter-quinone interactions and supports the development of selective inhibitors for hOCT1-3 and PMAT, contributing to more targeted drug design and improved therapeutic strategies.

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**P36**

A novel azobenzene paroxetine derivative and its interactions with biogenic amine transporters

Dreier D, **Belleza O**, Schlögl K, Kickinger S, Hellsberg E, Mayer F, Sandtner W, Mikšovsky P, Schittmayer M, Hu Y, Jäntsch K, Holy M, Ecker G, Sitte H, Mihovilovic M

The serotonin transporter (SERT) belongs to the solute carrier 6 family (SLC6) of membrane transporters which also include other transporters for norepinephrine, dopamine, and gamma-aminobutyric acid (GABA). These transporters facilitate the clearance of their biogenic amine substrates from the synaptic cleft,

thereby regulating neuromodulatory effects. SERT is critical for maintaining serotonin (5HT) homeostasis which impacts mood and cognition, and is therefore a target of antidepressants, e.g., paroxetine. Here, we report the development of a novel photoswitchable azobenzene paroxetine derivative and its pharmacological interaction profile. The azo-paroxetine derivative was switched between active (Z) and inactive (E) configurations, and 5HT uptake by SERT was inhibited more than 12 times more potently by the active (Z)-configuration. This activity was further supported by electrophysiological patch-clamp recordings and molecular docking studies. Meanwhile, there was no off-target activity observed at the human norepinephrine transporter (NET), human GABA-transporter (GAT) subtypes 1 and 3, and rat GAT1. Our results demonstrate the reversible light-dependent manipulation of SERT activity by azo-paroxetine. This photoswitchable compound can be applied as a tool for the selective, light-induced control of SERT activity in physiological investigations.

This project is supported by the Austrian Science Fund (FWF W1232, Molecular Drug Targets).

**P37**

Homoamphetamines: Structural Optimization of Monoamine Transporter Function to Mitigate Abuse Liability and Enhance Therapeutic Safety

**Sebastianelli-Schoditsch C**, zur Bonsen A, Kaiser D, Maulide N, Sitte H

Amphetamines act as psychostimulants by hijacking monoamine transporter function, driving the reverse transport of neurotransmitters into the synaptic cleft to amplify and prolong neural signaling. While clinically valuable for managing ADHD, narcolepsy, and PTSD, their therapeutic utility is hampered by substantial risks, including addiction, cardiovascular toxicity, and neuropsychiatric side effects such as hallucinations and anxiety. Structural alterations to the amphetamine framework—such as modifications to the phenethylamine core or stereochemistry—critically influence interactions with dopamine (DAT), norepinephrine (NET), and serotonin (SERT) transporters, dictating neurotransmitter release patterns and pharmacological selectivity. This study targets the development of homoamphetamines, a novel class of analogs engineered through strategic molecular design to optimize transporter specificity, thereby disentangling therapeutic benefits from adverse effects. By investigating partial efflux mechanisms and fine-tuning selectivity for DAT, NET, and SERT, we seek to identify analogs with diminished abuse liability and improved safety. Our approach integrates uptake-inhibition assays to quantify transporter binding affinity, efflux assays to characterize neurotransmitter release dynamics, and electrophysiological recordings to assess synaptic adaptations. Initial screening of six structurally modified analogs revealed distinct pharmacological signatures, with certain compounds exhibiting preferential activity at specific transporters, underscoring how tailored modifications can modulate release efficiency and selectivity. These insights demonstrate the potential of rational structural optimization to refine amphetamine pharmacology, paving the way for safer neurotherapeutics.

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**P38**

Serotonergic Modulation of Hippocampal Spatial Coding

**Arne Hansen**

Serotonin is involved in various brain functions underlying cognitive and physiological processes. A core endogenous function is to promote cognitive flexibility, enabling adaptive behavior under changing environmental demands. Growing evidence suggests that this flexibility, in part, relies on hippocampal

circuit function, allowing for dynamic updating of cognitive maps required for spatial navigation. Previous studies have shown serotonergic modulation of hippocampal activity both in vitro and in vivo, yet results remain inconclusive. Thus, the specific interplay between serotonergic tone and hippocampal spatial information processing remains unresolved. To investigate this relationship, we used a chemogenetic approach, expressing inhibitory designer receptors exclusively activated by designer drugs in the median raphe nucleus of mice to induce a global serotonin depletion during an open-field spatial exploration task. Simultaneously, we recorded calcium activity of dorsal CA1 pyramidal neurons using a miniature head-mounted microscope. Systemic serotonin depletion did not affect basic behavioral measures such as total exploration distance or speed. However, Bayesian decoding analysis of CA1 population activity revealed less accurate encoding of spatial information, reflected by increased decoding errors in serotonin-depleted mice. These findings underscore the important role of serotonergic modulation in hippocampal function even during basic spatial exploration. Whether this effect reflects local computations within the hippocampus that depend on serotonin, or serotonergic modulation of afferent information relayed to the hippocampus, remains to be determined. Future studies will also address to what extent more complex behaviors, such as novelty detection and cognitive flexibility, are affected, and which mechanisms underlie serotonin's modulation of hippocampal activity.

**P39**

In vivo multiplex monitoring of neuromodulators with multispectral GRAB sensors

**Cui Y**, Zheng Y, Dong H, Wang J, Cai R, Chen Z, Lavis L, Feng J, Li Y

Monoamines are essential neuromodulators that orchestrate fundamental brain functions. Norepinephrine (NE) plays critical roles in regulating arousal, attention, and stress responses, with its dysregulation implicated in anxiety and depression, in which many other neuromodulators are also involved such as serotonin (5-HT) and acetylcholine (ACh). Investigating the interplay between NE and other neuromodulators, requires simultaneous detection of multiple neuromodulators. Far-red fluorescence imaging emerges as an effective solution for multicolor detection, offering deeper tissue penetration, lower phototoxicity, and reduced background autofluorescence for in vivo studies. By leveraging the circularly permuted self-labeling protein cpHaloTag and a bright far-red dye-ligand conjugate (SiR650-HTL), we developed HaloNE1.0, a far-red NE sensor based on the GPCR activation based (GRAB) strategy. In both HEK293T cells and rat cortical neurons, HaloNE1.0 labeled with SiR650-HTL shows good fluorescent responses to NE, exhibiting optimal affinity, effective membrane trafficking, and molecular specificity. The sensor successfully captured optogenetically evoked NE release in the medial prefrontal cortex (mPFC) of freely moving mice. Combined with green and red GRAB sensors, we enabled simultaneous monitoring of NE, 5-HT, and ACh dynamics during sleep-wake cycles in vivo. This versatile approach paves the way for developing additional far-red or near-infrared (NIR) sensors targeting other neuromodulators, including 5-HT and endocannabinoids (eCBs), facilitating real-time, multiplex investigation of neuromodulator interactions in vivo.

**P40**

Next generation of GRAB sensors for monitoring spatiotemporal serotonin dynamics in vivo

**Wan J**, Deng F, Li G, Wang R, Wang Z, Li Y

The serotonergic system is pivotal in regulating various physiological and pathological processes and remains a crucial therapeutic target for numerous psychiatric disorders. Recent developments in genetically encoded, GFP-based serotonin (5-HT) sensors have provided valuable insights into serotonergic neurotransmission; however, their limited sensitivity and spectral profiles restrict their utility in resolving

5-HT signals under complex conditions. To address these constraints, we optimized green fluorescent G-protein-coupled receptor (GPCR)-activation-based 5-HT (GRAB5-HT) sensors and introduced a novel red fluorescent variant. These sensors demonstrate high specificity and sensitivity, as well as excellent spatiotemporal resolution, making them well-suited for in vivo monitoring of 5-HT dynamics. Leveraging these improved tools, we recorded subcortical 5-HT release in freely moving mice using fiber photometry. Additionally, we observed both uniform and gradient 5-HT release patterns within the mouse dorsal cortex under varying behavioral and physiological states with mesoscopic imaging. To further enable quantitative measurement, we created a fluorescence-lifetime-based 5-HT sensor. Unlike previously developed intensity-based sensors, this sensor leverages fluorescence lifetime—an intrinsic, intensity-independent parameter—to achieve artifact-free quantification of 5-HT concentrations. Together, these next-generation 5-HT sensors provide powerful new tools for probing serotonergic signaling with unprecedented precision, opening new avenues to investigate its roles in both health and disease.

**P41**

Near-infrared Optical Nanoprobes for Dynamic Neurochemical Imaging

**Jeong S**

Release and reuptake of neuromodulator is central to mood regulation and neuropsychiatric disorders, whereby imaging neurochemicals is of fundamental importance to study the neurochemistry signaling system. Recently, I presented a reversible near-infrared optical probe for serotonin that reports physiologically-relevant serotonin concentrations on relevant spatiotemporal scales, and is compatible with pharmacological tests. Synthetic molecular recognition for serotonin was conferred by evolving molecular recognition between single stranded DNA (ssDNA) and single-walled carbon nanotube (SWNT). To do so, we developed a high-throughput screening platform for evolution of serotonin molecular selectivity, in which systematic evolution of ligands by exponential enrichment is implemented on carbon nanotube surfaces, a process we've termed SELEC. Following the first SELEC result, I will represent the data analysis of DNA sequence library acquired from serotonin SELEC screening, which will shed the light for data-driven discovery of novel optical neurosensory. Our results suggest evolution of nanosensors could be generically implemented to rapidly develop other neuromodulator probes, and that these probes can image neuromodulator dynamics at spatiotemporal scales compatible with endogenous neuromodulation. Also, I will show the realtime near-infrared fluorescence imaging for the release/uptake of serotonin in neural cells. This presentation will show that our optical nanoprobes and microscopic system can be successfully applied to study the dynamic neurochemical events in various environments.

**P42**

Placental and Neonatal Serotonin (PlaNS) - A Prospective Birth Cohort Study in Zagreb, Croatia

**Perić M**, Horvatiček M, Bečeheli I, Žutić M, Nikolić B, Kesić M, Nakić Radoš S, Ivanišević M, Starčevič M, Hranilović D, Čičin-Šain L, Štefulj J

Placental and Neonatal Serotonin (PlaNS) is a prospective birth cohort study established with the primary aim of investigating how pregnancy-related risk factors relate to the placental and neonatal serotonin system and how placental and neonatal serotonin parameters predict offspring outcomes. From 2017 to 2023, 509 pregnant women were recruited at Clinical Hospital Center Zagreb, Zagreb, Croatia; 408 agreed to be contacted for prospective follow-up. Peripartum biological samples (maternal blood, cord blood, placenta) were collected, along with extensive demographic, clinical, psychological and lifestyle data. Laboratory analyses included blood metabolic parameters, platelet serotonin levels and platelet serotonin uptake activity in both maternal and cord samples. Follow-up data collection was completed 3 and 12

months postpartum and began 7-8 years after birth. Results to date indicate that maternal overweight/obesity and gestational diabetes mellitus (GDM) are associated with subtle changes in DNA methylation and/or expression of several serotonin-regulating genes in the placenta, with effects differing by fetal sex. Expression of the serotonin transporter gene in the placenta inversely predicted neonatal birth weight. Exposure to GDM was also associated with decreased substrate affinity of placental monoamine oxidase A, suggesting impaired placental 5-HT catabolism. Methylation of serotonin-regulating genes in cord blood cells was influenced by neonatal sex, with maternal metabolic parameters playing an additional role. The PlaNS study sheds light on how the prenatal environment shapes the placental and neonatal serotonin system and may contribute to a better understanding of serotonin-mediated pathways linking pregnancy risks to long-term outcomes in the offspring.

**P43**

Optogenetic activation of serotonergic neurons in the brain affects periodic phenomena

**Kanamaru M**

Serotonergic neurons have cell bodies in the raphe nuclei, extending from the midbrain to the medulla oblongata, and their fibers project throughout the brain. These neurons express more than 14 receptor subtypes and perform a wide range of functions. Although extensive research is available, the comprehensive role of serotonergic neurons in the brain is not fully understood. We performed optogenetic experiments, combining wireless fiber optics, whole-body plethysmography, wireless electroencephalography (EEG), and electromyography (EMG), in freely moving mice. The mice (Tph2-tTA::tetO-ChR2[C128S]-EYFP) were bred by crossing two mouse lines provided by RIKEN BRC (Tsukuba, Japan) through the MEXT National Bio Resource Project. We used yellow fluorescent protein-positive and -negative littermates. The anesthetized mice were implanted with the experimental apparatus. Each experiment was performed after a recovery period of at least 1 week. Blue light illumination of the median raphe nucleus elicited anxiety-like behavior and respiratory facilitation. Blue light illumination of the dorsal raphe nucleus (DRN) induced fast transition from non-rapid eye movement (NREM) sleep to active wakefulness. Furthermore, blue light illumination of the DRN altered masticatory patterns by increasing masticatory frequency and decreasing the root mean square amplitude of the EMG signal. In contrast, blue light illumination of the raphe obscurus nucleus produced little change. These findings suggest that activation of serotonergic neurons in the DRN contribute to a phase shift in periodic phenomena. The roles of serotonergic neurons of the brain in physiological functions are discussed.

