ISSR 2023

The 20th Meeting of the International Society for Serotonin Research

Meeting Handbook

ISSR 2023
Serotonin on the
Mexican Caribbean

International Society for Serotonin Research

Marriott Cancún Resort
Cancún, Mexico
April 23 – 27, 2023

Sponsors

This conference would not be possible without the generous support of our sponsors which enabled the meeting to be such a success.

Please wear badge during all sessions and events.

Cover photo credit: Noelle Anastasio

ISSR Officers and Council

President
2022-2024
Trevor Sharp, University of Oxford, UK

Immediate Past President
2019-2022
Anne M. Andrews, University of California Los Angeles, USA

Vice President-North America
2019-2024
John Neumaier, University of Washington, USA

Secretary
2022-2026
Noelle C. Anastasio, University of Texas Medical Branch at Galveston, USA

Treasurer
2022-2026
Lynette C. Daws, University of Texas Health San Antonio, USA

Immediate Past Secretary/Treasurer
2013-2022
Kelly A. Berg, University of Texas Health San Antonio, USA

Finance Committee
Judith R. Homberg, Donders Institute for Brain, Cognition, and Behaviour, Netherlands
Daniel Hoyer, University of Melbourne

Chairs Nomenclature Committee
Nicholas M. Barnes, University of Birmingham, UK
Daniel Hoyer, University of Melbourne, Australia

Councilors-Europe
2015-2018, 2019-2022
Natalia Alenina, Max Delbruck Centers for Molecular Medicine, Germany

2015-2018, 2019-2022
Valerie Compan, Genomic Functional Institute, France

2013-2016, 2017-2022
Andy McCreary, Janssen Prevention Center, Netherlands

2013-2016, 2017-2022
Adrian Newman-Tancredi, NeuroAct Communications, France

Councilors-North America

2017-2024
Chuck Nichols, Louisiana State University Health Sciences New Orleans, USA

2013-2016, 2017-2022
Kathryn Commons, Boston Children's Hospital, Harvard Medical School, USA

2014-2017, 2018-2022
Nancy Muma, University of Kansas, USA

Councilors-Rest of the World

2020-2023
Brian H. Harvey, North-West University, South Africa

2017-2024
Carlos M. Villallón, Cinevestav-IPN (Unidad Sur), Mexico

Associate Councilors-Europe

2013-2016, 2017-2022
Sebastian Fernandez, Institut du Fer à Moulin, France

2017-2022
Mariano Soiza-Reilly, Universidad de Buenos Aires-CONICET, Argentina

Associate Councilors-North America

2013-2016, 2017-2022
Adam Halberstadt, University of California San Diego, USA

2017-2024
Rheaclare Fraser-Spears, University of the Incarnate Word, USA

Associate Councilors-Rest of the World

2014-2017, 2018-2022
Silvina Diaz, Universidad de Buenos Aires-CONICET, Argentina

Webmasters

2022-Present
Sebastian Fernandez, Institut du Fer à Moulin, France
Mariano Soiza-Reilly, Universidad de Buenos Aires-CONICET, Argentina

The Serotonin on the Mexican Caribbean Organizing Committee Welcomes You!

Associate Professor
Noelle C. Anastasio
Department of Pharmacology & Toxicology
UT Medical Branch at Galveston, USA

Professor
Anne M. Andrews
Department of Chemistry & Biochemistry
University of California LA, USA

Professor
Kelly A. Berg
Department of Pharmacology
UT Health San Antonio, USA

Professor & Vice Chair
Kathryn A. Cunningham
Department of Pharmacology & Toxicology
UT Medical Branch at Galveston, USA

Professor
Lynette C. Daws
Cellular and Integrative Physiology
UT Health San Antonio, USA

Professor & Director
John Neumaier
Division of Psychiatric Neurosciences
University of Washington, USA

Professor
Trevor Sharp
Professor of Neuropharmacology
University of Oxford, UK

Professor
Carlos M. Villalon
Departamento de Farmacobiología
Cinvestav-IPN, Mexico

Program Committee

Natalia Alenina, Max-Delbruck-Centers for Molecular Medicine, Germany
Noelle C. Anastasio, University of Texas Medical Branch at Galveston, USA
Anne M. Andrews, University of California, Los Angeles, USA
Kelly A. Berg, University of Texas Health San Antonio, USA
Kathryn Commons, Boston Children's Hospital, Harvard Medical School, USA
Valerie Compan, Genomic Functional Institute, France
Kathryn A. Cunningham, University of Texas Medical Branch at Galveston, USA
Lynette C. Daws, University of Texas Health San Antonio , USA
Silvina Diaz, Universidad de Buenos Aires-CONICET, Argentina
Sebastian Fernandez, Institut du Fer à Moulin, France
Rheaclare Fraser-Spears, University of the Incarnate Word, USA
Adam Halberstadt, University of California San Diego, USA
Brian H. Harvey, North-West University, South Africa
Nancy Muma, University of Kansas, USA
Andy McCreary, Janssen Prevention Center, Netherlands
John Neumaier, University of Washington, USA
Adrian Newman-Tancredi, NeuroAct Communications, France
Chuck Nichols, Louisiana State University Health Sciences Center, USA
Trevor Sharp, University of Oxford, UK
Mariano Soiza-Reilly, CONICET - University of Buenos Aires, Argentina
Carlos M. Villalón, Cinvestav-IPN (Unidad Sur), Mexico
Noelia Weisstaub, Instituto de Neurología Cognitiva y CONICET, Argentina

ISSR President's Welcome

A warm welcome from the President of the International Society for Serotonin Research

Dear colleagues,

As the latest President of ISSR it gives me great pleasure to welcome you all to the 20th meeting of the International Society for Serotonin Research. The meeting location is the impressive Marriott Cancún Resort on the beautiful Caribbean coast of Mexico. It is five, pandemic-disrupted years since our last meeting in Cork, but the wait will be worth it!

The programme includes some of the world's leading serotonin researchers focusing on cutting edge topics of serotonin research, ranging from neuropharmacology and receptor signalling, all the way through to development and function of neural circuits and therapeutic applications. Each day will start with a pair of parallel symposia (6 in total), and this will be followed by a mix of plenary lectures and symposia, short communications, and posters.

I'm delighted to welcome Lynette Daws, René Hen, and Kjell Fuxe as our three plenary lecturers; for sure to hear about their pioneering work in the serotonin field will be a meeting highlight. In addition, we have seventeen talented early career researchers giving oral presentations, and many more contributing to poster presentations that will be in position throughout the meeting.

The program also includes several social events commencing with the opening reception on Sunday at 6 pm in the hotel's Seaside Garden. Here we will have the chance to interact with our very special community of serotonin researchers, renew old friendships and make new ones. This is followed by another Networking Social in the hotel on Tuesday, and the meeting will close with a Gala Dinner on Wednesday.

We are very grateful to our sponsors whose support is as ever, vital to the success of our meetings. The theme of the 2023 meeting "Serotonin on the Mexican Caribbean" gives a strong hint that the meeting has more to it than an exchange of ideas and new discoveries about serotonin! We have a unique opportunity on the days either side of the meeting to experience Mexican culture and ancient history and explore the stunning coastline and fascinating towns of the Yucatan Peninsula. I look forward to seeing you all in Cancún and hope you have a wonderful meeting.

Yours truly,

Trevor Sharp
ISSR President


Serotonin on the Mexican Caribbean
Marriott Cancún Resort and Spa, Cancún, Mexico
April 23-27, 2023
Translating Serotonin
•Preclinical and clinical developments in psychedelic therapy for psychiatric disorders
• SERT and beyond in treatment of depression
•Mining 5-HT for precision medicine for substance use disorders
• Towards a better understanding of how 5-HT modulates impulsivity
•Novel class of 5-HT2C agonists with therapeutic promise and novel signaling mechanisms
Connecting Serotonin
•Recent advances in molecular neuroimaging of the 5-HT system
•Development and plasticity of serotonergic circuitry
•Physiology & behavioral functions of dorsal raphe 5-HT neurons
•Lipid dynamics and 5-HT signaling in the brain: From molecular interactions to behaviour
Building Serotonin
• Serotonylation: new vistas of receptor-independent 5-HT signaling
•Physiological relevance of 5-HT receptor functional crosstalk
•Are cerebral functions of 5-HT4 receptors evolutionarily conserved?
• The fruit fly: An important model for the study of 5-HT neurobiology
www.serotoninclub.org/issr2023

Travel Award Recipients

Oliver John Belleza, Medical University of Vienna, Austria
Lindsay Cameron, University of California Davis, USA
Briana Chen, Columbia University Irving Medical Center, USA
Rebecca Coray, University of Zurich, Switzerland
Thomas Flanagan, Louisiana State University Health Science Center New Orleans, USA
Rocio Foltran, Instituto de Biologia Celular y Neurociencia, Argentina
Joanna Golebiowska, Maj Institute of Pharmacology Polish Academy of Sciences, Poland
Elizabeth Kitto, University of Michigan, USA
Felix Mayer, University of Copenhagen, Denmark
Carina Meinke, Florida Atlantic University, USA
Christina Merritt, University of Texas Medical Branch at Galveston, USA
Lluis Miquel-Rio, Spanish National Research Council, Spain
Nako Nakatsuka, Institute for Biomedical Engineering, Switzerland
Marco Niello, Medical University of Vienna, Austria
Rebecca Ravenelle, Columbia University Irving Medical Center, USA
Justin Saunders, Virginia Commonwealth University School of Medicine, USA
Faranak Vahid-Ansari, University of Ottawa Brain and Mind Research Institute, Canada

Honorary Irvine Page Plenary Lecture

Lynette C. Daws, Ph.D.
Frost Bank Distinguished Professor in
Biomedical Research
Departments of Cellular & Integrative Physiology and Pharmacology
University of Texas Health Science Center at San Antonio

Lynette (Lyn) Daws obtained her PhD in neuropharmacology under the mentorship of Drs. David Overstreet and Joe Orbach at the Flinders University of South Australia, Adelaide, Australia. Using animal models, she investigated the role of muscarinic and serotonergic receptors in depression. Following her interest in serotonin, depression and related psychiatric disorders, in 1994 Lyn joined the laboratory of Dr. Alan Frazer at the University of Texas Health Science Center in San Antonio for her post-doctoral studies. After training in electrochemical techniques to measure neurotransmitters in brain in real time, at the Marine Biological Laboratory, Woods Hole, Massachusetts, Lyn established high-speed chronoamperometry in the Frazer lab to measure clearance of serotonin from extracellular fluid in brain *in vivo*. Lyn was quick to discover that by applying exogenous serotonin to regions of the limbic forebrain implicated in mood disorders, she could interrogate mechanisms contributing to serotonin clearance dynamics and gain new insight into mechanisms of psychiatric diseases. She found that serotonin is efficiently removed from extracellular fluid *in vivo* by the norepinephrine transporter (NET), particularly in brain regions where expression of NET is greater than that of the high-affinity serotonin transporter (SERT). This was a leading observation that introduced the concept of transporter promiscuity and its importance for regulating biogenic amine neurotransmission. During this time, she was also the first to show that activity of SERT is regulated by the 5-HT_{1B} autoreceptor *in vivo*. Her postdoctoral research provided an important expansion of contemporary models of how diverse transport mechanisms intricately regulate biogenic amine neurotransmission is models of psychiatric illness.

Lyn subsequently returned to Australia as a Research Fellow for 18 months in the Department of Clinical and Experimental Pharmacology at The University of Adelaide before returning to San Antonio in 1998 as a Research Assistant Professor. She established her own laboratory in 2002 and quickly rose through the ranks to full tenured Professor in 2011. She is currently the Frost Bank Distinguished Professor in Biomedical Research. Lyn harnesses the combined power of *in vivo* electrochemical, biochemical, behavioral, pharmacological, and genetic approaches to her research. Lyn's groundbreaking studies have revealed organic cation transporters (OCTs), particularly OCT3, as crucial regulators of biogenic amine signaling. These transporters have low affinity for biogenic amines but a very high capacity to transport them. She found that OCT3 buffers the ability of selective serotonin reuptake inhibitors (SSRIs) to increase extracellular serotonin and produce antidepressant-like effects, providing a mechanism for the now well-established lack of therapeutic efficacy of SSRIs in many patients suffering from depression.

In collaboration with Dr. Harald Sitte and colleagues at the University of Vienna, Austria, Lyn found that the actions of amphetamine to cause dopamine release and produce rewarding and locomotor stimulant effects are highly OCT3-dependent. These ongoing studies provide a mechanistic basis for why these actions of amphetamine are not lost in dopamine transporter (DAT) knockout mice, which was considered surprising at the time since DAT was thought to be the primary target underlying amphetamine addiction. Most recently Lyn found that ethanol acts at OCT3 to inhibit monoamine clearance. Through this mechanism, ethanol potentiates the ability of cocaine (a DAT, NET and SERT blocker) to inhibit monoamine uptake and produce rewarding effects. Her exciting research indicates that OCT3 is a promising target for therapeutic intervention in a number of psychiatric and substance use disorders.

Lyn has been continuously funded by the National Institutes of Health since 2001 and has been the recipient of numerous other awards, including two NARSAD Young Investigator and two NARSAD Independent

Investigator Awards. She has trained many students and post-doctoral fellows who have gone onto successful careers. She publishes in top journals including Proceedings of the National Academy of Science, Molecular Psychiatry, Journal of Neuroscience and Neuropsychopharmacology. Among her many roles and activities, Lyn is currently the editor in chief of Pharmacological Reviews, a fellow of the American College of Neuropsychopharmacology and is co-founder and former President of the International Transmembrane Transporter Society. Of special note, Lyn has a long association with the International Society for Serotonin Research (Serotonin Club), serving as councilor (2009-2010), Vice President (2011-2014), President (2015-2016), Immediate Past President (2017-2019), and is the current Treasurer (2022-present).

Honorary Maurice Rapport Plenary Lecture

Kjell Fuxe, MD, Ph.D.
Professor Emeritus
Karolinska Institutet

Dr. Kjell Fuxe identified the dopamine, noradrenaline and serotonin brainstem neurons in his thesis in 1965 and related papers (1964 -1966) using the Falck-Hillarp histological-fluorescence method. The monoamine innervation of the brain and the spinal cord appeared to take place through an extensive collateralization of the ascending and descending catecholamine and serotonin pathways from the brainstem. As to the serotonin neurons, what stands out in his memories is the unique beauty of the serotonin nerve cell groups in the median and dorsal raphe of the midbrain. When the images created by the serotonin fluorescence in these raphe and para-raphe neurons reached his eyes for the first time in the darkness of the microscopy room they changed his life.

In 1967, with Urban Ungerstedt, they found after intraventricular 5-HT injections that 5-HT accumulated in central 5-HT neurons. The overall analysis indicated the existence of a 5-HT uptake-concentration mechanism in the serotonin neurons that may be a target for antidepressant drugs. They then approached Arvid Carlsson for a collaboration to test the hypothesis. In 1968, our two groups found that imipramine could block the 5-HT uptake mechanism. The same was true also for other classical antidepressant drugs. The Stockholm group also approached Hans Corrodi in Goteborg to study the effects of imipramine on 5-HT turnover. In 1968, they found that known antidepressant drugs could reduce 5-HT turnover in the brain. It could be part of a negative feedback mechanism to reduce serotonin transmission. This work initiated the search for serotonin selective reuptake inhibitors (SSRI).

In 1968, together with the Anden's group in Goteborg, they also obtained indications that hallucinogenic lysergic acid diethylamide (LSD) activated 5-HT receptors.

In 1970s, they introduced 6-hydroxytryptamine as a new tool in catecholamine and serotonin fluorescence histochemistry. It became possible to have an improved visualization of the serotonin nerve terminal networks. However, in 1981 the 5-HT immunohistochemistry turned out to be the best approach to show the serotonin nerve terminal networks in an excellent way (Steinbush 1981). In 1974, they published a paper on the theory, practice and application of the Falck-Hillarp technique in Journal of Histochemistry and Cytochemistry.

In 1970s, 5,6-Dihydroxytryptamine and 5,7-Dihydroxytryptamine were introduced as new tools in the mapping of central 5-HT neurons and in producing their selective degeneration. This work became possible through a close collaboration with an outstanding biochemist John Daly. He played a great role in our work together on understanding the multiple functions of Serotonin neurons. Also the important role of Barry Everitt, Peter Lidbrink, K.Hole and K.Kiianmaa should be recognized.

In this period together with especially Nils-Erik Anden, they found that also hallucinogenic indolamines like psilocybin and dimethyltryptamine, and hallucinogenic phenylethylamines may activate postjunctional 5-HT receptors in line with our d-LSD data from 1968. We suggested that such actions at postjunctional 5-HT receptor may participate in introducing their hallucinogenic actions. Dr. Fuxe's colleague and friend George Aghananian, a pioneer in neuropsychopharmacology, had instead, data suggesting that presynaptic 5-HT receptors were involved in mediating the hallucinogenic effects. Today there is evidence that 5-HT2A receptors are involved.

In 1977, we found that the antidepressant drugs amitriptyline and nortrypyline had affinity for the ^{3}H-d-LSD binding sites in the cerebral cortex, while lacking clear effects on the 3H 5-HT binding. Both these drugs

blocked d-LSD -induced head twitches. These results indicated the possible existence of two types of 5-HT receptors (removed by the reviewer). In 1979, Peroutka and Snyder produced a significant paper with data showing two types of 5-HT receptors. The same year we published with Sven-Ove Ogen a paper in Journal of Neural Transmission representing an important continuation of the 1977 paper published in Neuroscience letter. A substantial number of antidepressant drugs, like amitriptyline and nortriptyline, were found to block certain types of 5-HT receptors that were labeled by ^{3}H-d- LSD binding sites. D-LSD was previously indicated to activate postjunctional 5-HT receptors (Anden et al. 1968). We found that the degree of inhibition of d-LSD and 5-HTP induced head twitches in mice by some antidepressant drugs tested was highly correlated to their affinity for the ^{3}H d-LSD binding sites. Instead, these antidepressant drugs had a lower affinity for the ^{3}H-5-HT binding sites (Ogren et al. 1979). These results supported our 1977 paper and the 1979 paper by Peroutka and Snyder that two types of 5-HT receptors can exist. Furthermore, antidepressant drugs that block the d-LSD linked 5-HT receptor may produce antidepressant effects by blocking these types of 5-HT receptors.

In the abstract we have already described the impact of the serotonin heteroreceptor complexes and their allosteric receptor-receptor interactions in understanding major depressive disorder and its treatment. The heteroreceptor complexes also have a fundamental role in learning and memory. Looking into the future of novel approaches to understand major depressive disorder, it seems to me important to understand the role of glucocorticoid receptors in modulating the serotonin heteroreceptor complexes, especially in relation to stress.