**P44**

Serotonergic neuron activity promotes oligodendrogenesis in the dorsal raphae

**Rogers A**, Yalçın B, Herrera Castaneda E, Drexler R, Monje-Deisseroth M

Serotonergic circuitry modulates diverse neural pathways contributing to a variety of behavioral outputs such as anxiety, locomotion, sociability, and fear due to its vast network of projections targeting widespread brain regions. Such neural network complexity also requires intricate tuning of serotonergic transmission to allow for these heterogeneous behavioral responses. A recently highlighted mechanism of neural circuit plasticity is activity-regulated oligodendroglial changes, including proliferation of oligodendroglial precursors (OPCs), generation of new oligodendrocytes, and adaptive myelination that tunes circuit function and contributes to behavior. However, it is not known to what extent serotonergic axons are myelinated, or if they exhibit myelin plasticity. We hypothesize that activity-regulated myelin changes on serotonergic axons tune serotonergic circuit functions that modulate behavioral outputs. Using an in vivo optogenetic strategy, we demonstrate that serotonergic neuronal activity promotes OPC proliferation and oligodendrogenesis,

along with increased myelin protein intensity. This effect is specific to the dorsal raphae but is not seen in regions to which serotonergic neurons project. Incubation of mouse OPCs with serotonin in vitro does not lead to increased OPC proliferation, suggesting that serotonin itself is not acting on the OPCs to elicit more proliferation. Genetic blockade of oligodendrogenesis leads to behavioral changes, including increased open arm exploration in the Elevated Plus Maze and increased active coping in the Forced Swim Test. Our results highlight a previously underappreciated role for serotonergic neuron activity in modulating oligodendroglia in a brain-region specific manner, and point to behavioral consequences of myelin plasticity in behaviors associated with serotonergic signaling.

**P45**

Neuronal Dynamics of Serotonin Pathways in the Regulation of Anxiety and Exploration-related behaviors

**Costa A**, Mlost J, Juhlin L, Pollak Dorocic I

Serotonin (5-HT) plays a crucial role in both anxiety and exploration. However, the specific contributions of different dorsal raphe nucleus (DRN) 5-HT projections to these behaviors remain unclear. To address this, our study focuses on the role of three key forebrain targets of DRN 5-HTergic projections: the basolateral amygdala (BLA), the lateral hypothalamic area (LHA), and the ventral tegmental area (VTA). Using in vivo fiber photometry and chemogenetics, we recorded and manipulated projection-specific 5-HT neuron activity during the Elevated Plus Maze (EPM), Novel Object Recognition, and Three-Chamber Social Recognition test. We also used unsupervised learning behavioral classification to uncover subtle behavioral effects of projection-specific manipulations in the Open Field Test. We found that DRN 5-HT neurons increase activity during both exploratory and high-anxiety states in the EPM. While all projections are activated by novelty, the BLA is predominantly activated by novel stimuli but not familiar. Chemogenetic inhibition of BLA enhanced open arm time in the EPM and abolished preference for novel objects; LHA inhibition enhanced preference for novel stimuli; and VTA inhibition had no significant effect. These findings suggest that 5-HT modulation in the BLA promotes the avoidance of threatening situations in anxiety-like contexts but facilitates responses to novelty, while the LHA facilitates exploration and engagement with novel stimuli. 5-HT modulation in the VTA does not appear to be essential for the regulation of anxiety or exploratory behaviors. These findings highlight the complex and context-dependent role of distinct DRN 5-HT pathways in regulating anxiety, exploration, and responses to novelty.

**P46**

Spatio-molecular Organization of the Dorsal Raphe Nucleus and Transcriptional Effects of SSRI Treatment

**Henningson C**, Mlost J, Pollak Dorocic I

The Dorsal Raphé (DR), a major forebrain serotonergic nucleus, regulates physiological and emotional functions. Recent studies have identified subpopulations of DR serotonin (5-HT) neurons based on gene expression, but no unbiased spatial mapping exists. Selective Serotonin Reuptake Inhibitors (SSRIs) act directly on serotonergic neurons but it is unknown if these subpopulations are differently affected. This study explores the DR's spatio-molecular organization and the impact of SSRIs on its transcriptome.

Using spatial transcriptomics (ST) and single-molecule fluorescent in-situ hybridization (smFISH), we analysed the DR in mice. ST enabled unbiased survey of gene expression, while smFISH provided single-cell resolution for key markers. ST revealed six subclusters along the rostral-caudal axis, each with distinct molecular signatures, including genes associated with specific 5-HT subpopulations.

Temporal effects of SSRIs were examined using acute and chronic treatment. Functional categories of

genes were dynamically affected during treatment, including receptors and kinases. Notably, the neuropeptides Pdyn (Prodynorphin) and Trh (Thyrotropin-Releasing Hormone), co-expressed in 5-HT neurons, exhibited opposing responses to SSRIs. Acute SSRI treatment increased Pdyn expression in the midline DR, while chronic treatment led to a significant rise in Trh expression in the lateral wings.

These results reveal the complex spatio-molecular organization of the DR and highlight transcriptional changes induced by SSRIs. The opposing expression patterns of Pdyn and Trh may contribute to SSRIs' delayed therapeutic effects. Our findings suggest candidate genes, pathways, and cell types for future studies aimed at understanding depression and identifying novel pharmacological targets.

**P47**

Investigating the 5-HT2A Receptor and BDNF-Mediated Effects of Serotonergic Psychedelics on Mitochondrial Dynamics in Rodent Cortical Neurons

Fanibunda S, **Mazumder I**, Singla A, Kukkemane K, Suryavanshi S, Janakiraman B, Mendon S, Vuruputuri M, Sharma M, Vaidya A, Vaidya V

Serotonergic psychedelics exhibit rapid antidepressant and anxiolytic effects, yet their mechanisms remain elusive. Notably, mitochondrial dysfunction is implicated in the development of neuropsychiatric disorders, including major depression, making mitochondria increasingly relevant targets for both understanding and treating these conditions. Our earlier research has shown that serotonin, acting through the 5-HT2A receptor, significantly boosts the creation and operation of mitochondria in the neocortex (Fanibunda et al., 2019; 2021). Building on this, we propose that serotonergic psychedelics, which work at least partially through the 5-HT2A receptor, might affect mitochondria in crucial limbic brain areas, leading to beneficial changes in neuronal plasticity and mood-related behaviors.

Serotonergic psychedelics, including DOI (2,5-dimethoxy-4-iodoamphetamine), have garnered significant interest for their potential neuroprotective and neuroplastic effects mediated via 5-HT2A receptor signaling. Here, we demonstrate both in-vitro and in-vivo, that DOI enhances mitochondrial biogenesis and function in rodent cortical neurons of the prefrontal cortex through a 5-HT2A receptor-mediated recruitment of the BDNF-SIRT1-PGC-1α axis. This led to improved mitochondrial respiratory capacity, oxidative phosphorylation efficiency, and elevated cellular ATP levels.

Mechanistically, the neurotrophic factor Brain-derived Neurotrophic Factor (BDNF) was identified as a critical downstream target of 5-HT2A receptor activation. DOI-induced mitochondrial biogenesis and function were mediated via 5-HT2A receptor dependent release of brain derived neurotrophic factor (BDNF) and signaling via TrkB, resulting in activation of the SIRT1-PGC-1α pathway. We are now investigating how these mitochondrial changes contribute to the effects of psychedelics on neuronal plasticity and mood.

**P48**

Aripiprazole may be a partial agonist at 5-HT2A receptor coupled with G(alpha)q/11 proteins in rat cerebral cortical membranes

**Odagaki Y**, Kinoshita M, Honda M, Meana J, Callado L, García-Sevilla J, Palkovits M, Borroto-Escuela D, Fuxe K

Functional activation of heterotrimeric guanine nucleotide-binding proteins (G-proteins) via G-protein-coupled receptors (GPCRs) has been extensively studied by using a guanosine-5'-O-(3-[$^{35}$S]thio)triphosphate ([$^{35}$S]GTPγS) binding assay. However, the conventional method is

applicable exclusively to Gi/o family without discrimination among G-protein subtypes. The present study aimed at re-establishing a new method termed "[$^{35}$S]GTPγS binding/immunoprecipitation assay" by searching a most suitable anti-Gαq/11 antibody instead of the previously utilized, but now withdrawn, antibody. In initial screening of multiple commercially available anti-Gαq/11 antibodies, one antibody was hit as promising when 5-HT was used as an agonist. Subsequent to determination of the optimal experimental conditions in rat and post-mortem human brain membranes, stimulatory effects of a variety of agonists were determined. Among these, carbachol was the most efficient agonist, followed by 5-HT. The concentration-dependent increase in [$^{35}$S]GTPγS binding to Gαq/11 elicited by 5-HT was determined in rat cerebral cortical membranes. Aripiprazole, an atypical antipsychotic drug, was also shown to hehave as a weak agonist in this experimental system. The increase in [$^{35}$S]GTPγS binding to Gαq/11 evoked by aripiprazole was inhibited potently by MDL100907, a selective 5-HT2A receptor neutral antagonist, but not by sulpiride or WAY-100135, indicative of involvement of 5-HT2A receptor. Although it is usually supposed that aripiprazole is an antagonist at 5-HT2A receptor, these results raise a possibility that this atypical antipsychotic agent acts as a weak 5-HT2A receptor agonist in the central nervous system.

**P49**

Gα-Proteins as Novel Players in Mood Regulation: Exploring Serotonin Transporter Regulation and Serotonin Dynamics in C. elegans

**Arrasz N**, Fitzsimons A, Blacque O, Haase J

Serotonin transporter (SERT) is essential for serotonin (5-HT) uptake from the synaptic cleft, facilitating serotonin recycling. Imbalances in 5-HT homeostasis are implicated in conditions such as depression, making SERT a primary target for therapeutics. Prior research suggests that Gα-proteins may inhibit SERT function. We aim to elucidate the mechanism of Gα-proteins' influence on SERT in vitro and introduce the nematode Caenorhabditis elegans as a model system to study SERT regulatory mechanisms. By comparing the uptake kinetics of rat SERT (ratSERT) and C. elegans SERT (ceSERT) in HEK cells, we observed that ceSERT exhibits lower Km and Vmax values, indicating distinct functional characteristics. RatSERT showed greater sensitivity to the antidepressants Escitalopram and Paroxetine compared to ceSERT, while responses to Fluoxetine, Imipramine and Ibogaine were similar. These findings suggest that structural differences influence inhibitor affinity between the two SERT types. Our ongoing research explores the impact of Gα-proteins on SERT function in Gα-knockout HEK cells. Preliminary results indicate that ratSERT demonstrates increased uptake activity in the absence of Gα-proteins, supporting the hypothesis that Gα-proteins inhibit SERT. We currently investigate how Gα-proteins affect SERT sensitivity to inhibitors and assess the physiological effects of Gα-proteins in C. elegans regarding on 5HT uptake. This research aims to enhance our understanding of Gα-proteins' role in SERT function and serotonin dynamics, potentially guiding new therapeutic developments for mood disorders.

The project is funded by the School of Biomolecular and Biomedical Science, University College Dublin.

**P50**

A prospective code for value in the serotonin system

**Harkin E**, Grossman C, Cohen J, Béïque J, Naud R

The in vivo responses of dorsal raphe nucleus serotonin neurons to emotionally salient stimuli are a puzzle. Existing theories centring on reward, surprise, salience and uncertainty individually account for some aspects of serotonergic activity but not others. Merging ideas from reinforcement learning theory with recent insights into the filtering properties of the dorsal raphe nucleus, here we find a unifying perspective in a prospective code for value. This biological code for near-future reward explains why serotonin neurons are activated by both rewards and punishments, and why these neurons are more strongly activated by

surprising rewards but have no such surprise preference for punishments—observations that previous theories have failed to reconcile. Finally, our model quantitatively predicts in vivo population activity better than previous theories. By reconciling previous theories and establishing a precise connection with reinforcement learning, our work represents an important step towards understanding the role of serotonin in learning and behaviour.

**P51**

Mutated (D100A) 5-HT4-serotonin receptors in the mouse atrium

**Neumann J**, Gergs U

Serotonin exerts a positive inotropic effect in the human atrium via 5-HT4-serotonin receptors. It is already known that serotonin failed to bind to a mutated the 5-HT4-serotonin receptor (D100A, i.e. aspartate to alanine in amino acid 100 in the protein sequence) and to stimulate cAMP levels in the receptor-transfected cells. We now tested the hypothesis that a 5-HT4-serotonin receptor antagonist or serotonin itself might act oppositely on force of contraction in D100A human 5-HT4-serotonin receptors in the mammalian heart. To this end, force of contraction (FOC) was measured in isolated left atrial preparations (LA) from mice with heart-specific overexpression of the human mutated (D100A) 5-HT4-serotonin receptor (5-HT4m-TG). Serotonin 1 µM failed to increase whereas GR125487, an inverse agonist at human 5-HT4-serotonin receptors and cisapride, an agonist at human 5-HT4-serotonin receptors increased FOC in LA from mice from 5-HT4m-TG. However, serotonin and cisapride increased and GR125487 failed to increase FOC in LA mice with cardiac specific overexpression of the human unmutated 5-HT4-serotonin receptors (5-HT4-TG). As a control, we noted that serotonin cisapride and GR125487 failed to increase FOC in LA from wild type mice (WT), respectively. These data suggest that not only in vitro but also in vivo a single amino acid mutation (D100A), supposedly at the receptor binding site of the human 5-HT4-receptor is able to alter agonist- and antagonist-mediated contractile function.

**P52**

Positive inotropic effects of felcisetrag in isolated human atrial preparations

Rayo Abella L, Hofmann B, Gergs U, Kirchhefer U, **Neumann J**

We hypothesized that felcisetrag might act as an agonist on human cardiac 5-HT4-serotonin receptors. We measured its effects in the organ bath (felcisetrag 0.1 – 100 nM) cumulatively applied on the force of contraction (FOC) in isolated, electrically stimulated (1 Hz) right atrial preparations (HAP) obtained from adult patients with coronary heart disease during cardiac surgery. We noted an increase in FOC in a concentration- and time-dependent fashion with felcisetrag alone. After pre-stimulation of FOC with 1 µM serotonin, felcisetrag (100 nM) reduced FOC in HAP. Felcisetrag was less effective than 1 µM serotonin to raise FOC in HAP. Felcisetrag alone increased FOC and beating rate in left (LA) or right atrial (RA) preparations, respectively, from mice with cardiac overexpression of the human 5-HT4-serotonin receptor (5-HT4-TG) but not in LA or RA from wild type (WT) littermate mice. After stimulation of the 5-HT4 –serotonin receptor with serotonin (1 µM), felcisetrag exerted negative inotropic or negative chronotropic effects in LA or RA, respectively. This suggests that in LA and RA from 5-HT4-TG, felcisetrag acts as a partial agonist at the 5-HT4-serotonin receptor. The contractile effects of felcisetrag in LA from 5-HT4-TG and in HAP were reversed by 1 µM GR125487, a 5-HT4-serotonin receptor antagonist. We conclude that felcisetrag acts as a partial agonists at 5-HT4-serotonin receptors in HAP. It remains to be elucidated whether these cardiac effects are detrimental or beneficial for the patient.

**P53**

The role of organic cation transporter 1 in determining serotonin levels in peripheral circulation

**Morof F**, Meyer-Tönnies M, Knospe F, Moritz E, Rönnpagel V, Jedlitschky G, Tzvetkov M

About 95% of the serotonin present in the human body is found outside the CNS. Peripheral serotonin is synthesized in enterochromaffin cells of the small intestine. After release into the bloodstream, it is mainly taken up into platelets by the serotonin transporter SERT (SLC6A4), or metabolized in hepatocytes by monoamine oxidase A. The organic cation transporter 1 (OCT1; SLC22A1) may facilitate the hepatic uptake of serotonin and could represent a rate-limiting step in the regulation of systemic serotonin concentrations. OCT1 is genetically highly variable. Four common loss-of-function polymorphisms cause partial or full OCT1 deficiency in up to 2% of Europeans. We investigate the impact of OCT1 deficiency on the distribution of peripheral serotonin in humans and mice. In a cohort of 120 healthy volunteers, endogenous serotonin levels were quantified in whole blood, serum, platelet-rich plasma (PRP), platelet-poor plasma (PPP), and platelets, and were related to OCT1 and SERT/5HTTLPR-genotypes. Additionally, serotonin levels were compared between Oct1/Oct2 knockout and wild-type mice. The serotonin concentration in blood varied 5-fold from 60,8 to 301,6 ng/ml. More than 98% of circulating serotonin is stored in platelets. Poor OCT1 transporters showed significantly increased serotonin levels in whole blood, PRP and in platelets, but not in PPP. These findings were confirmed in the mouse model. Our results support a role for OCT1 in determining serotonin levels in systemic circulation. The physiological implications of these elevated serotonin levels should be further investigated.

**P54**

Sex-Specific Effects of Exercise on Serotonin Dynamics During Stress: Potential Role of GABAergic Inhibition in the Dorsal Raphe

**Mellert S**, Amat J, Levy E, Solomos N, Greenwood B, Baratta M

Exercise is a potent stress resistance factor that reduces the incidence and severity of stress-related disorders. Indeed, preclinical models demonstrate that voluntary wheel running (VWR) prevents the typical depressive- and anxiety-like behavioral outcomes of inescapable stress (IS) in rats. Importantly, females are more responsive than are males to the effects of exercise, attaining stress resistance after only 3 weeks of VWR compared to 6 weeks of VWR required in males. In sedentary rats, IS potently activates serotonergic neurons in the dorsal raphe nucleus (DRN), leading to behaviors such as exaggerated fear. In males, six weeks of VWR confers stress resistance by constraining the DRN serotonin response to IS. However, whether 3 weeks of VWR similarly attenuates the DRN serotonin response to IS in females, and the underlying mechanism of constraint, remain unknown. Microdialysis revealed that 3 weeks of VWR attenuated stress-induced serotonin efflux in the DRN in females but did not produce a similar effect in males. These findings provide a neurochemical basis for the previously observed sex differences in the time course of exercise-induced behavioral stress resistance. Further investigation of DRN GABAergic neurons, which inhibit serotonin activity, using RNAscope co-labeling of vesicular GABA transporter and cfos mRNAs revealed that 3 weeks of VWR potentiated GABAergic activity in the DRN during IS in females. Together, these data indicate that exercise more readily constrains the DRN serotonin response to stress in females than in males and identifies GABAergic activity in the DRN as a potential mediator of sex-specific exercise-induced stress resistance.

**P55**

Identification of the Amitriptyline Binding Site in the Human Serotonin Transporter by CryoEM and Computational Studies.

**Henry K**, Dang P, Curtis M, Kratcha E, Kurre D, Vaughan R, Alam A

The human serotonin transporter (hSERT) is the target of many clinical compounds, including tricyclic antidepressants (TCAs). Here, we used CryoEM to determine the structure of full-length, native hSERT complexed with the TCA amitriptyline (AMI) at 3.7Å resolution. The hSERT protein generated from the expression construct was functionally and pharmacologically consistent with native hSERT. The AMI-SERT complex was found to be in the occluded conformation with AMI bound at the orthosteric binding site (S1) but not the allosteric site (S2) when utilizing 10μM AMI (a concentration 25-fold higher than the therapeutic window). The AMI binding to S1 was corroborated independently using several computational ligand docking approaches. These results, combined with prior structural studies, further support a key distinction: Unlike SSRIs (e.g., citalopram) that are capable of binding both S1 and S2 sites simultaneously, TCAs like AMI likely interact exclusively with the orthosteric site at clinically relevant concentrations.

**P56**

Serotonin:Glutamate Synergy Bridges High Impulsivity and Reward in Preclinical Studies

**Anastasio N**, LeDay L, Davis-Reyes B, Chapman H, Hommel J, Cunningham K

Dysfunction in serotonin (5-HT) and glutamate (Glu) signaling within the raphe-accumbens circuit is a driver of the cognitive and/or behavioral dimensions underlying impulse control and reward-related behaviors. Vesicular glutamate transporter 3 (VGLUT3) is localized on heterogeneous neurons which co-release Glu and 5-HT within the raphe-accumbal circuit, presenting a novel target in unmasking disorders and behaviors governed by impulsivity. We hypothesized that an imbalance in nucleus accumbens (NAc) VGLUT3 homeostasis marks high trait impulsivity and concomitant non-homeostatic feeding (i.e. beyond basic caloric needs with hedonic components). Outbred male Sprague Dawley rats were identified as high (HI) or low (LI) impulsive using the one-choice serial reaction time (1-CSRT) task. Following behavioral assessments, NAc synaptosomal VGLUT3 protein levels were analyzed via capillary electrophoresis-based immunoblotting. Accumbal VGLUT3 was knocked down in the NAc and effects on motor impulsivity, feeding behaviors, and body composition analyzed. HI rats expressed higher NAc synaptosomal VGLUT3 protein vs. LI rats ($p<0.05$); there was a positive correlation between VGLUT3 expression and impulsivity ($r=0.643$, $p=0.02$). Knockdown of NAc VGLUT3 decreased impulsivity and high fat food intake vs. baseline in HI rats ($p<0.05$). There were no differences between % fat mass or % lean mass in control vs. VGLUT3 knockdown in HI rats. Thus, an imbalance in VGLUT3 may shift 5-HT:Glu signaling within the raphe-accumbens circuit and contributes to an aberrant neurobiology underlying disorders characterized by a loss of impulse control.

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**P57**

Serotonergic modulation of social cognition in mice

**Niello M**, Ciccocioppo E, Pacinelli G, Managò F, Walle R, Sitte H, Papaleo F

Social cognition refers to the processing of social information and it is impaired in numerous neuropsychiatric disorders. Research on social cognition has been largely restricted to clinical settings and preclinical studies have primarily focused on their prosocial effects, limiting our understanding of the mechanisms underlying social interaction. Serotonin (5HT) is classically associated with the processing of social information and serotonergic neurons project to the medial prefrontal cortex (mPFC), a fundamental hub of socio-cognitive functions. However, the role of 5HT dynamics in social cognition, the related circuits, and their pharmacological manipulation are still unexplored. Here, we combine recently developed behavioral tasks to assess social cognition in mice with circuit manipulations, fiber photometry, and in vivo pharmacology. We observed that manipulating the 5HT system with serotonin-releasing agents vastly impacts different aspects of social cognition in mice, going beyond “prosocial” effects. Consistently, 5HT manipulation impacts mPFC local circuits commonly engaged in socio-cognitive processes. Our findings underscore the pivotal role of the serotonergic system in modulating distinct domains of social cognition, providing a foundation for innovative therapeutic approaches to neuropsychiatric disorders.

**P58**

Placental serotonin causes transcriptional and compositional changes in brain

**Romanov R**

Here we argue that placental serotonin (5-HT) can work as a transgenerational non-genetic factor of fetal neurodevelopment. Our results show that in pregnant rodents stress causes the physiological increase of 5-HT levels that lead to changing the balance of hormones and behavioral patterns of coping strategies in the adulthood of their offspring. However, the effects of increased serotonin levels on the developing brain remain unexplored. To address the hypothesis that an increase in serotonin levels during the gestational period leads to enduring alterations in the transcriptional and compositional profiles of brain cells we focused on the hypothalamus since exactly this brain region is involved in regulating hormone secretion, metabolism, and homeostasis. Moreover, the hypothalamus plays a crucial role in coordinating adaptive responses to environmental cues including social behavior. As a model to increase the serotonin, we used pregnant rats fed with 5-HT precursor during a period of intensive neurogenesis (E11 – E14). After analysis of scRNA-seq datasets obtained from control and experimental animals at different stages (E20, P28), we revealed trajectory shifts leading to the compositional changes including specific populations of neurons and glia. The single-cell analysis of cis-regulatory elements confirmed that serotonin precursor administration causes massive rearrangement of epigenetic profiles and tuning of developmental programs. Particularly, our investigation encompassed the identification of gene programs associated with WNT signaling and shift in neurogenesis-gliogenesis balance. Our data suggest that placental serotonin non-genetically directs brain development highlighting its relevance in the context of neurodevelopmental disorders and long-term health outcomes in offspring.

**P59**

An immediate early gene and glutamate response is not necessary for the medicinal and neuroplasticity promoting effects of non-hallucinogenic 5-HT2A receptor agonists

**Isak Aarrestad**

Cortical atrophy and dysfunction have been implicated in a wide variety of psychiatric illnesses including depression, anxiety and post-traumatic stress disorder. After a single dose, psychedelics have been shown to promote robust neuroplasticity in the prefrontal cortex (PFC) and rescue atrophy in the cortical circuits underlying these conditions. Recently, analogues of these compounds were synthesized that lack hallucinogenic potential but retain efficacy in pre-clinical models of psychiatric disorders. These compounds present a scalable alternative to guided psychedelic therapy, however, the field has yet to discover how similar their mechanism is to that of their hallucinogenic counterparts. Here, we demonstrate that non-hallucinogenic psychedelic analogues promote neuroplasticity through the same 5-HT2A receptor mediated biochemical pathway as hallucinogenic agonists. We find that, while both classes of compound produce robust neuroplasticity and calcium responses in the prefrontal cortex, the non-hallucinogenic agonist TBG does so without evoking a glutamate or immediate early gene response.