Honorary Paul Vanhoutte Lecture

René Hen, Ph.D.
Professor of Pharmacology (in Psychiatry) and Neuroscience
Director, Division of Integrative Neuroscience
Columbia University

Dr. René Hen has made several fundamental contributions to the study of the neurobiology of affective behavior.

In his predoctoral work at Strasbourg with Pierre Chambon, René carried out an important work on the control of gene transcription in viruses. As a postdoctoral fellow in Richard Axel's laboratory, René developed an interest in neurobiology in general, and specifically in seven trans-membrane spanning receptors that are coupled to G-proteins.

After returning to Strasbourg to set up his own laboratory, René used a variety of experimental approaches to clone the genes encoding Drosophila serotonin, tyramine, and tachykinin receptors. As genetic engineering of mice became more practical, he moved back to the vertebrates, rapidly cloning the genes for several mouse serotonin receptors, including two novel subtypes. As the number of known transmitter genes continued to increase during this period, it became apparent that conventional pharmacological approaches would only rarely provide the precision required to selectively block a given receptor subtype. Thus René began to use gene knockout techniques to determine the effects of various serotonin receptors on behavior. He generated the first knockout of a neurotransmitter receptor with a clear behavioral phenotype in 1994, showing that knocking out the 5HT-1B receptor led to an increase in aggressive and impulsive behavior. It thus became apparent that this general approach could, in an animal model, allow one to study with precision the various receptor subtypes that mediate specific aspects of behavior that contribute to complex pathological behavioral traits in humans. In just a short time René had become one of the world leaders in the study of the functional role of vertebrate serotonin receptors. Because of his desire to study the function of these receptors in their neurobiological context, René decided to move his laboratory to Columbia where neurobiology was more strongly represented.

Since moving to Columbia, René has continued to prove that he is not just someone who clones a gene and then jumps on to the next one. Rather, he is a neurobiologist who asks fundamentally important questions in the context of cell biology, pharmacology, genetics and behavior. In his work here he initially continued to use conventional knockout technology on specific serotonin receptors to study their role in behavior – particularly in aspects of behavior that are found in various mood disorders. But knockout mice can exhibit developmental defects and compensatory changes secondary to the primary genetic manipulation. Moreover, because the changes in receptor expression are global, it is difficult to pin down the neural locus underlying a genetically-engineered behavioral change. To overcome these complications, René has been on the forefront of developing techniques for producing temporally and spatially selective changes in gene expression. An example of this approach is his study with his student, Ai Yamamoto, of the temporally regulated over-expression of the huntingtin gene. This innovative study demonstrated that development of a therapy that eliminates expression of the defective protein could be expected not only to block the progress of the disease but to reverse it as well. This result has tremendous implications for understanding the pathological mechanisms underlying the disease, as well for guiding efforts at therapeutic treatment. More generally, this study has served as a new paradigm for the concept of studying disease reversibility in other neurodegenerative disorders.

Throughout René's career at Columbia, he has continued to elaborate on the theme that single gene mutations can influence complex behaviors such as aggression, impulsivity, vulnerability to drugs of abuse, fear or anxiety, and attention deficit disorder. Using gene-targeting approaches to affect various serotonin receptors, a substance P receptor, and transporters for monoamines, he has generated animals with a

number of behavioral abnormalities that are reminiscent of various features of human psychiatric diseases. His studies have become progressively more analytical during this period as he has begun to explore in depth the specific cellular and molecular changes that mediate the steps between changes in gene expression and changes in behavior. A prime example of this mechanistic approach is the study with Santarelli in which it was shown that the anxiolytic effects of knocking out the neurokinin-1R receptor (NK1R) may be explained by an increased output of serotonergic neurons in the dorsal raphe and a desensitization of 5HT1A auto-receptors. This study validates a central assumption underlying René's research. Although heritable psychological disorders are likely to be polygenic and to consist of a complex of symptoms, single gene manipulations that alter transmitter systems and generate a subset of symptoms may elucidate mechanisms underlying normal brain function and disease etiology and suggest potential new therapeutic approaches.

With his postdocs Gross and Santarelli, René has made a major contribution to the study of serotonin-reuptake inhibitors (SSRIs). First, they demonstrated that turning off the 5-HT1A receptor in knockout mice made the mice non-responsive to the SSRI fluoxetine in the novelty-suppressed feeding paradigm, suggesting that 5-HT1A receptors are required to mediate the behavioral effects of SSRIs. Next, they characterized an inducible knockout of the 5-HT1A receptor in which receptor expression is spatially restricted to the forebrain and can be turned on and off at will. These forebrain-rescue mice, in which the receptor has been turned on in young animals, have normal levels of anxiety-related behaviors, suggesting that it is the lack of forebrain receptors that is responsible for the increased fearful behavior of the knockout mice. This is an important result and is the first example of the rescue of a complex emotional phenotype by the conditional and tissue-specific expression of a gene. They then went on to show that the rescue phenotype is not dependent on the presence of the receptor in the adult, but rather requires receptor expression at an earlier stage in development. This second result argues for a fundamental shift in the way that we view the role of G-protein coupled receptors in emotional behavior -- more weight must be given to the role played by these receptors in establishing proper neural circuitry during development.

Over the past twenty years René, leading an international team of investigators, has carried the study of antidepressant drugs to a new level. Various chronic antidepressant treatments previously had been found to increase adult hippocampal neurogenesis, but the functional significance of this phenomenon had remained unclear. In a series of seminal studies starting with a landmark publication in *Science* (Santarelli et al, 2003), using genetic and radiological methods, René and collaborators found that disrupting antidepressant-induced neurogenesis blocks behavioral responses to antidepressants. They found that 5HT1A receptor-null mice are insensitive to the neurogenic and behavioral effects of fluoxetine. Furthermore, X-irradiation of a restricted region of the mouse brain containing the hippocampus prevents the neurogenic and behavioral effects of two classes of antidepressants. These exciting findings provide the best evidence yet that the behavioral effects of chronic antidepressants may be mediated by the stimulation of neurogenesis in the hippocampus.

René Hen's unusual combination of creativity and productivity, together with his rigorous training in molecular biology, have led him to become an internationally recognized leader in studies of the neural mechanisms of behavior. As such, he has received in 2019 the Goldman-Rakic Prize for Outstanding Achievement in Cognitive Neuroscience.

ISSR 2023
Serotonin on the Mexican Caribbean

"Serotonin on the Mexican Caribbean"

	Sunday	Monday	Tuesday	Wednesday	Thursday
	April 23, 2023	April 24, 2023	April 25, 2023	April 26, 2023	April 27, 2023
7:00		*Registration*	*Registration*	*Registration*	*Departure Day*
7:30		*Travel Awardee Breakfast (by invitation only) Maya 5-8*			
8:00		**Parallel Symposia 1 /2 (90 min)**	**Parallel Symposia 3/4 (90 min)**	**Parallel Symposia 5/6 (90 min)**	
8:30		Nautiyal Towards a better understanding of how serotonin regulates impulsivity — Maya 1-3 Dacks/Krantz The fruit fly: An important model for the study of 5HT neurobiology — Maya 4	Compan Are cerebral functions of serotonin 4 receptors evolutionarily conserved? — Maya 1-3 Kirby Physiology and behavioral functions of DR 5HT neurons — Maya 4	Hasse/Müller Lipid dynamics and serotonin signaling in the brain: From molecular interactions to behaviour — Maya 1-3 McCorvy Novel class of 5-HT2C receptor agonists with therapeutic promise and novel signaling mechanisms — Maya 4	
9:00					
9:30		Coffee Break (30 min)	Coffee Break (30 min)	Coffee Break (30 min)	
10:00		**Plenary symposium #1 (90 min)** Nichols/Rasenick Preclinical and clinical developments in psychedelic therapy for psychiatric disorders Maya 1-4	**Plenary symposium #4 (90 min)** Alenina Serotonylation: New vistas of receptor independent signaling Maya 1-4	**Short talks session 1 (90 min)** Maya 1-4	
10:30					
11:00					
11:30		LUNCH Provided (60 min) Maya 5-8	LUNCH 90 min (on own)	LUNCH 90 min (on own)	
12:00			Nomenclature Committee Meeting (virtual, link separate) Maya 5-8	Business meeting (LUNCH provided, members only, 60 min) Maya 1-4	
12:30		Travel Awardee Short Talks Session (60 min) Sponsored by LifeInSightBiotherapeutics and British Pharmacological Society Maya 1-4			
1:00			**Plenary symposium #5 (90 min)** Sitte/Daws SERT and beyond in treatment of depression Maya 1-4	**Short talks session 2 (90 min)** Maya 1-4	
1:30	Registration	**Plenary symposium #2 (90 min)** Cunningham Mining serotonin for precision medicine for substance use disorders Maya 1-4			
2:00					
2:30			Coffee Break (30 min)	Coffee Break (30 min)	
3:00		Coffee Break (30 min)	**Plenary symposium #6 (90 min)** Anastasio Physiological relevance of serotonin receptor functional crosstalk Maya 1-4	**Plenary symposium #7 (90 min)** Hansen/Sharp Recent advances in molecular neuroimaging of the serotonin system Maya 1-4	
3:30		**Plenary symposium #3 (90 min)** Pasqualetti/Dymecki Development and plasticity of serotonergic circuitry Maya 1-4			
4:00					
4:30	Welcome and lecture intro (30 min)		short break Introduction to lecture (15 min)	short break Introduction to lecture (15 min)	
5:00	Page Lecture-Daws (60 min) Maya 1-4	Poster Session Refreshments *posters will be accessable for viewing throughout conference* Maya Foyer	Vanhoutte Lecture-Hen (60 min) Maya 1-4	Rapport Lecture-Fuxe (60 min) Maya 1-4	
5:30					
6:00	Opening Reception Seaside Garden at JW Marriott		*Networking Social (self-paid)* JW Marriott Lobby Bar	Break	
6:30					
7:00				Gala Reception/Dinner and Awards Maya 5-8	
7:30	Dinner on own	Dinner on own	Dinner on own		
8:00					
9:00					

WiFi: MarriottBonvoy (no passcode needed)

Access available in conference rooms as well as guest rooms.