**P60**

Role of Epithelial 5HT4 Receptor in Gastrointestinal Motility and Visceral Pain

**Chalystha Yie Qin Lee**

Gastrointestinal (GI) serotonin (5HT) increases motility and decreases visceral pain, largely through the $5HT_4$ receptor ($5HT_4R$). Systemic $5HT_4R$ agonists are thus a primary treatment for chronic constipation and constipation-predominant irritable bowel syndrome (IBS-C), but are effective in only 50% of patients. $5HT_4Rs$ are located in the brain but also the GI epithelium and the enteric nervous system (ENS), suggesting that this lack of efficacy could be secondary to off-target effects. We found that antagonism of GI epithelial SERT promotes an anti-nociceptive phenotype. We thus hypothesized that the beneficial mood and pain phenotypes elicited by gut 5-HT (but not GI motility) are due to $5HT_4R$ agonism exclusively targeted to the gut epithelium. We first developed a mouse model with deletion of $5HT_4R$ limited to the GI epithelial layer, Villin-cre::$5HT_4$RFL/FL. GI function was evaluated through invivo and exvivo motility assays. Spontaneous mood- and pain-related behaviors were monitored, and intestinal pain sensitivity was measured using the visceromotor response (VMR) assay. Villin-cre::$5HT_4$RFL/FL showed $5HT_4R$ presence in the brain, ileum and colon ENS layers, but not in the GI epithelial layer. Villin-cre::$5HT_4$RFL/FL mice displayed significantly faster colonic motility. Analysis of ENS motility function by measurement of colonic migrating motor complexes (CMMC), showed fewer CMMCs in villin-cre::$5HT_4$RFL/FL mice. Male villin-cre::$5HT_4$RFL/FL mice exhibited increased anxiolytic grooming behaviour, and both male and female villin-cre::$5HT_4$RFL/FL mice demonstrated visceral hypersensitivity indicating decreased intestinal pain threshold. In conclusion, these changes indicate that $5HT_4R$ specific to the epithelial layer may play an important role in 5HT activity in pain, motility, and mood.

**P61**

Endogenous serotonin signaling in prefrontal cortex: frequency dependence, plasticity, and perturbation by chronic SSRI treatment

**Saige Power**

Serotonin is an important regulator of mood, emotion, and executive function. Despite strong effects of exogenous serotonin and related agonists, the behavioural and neurophysiological effects of axonally-released serotonin are elusive and have only begun to be characterized. Here, we use optogenetics and whole cell recording in brain slices from transgenic mice to probe the impact of endogenous serotonergic transmission in medial prefrontal cortex. We find this signaling shows frequency dependent plasticity, with higher-frequency stimulation of serotonin afferents strongly depressing subsequent responses. Examining regulatory mechanisms at the terminal and target sides of the 'synapse' highlights the frequency-dependence of serotonergic regulation. Control mechanisms, including the serotonin transporter and receptor mediated suppression, are biased towards high frequency signaling and exert negligible effects on lower frequency transmission. This profile raises the question of how serotonin regulation in prefrontal cortex is perturbed by chronic exposure to selective serotonin reuptake inhibitors (SSRIs). We discover that axonally-released serotonin continues to display typical baseline response characteristics, despite prominent downregulation of the serotonin transporter from SSRI treatment of weeks to months. This surprising adaptation of serotonin signaling results from changes in the frequency-dependent profile of key regulatory mechanisms. Our optogenetic investigation probes the tightly regulated nature of serotonin signalling and the mechanisms that underlie its plasticity. Taken together, the findings give new strategies to best engage the serotonergic system and suggest adjuvant treatments to enhance neurotransmission.

**P62**

Ungating the Gated-Pore Mechanism of the Serotonin Transporter

**Ralph Gradisch**

The presynaptically located serotonin transporter (SERT) utilizes the sodium gradient as a driving force for the reuptake of serotonin (5-HT), hence fine-tuning serotonergic signalling. Importantly, SERT is pharmacologically targeted for the treatment of neuropsychiatric diseases. High-resolution structures of SLC6 members, including SERT, revealed their conserved three-dimensional architecture and highlighted the structural elements enabling the alternating access mechanism. The transport cycle is initiated upon binding of 5-HT and requires structural rearrangements to transition from the outward-open state to the occluded and, subsequently, the inward-open state. Movements of the dynamic bundle domain towards and away from the rigid scaffold domain regulate the gates above and below the central substrate binding site (S1) to facilitate either extracellular or intracellular accessibility to the S1. The "gated pore" and "rocking bundle" mechanisms ensure that the S1 can only be accessed from one side of the membrane, but never from both simultaneously. This feature discriminates channels from transporters. However, not all features of SERT can be explained by these models. Electrophysiological recordings revealed excessive ion flux in the presence of substrate, surpassing the proposed transporter stoichiometry. Although much smaller than channel-mediated currents, this high-conducting state is reminiscent of a ligand-gated channel. This study demonstrates that a single point mutation above the S1 is sufficient to render a transporter a ligand-gated pore. By integrating molecular dynamics simulations with experimental approaches, we unravelled SERT's sealing mechanism. Besides adding another layer of complexity to the alternating access model, this study highlights the extremely fine line separating transporters and channels.

**P63**

Real-Time Analysis of Serotonin Dynamics in Human-Derived 3D Organoids and Spheroids Using Fast-Scan Cyclic Voltammetry

**Bettina Bohl**

Serotonin (5HT) is predominantly produced in two key organ systems: the brain and the gut. In the brain, 5HT plays a crucial role in regulating various processes, including mood and sleep. In the gut, 5HT is secreted by enterochromaffin cells in the mucosa and neurons of the enteric nervous system regulating metabolic functions and gut motility, respectively. Dysregulation of 5HT in both systems is linked to pathological conditions, notably major depressive disorder and irritable bowel syndrome. Despite this impact of 5HT on human health, our understanding of its release and reuptake dynamics remains limited. This gap is partly due to the lack of suitable model systems and sensors. To address this, we investigated 5HT dynamics in human brain and gut models using human stem cell-derived culture models and fast-scan cyclic voltammetry (FSCV). Stem cells provide a unique opportunity to study otherwise inaccessible tissues, such as the brain and the gut, and to target specific, rare cell populations within complex tissues through introduction of reporter lines—for example, for enterochromaffin cells. In this study, we developed serotonergic neuronal spheroids, and gut organoids incorporating a fluorescent reporter specific to enterochromaffin cells. Using FSCV, we analyzed 5HT release from serotonergic neurons and enterochromaffin cells in response to electrical and chemical stimulation, respectively. We performed an in-depth biochemical characterization of these systems and then investigated the impact of selective serotonin reuptake inhibitors on 5HT. In sum, we present exciting new tools to study 5HT dynamics in human derived neuronal and gut systems.

**P64**

Protocadherin-alphaC2 is required for fluoxetine-induced serotonin re-innervation and behavioral recovery after stroke

**Sara Asgharzadeh**

Introduction: Serotonin (5-HT) regulates behavior and cognition through brain-wide projections and exhibits neuroplasticity, enabling regeneration after injury. How 5-HT neurons regrow after injury remains unclear. Protocadherin-αC2 (PcdhαC2) gene is crucial for development of serotonergic innervation, with its loss causing tangled serotonin axons. However, its role in adulthood regeneration is unknown. Using a mouse injury model of post-stroke depression (PSD), we previously found that chronic fluoxetine (FLX) treatment induces recovery of anxiety- and depression-like phenotypes and of serotonin innervation. We hypothesized that PcdhαC2-mediated axonal regrowth is essential for FLX-induced recovery.

Methodology: Using tamoxifen-inducible TPH2-ACON mice, we selectively knocked out Pcdhα in adult 5-HT neurons to generate Pcdhα-cKO mice. Stroke was then induced by endothelin-1 microinjection in the left medial prefrontal cortex and verified by MRI. PSD mice were treated with FLX/vehicle for 3-weeks and during behavioral and cognitive tests. Brains collected for immunofluorescence staining of PcdhαC2, FosB and SERT to assess brain-wide activity and 5-HT innervation.

Results: Pcdhα-WT PSD mice treated with FLX showed significant behavioral recovery, increased PcdhaC2 expression in 5-HT neurons, restored serotonergic innervation, and increased FosB in dorsal raphe. In contrast, PSD mice displayed tangled serotonergic axons in hippocampus and mPFC, with FLX treatment showing no improvement or worsened axonal tangling and more severe behavioral phenotypes. These findings show that PcdhαC2 is critical for FLX-induced serotonergic recovery from PSD.

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**P65**

The non-hallucinogenic serotonin 1B receptor is involved in the persisting behavioral effects and neural mechanisms of psilocybin in mice

**Sixtine Fleury**

Recent studies highlight the strong potential of psychedelic therapies for psychiatric disorders, with numerous clinical trials exploring psilocybin's effects on major depressive disorder. The persisting clinical effects of psychedelic therapies are most attributed to activation of the serotonin 2A receptor (5-HT2A R) based on its role in the acute hallucinatory effects. However, psilocin, the active metabolite of psilocybin, binds to many serotonin subtypes. Investigating the role of other 5-HTRs is crucial to comprehend psilocybin's lasting clinical effects. We hypothesize that psilocybin influences depressive-like behaviors via 5-HT1BRs, non-hallucinatory serotonin receptors previously implicated in mediating depressive phenotypes and neural plasticity. We first established a protocol to test psilocybin's effects on anxiety-like behavior and anhedonia. We then used transgenic mouse models to assess the role of 5-HT1BR in mediating these behavioral responses. Our results show that psilocybin reduced anxiety and decreased anhedonia in female mice treated with chronic corticosterone. Interestingly, mice lacking 5-HT1BR showed no significant reversal in cort-induced anhedonia, and no reduced anxiety in the elevated plus maze and novelty-suppressed feeding test compared to saline-treated knockout mice. Next, we used c-fos labeling to quantify whole-brain neural activity following psilocybin administration in controls and mice lacking 5-HT1BR to compare neural activity and assess functional connectivity across brain regions. Whole brain c-fos analysis shows that 5-HT1BR expression influences brain-wide activity following psilocybin administration in regions regulating emotional processing and cognitive function, like the amygdala and basal ganglia. Overall, this research suggests that psilocybin induces antidepressant effects, which the serotonin 1B receptor may partially modulate.

**P66**

The medullary 5-HTergic neuron subtype called Tac1-Pet1 augments breathing during quiet wake and counters morphine-induced respiratory depression

**Kathryn Lehigh**

Brainstem serotonin (5-HT)-producing neurons modulate breathing, with distinct 5-HTergic neuron subtypes contributing to specific respiratory functions (Brust et al., 2014; Okaty et al., 2019). The Tac1-Pet1 neuron subtype, named by gene expression, distributes soma across raphe obscurus (ROb), raphe magnus (RMg), and lateral paragigantocellularis. Tac1-Pet1 neurons innervate brain and spinal cord regions involved in respiratory motor output (i.e., hypoglossal, phrenic, nucleus ambiguus) and nuclei critical for respiratory rhythm generation and modulation (i.e., preBotzinger, parabrachial). Tac1-Pet1 neurons are required for mounting a full respiratory response to hypercapnia (Hennessy et al., 2017). Until now, their sufficiency to drive breathing had not been assessed, though our axonal projection and functional data, as well as prior work on 5-HTergic ROb neurons, suggest that activating these neurons may augment breathing (Depuy et al., 2011; Pilowsky et al., 2014). We combined our mouse intersectional genetic platform with DREADD chemogenetics to activate Tac1-Pet1 neurons and measure respiration via awake whole-body plethysmography. We found that acute activation of Tac1-Pet1 neurons increases respiratory rate and minute ventilation – comparable to activating Pet1+ neurons en masse. Initial work using focal viral DREADD injections to infect ROb 5HTergic neurons has shown a similar increase in respiratory rate after CNO-hM3Dq triggered neuron excitation, indicating brain specificity of the respiratory phenotype—in alignment with prior optostimulation of ROb 5HTergic neurons (Depuy et al., 2011). Given the sufficiency of

Tac1-Pet1 neurons to increase minute ventilation, we are exploring the capability of Tac1-Pet1 neurons for augmenting breathing under conditions of drug-induced respiratory depression and assessing Tac1-Pet1 neuron modulation of motor output (upper airway tone and diaphragm EMG) and respiration across sleep-wake states. Preliminary findings demonstrate acute activation of Tac1-Pet1 neurons can counter opiate-induced disordered breathing.

**P67**

CART Peptide Modulation of Serotonergic Activity: A Key Driver of Anxiety

**Nagalakshmi Balasubrama**

The aftermath of COVID-19 has led to a 33% increase in cases of major depressive disorder (MDD), with many patients exhibiting resistance to current monoamine-based treatments. This underscores an urgent need for alternative therapeutic strategies. In this study, we have identified a novel circuit that modulates dorsal raphe nucleus (DRN) serotonin (5HT) function via a small neuropeptide – CART (cocaine- and amphetamine-regulated transcript). Previous studies have shown reduced CART levels in MDD patients, and individuals with mutations in the CART gene exhibit elevated anxiety/stress disorders. Our initial findings reveal CART projections to the DRN, along with decreased c-fos activity in ventral 5HT-DRN neurons following central administration of CART peptide. Moreover, intracranial administration of CART into the DRN induced anxiogenic effects in male C57BL/6J mice. This anxiogenic effect was accompanied by a reduction in 5HT-DRN activity and release, as demonstrated using in vivo fiber photometry. We observed CART inputs to the DRN from various subcortical nuclei, with the Edinger-Westphal centrally projecting nucleus (EWcp) showing heightened responsiveness to acute restraint stress. Interestingly, chemogenetic activation of DRN-projecting CART-EWcp neurons recapitulated anxiogenic effects observed with intra-DRN CART infusion in males, but not females, suggesting a sex-specific role for this pathway. Chemogenetic stimulation of CART-EWcpDRN pathway inhibited 5HT neurons via the recruitment of GABAergic neurons. In summary, this study highlights CARTEWcpDRN circuit as a critical driver of anxiety-like behavior by promoting feedforward inhibition of 5HT neurons in a sex-specific manner. These findings identify this circuit as a promising therapeutic target for depression.

**P68**

Acute effects of MDMA, MDA and their prodrugs Lysine-MDMA and Lysine-MDA in healthy participants

**Isabelle Straumann**

3,4-Methylenedioxymethamphetamine (MDMA) is used recreationally, in research, and in MDMA-assisted psychotherapy. The effects of its psychoactive metabolite 3,4-methylenedioxyamphetamine (MDA), have never been directly compared to the effects of MDMA in humans. Prodrugs like Lysine-MDMA and Lysine-MDA were developed to enhance tolerability and reduce abuse potential. We compared acute responses to MDMA (100 mg), MDA (93.9 mg), Lysine-MDMA (171.7 mg), Lysine-MDA (165.6 mg), and placebo dosed equimolarly and in a counterbalanced order. Outcome measures included acute subjective and autonomic effects, and pharmacokinetics. MDA and MDMA induced effects of comparable intensity. MDA induced more subjective stimulant-like effects, more negative “bad drug” effects and tended to produce slightly more fear and visual changes. The effect duration (mean ± SEM) of MDA was 6.6 ± 0.7 hours and longer compared to the effect duration of MDMA of 3.7 ± 0.4 hours. Lysine-MDA did not induce different effects than MDA other than a slightly later effect onset and a longer time to maximal effect. The plasma elimination half-life (geometric mean ± SEM) of MDMA and MDA was 7.3 ± 0.7 and 8.4 ± 0.4 hours, respectively. When Lysine-MDMA was given, no MDMA could be measured in the blood samples and no subjective or autonomic effects occurred. MDMA and MDA produce similar acute subjective and autonomic

effects. MDA produced more stimulant-type effects and acted longer than MDMA. Lysine-MDA represents a functional slow-release prodrug form of MDA. Lysine-MDMA did not release MDMA due to the tertiary amine structure and is therefore not a functional prodrug of MDMA.

**P69**

Molecular Determinants of Serotonin 5-HT2C Receptor Non-canonical Signaling

**Emma Bonniwell**

The serotonin 5-HT2C receptor, a G protein coupled receptor (GPCR), is a promising therapeutic target for addiction, obesity, anxiety, and certain forms of epilepsy. 5-HT2C is also a top target activated by serotonergic psychedelic drugs like LSD and psilocin/psilocybin, which demonstrate rapid-acting and durable antidepressant activity in clinical studies. Although 5-HT2C is known to canonically couple to Gq/11 pathways, previous studies have suggested that GPCRs, like 5-HT2C, can activate other (non-canonical) G protein subtypes. It remains unclear which 5-HT2C signaling pathway(s) is responsible for therapeutic effects for agonists, such as psychedelics or selective 5-HT2C agonists like lorcaserin (Belviq), which was recently withdrawn for increased cancer risk. Therefore, the purpose of this study was to profile 5-HT2C G protein coupling using biosensor and signaling technologies, identify how 5-HT2C RNA editing isoforms affects coupling, and to profile 5-HT2C agonists toward identifying structure-function relationships that confer non-canonical signaling. Our results reveal that unlike 5-HT2A and 5-HT2B, 5-HT2C promiscuously signals via canonical (Gq/11/15) and non-canonical pathways (Gi/o/z, G12/13, β-arrestin1/2), and that RNA editing of intracellular loop 2 (ICL2) leads toward Gq/11 preference. Furthermore, using chimeric and structural approaches, we reveal that ICL2, intracellular loop 3 (ICL3) and the C-terminus (CT) regions of 5-HT2C are responsible for mediating non-canonical G protein coupling. Finally, psychedelics, including LSD and psilocin, show preference for 5-HT2C Gq/11 signaling over other non-canonical G protein signaling, suggesting psychedelic avoid ‘off-target’ non-canonical signaling pathways. Ultimately, this work will lead toward the fine-tuned design of 5-HT2C pathway-selective agonists for several neurological diseases.

**P70**

Marked Sex Differences are Observed in Heroin Acquisition and Affective States in Rats, but Converge to Similar Levels of Footshock Stress-Induced Reinstatement

**Claire Deckers**

Negative affective states associated with stress are known to trigger the development of heroin addiction and relapse. The sexually dimorphic dorsal raphe nucleus (DRN)- serotonin (5-HT) system contributes to affective components of drug use and is known to be impacted by experience of stress. Stress results in the release of corticotropin-releasing factor (CRF), which indirectly dampens DRN 5-HT activity. We hypothesize that this dampening promotes negative reinforcement-driven heroin seeking behaviors, and further that females will be more vulnerable to stress-induced heroin seeking. In baseline studies, ultrasonic vocalizations (USVs) reflecting negative (22 kHz) and positive (50 kHz) affective states were recorded during heroin self-administration and footshock stress-induced reinstatement in rats. Females showed escalated heroin self-administration, but this was not linked to gonadal hormones. Males emitted more 50 kHz calls, suggesting positive reinforcement-driven intake. We observed successful stress-induced reinstatement in both males and females, but females displayed greater “frontloading” of active lever pressing. Additionally, all females met reinstatement criteria, compared to 77% of males. Ex-vivo patch clamp electrophysiology of DRN 5-HT cells following reinstatement revealed no sex differences in inhibitory post-synaptic current (IPSC) amplitude, but greater IPSC frequency in females, indicating greater

GABAergic inhibition of 5-HT cells in response to stress. These findings indicate sex-specific vulnerabilities to the development of heroin use disorders, potentially driven by differential DRN 5-HT contributions. Future studies will focus on direct assessment of the role of DRN 5-HT systems in these behaviors using complementary chemogenetic, fiber photometric, and electrophysiological approaches.

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**P71**

5-HT4 receptor activation reverses stress-induced dopamine system dysfunction

**Olivia Yang**

Psychosis is a debilitating condition characterized by hallucinations and delusions. While often associated with schizophrenia, psychosis is also prevalent in approximately 64% of patients with post-traumatic stress disorder (PTSD). Despite its high occurrence, psychosis in PTSD remains understudied, limiting therapeutic options. Psychotic symptoms are widely believed to result from heightened mesolimbic dopamine transmission. Specifically, increases in dopamine neuron population activity, defined as the number of spontaneously firing dopamine neurons in the ventral tegmental area (VTA), have been linked to the development of psychosis. We have previously demonstrated that acute foot shock stress produces profound increases in dopamine population activity by dysregulation of a multisynaptic circuit comprising the ventral hippocampus, ventral pallidum, and nucleus accumbens (NAc). The 5-HT4 receptor (5-HT4R) presents a pharmacological target of interest for modulating mesolimbic dopamine activity due to its expression in key brain regions of this circuit. In this study, we examined the effects of 5-HT4R activation on stress-induced dysregulation of the dopamine system. Using in vivo extracellular electrophysiology, we found that the 5-HT4R agonist, BIMU8 (1 mg/kg i.p.), completely restored normal dopamine neuron population activity in stressed animals. Further, intra-NAc delivery of BIMU8 replicated the effects of systemic administration, indicating that BIMU8 may exert its effects via NAc-associated pathways. Finally, we performed RT-PCR to determine changes in 5-HT4R mRNA before and after stress. Taken together, this study examines 5-HT4R activation as a novel mechanism to reverse stress-induced dopamine system dysfunction relevant to PTSD and comorbid psychosis.

**P72**

Astrocytes respond to serotonin and regulate serotonin-induced synaptic transmission in the basolateral amygdala

**Jacob Noeker**

Serotonin is a neuromodulator involved in many physiological processes, including cognition and emotional responses, and it has been implicated in psychiatric diseases such as anxiety and PTSD. While the investigation into serotonin signaling has focused on neurons, the effects of serotonin on astrocyte activity remain largely unknown. Using two-photon Ca2+ imaging, electrophysiology, pharmacology, chemogenetic, and optogenetic approaches in amygdala brain slices, we have investigated whether astrocytes in the basolateral amygdala respond to serotonin and the consequent neuromodulatory effects of serotonin-mediated astrocyte activation. We have found that astrocytes respond to exogenous and synaptically released serotonin with intracellular Ca2+ elevations. This serotonin-evoked astrocyte Ca2+ signal is associated with a long-lasting synaptic depression of excitatory synaptic transmission, which was mediated by activation of presynaptic adenosine A1 receptors. In contrast, serotonin elicited a long-term potentiation of synaptic transmission when the astrocyte Ca2+ signal was prevented in either IP3R2-/- mice or in the presence of A1 or 5HT2A receptor antagonists. We conclude that astrocytes in the basolateral amygdala respond to serotonin with intracellular Ca2+ elevations, which stimulate the release of the gliotransmitter ATP/adenosine that depresses excitatory synaptic transmission through the activation of

presynaptic A1 receptors. Furthermore, in the absence of serotonin-evoked astrocyte calcium signaling, serotonin elicits a potentiation of synaptic transmission. Therefore, astrocytes respond to serotonin and control the sign of serotonin-induced synaptic regulation.

**P73**

Investigation of 5-HT2A receptor localization using super resolution microscopy

**Blake Fordyce**

5HT2A serotonin receptors are widely known for their role in mediating the effects of psychedelics in a variety of species. As part of a large family of transducer-dependent, seven transmembrane receptors known as G-protein coupled receptors (GPCRs), the 5HT2A receptor is known to recruit G-proteins and -Arrestin at the intracellular face and can exhibit biased signaling towards these transducers on a ligand-dependent basis. Together, ligand type and transducer recruitment stabilize the receptor into one of multiple potential conformations. Interestingly, psychedelic 5HT2A agonists exhibit varying levels of G-protein and β-Arrestin recruitment. Due to this wide variability, it remains unclear how psychedelics affect 5HT2A conformations, activation, and signaling. This spectrum of conformational states results in distinct receptor trafficking patterns, influencing both short- and long-term cellular signaling. Single-domain antibodies known as nanobodies have been used to allosterically modulate conformational states of GPCRs. In previous work, our lab identified nanobodies with nanomolar affinity for the intracellular loop 3 (ICL3) of the Kappa Opioid receptor (KOR), which promote either a ligand-dependent inactive (Nb6) or active (Nb39) state conformation. To explore conformational changes in response to psychedelics, I employ custom bioengineered 5HT2A receptors containing the KOR ICL3 chimera, enabling allosteric modulation using this nanobody-based system. Blinking, photoactivatable, and/or photoconvertible fluorescent proteins are fused to proteins of interest (5HT2AR-KOR-ICL3, Nb6, etc.) to enable super-resolution microscopy for detecting receptor conformational states. Investigating psychedelic-induced 5HT2A-mediated conformational shifts and recruitment patterns in living cells. This work provides valuable insights into potential therapeutic cellular processes.

**P74**

Overexpression of α-synuclein in serotonin neurons alters the activity and connectivity profile of the mouse medial prefrontal cortex. Relation to anxiety disorders in PD

**Maria Sancho Alonso**

Parkinson's disease (PD) is a motor disorder, although it is now recognized that non-motor symptoms can precede the motor manifestations of PD and are an importat component at all disease stages. Among these, depression and anxiety are the most prevalent symptoms and cause a higher symptom burden in women than in men. Abnormalities in the serotonin (5-HT) system are generally considered a risk factor for mood disorders, and aggregates of α-synuclein (α-Syn) have been described in the 5-HT raphe nuclei of patients with PD and depression. To investigate the role of the ventromedial prefrontal cortex (vmPFC)-dorsal raphe nucleus (DR) circuit in depressive/anxiety disorders in PD, female mice overexpressing the mutant A53T form of human alpha-synuclein (h-α-Syn) in the DR induced by AAV vector were studied. The anxious/depressive phenotype was examined using several behavioral tests. Neuronal activity of infralimbic cortex (IL) and prelimbic cortex (PL) in awake mice was assessed by single-unit recordings and LFPs, using a multichannel probe and a virtual reality corridor. Functional and structural connectivity of interconnected brain areas was analyzed using functional magnetic resonance imaging (fMRI). Results showed that female mice overexpressing h-α-Syn in 5-HT neurons develop an anxiety-like phenotype. In parallel, h-α-Syn overexpression in the 5-HT system changed the firing rates

(spike width and firing frequency) of pyramidal and GABAergic neurons (somatostatin and parvalbumin interneurons) in the PL and IL, as well as brain connectivity. This may contribute to neuropsychiatric symptoms in PD. Ketamine treatment reversed some of these functional alterations and the anxiety-related phenotype.