Program Details

Sunday April 23, 2023

13:30-16:30
Maya Hall
Registration

16:30-17:00
Maya 1-4
Welcome and Introduction to Lecture
Trevor Sharp, ISSR President

17:00-18:00
Maya 1-4
Page Lecture
More Than One Way to Transport Serotonin: A journey Through the Years
Lynette Daws, University of Texas Health San Antonio

Serotonin is an essential modulator of a variety of physiological and neural processes. Imbalances in serotonin signaling are strongly linked to a number of psychiatric disorders including depression and anxiety disorders. The strength and duration of signaling is tightly controlled by the serotonin transporter (SERT), the high-affinity transport mechanism for serotonin. Not surprisingly then, SERT is also the major target for many psychotherapeutic drugs (e.g., selective serotonin reuptake inhibitors [SSRIs]) and psychostimulants (e.g., 3,4-methylenedioxymethamphetamine [MDMA]). Until the late 1990s it was generally accepted that there is only one transporter for each neurotransmitter, SERT for serotonin, the dopamine transporter (DAT) for dopamine and so on. The idea of transporter "promiscuity" was not widely accepted. This presentation will tell the story of how a secondary transporter for serotonin was first identified in the 1960s, then largely forgotten until the 2000s. The journey begins with identifying the norepinephrine transporter as a very capable transporter of serotonin and ends with studies revealing organic cation transporters (OCTs), particularly OCT3, as important mechanisms for serotonin transport. These transporters, though have low affinity to transport serotonin, have a very high capacity to do so. Our findings point to OCT3 as a promising therapeutic target for psychiatric and substance use disorders.

18:00-19:30
Seaside Garden
Opening Reception

Dinner on own

Monday April 24, 2023

07:00-08:00 Maya Hall	**Registration**
07:00-08:00 Maya 5-8	**Travel Awardee Breakfast (by invitation only)**
08:00-09:30 Maya 1-3	**Parallel Symposia 1: Towards a Better Understanding of How Serotonin Modulates Impulsivity** *Chair: Katherine Nautiyal, Dartmouth College*

While dopamine is generally thought of as the major modulator of impulsivity and risky decision making, a large body of literature now shows that serotonin has an important role in regulating these behaviors. The multi-dimensional aspects of these behavioral systems coupled with the complexities of serotonin signaling make this topic timely especially as new behavioral and neuroscience measures emerge. The goal of this symposium is to discuss the scope and extent of serotonin control over these behavioral and cognitive systems, to highlight advances in our understanding of the neural circuit and genetic mechanisms of this control, and to look to the impact of serotonin signaling throughout the brain.

08:00-08:25	**Differential effects of 5-HT2C antagonists across risk-based versus effort-based decision-making tasks in the rat** Kelly Hrelja
08:25-08:50	**Using the GRAB-5-HT sensor to identify what aspects of action and reward are encoded by serotonin** Katherine M. Nautiyal
08:50-09:15	**Serotonergic modulation of semantic drug cue-elicited brain connectivity in addictions** James Bjork
09:15-09:30	**Chronic desipramine induces norepinephrine neuroplasticity and behavioral recovery in a fluoxetine-resistant mouse model of depression** Farank Vahid-Ansari

9:30-10:00 Maya Hall	**Coffee Break**

08:00-09:30
Maya 4

Parallel Symposia 2: The Fruit Fly: An Important Model for the Study of Serotonin Neurobiology
Chairs: Andrew Dacks, West Virginia University
David Krantz, University of California Los Angeles

The fruit fly, *Drosophila melanogaster*, has been an important model system for over 100 years that has been critical for our current understanding of modern human biological and disease processes. Drosophila express five different serotonin receptors throughout the brain and body where they mediate conserved processes to their mammalian counterparts: 5-HT_{1B}Dro and 5-HT_{1B}Dro are homologs to mammalian 5-HT_{1A} receptors, 5-HT_{2A}Dro is a homolog to mammalian 5-HT_2 receptors, 5-HT7Dro is a homolog to the mammalian 5-HT_7 receptor, and the 5-HT_{2B}Dro is a GPCR responsive to serotonin that couples to Gq. Conserved roles of serotonin in the fly include developmental processes, CNS function, behaviors, and physiological processes. The first talk by Dr. Charles Nichols will discuss the role of individual 5-HT receptors in the brain with respect to complex behaviors relevant to psychiatric disorders including social interaction, drug abuse, and depression. The second talk by Dr. Andrew Dacks will explore the consequences of serotonin receptor diversity for odor coding and an instance in which serotonin receptor expression is organized in a stimulus specific, rather than a cell-class specific, manner. The third talk by Dr. David Krantz at UCLA will focus on the effects of serotonin transporter mutants on behavior and transcription in the CNS.

08:00-08:25 **The role of serotonin and its receptors in behaviors relevant to human psychiatric disorders in *Drosophila melanogaster***
Charles D. Nichols

08:25-08:50 **Stimulus-specific modulation by serotonin in the olfactory system**
Andrew M. Dacks

08:50-09:15 **The effects of serotonin transporter mutants on behavior and transcription in the CNS**
David Krantz

09:15-09:30 **Utilizing genetically encoded sensors for serotonin and microdialysis in freely moving mice for the identification of novel serotonin-releasing agents**
Felix Mayer

9:30-10:00
Maya Hall

Coffee Break

10:00 - 11:30
Maya 1-4

Plenary Symposium 1: Preclinical and Clinical Developments in Psychedelic Therapy for Psychiatric Disorders
Chairs: Charles D. Nichols, LSU Health Sciences Center
Mark Rasenick, University of Illinois Chicago

The resurgence of interest in the field of psychedelics has primarily focused on clinical use for the treatment of depression. Although this is an important area for study, there have also been important advances in the preclinical arena. These include mechanistic studies in cell-based and rodent models, and drug development efforts to identify novel molecules with improved pharmacological profiles over currently used 5-HT2A receptor agonists in the clinic. In this symposium we will span preclinical to clinical, and highlight important recent advances in each area. In the first talk, Dr. Mark Rasenick will present data regarding potential cellular and molecular mechanisms underlying the rapid anti-depressant effects of serotonergic agents like psilocybin. In the second talk, Dr. Kevin Murnane will present his research toward the development of "non psychedelic" psychedelics that retain therapeutic potential but minimize behavioral effects. The final presentation will be from Dr. Matt Johnson, who will discuss recent clinical developments using psilocybin to treat depression and substance use disorder in human patients.

10:00-10:25 **Cellular Features of Psychedelic Antidepressant Action**
Mark Rasenick

10:25-10:50 **Development of novel serotonin 2A receptor activators with reduced psychoactivity for mental health substance use disorders**
Kevin Murnane

10:50-11:15 **Psilocybin in the treatment of depression and substance use disorders**
Matthew W. Johnson

11:15-11:30 **Development and mechanistic understanding of non-hallucinogenic psychedelic analogs**
Lindsay Cameron

11:30-12:30
Maya 5-8

Lunch provided.

12:30- 13:30
Maya 1-4

Travel Awardee Short Talks Session:
Sponsored by LifeinSight Biotherapeutics and British Pharmacological Society
Chair: John Neumaier, University of Washington

12:30-12:45 **Novel fluorescent probes for imaging the serotonin transporter**
Oliver John Belleza

12:45-13:00 **Neuronal correlates of declarative memory impairments in a condition of chronic serotonin deficiency**
Rebecca Coray

13:00-13:15 **The pro-longevity gene flavin-containing monooxygenase 2 modulates serotonin metabolism to regulate exploratory behavior in *C. elegans.***
Elizabeth S. Kitto

13:15-13:30 **Serotonin transporter (SERT) Ala276 mouse: Novel model to assess the biochemical, physiological, and behavioral impact of SERT Thr276 phosphorylation *in vivo***
Carina Meinke

13:30 – 15:00
Maya 1-4

Plenary Symposium 2: Mining Serotonin for Precision Medicine for Substance Use Disorders
Chair: Kathryn A. Cunningham, University of Texas Medical Branch

Substance use disorders (SUDs) develop in the context of risk vulnerability and result in clinically significant impairment. While treatment has a clear goal of reducing or eliminating drug intake, risk for continued relapse is promoted by disruption of executive, reward processing and activation of stress circuitry, with its psychopathology marked by craving, impulsivity, and drug cue sensitivity. This complex clinical profile has no single etiology to predict an SUD, its progress or resolution, culminating in profound heterogeneity in SUD clinical trials and poor signal detection, hindering the progress of therapeutic development. 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 ultimately inform refined, personalized pharmacotherapeutic intervention for the treatment of SUDs. The present panel focuses on recent preclinical, translational and clinical research directed to identify serotonergic mechanisms that may be purposed as pharmacodynamic and/or predictive biomarkers in medications development studies.