**P75**

Lateral hypothalamus promotes compulsive-like behavior through disinhibition of serotonin cells

**Renata Sadretdinova**

The serotonergic dorsal raphe nucleus (DRN) is implicated in obsessive-compulsive disorder (OCD). However, the mechanisms linking serotonin (5-HT) dysregulation to this condition remain elusive. Here, we investigate how the lateral hypothalamic area (LHA), a major presynaptic partner of the DRN, regulates DRN neuronal activity and translates it into behavior in mice. We first identified the postsynaptic target cell types for LHA axons in the DRN (DRN_LHA neurons). Using an anterograde transsynaptic tracing and immunostaining, we found that only ~15% of DRN_LHA neurons were serotonergic. Single-cell RNA sequencing further characterized the DRN_LHA population, identifying a substantial proportion of non-5-HT neurons as GABAergic. Combining optogenetic stimulation and ex vivo electrophysiology, we found that the LHA sends mixed excitatory and inhibitory inputs to DRN_LHA neurons, with a predominant inhibitory component. The same approach showed that DRN_LHA neurons, in turn, densely synapse onto local 5-HT neurons, with an overall strong inhibitory drive, suggesting that the LHA regulates 5-HT neurons via inhibition of local GABAergic interneurons. To explore behavioral implications, we chronically silenced DRN_LHA neurons using tetanus toxin (TeLC). TeLC-expressing mice displayed increased mobility, stereotypical circling, sucrose and water consumption, and nest shredding compared to controls, indicative of compulsive-like traits. In these mice, we observed increased cFos expression and a reduced frequency of spontaneous inhibition in 5-HT neurons. Overall, we described an indirect pathway that allows the LHA to control 5-HT neurons via a disinhibition mechanism. Chronic disinhibition of this circuit promotes compulsive-like behaviors, highlighting the potential involvement of LHA-DRN dysregulation in OCD pathophysiology.

**P76**

Biosensor evidence that glutamate co-released from 5-HT neurons modulates reward prediction error signals

**L. Sophie Gullino**

Many 5-hydroxytryptamine (5-HT) neurons express the vesicular glutamate transporter 3 (VGLUT3) co-releasing 5-HT and glutamate. These neurons are activated by reward and excite dopamine neurons innervating the nucleus accumbens (NAc). Thus, glutamate co-release from 5-HT neurons was hypothesised to impact NAc dopamine release and reward processing. Fibre photometry and the dopamine biosensor dLight1.1 were used to measure dopamine transients in the NAc of transgenic mice with VGLUT3 deletion in 5-HT neurons (VGLUT3 cKO5-HT, n=6) and controls (n=6). Mice progressed through operant tasks, where nose-poking an illuminated port triggered reward delivery, preceded by an auditory cue. Early in training, both groups learned to collect rewards and dopamine signals scaled with reward size ($F(1,11)=0.742$, $p=0.408$). Surprisingly, VGLUT3 cKO5-HT mice showed increased dopamine signals to reward-predictive cues compared to controls ($F(1,11)=7.04$, $p=0.023$). However, as training progressed with different inter-trial intervals (ITI), probabilistic reward delivery, or changed port location, VGLUT3 cKO5-HT mice displayed reduced dopamine signals to reward delivery, compared to controls (ITI: $F(1,8)=7.240$, $p=0.028$; probabilistic: $F(1,10)=5.88$, $p=0.036$; novel port: $t(10)=-2.452$, $p=0.034$). This was not caused by

overt changes in motivation as the number and latency of rewards collected did not differ between groups. In summary, early in training VGLUT3 cKO5-HT mice showed increased dopamine signalling in the NAc to reward-predictive cues, but further training led to a decrease in dopamine to the reward. These changes in dopamine transients are typical of associative reward-based learning but occurred faster in VGLUT3 cKO5-HT mice, suggesting that glutamate co-released from 5-HT neurons modulates reward prediction error signals.

**P77**

Acute but not Chronic Psilocybin Treatment Reduced Compulsive-like Behaviours in SAPAP3 Knockout Mice

**James Gattuso**

Psilocybin, a serotonergic psychedelic, demonstrates potential as a treatment for obsessive-compulsive disorder (OCD) and related symptoms (including anxiety and social behaviours). Utilizing SAPAP3 knockout (KO) mice, a well-validated mouse model of OCD, we assessed the effects of both acute (1 mg/kg, intraperitoneal) and chronic (0.1–1 mg/kg, oral gavage) treatment with psilocybin on (i) immediate behavioural outcomes (i.e. locomotor activity and the head-twitch response) and (ii) possible therapeutic effects on anxiety- and compulsive-like behaviours as well as sociability. Psilocybin administration induced a hyper-locomotor effect in WT but not in SAPAP3 KO animals suggesting differences in in-vivo serotonergic receptor signalling between genotypes. Psilocybin (1 mg/kg) reliability increased the number of head-twitches in mice (regardless of the genotype) indicating its hallucinogenic potential at this dose. Acute psilocybin reduced compulsive grooming behaviours in male KO mice for up to 1 week and significantly reduced grooming in female KO and wild-type (WT) mice. Chronic psilocybin, however, did not improve anxiety-like, depressive-like, or compulsive-like behaviours, nor did it affect the social deficits exhibited by SAPAP3 KO mice. This combined evidence highlights acute psilocybin as a promising candidate for reducing compulsive behaviours, while chronic administration offers limited benefits. Future studies should further investigate underlying molecular mechanisms and dose optimization, to enhance therapeutic outcomes.

**P78**

Sex-dependent synergy of serotonin reduction with early life stress to produce adult depressive-like and anxiety phenotypes

**Rocio Beatriz Foltran**

Early-life stress elicits anxiety and depressive-like behaviors both in humans and in rodents. At the same time, serotonin deficits have been linked to the origin of these mental disorders, and drugs enhancing brain serotonin levels are the first line treatment for such neuropsychiatric conditions. Here we sought to investigate the role of serotonin in a maternal separation model of early-life stress in mice. C57BL/6 pups were subjected to the maternal separation (MS) protocol (3h/day) during a postnatal critical period (P2-P14) while receiving daily injections of the tryptophan hydroxylase inhibitor para-chlorophenylalanine (PCPA, 10mg/kg/day s.c.). We analyzed the synaptic innervation of the prefrontal cortex to the dorsal raphe nucleus (PFC-to-DRN) circuit by high-resolution microscopy (array tomography), and serotonin cell activation by c-fos immunohistochemistry. Lastly, emotional responses to stress were evaluated in a battery of behavioral paradigms at adulthood (from P80). Our study showed that male mice were more susceptible to the MS exposure, resulting in long-lasting higher levels of anxiety and depressive-like behaviors. Interestingly, in females, MS had milder effects on emotional responses to stress that became apparent only when serotonin was concomitantly depleted. Behavioral findings could relate to changes in the

PFC-to-DRN circuit connectivity and DRN serotonin neuronal responses to an acute stress. Our results emphasize the emotional alterations caused by early-life stress and the interaction with the serotonin transmission, in the search for understanding a main risk factor for the development of psychiatric disorders.

**P79**

Modulation of dorsal raphe nucleus connectivity and serotonergic signalling to the insular cortex in the prosocial effects of chronic fluoxetine

**Jennyfer Payet**

The serotonin reuptake inhibitor, fluoxetine, is commonly prescribed for anxiety disorders including social anxiety, and can alter social withdrawal. Despite its effectiveness, fluoxetine has a delayed therapeutic onset, often following an initial exacerbation in anxiety and avoidance behaviour. Adaptive changes in serotonergic neurotransmission likely mediate this delayed effect, although the exact mechanisms are still unclear. In this study we investigated the functional circuitry underlying the biphasic effects of fluoxetine on social approach-avoidance behaviour, and explored the place of serotonergic dorsal raphe nucleus (DR) ensembles in this network, using c-Fos-immunoreactivity as a correlate of activity. Highly anxious BALB/c mice were exposed to 12 days of fluoxetine-treated (18 mg/kg/day) or untreated drinking water, followed by an i.p. injection of fluoxetine or saline before social behaviour testing. Graph theory-based network analysis revealed brain-wide functional connectivity changes associated with acute and chronic fluoxetine, and identified neuronal populations in the insular cortex (IC) and DR as hub nodes central to the prosocial effects of chronic fluoxetine. Retrograde tracing was then employed to determine the role of serotonergic projections to the IC. Chronic fluoxetine increased activation in insula-projecting serotonergic neurons in the ventral DR, which was associated with increased social behaviour. Lastly, using a virally-delivered Tet-Off platform for temporally-controlled neuronal activation, we identified independent but interconnected serotonergic ensembles in the DR involved in the prosocial effects of chronic fluoxetine. These findings suggest that adaptive changes in functional connectivity, likely mediated by increased serotonergic signalling to the IC, underlie the effects of fluoxetine on social approach behaviour.

**P80**

Multimodal neuroimaging for PK/PD profile of NLX-204, a biased 5-HT1A receptor agonist

**Violette Richin**

Background: NLX-204, a highly selective biased agonist for serotonin 5-HT1A receptors, shows strong affinity (pKi: 10.19) and preferentially elicits ERK1/2 phosphorylation, a response linked to antidepressant activity. Preclinical studies in rodent models confirm its potential as an effective treatment for depression but it has not been characterized by means of neuroimaging techniques.

Methods: NLX-204 was radiolabeled with fluorine-18 to evaluate its brain distribution and pharmacokinetics using PET imaging in rats. Its pharmacodynamics were further explored through a dose-response study in awake, freely moving rats using functional ultrasound (fUS) imaging.

Results: The radiolabeling of NLX-204 was completed with high radiochemical purity and good molar activity. The in vivo evaluation of its binding was found to be repeatable. In vitro, [18F]NLX-204 showed high binding in hippocampus, septum and cingulate cortex, with moderate binding in frontal, parietal and occipital cortices and a low binding in thalamus and striatum. In vivo, distribution of [18F]NLX-204 labeling in rat brain was highest in the brainstem and thalamus-hypothalamus. In fUS studies, NLX-204 (0.16-0.64

mg/kg s.c.) induced robust and dose-dependent increases of brain activity in cortical regions and in the hippocampus.

Conclusions: This unique combination of PET and fUS brain imaging data demonstrates that NLX-204 engages 5-HT1A receptors in rat brain, eliciting robust activation in specific cortical regions. These results support the potential of NLX-204 as a promising candidate for treatment of mood disorders.

Funding sources: Violette Richin's PhD is funded by Neurolixis.

**P81**

Perinatal fluoxetine exposure and lifelong behavioral alterations: shedding light on dynamics of sensitive periods

**Maria Teresa Gallo**

Brain development follows timed processes and any deviation from these sequences can disrupt brain formation and function and therefore lead to pathological conditions that often do not manifest until adulthood. Accordingly, we previously demonstrated that fluoxetine (FLX) exposure in early life leads to long-lasting behavioral alterations, with male rats developing an anhedonic-like behavior while females showing cognitive deficits. Here, we examined if changes in sensitive period dynamics, specific windows of development, may underlie these pathological-like behaviors. We evaluated the expression of parvalbumin-positive (PV+) interneurons and the formation of perineuronal nets (PNNs), in the prefrontal cortex and dorsal hippocampus of adolescent rats. Indeed, PNNs are specialized extracellular matrix structures that enwrap PV+ interneurons, regulating neuronal plasticity and stabilizing synaptic connections during critical periods of brain development. To these aims, male and female Wistar rats were exposed to FLX at a dose of 15 mg/kg/day, during gestation (prenatal-FLX) and lactation (postnatal-FLX). Our results revealed that the period of FLX exposure shapes PV+ interneuron expression and PNNs formation in a brain region and sex-specific manner, suggesting that FLX exposure during critical developmental windows alters the dynamics of sensitive periods. By measuring "trigger" (e.g., Bdnf, Bmal1, Gad67) and "brake" genes (e.g., Ncan, Bcan, Sem3a) we found that the FLX exposure affected the opening and closure of sensitive periods. Our findings indicate that the sex-dependent behavioral alterations due to perinatal FLX administration are likely the result of changes in the dynamics of sensitive periods supporting the role of neuroplasticity in the development of neuropsychiatric conditions.

**P82**

Modeling the Neuroprotective Role of Estrogen and Progesterone in Brain Inflammation and Serotonin Regulation

**Beatrice Baumberger**

Neuroinflammation has emerged as a critical factor in the etiology of depression, with the effects of inflammation on serotonin signalling playing a central role. While much is known about the neuroprotective properties of estrogen and progesterone, the specific impact of these hormones on brain inflammation and serotonin levels throughout the female lifecycle remains poorly understood. Using a novel mathematical model, I investigate how fluctuations in these hormones during the menstrual cycle, pregnancy, and menopause influence brain inflammation and downstream serotonin regulation. I demonstrate how the fluctuation in estrogen and progesterone levels, specifically the ratio between the two hormones affect neuroinflammation by modulating histamine levels, a key mediator of inflammatory responses. By suppressing excessive histamine activity, these hormones indirectly restore serotonin levels mitigating

depression symptoms. The model highlights distinct patterns of neuroprotection, including enhanced neuroprotective capabilities during pregnancy, and increased inflammatory susceptibility during menopause. I will present key features of the model, including predictions about hormone-mediated modulation of histamine and serotonin balance, and discuss the implications for understanding brain health across the female lifespan. This work lays the foundation for integrating computational tools with experimental approaches to elucidate sex hormone contributions to brain resilience and inflammation.

**P83**

Early-life stress alters development of prefrontal circuits modulating dorsal raphe serotonin neurons: Implications for maladaptive adult emotional behavior.

**Carla Veronica Arganaraz**

The vulnerability to stress and mood disorders is thought to have a developmental origin. Converging evidence indicates that prefrontal cortex (PFC) circuits engaged in cortico-limbic top-down control are key in the developmental etiology of mood disorders. The neural circuit connecting the PFC to the dorsal raphe nucleus (DRN) is critically involved in stress-coping responses and mood control, and represents the main source of brain serotonin (5-HT). During mouse development there is a critical period [postnatal days (P) 2 to 14] when environmental factors can influence neurodevelopmental trajectories with long-lasting consequences for adult life. The early-life stress of maternal separation (MS) is a validated model that causes adult emotional alterations. We investigate how the early PFC-to-DRN circuit is formed and refined, and how dysregulation of its neurodevelopment is affected in the MS model. We evaluated alterations in the synaptic connectivity of the PFC-to-DRN circuit using the high-resolution microscopy technique Array Tomography and the activation of DRN 5-HT neurons was assessed by cFos immunostaining. To investigate possible physiological correlates accompanying morphological changes we performed ex-vivo patch clamp recordings on both 5-HT and GABA DRN neurons of MS mice at these different developmental ages. Our work indicates that maternally-separated mice have alterations in the PFC-to-DRN circuit and 5HT neuron stress-dependent activation. Overall, we established the first two weeks of postnatal life as a critical stage in the maturation of the PFC-DRN circuit, revealing maladaptations on this circuit caused by MS as possible neural substrates contributing to an increased adult vulnerability to psychiatric disease.

**P84**

Role of β-arrestin-2 in psychedelic drug-induced head-twitch responses and expression of plasticity-related genes in mice

**Aurelija Ippolito**

Psychedelic drugs are in advanced clinical trial for the treatment of major depression. There is much current interest in whether hallucinogenic and antidepressant effects of psychedelics are pharmacologically separable. A potential mechanism is biased agonism at the 5-HT2A receptor whereby preferential signalling through Gq or ß-arrestin-2 pathways drives the psychedelic versus antidepressant effect. Here we investigated the role of ß-arrestin-2 signalling in mouse proxies of psychedelic drug-induced hallucinogenic and antidepressant effects, the head-twitch response (HTR) and expression of plasticity genes, respectively. Wildtype and ß-arrestin-2 knockout mice were administered psilocin (1mg/kg), DOI (2mg/kg) or vehicle. The HTR was then scored for 20 min, and after 60 min cortical tissue was extracted for RT-qPCR analysis of plasticity gene mRNA (cFos, Egr1, Egr2, Arc). Data were analysed using a linear mixed-effects model, followed by a Type III ANOVA and a post-hoc Tukey's test. In wildtype mice both psilocin and DOI evoked a HTR and increased plasticity gene expression compared to vehicle. The HTR to psilocin was modestly reduced (-20%) in ß-arrestin-2 knockout mice but that of DOI was unchanged.

Plasticity gene responses to psilocin and DOI were not different in ß-arrestin-2 knockout versus wildtype mice. ß-arrestin-2 knockout altered neither ß-arrestin-1 nor 5-HT2A receptor expression. In conclusion, ß-arrestin-2 knockout had little or no effect on the HTR and plasticity gene response to psilocin and DOI. These data do not support the contention that the hallucinogenic and antidepressant actions of psychedelic drugs are separable from a 5-HT2A receptor signalling pathway perspective.

**P85**

The microbiome and serotonin immune interactions

**Lewis Yu**

Serotonin (5-hydroxytryptamine, 5-HT) is a hormone and neurotransmitter that modulates diverse biological functions such as food intake, sleep, and a variety of complex behaviors. While commonly associated with neurotransmission, 5-HT is also an important chemical signal outside of the nervous system and is increasingly implicated as a key signaling factor for regulating immune function. Under homeostatic conditions, enterochromaffin cells (ECs) in the gut enhance 5-HT synthesis and release in response to microbial metabolites produced by indigenous spore forming (Sp) microbiota. EC-derived 5-HT is secreted basolaterally into the gut mucosa where enteric immune cells are poised to sense and respond to 5-HT through various 5-HT receptors. In particular, resident dendritic cells (DCs), the primary antigen presentation cell involved in lymphocyte maturation, highly express 5-HT receptor subtype 7 (5-HT7). Interestingly, select gut bacterial species identical to Sp microbiota have been shown to promote the lymphocyte maturation by inducing regulatory Th (Treg) cells, but whether 5-HT-5-HT7 signaling is involved in maintaining tolerogenic programs remains unclear. We demonstrate that i) microbiome depleted mice exhibit dampened 5-HT synthesis and weak Treg induction but are restored upon colonizing with Sp microbiota, ii) altering 5-HT bioavailability modulates Treg development, iii) blocking 5-HT7 signaling using pharmacologic and genetic (DC-specific) approaches reduce Tregs, and iv) the chemotactic and tolerogenic potential of DCs is modulated by the microbiome through 5-HT-5-HT7 signaling. Collectively, our study demonstrates that microbial induction of intestinal Tregs is regulated by 5-HT-5-HT7 signaling in DCs and represents a novel mechanism for microbiome-immune interactions in the gut.

MEETING
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# Additional Information and Directions

## Welcome Dinner (Heurigen Restaurant Fuhrgassl-Huber)

Please be reminded that travel from the University Clinic of Dentistry to the **Welcome Dinner (Heurigen Restaurant Fuhrgassl-Huber, Neustift am Walde 68, 1190 Vienna)** is at your own expense and is to be organized on your own.

Therefore, we put together this short guide on how to get there (what tram or bus to use and where to buy tickets). Public transportation is efficient and easy to use – and cheap. However, it is also possible to use Uber and/or Taxi services (~10-20 min faster than public transportation).

Please be reminded that we will start as soon as the majority of participants have arrived at the Heurigen Restaurant - between 6:30 and 7:00 p.m. - with the Welcome address of a representative of the Mayor of Vienna. We look forward to welcoming you there!

## Possibility 1 to get there (Tram --> Bus)

**Von: Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien**
**Nach: Weingut Fuhrgassl-Huber, 1190 Wien (Essen und Trinken)**

Abfahrt: 07.07.2025, 18:12 Ankunft: 07.07.2025, 18:48 Dauer: 0:36 h Umsteigen: 1x

| Haltestelle | Uhrzeit | Gleis / Steig | Verkehrsmittel | Dauer |
|---|---|---|---|---|
| Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien | Ab 18:12 | | Fußweg | 0:07 h |
| Wien Spitalgasse | An 18:19 | | | |
| Wien Spitalgasse | Ab 18:19 | | Straßenbahn 37 Richtung: Wien Hohe Warte | 0:05 h |
| Wien Nußdorfer Straße | An 18:24 | | | |
| Wien Nußdorfer Straße | Ab 18:24 | | Umsteigezeit | 0:02 h |
| Wien Nußdorfer Straße | An 18:26 | | | |
| Wien Nußdorfer Straße | Ab 18:26 | | Stadtbus 35A Richtung: Wien Salmannsdorf | 0:20 h |
| Wien Neustift am Walde | An 18:46 | | | |
| Wien Neustift am Walde | Ab 18:46 | | Fußweg | 0:02 h |
| Weingut Fuhrgassl-Huber, 1190 Wien (Essen und Trinken) | An 18:48 | | | |

## Possibility 2 to get there (Bus --> Bus)

**Von: Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien**
**Nach: Weingut Fuhrgassl-Huber, 1190 Wien (Essen und Trinken)**

Abfahrt: 07.07.2025, 18:18 Ankunft: 07.07.2025, 18:55 Dauer: 0:37 h Umsteigen: 1x

| Haltestelle | Uhrzeit | Gleis / Steig | Verkehrsmittel | Dauer |
|---|---|---|---|---|
| **Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien** | Ab 18:18 | | **Fußweg** | **0:07 h** |
| **Wien Spitalgasse** | An 18:25 | | | |
| **Wien Spitalgasse** | Ab 18:25 | | **Stadtbus 40A** Richtung: Wien Döblinger Friedhof/Felix-Dahn-Str. | **0:09 h** |
| **Wien Währinger Park** | An 18:34 | | | |
| **Wien Währinger Park** | Ab 18:36 | | **Stadtbus 35A** Richtung: Wien Salmannsdorf | **0:17 h** |
| **Wien Neustift am Walde** | An 18:53 | | | |
| **Wien Neustift am Walde** | Ab 18:53 | | **Fußweg** | **0:02 h** |
| **Weingut Fuhrgassl-Huber, 1190 Wien (Essen und Trinken)** | An 18:55 | | | |

**Possibility 3 to get there (Tram --> Bus)**

**Von: Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien**
**Nach: Weingut Fuhrgassl-Huber, 1190 Wien (Essen und Trinken)**

Abfahrt: 07.07.2025, 18:27 Ankunft: 07.07.2025, 19:03 Dauer: 0:36 h Umsteigen: 1x

| Haltestelle | Uhrzeit | Gleis / Steig | Verkehrsmittel | Dauer |
|---|---|---|---|---|
| Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien | Ab 18:27 | | Fußweg | 0:07 h |
| Wien Spitalgasse | An 18:34 | | | |
| Wien Spitalgasse | Ab 18:34 | | Straßenbahn 38 Richtung: Wien Grinzing | 0:10 h |
| Wien Gatterburggasse | An 18:44 | | | |
| Wien Gatterburggasse | Ab 18:44 | | Umsteigezeit | 0:03 h |
| Wien Gatterburggasse | An 18:47 | | | |
| Wien Gatterburggasse | Ab 18:47 | | Stadtbus 35A Richtung: Wien Salmannsdorf | 0:14 h |
| Wien Neustift am Walde | An 19:01 | | | |
| Wien Neustift am Walde | Ab 19:01 | | Fußweg | 0:02 h |
| Weingut Fuhrgassl-Huber, 1190 Wien (Essen und Trinken) | An 19:03 | | | |