13:30-13:50	**Adaptive clinical trials to optimize efficacy of the SSRI citalopram for the treatment of cocaine use disorder** Joy Schmitz
13:50-14:10	**5-HT_{2A}R and 5-HT_{2C}R dynamics and neurocircuitry in cocaine use disorder: Unmasking a relapse biosignature** Kathryn A. Cunningham
14:10-14:30	**Preclinical models identify serotonergic interventions to treat opioid use disorder with comorbid alcohol use** Jamie Peters
14:30-15:00	**Loss of accumbal 5-HT_{2C}R blunts efficacy of lorcaserin to suppress oxycodone intake** Christina Merritt

15:00-15:30
Maya Hall

Coffee Break

15:30 – 17:00
Maya 1-4

Plenary Symposium 3: Development and Plasticity of Serotonergic Circuitry
Chairs: Massimo Pasqualetti, University Di Pisa
Susan Dymecki, Harvard University

Early alterations in 5-HT signaling have been implicated in neuropsychiatric disorders. 5-HT modulation of neural circuitry is determined, in part, by intrinsic developmental programs and can be fine-tuned by 5-HT-induced plasticity mechanisms. In this symposium, the speakers will present new findings on the development, plasticity, and behavioral impact of serotonergic signaling and connectivity. The presentations will highlight new research advancing our understanding of the development of serotonergic neuron subtypes, the expansive connectivity of serotonergic neurons, and how this connectivity can be selectively modified in response to its own endogenous transmitter and protected transcriptomically.

15:30-15:55	**Embracing differences: heterogeneity in the brain serotonergic neuronal system across lifespan** Susan Dymecki
15:55-16:20	**Early-life vs adulthood Fluoxetine-induced change of 5-HT homeostasis: distinct impact on serotonergic fiber wiring** Massimo Pasqualetti
16:20-16:45	**Safeguarding adult axons and synapses against degeneration** W. Clay Spencer
16:45-17:00	**Comparison of social behaviour in rats with life-long and acute genetic depletion of brain serotonin** Joanna Golebiowska

17:00-19:30 **Poster Session** (Posters will be accessible for viewing throughout the conference)
Maya Foyer **Refreshments and appetizers provided.**

Dinner on own

Poster Presentations	Number
Oliver John Belleza Novel fluorescent probes for imaging the serotonin transporter	1
Lindsay Cameron Development and mechanistic understanding of non-hallucinogenic psychedelic analogs	2
Briana Chen Simultaneous targeting of 5-HT_4Rs and NMDARs exerts additive effects against stress	3
Rebecca Coray Neuronal correlates of declarative memory impairments in a condition of chronic serotonin deficiency	4
Thomas Flanagan 5-HT_2 receptor activation differentially impacts linker histone H1.5 kinetics and induces the expression of factors relevant for global chromatin architecture	5
Rocio Foltran What happens when mice lack serotonin? Consequences in behavior and in the BDNF pathway in two models of hyposerotonergic mice	6
Joanna Golebiowska Comparison of social behavior in rats with life-long and acute genetic depletion of brain serotonin	7
Elizabeth Kitto The pro-longevity gene flavin-containing monooxygenase 2 modulates serotonin metabolism to regulate exploratory behavior in *C. elegans*	8
Felix Mayer Utilizing genetically encoded sensors for serotonin and microdialysis in freely moving mice for the identification of novel serotonin-releasing agents	9
Carina Meinke Serotonin transporter (SERT) Ala276 mouse: Novel model to assess the biochemical, physiological, and behavioral impact of SERT Thr276 phosphorylation in vivo	10
Christina Merritt Loss of Accumbal 5-HT2CR Blunts Efficacy of Lorcaserin to Suppress Oxycodone Intake	11
Lluis Miquel-Rio Humanized mice overexpressing α-synuclein in serotonin neurons evoke a depressive phenotype. Reversal by conjugated antisense therapy	12
Nako Nakatsuka *Ex Vivo* nanoscale serotonin mapping with electrophysiology	13
Marco Niello Enantiomer-specific pharmacology of cathinones shapes their potential as a scaffold for novel therapeutic agents	14
Rebecca Ravenelle Activation of serotonin input to the dorsal BNST leads to sex differences in fear learning	15
Justin Saunders Glucocorticoid receptor dysregulation underlies 5-HT_{2A}R-dependent synaptic and behavioral deficits in a mouse neurodevelopmental disorder model	16
Faranak Vahid-Ansari Chronic desipramine induces norepinephrine neuroplasticity and behavioral recovery in a fluoxetine-resistant mouse model of depression	17

Tuesday April 25, 2023

07:00-08:00 Maya Hall	**Registration**
08:00-09:30 Maya 1-3	**Parallel Symposia 3: Are Cerebral Functions of Serotonin 4 Receptors Evolutionarily Conserved?** *Chair: Valerie Compan, University of Nimes*

The cerebral distribution of serotonin (5-HT, 5-hydroxytryptamine) 4 receptors (5-HT_4Rs) seen in rodents is conserved in humans, with the highest levels in the nucleus accumbens, a critical brain area of the reward system; and, the lowest in the cerebral cortex. 5-HT_4Rs mediate a rare (may be unique) positive feedback effect on the dorsal raphe (DR)-5-HT cells, not from the DR (they are absent) but from the ventral part of the medial prefrontal cortex. In 1975, L. Descarries described that 5-HT binds receptors (5-HTRs), more often located at 100 □m than at 20 nm (synaptic transmission) from the site of 5-HT release, introducing the *volume transmission*. The preponderant 5-HT volume transmission extends the ubiquitous distribution of the serotonergic system, supporting its multiple functions; all physiologically interrelated, from habituation, memory, moving etc., to; protecting survival (antidepressant-like behavior), likely for critically contributing to adaptive and adapted responses to stress (environmental changes). The phylogenetically old serotonergic system then appears as a *continued red line* underlying crucial functions, which appear *sophisticated* to the point of a 5-HT-independent action of some 5-HTRs, including 5-HT_4Rs, to evoke constitutive activity. Consistently, the present symposium highlights the conservation of specific functions (memory, food intake, adaptive response to stress) of 5-HT_4Rs seen in simpler transgenic (or not) animal models and humans. Two decades of our studies suggest that inhibition of eating, abilities to adapt stress, self-preserve (antidepressant-like) and to prevent fear, learn and memorize and, to react to novelty involve 5-HT_4Rs. Compensatory high levels of 5-HT_4Rs and 5-HT depletion were detected in brains in rodents and humans with obesity (high 5-HT_4R constitutive activity reduces food intake), memory impairments and finally with, inabilities to remind affective words in healthy humans; suggesting an evolutionary specific implication of one of the ten (only four in mice) splice variants of *Htr*4 gene in humans, consistent with the implication of 5-HT_4Rs in dendritic spines growth. In the actual scientific context, our studies of 5-HT_4Rs' functions suggest that excessive formation of synapses to the detriment of volume transmission implements repetitive behaviors, while the equilibrium between the volume and the synaptic transmission would favor adaptive (*flexible*) behavior.

08:00-08:25	**Serotonin 4 receptors in human brains with obesity, depression and memory impairments** Gitte Knudsen
08:25-08:50	**Stimulation of serotonin 4 receptor prevents fear and depressive-like behavior in animal models** Christine Denny
08:50-09:15	**Functional maturation of dendritic spines involves changes in the cytoskeleton through activation of G13/RhoA signaling pathway induced by stimulation of 5-HT_4 receptors** Evgeni Ponimaskin
09:15-09:30	**Simultaneous targeting of 5-HT4Rs and NMDARs exerts additive effects against stress** Briana Chen

9:30-10:00 Maya Hall	**Coffee Break**

08:00-09:30
Maya 4

Parallel Symposia 4: Physiology and Behavioral Functions of Dorsal Raphe Serotonin Neurons
Chair: Lynn Kirby, Temple University

This symposium will focus on links between the physiology of raphe serotonergic neurons and animal behavior. The speakers use a range of physiological measurements, neuronal manipulations, and behavioral paradigms to study the functions of serotonergic neurons and their synaptic partners. The three talks will span a range of *ex vivo* and *in vivo* electrophysiological measurements, optogenetic and chemogenetic manipulations, and behavioral tasks in rodents. Dr. Kirby will present behavioral pharmacology, chemogenetic and *ex vivo* electrophysiology data showing a critical role for corticotropin releasing-factor-5-HT circuits in the dorsal raphe nucleus in models of both stress-induced negative affect and stress-modulated opioid and alcohol self-administration. Together, these findings support the hypothesis that 5-HT dorsal raphe circuits contribute to both reward potency and negative affective responses that motivate opioid- and alcohol-taking. Dr. Béïque will describe electrophysiological, behavioral and optogenetic experiments used with computational approaches that seeks to study processing features of the raphe. Using the inputs from the prefrontal cortex and lateral habenula as comparative testbeds, he will present data that identifies neural strategies that implements selection and classification of competing inputs and that explores their roles in modulating goal-directed behaviors. Dr. Amilhon will present work using electrophysiology, optogenetic tools and calcium sensors to elucidate neural circuits that contribute to anxiety. She has identified a critical role for ventral hippocampal-projecting raphe 5-HT neurons in the expression of anxiety in female but not male mice and modulation of associated hippocampal oscillations. Dr. Ravenelle will describe electrophysiology, optogenetic and behavioral pharmacology experiments elucidating serotonergic circuits involved in fear learning in male and female mice. She will show data to suggest greater sensitivity of females to serotonin-induced increases in fear learning and involvement of raphe-extended amygdala circuits and 5-HT_{2C} receptors in these sex-specific effects. Taken together, the four talks will provide attendees with an update on the field's understanding of serotonin's multiple functions, from synapses to computation and behavior.