**How to get from the Bus/Tram Stop to the Heurigen Restaurant:**

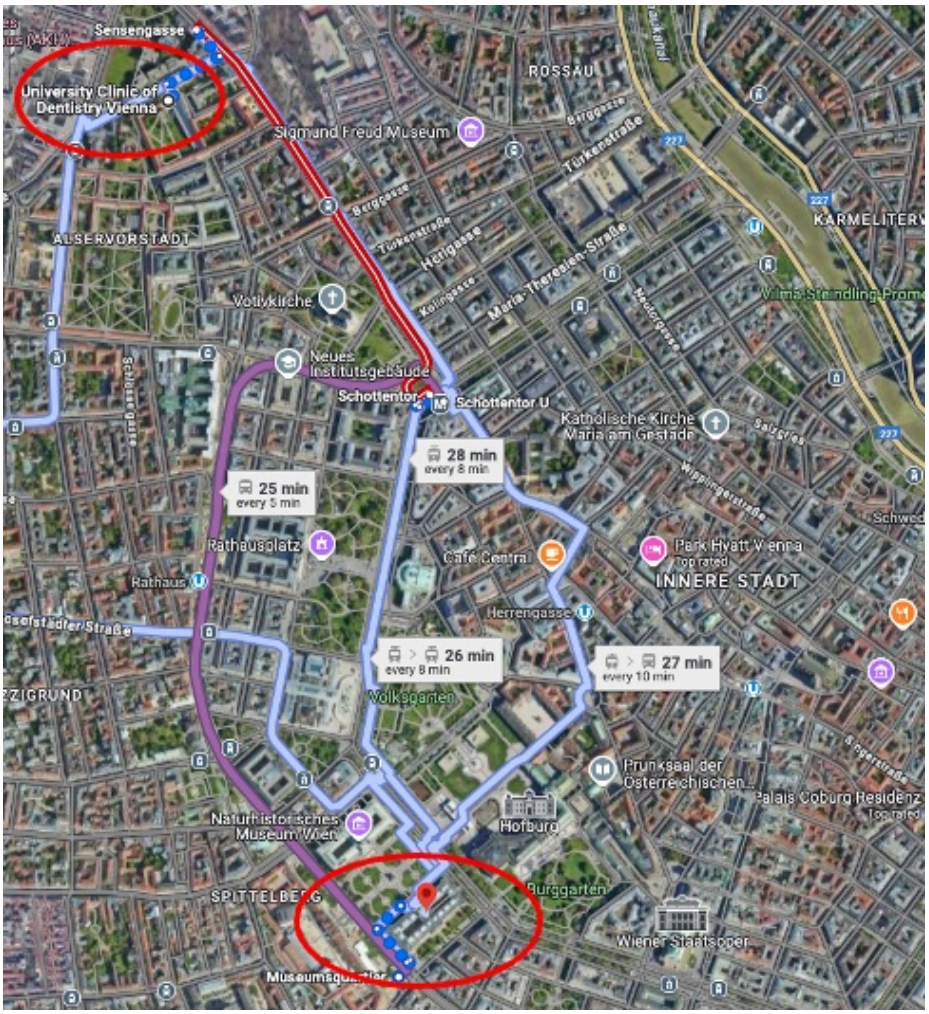

## Farewell Dinner (Kunsthistorisches Museum)

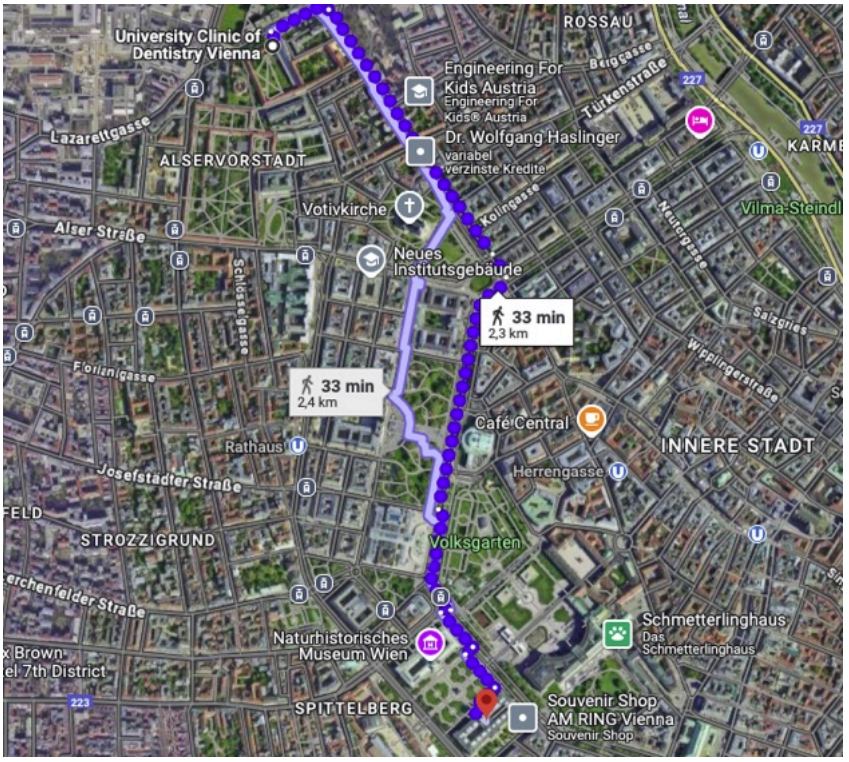
Please be reminded that travel from the University Clinic of Dentistry to the **Farewell Dinner (Kunsthistorisches Museum, Maria-Theresien-Platz, 1010 Vienna)** is at your own expense and is to be organized on your own.

Therefore, we put together this short guide on how to get there (what tram or bus to use and where to buy tickets). Public transportation is efficient and easy to use – and cheap. However, it is also possible to use Uber and/or Taxi services (~5-10 min faster than public transportation).

Please be reminded that we can only enter the Museum from 7 p.m. onwards and will then have Aperitif and Canapés - followed by a guided tour through the Museum (in several small groups) - the tours start at around 7:30. After arriving at the tables, we will then have some Farewell addresses and from one of our sponsors and the festivity may begin! We look forward to welcoming you there!

Please see two maps on the next page for an overview of how to get there: Upper map shows possibilities by public transportation. Lower map shows the walking option, which will take around half an hour - and will actually lead you by some of the finest pieces of architecture that Vienna has to offer on the Ringstrasse - the New University, the Burgtheater, the Townhall, the Austrian Parliament, Heldenplatz with the Hofburg - and the two large Museums, one for natural history, opposite the Fine Arts Museum or Kunsthistorisches Museum - where our Farewell Dinner will take place. Obviously, you will see these buildings also when you go by tram…


Sensengasse
University Clinic of Dentistry Vienna
ROSSAU
Sigmund Freud Museum
Türkenstraße
ALSERVORSTADT
Votivkirche
Neues Institutsgebäude
Schottentor
Schottentor U
Katholische Kirche Maria am Gestade
28 min
every 8 min
25 min
every 5 min
Rathausplatz
Café Central
Park Hyatt Vienna
Top rated
INNERE STADT
Rathaus
Herrengasse
26 min
every 8 min
27 min
every 10 min
Volksgarten
Prunksaal der Österreichischen...
Palais Coburg Residenz
Naturhistorisches Museum Wien
Hofburg
SPITTELBERG
Burggarten
Wiener Staatsoper


University Clinic of Dentistry Vienna
ROSSAU
Engineering For Kids Austria
Engineering For Kids® Austria
Dr. Wolfgang Haslinger
variabel verzinste Kredite
Lazarettgasse
ALSERVORSTADT
Votivkirche
Alser Straße
Neues Institutsgebäude
33 min
2,3 km
33 min
2,4 km
Café Central
INNERE STADT
Rathaus
Herrengasse
Josefstädter Straße
STROZZIGRUND
Volksgarten
Schmetterlinghaus
Das Schmetterlinghaus
Naturhistorisches Museum Wien
SPITTELBERG
Souvenir Shop
AM RING Vienna
Souvenir Shop

**Possibility 1 to get there (Tram --> Tram)**

## Von: Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien
## Nach: Maria-Theresien-Platz, 1010 Wien

Abfahrt: 11.07.2025, 18:21 Ankunft: 11.07.2025, 18:46 Dauer: 0:25 h Umsteigen: 1x

| Haltestelle | Uhrzeit | Gleis / Steig | Verkehrsmittel | Dauer |
|---|---|---|---|---|
| **Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien** | Ab 18:21 | | **Fußweg** | **0:04 h** |
| **Wien Sensengasse** | An 18:25 | | | |
| **Wien Sensengasse** | Ab 18:25 | | **Straßenbahn 40** Richtung: Wien Schottentor U (Tiefgeschoß) | **0:04 h** |
| **Wien Schottentor U (Tiefgeschoß)** | An 18:29 | A | | |
| **Wien Schottentor U (Tiefgeschoß)** | Ab 18:29 | | **Umsteigezeit** | **0:04 h** |
| **Wien Schottentor** | An 18:33 | | | |
| **Wien Schottentor** | Ab 18:34 | C | **Straßenbahn 2** Richtung: Wien Dornbach | **0:04 h** |
| **Wien Parlament** | An 18:38 | | | |
| **Wien Parlament** | Ab 18:38 | | **Fußweg** | **0:08 h** |
| **Maria-Theresien-Platz, 1010 Wien** | An 18:46 | | | |

**Possibility 2 to get there (Tram --> Tram)**

## Von: Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien
## Nach: Maria-Theresien-Platz, 1010 Wien

Abfahrt: 11.07.2025, 18:21 Ankunft: 11.07.2025, 18:46 Dauer: 0:25 h Umsteigen: 1x

| Haltestelle | Uhrzeit | Gleis / Steig | Verkehrsmittel | Dauer |
|---|---|---|---|---|
| **Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien** | Ab 18:21 | | **Fußweg** | **0:04 h** |
| **Wien Sensengasse** | An 18:25 | | | |
| **Wien Sensengasse** | Ab 18:25 | | **Straßenbahn 40** Richtung: Wien Schottentor U (Tiefgeschoß) | **0:04 h** |
| **Wien Schottentor U (Tiefgeschoß)** | An 18:29 | A | | |
| **Wien Schottentor U (Tiefgeschoß)** | Ab 18:29 | | **Umsteigezeit** | **0:04 h** |
| **Wien Schottentor** | An 18:33 | | | |
| **Wien Schottentor** | Ab 18:34 | C | **Straßenbahn 2** Richtung: Wien Dornbach | **0:04 h** |
| **Wien Parlament** | An 18:38 | | | |
| **Wien Parlament** | Ab 18:38 | | **Fußweg** | **0:08 h** |
| **Maria-Theresien-Platz, 1010 Wien** | An 18:46 | | | |

**Possibility 3 to get there (Tram --> Tram)**

**Von: Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien**
**Nach: Maria-Theresien-Platz, 1010 Wien**

Abfahrt: 11.07.2025, 18:26 Ankunft: 11.07.2025, 18:52 Dauer: 0:26 h Umsteigen: 1x

| Haltestelle | Uhrzeit | Gleis / Steig | Verkehrsmittel | Dauer |
|---|---|---|---|---|
| Sensengasse 2A Zahnklinik d.Univ.Wien, 1090 Wien | Ab 18:26 | | Fußweg | 0:04 h |
| Wien Sensengasse | An 18:30 | | | |
| Wien Sensengasse | Ab 18:30 | | Straßenbahn 37 Richtung: Wien Schottentor U (Tiefgeschoß) | 0:04 h |
| Wien Schottentor U (Tiefgeschoß) | An 18:34 | A | | |
| Wien Schottentor U (Tiefgeschoß) | Ab 18:34 | | Umsteigezeit | 0:04 h |
| Wien Schottentor | An 18:38 | | | |
| Wien Schottentor | Ab 18:40 | C | Straßenbahn 2 Richtung: Wien Dornbach | 0:04 h |
| Wien Parlament | An 18:44 | | | |
| Wien Parlament | Ab 18:44 | | Fußweg | 0:08 h |
| Maria-Theresien-Platz, 1010 Wien | An 18:52 | | | |

## Ticketing in Vienna

A single fare ticket in Vienna costs €2.40, valid for one journey up to 80 minutes from validation, including transfers - but no interruptions or round trips (wienerlinien.at).
If bought onboard (tram or bus), it's slightly more at €2.60 (introducingvienna.com).

**Where to buy it**
You can purchase a single ticket:
Ticket machines at U bahn station entrances and some in trams/buses (wienerlinien.at)
WienMobil app or website — purchase and display digitally; validated immediately (wienerlinien.at)
Tobacconists ("Tabak Trafik") showing the blue WL logo (wienerlinien.at)
Wiener Linien info offices and ÖBB machines (especially at the main train station) (reddit.com)

**Important Note:** Not all onboard machines accept credit cards—rely on station or app purchases instead. However, all public transportation vehicles are equipped with coin operated vending machines.

**Validation rules**

Paper ticket: Must be stamped once in the blue validator before boarding metro, tram, or bus (wien.gv.at).
Digital ticket (app, PDF): automatically valid—no stamping required.

Stamping more than once isn't necessary and doesn't extend validity.

**Fines & enforcement**

Random ticket inspections are common—this is an open system with no turnstiles (gretewalz.com).
Traveling without a validated ticket can result in a €105 fine (within 14 days) or €145 afterward (virtualvienna.net).
Even forgetting to stamp a valid ticket has led to complaints about hefty fines (~€210).

**Quick tips**

| Scenario | What to know |
|---|---|
| **Buying in advance** | Head to any metro station entrance and use machines, or buy via the WienMobil app to avoid queues (reddit.com, reddit.com). |
| **Boarding a tram/bus** | If you don't have a ticket, buy from the onboard machine—but beware of the €2.60 fare. |
| **Using the app** | Digital tickets are all stamped automatically—just show on screen during inspection. |

**Summary**

**Price:** €2.40 single ticket; onboard €2.60
**Validity:** 80 minutes with transfers
**Purchase:** Machines, app, tobacconists, info offices
**Validation:** Stamp paper tickets once; app tickets auto-validate
**Enforcement:** Random checks; fines ~€105+

If you're staying longer or using public transit often, check out 24/48/72 hour passes (€8 €17), the Vienna City Card, or EasyCityPass, which bundle transport with tourist benefits (homepage.univie.ac.at, wienerlinien.at, reddit.com, gretewalz.com).

So for a single trip, get the €2.40 ticket, stamp it once (or use the app), and you're all set. Enjoy exploring Vienna - just keep your ticket ready! 😊