08:00-08:25	**CRF-5-HT interactions and motivation for stress-induced opioid and alcohol self-administration** Lynn Kirby
08:25-08:50	**Synaptic and network strategies in the dorsal raphe that supports dynamical classification of long-range inputs** Jean-Claude Béïque
08:50-09:15	**Serotonergic modulation of the ventral hippocampus underlies sex-related differences in anxiety** Bénédicte Amilhon
09:15-09:30	**Activation of serotonin input to the dorsal BNST leads to sex differences in fear learning** Rebecca Ravenelle

9:30-10:00
Maya Hall

Coffee Break

10:00-11:30 Maya 1-4	**Plenary Symposium 4: Serotonylation: New Vistas of Receptor-Independent Serotonin Signaling** *Chair: Natalia Alenina, Max Delbruck Center Molecular Medicine*

Although serotonin (5-HT) is a molecule with diverse functions, such as acting as a morphogen during development, an autacoid in the periphery, and a neurotransmitter in the central nervous system, for decades most of its actions have been thought to be mediated via its interactions with specific membrane bound receptors. However, receptor-independent actions of serotonin were additionally discovered in 2003 – i.e., the covalent bonding of the amino group of serotonin to glutamine resides in certain cytosolic proteins, a reaction catalyzed by the enzyme Transglutaminase (referred to as serotonylation). Since then, multiple physiological functions for this posttranslational modification have been identified, including roles in hemostasis, insulin secretion and vascular processes. Most recently, it was discovered that nuclear proteins, such as histones, can be serotonylated, providing evidence that serotonin is an important direct regulator of transcriptional activity. In this symposium, we will provide a comprehensive overview of serotonylation functions, focusing on recent discoveries of intranuclear serotonylation, its roles in cellular differentiation and embryonic development and serotonin's role in control of neuroendocrine tissue development. Michael Bader (Berlin, Germany) will give a historical and biochemical overview of the process and highlight roles for serotonylation in the cardiovascular system. Jennifer Chan (New York, USA) will provide an extensive description of recent findings of histone serotonylation and its role in epigenetic regulation, modulation of chromatin structure and transcriptional activity. Igor Adameyko (Stockholm, Sweden) will present recent data about how maternal-to-embryonic serotonin controls the numbers of neuroendocrine chromaffin cells in vertebrate adrenal glands and converts the amount of maternal stress during pregnancy into the behavioral differences in progeny. Rocío Foltran (Buenos Aires, Argentina) will show how hyposerotonergia affects neuronal survival, BDNF levels and behavior in mice.

10:00-10:25	**Serotonylation in the cardiovascular system** Michael Bader
10:25-10:50	**Histone H3 serotonylation: a novel epigenetic mediator of placental and brain regulation** Jennifer Chan
10:50-11:15	**The role of serotonin in controlling neuroendocrine tissue development and transgenerational adaptations** Igor Adameyko
11:15-11:30	**What happens when mice lack serotonin? Consequences in behavior and in the BDNF pathway in two models of hyposerotonergic mice** Rocío B Foltran

11:30-13:00	**Lunch on own**
11:45-12:45 Maya 5-8	**Nomenclature Committee Meeting: virtual only and link sent separately by chair of committee (please bring own laptop and headphones)**

13:00 – 14:30
Maya 1-4

Plenary Symposium 5: SERT and Beyond in Treatment of Depression
Chairs: Harald Sitte, Medical University of Vienna
Lyn Daws, University of Texas Health San Antonio

The serotonin transporter (SERT), the high-affinity clearance mechanism for serotonin, has been the subject of intense research for many decades due to its important role in regulating the strength and duration of serotonin signaling, and as a target for psychotherapeutic drugs. Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed treatment for a host of psychiatric diseases, primary among these, depression. However, SSRIs leave many patients without symptom relief, underscoring a need to improve upon existing SSRIs. Speakers in this symposium will discuss exciting new advances to this end. Dr. Claus Loland will present the structural basis for the inhibition of SERT by the antidepressant drug vilazodone. Vilazodone binds with low nanomolar affinity to an allosteric site distinct from the canonical substrate binding site. The cryo-EM structure of the SERT:vilazodone complex is substantiated by pharmacological assays showing that vilazodone is a non-competitive inhibitor of serotonin uptake and that mutation of residues in the proposed vilazodone binding site decreases its affinity. Insertion of the fluorescent unnatural amino acid L-Anap was used as a reporter for the SERT conformational changes during vilazodone binding. Dr. Harald Sitte will introduce organic cation transporter 3 (OCT3), a low-affinity, high-capacity transporter for monoamines and its role as a regulator of monoaminergic neurotransmission. He will discuss new data showing how the tertiary and quaternary arrangement of OCT3 is impacted by compounds and constituents of the plasma membrane, which also regulate their functional activity. These new insights may reveal important new possibilities for the development of novel therapeutics. Dr. Parastoo (Parry) Hashemi revisits the monoamine hypothesis of depression by measuring *in vivo* serotonin dynamics with voltammetry in mice. She finds that ambient serotonin levels are robustly lower in animals with behavioral phenotypes of depression, and that this is a consequence of increased extracellular histamine, arising from neuroinflammation, which inhibits serotonin release via inhibitory H3 heteroreceptors on serotonin terminals. Moreover, she finds that agents with antidepressant activity also inhibit histamine uptake, which can explain the variable clinical efficacy of SSRIs, as a consequence of individual levels of inflammation. Her work highlights the merits of the monoamine hypothesis of depression via focus on a lesser-known monoamine, histamine.

13:00-13:25	**Allosteric binding of the antidepressant vilazodone to the serotonin transporter** Claus Juul Loland
13:25-13:50	**The structural basis of organic cation transporter 3 inhibition** Harald Sitte
13:50-14:15	**Inflammation mediated histaminergic inhibition of serotonin: Not yet time to give up on the monoamine hypothesis of depression** Parastoo Hashemi
14:15-14:30	**Enantiomer-specific pharmacology of cathinones shapes their potential as a scaffold for novel therapeutic agents** Marco Niello

14:30-15:00
Maya Hall

Coffee Break

15:00 – 16:30
Maya 1-4

Plenary Symposium 6: Physiological Relevance of Serotonin Receptor Functional Crosstalk
Chair: Noelle Anastasio, University of Texas Medical Branch at Galveston

Functional crosstalk between G protein coupled receptors (GPCRs) in cells promote pharmacological diversity and tailored cellular responsivity. This symposium will discuss the convergent evidence that functional crosstalk for serotonin (5-HT) receptors exist *in vivo*, including in complexes with receptors responding to other natural ligands than 5-HT. The 5-HT_{2A} receptor (5-HT_{2A}R) and 5-HT_{2C} receptor (5-HT_{2C}R) in the central nervous system are implicated in a range of normal behaviors (e.g., appetite, sleep) and physiological functions (e.g., endocrine secretion) while dysfunctional 5-HT_{2A}R and/or 5-HT_{2C}R are implicated in neuropsychiatric disorders (e.g., addiction, obesity, schizophrenia). Preclinical studies suggest that the 5-HT_{2A}R and 5-HT_{2C}R may act in concert to regulate the neural bases for behavior. Dr. Allen will discuss CRISPR/Cas9 strategies being used to elucidate molecular signaling pathways engaged by the 5-HT_{2A}R and 5-HT_{2C}R. These studies highlight the involvement of β-arrestins as key adaptor proteins that profoundly control the trafficking and duration of 5-HT_2R signaling to agonists, including serotonin and psychedelics. Dr. Noelle Anastasio will discuss the physical interaction between the 5-HT_{2A}R and 5-HT_{2C}R detected in heterologous cellular systems, rat brain with a focus on the receptor interfaces which mediate this interaction and may influence intracellular signaling. The metabotropic glutamate 2 receptor 2 (mGluR2) and the 5-HT_{2A}R are GPCRs that are speculated to play a pivotal role in processes related to cognition, perception, and mood. Unbalanced levels of Gq-coupled serotonin 5-HT2A and of Gi/o-coupled mGluR2receptors are involved in psychosis. Pioneering work by members of the Gonzalez-Maeso lab demonstrated using various approaches that 5-HT_{2A}R and mGluR2 can associate in heteromeric complex in *in vivo* cortical neurons. This receptor heteromer displays a remarkable inverse cross-regulation to likely explain the convergent effects of mGluR2- and 5-HT_{2A}R-targeted antipsychotic compounds (and of pro-psychotics and hallucinogens), which, in summary, regulate the Gi/o-Gq-coupling balance of these heterodimers. Hence, selecting receptor complex-specific responses of specific heterocomplexes may constitute an emerging strategy to improve therapeutic strategies.

15:00-15:25	**CRISPR/Cas9 approaches to define signaling mechanisms and crosstalk between 5-HT_2 receptors** John A. Allen
15:25-15:50	**Interrogation of the 5-HT_{2A}R:5-HT_{2C}R protein:protein interface** Noelle C. Anastasio
15:50-16:15	**Co-translational assembly of 5-HT_{2A}R and mGluR2 in mammalian cells** Somdatta Saha
16:15-16:30	**5-HT_2 Receptor Activation Differentially Impacts Linker Histone H1.5 Kinetics and Induces the Expression of Factors Relevant for Global Chromatin Architecture** Thomas Flanagan

16:30-16:45 **Short Break**

16:45-17:00 Maya 1-4	**Introduction to Lecture** *Trevor Sharp, ISSR President*
17:00-18:00 Maya 1-4	**Vanhoutte Lecture** **Serotonin, Neurogenesis, Overgeneralization, and Depression** *René Hen, Columbia University*

Depression and anxiety disorders are debilitating illnesses that affect more than 350 million people worldwide. The most common treatments for these disorders are SSRIs (selective serotonin reuptake inhibitors), which block the serotonin transporter and thereby increase serotonin levels in many brain regions. However, about 50% of patients who take SSRIs do not fully respond and among those who respond a significant fraction experiences various side effects such as sexual dysfunction. In addition, SSRIs have a delayed onset of therapeutic efficacy of several weeks. There is therefore a considerable need for better and faster acting antidepressants. One way to develop novel antidepressants is to understand how SSRIs work and why they take so long to be effective and then to target directly the underlying mechanisms. We have shown that SSRIs stimulate neurogenesis in the ventral dentate gyrus of the hippocampus and that the resulting young neurons are critical for stress resilience and for some of the behavioral effects of antidepressants. Specifically, we have shown that adult-born granule cells facilitate pattern separation which may in turn mitigate the overgeneralization often observed in mood and anxiety disorders. We have also shown that the ventral hippocampus contains a specialized population of cells that encode “negative valence” and that project to the hypothalamus and amygdala. We are proposing that chronic SSRIs decrease the activity of these “negative valence cells”, resulting in a decrease in anxiety and depression related behaviors. We hope that by inhibiting these cells either directly or indirectly via a stimulation of neurogenesis, we will be able to develop faster acting antidepressants and possibly compounds that are active in treatment resistant depression.

18:00-19:30 JW Marriott Lobby Bar	**Networking Social (self-paid)**

Dinner on own

Wednesday April 26, 2023

07:00-08:00
Maya Hall
Registration

08:00-09:30
Maya 1-3
Parallel Symposia 5: Lipid Dynamics and Serotonin Signaling in the Brain: From Molecular Interactions to Behaviour
Chair: Jana Haase, University College Dublin
Christian Müller, University of Erlangen

Serotonergic signaling in the brain is determined by the expression and activity of synthetizing enzymes, transporters, and receptors, which are mainly located within or near lipid membranes. However, the role of the membrane in the function of these proteins has been largely ignored until recently when we have started to engage with the complex interplay between membrane components and membrane protein function to understand the temporal-spatial organisation, which is essentially determined by the lipid landscape. The lipid-shaped synaptic membrane is far from a static and constant environment but emerged as highly dynamic during normal plasticity as well as a source of dysfunction and behavioural pathologies. This has a direct and pronounced effect on serotonergic signaling components and, thus, the neurobiology of all serotonergic systems. In this symposium we discuss how different lipid classes shape the function of key proteins in the serotonergic system as well as effects on whole system activity. The symposium will explore the role of cholesterol, sphingolipids and lipid signaling molecules in serotonergic neurotransmission by bringing together current research using clinically relevant animal models of acute and chronic lipid manipulations and highlight recent advances towards the elucidation of molecular mechanisms underlying the bidirectional interaction of serotonin system proteins with their lipid environment.

08:00-08:25 **The role of a specific cholesterol site for monoamine transporter function, folding and pharmacology**
Steffen Sinning

08:25-08:50 **Linking sphingolipid dynamics to sex specific serotonin transporter regulation**
Jana Haase

08:50-09:15 **Sphingolipid control of serotonergic activity – a pathway into depression and back**
Christian P. Müller

09:15-09:30 **Humanized mice overexpressing α-synuclein in serotonin neurons evoke a depressive phenotype: Reversal by conjugated antisense therapy**
Lluis Miquel-Rio

9:30-10:00
Maya Hall
Coffee Break

08:00-09:30
Maya 4
Parallel Symposia 6: Novel Classes of 5-HT2C Agonists with Therapeutic Promise and Novel Signaling Mechanisms
Chair: John McCorvy, Medical College of Wisconsin

This panel will provide a survey of the 5-HT_{2C}-selective agonist landscape (McCorvy), a novel selective 5-HT_{2C} agonist currently in clinical trials (Vasilkevich), and detail non-canonical G protein 5-HT_{2C} signaling mechanisms that may be important toward clinical efficacy (Bonniwell).

08:00-08:25 **Surveying the 5-HT_{2C}-selective agonist landscape: What's selective and what is not?**
John D. McCorvy

08:25-08:50 **BMB-101: A selective 5-HT_{2C} agonist in clinical trials with therapeutic utility**
Alex Vasilkevich

08:50-09:15 **Investigating 5-HT_{2C} Non-Canonical Signaling Profiles**
Emma M. Bonniwell

09:15-09:30 **Glucocorticoid receptor dysregulation underlies 5-HT2AR-dependent synaptic and behavioral deficits in a mouse neurodevelopmental disorder model learning**
Justin Saunders

9:30-10:00
Maya Hall
Coffee Break

10:00 - 11:30 Maya 1-4	**Short Talks Session 1** *Chair: Noelle C. Anastasio, University of Texas Medical Branch at Galveston*
10:00-10:15	**Selective refinement of glutamate and GABA synapses on dorsal raphe 5-HT neurons during postnatal life** Mariano Soiza-Reilly
10:15-10:30	**Serotonin modifies the profile of genes controlling cellular lipid and cholesterol metabolism in human monocyte-derived macrophages through 5-HT$_7$** Israel Rios
10:30-10:45	**Delineating a serotonin receptor pathway for weight-loss therapy** Chen Liu
10:45-11:00	**Targeting intestinal mucosal serotonin alone is critical for modulation of mood** Lin Y. Hung
11:00-11:15	**Modulation of ultra-slow calcium oscillation in the dentate gyrus during non-REM sleep** Yueqing Peng
11:15-11:30	**Looking from the other side – What converts a full substrate to a partial substrate, releaser or even a blocker at the human serotonin transporter?** Ralph Gradisch
11:30-13:00	**Lunch on own**
12:00-13:00 Maya 1-4	**Business Meeting (Lunch provided. Members only)**
13:00 - 14:30 Maya 1-4	**Short Talk Session 2** *Chair: Noelle C. Anastasio, University of Texas Medical Branch at Galveston*
13:00-13:15	**Molecular mode of action of new ecstasy analogues: methylenedioxyphenyl group bioisosteric replacement** Ana Sofia Alberto e Silva
13:15-13:30	**Experimental and theoretical insights into the self-organization of the brain serotonergic matrix** Skirmantas Janušonis
13:30-13:45	**Serotonin 2A- or 2C-like discriminative stimulus effects of novel 4-phenyl-2-dimethylaminotetralins (4-PATs) in nonhuman primates** Stephen J. Kohut
13:45-14:00	**Functional impact of PIP$_2$ on the serotonin transporter (SERT)** Maria Cristina Fenollar-Ferrer
14:00-14:15	**Organic cation transporter 3 contributes to serotonin clearance in basolateral amygdala and sex-dependently modulates fear-related behaviors** Lauren E. Honan
14:15-14:30	**Medial prefrontal serotonergic input regulates cognitive flexibility behavior in mice** Nuno D. Alves/Mark Ansorge
14:30-15:00 Maya Hall	**Coffee Break**

15:00 – 16:30 **Plenary Symposium 7: Recent Advances in Molecular Neuroimaging of the Serotonin System**
Maya 1-4 *Chairs: Hanne Demant Hansen, Harvard University*
Trevor Sharp, University of Oxford

Neuroimaging with positron emission tomography (PET) is one of the most powerful tools for capturing 5-HT transmission in the living human brain and studying its role in the pathophysiology and treatment of psychiatric disorder. Currently PET radioligands are available to detect many of the 5-HT receptor subtypes in human brain, as well as the 5-HT transporter and 5-HT synthesis, but progress continues to exploit the utility of the technology and improve spatiotemporal resolution. This symposium brings together international experts in the field to discuss recent advances in the development of PET radioligands and PET technology, and their application in psychiatry especially in relation to major depressive disorder (MDD). Prof. Rupert Lanzenberger will present clinical studies performed with ultrafast functional PET and multimodal imaging in psychiatric patients and healthy controls. Results are ranging from new approaches to quantify the serotonin transporter occupancy by SSRIs in association with efflux transporters to imaging-genetics and transcriptomics for precision pharmacotherapy in psychiatry. Prof. Gitte Moos Knudsen will discuss the current status regarding the utility of PET to measure release of 5-HT, using examples of studies which follow the displacement of PET radioligands by 5-HT releasing agents in healthy human volunteers and patients with MDD. Finally, Dr. Hanne D. Hansen will showcase preclinical and clinical experiments that demonstrate how hybrid neuroimaging approaches based on the combination of PET and magnetic resonance imaging, opens up possibilities to test the mechanism of novel drugs by evaluating their blood-brain barrier passage, regional 5-HT receptor occupancy and functionality. Overall, this symposium will overview both preclinical and clinical studies to provide a perspective on the current state and future application of PET technology to understand the complex role of the 5-HT system in normal and dysfunctional brain function.

15:00-15:25 **Functional PET, imaging (epi)genetics and transcriptomics**
Rupert Lanzenberger

15:25-15:50 **Measuring 5-HT release with PET in healthy volunteers and patients with major depressive disorder**
Gitte Moos Knudsen

15:50-16:15 **Functional characterization of drugs targeting the serotonin system using simultaneous PET/MR imaging**
Hanne Demant Hansen

16:15-16:30 ***Ex Vivo* Nanoscale Serotonin Mapping with Electrophysiology**
Nako Nakatsuka

16:30-16:45 **Short Break**

16:45-17:00 Maya 1-4	**Introduction to Lecture** *Trevor Sharp, ISSR President*
17:00-18:00 Maya 1-4	**Rapport Lecture** **Understanding the fundamentals of the heteroreceptor complexes and the role of serotonin heteroreceptor complexes in major depressive disorders and its treatment** *Kjell Fuxe, Karolinska Institutet*

After the discovery of the 5-HT neurons in the raphe and para-raphe regions in the lower brain-stem in the 1960s with ascending and descending projections to the tel- and diencephalon and the spinal cord as well as local projections in the brainstem[1,2], it became of interest to understand their functions and role in mental and neurological diseases. Based on biochemistry and the Falck-Hillarp histochemical technique[3,4] it became clear that 5-HT nerve terminal net-works existed in the limbic regions[5]. These findings indicated a potential role in major depressive disorders. In 1967-1968[6] indications were obtained for the existence of a 5-HT reuptake mechanism in the serotonin neurons of the brain, which was reduced in activity by the antidepressant drug imipramine[7]. It was the beginning of the SSRI area in the treatment of depression. In 1977-1979[8-10], evidence was obtained that the antidepressant drugs amitriptyline and nortriptyline had a preferential affinity for the d- LSD binding sites, later on recognized as the 5-HT2A receptor, versus the 5-HT binding sites. The degree of blockade of head-twitches by the antidepressant drugs was highly correlated to their affinity for the d-LSD binding sites. These results taken together indicated the potential existence of different 5-HT receptor subtypes[10] (removed by the reviewer in the 1977 paper, Fuxe et al., Neuroscience Letters). Furthermore, it seemed that certain antidepressant drugs may exert their therapeutic effect by blockade of a certain 5-HTR subtype. Now we know that there exist large numbers of 5-HTR subtypes in the CNS. Some should be blocked while others should be activated.

In 1980-1983, our concept of physical allosteric receptor-receptor interactions was introduced[11], representing a new intramembrane integrative mechanism. In 1993, our hypothesis was introduced that the changes we had observed in affinity and Bmax values in membrane preparations were made possible through the formation of homodimers and heterodimers[12]. The heterodimer is built up of a distinct receptor “A” and receptor “B” formed by an increased affinity for each-other. It leads to the formation of a receptor interface in which hotspots give its strength and over which the allosteric waves can pass to the other receptor protomer and produce the allosteric binding modulation and the modulation of the density of the heterodimer and in higher order heteroreceptor complexes [13-15]. Pro-triplet amino acid homologies play a significant role in creating the receptor interface based on bioinformatic analysis[16]. Electrostatic receptor-receptor interactions in intracellular domains also play significant role[17,18]. Major neurochemical techniques used are the proximity ligation assay (PLA) and BRET[19,20].

It is proposed that in MDD the major disturbance may be in distinct highly vulnerable heteroreceptor complexes in the emotional networks of the brain[21-24]. It includes especially the serotonin heteroreceptor complexes and their balance with other types of heteroreceptor complexes, especially serotonin hetero and homo receptor complexes. We have found in the pyramidal cell layer of the hippocampus highly interesting 5-HT1A-5-HT2A[25], FGFR1-5-HT1A[26], and GalR1-GalR2[27] heteroreceptor complexes, and indications of 5-HT1A-GalR1-GalR2 heteroreceptor complexes exist. The 5-HT1A receptor is a hub receptor[28]. The depressant effects of 5-HT2A protomer can involve an allosteric inhibition of the 5-HT1A receptor protomer in the hippocampus and the frontal lobe[25]. The N terminal galanin fragment (1-15) found in discrete brain regions in 1992[29] and later on found to target the GalR1-GalR2 heteroreceptor, causing depressive effects and anxiety involving the hippocampus. However, in the 5-HT1A-GalR1-GalR2 heteroreceptor complexes, the Galanin fragment in contrast enhances the anti-depressant effects of 5-HT1A agonist and SSRIs[27,30-32]. It is probably related to the ability of 5-HT1A receptor activation to inhibit the GalR1signaling over Gi/o removing its inhibition of the GalR2 signaling setting free Gq activation and antidepressant actions.

The FGFR1-5-HT1A heteroreceptor complex in raphe-hippocampal system represents a novel highly interesting target for antidepressant drugs and offers a novel strategy for treatment of MDD[21,26]. Combined treatment with 5-HTR1 agonist and FGFR1 agonists like FGF2 and Sun 11602 reduced the amplitude of the GIRK channel, induced by the 5-HT1A receptor activation[33]. In the case of the 5-HT1autoreceptor it leads to

reduced hyperpolarization of the serotonin dorsal raphe neurons and increased firing of the ascending 5-HT neurons to the hippocampus and antidepressant effects. Thus, the allosteric inhibition by the FGFR1 protomer of the 5-HT1A protomer leads to reduced opening of the GIRK channels reducing the hyperpolarization and likely increasing the firing of the raphe-hippocampal serotonin neurons. Something goes wrong in the FSL rats, representing a genetic rat model of MDD[33]. Here the allosteric inhibition by FGFR1 protomer of the ability of the serotonin 5-HT1A autoreceptor function in the dorsal raphe to open the GIRK channels is lost.

In 2013, evidence was obtained for the existence of D2R-OXTR heteroreceptor complexes in the ventral and dorsal striatum enhancing the signaling of each other and with D2R and OXTR agonists increasing their densities[34]. It may be that these complexes have a major role in emotional networks, especially social attachment. The combined activation of the two receptor protomer may reduce dysfunction in MDD[22,23,35]. In 2019 also OXTR-5-HT2A heterocomplexes were demonstrated in cellular models using flow cytometry-based FRET and in distinct limbic circuits relevant for social interactions using PLA[36]. In cellular models the 5-HT2A receptor protomer attenuated the oxytocin receptor signaling via G alpha q involving allosteric constitutive inhibition of calcium influx. In 2021, OXTR-5-HT2C heteroreceptor complexes were also found in cellular models and in the limbic system of the rat using the same methodologies as above[37]. In HEK cells the oxytocin induced influx of calcium influx was markedly blocked by the expression of the OXTR-5-HT2C heteroreceptor complex. The in vivo correlates remain to be established.

Learning and memory. The synaptic heteroreceptor complexes can represent the molecular basis of learning and memory[14,38]. It is based on the reorganization of homo and heteroreceptor complexes in the postsynaptic membrane[39]. The presynaptic heteroreceptor complexes have instead a role to facilitate the pattern of transmitter release to be learned by the postsynaptic heteroreceptor complexes through their reorganization[38].

Future research on serotonin and other types of heteroreceptor complexes

Promised land for drug development in mental and neurological disorders[40].

Hetero bivalent drugs and interface interfering peptides[16,26].

For MDD there exist multiple serotonin heteroreceptor complexes in key brain circuits[22-24,37,41]. Which are the most vulnerable in MDD and in animal models of MDD? They can be targets for novel antidepressant drugs, which also can enhance the actions of known antidepressant drugs.

References

[1]Dahlstroem, A. *et al. Acta Physiol Scand Suppl*, SUPPL 232:231-255,(1964); [2]Fuxe, K. *Acta Physiol Scand Suppl*, SUPPL 247:237+,(1965); [3]Anden, N. E. *et al. Life Sci* **4**, 1275-1279,(1965); [4]Fuxe, K. *et al. Brain Res Rev* **55**, 17-54,(2007); [5]Steinbusch, H. W. *et al. Acta histochemica. Supplementband* **24**, 107-122,(1981); [6]Fuxe, K. *et al. The Journal of pharmacy and pharmacology* **19**, 335-337,(1967); [7]Carlsson, A. *et al. The Journal of pharmacy and pharmacology* **20**, 150-151,(1968); [8]Fuxe, K. *et al. Neurosci Lett* **6**, 339-343,(1977); [9]Ogren, S. O. *et al. J Neural Transm* **46**, 85-103,(1979); [10]Peroutka, S. J. *et al. Mol Pharmacol* **16**, 687-699,(1979); [11]Fuxe, K. *et al. Journal of neural transmission*, 165-179,(1983); [12]Zoli, M. *et al. Mol Neurobiol* **7**, 293-334,(1993); [13]Fuxe, K. *et al. Curr Opin Pharmacol* **10**, 14-22,(2009); [14]Fuxe, K. *et al. Neuroscience Discovery* **2**,(2014); [15]Fuxe, K. *et al. Neuropsychopharmacology* **41**, 380-382,(2016); [16]Borroto-Escuela, D. O. *et al. Frontiers in pharmacology* **9**, 829,(2018); [17]Tarakanov, A. O. *et al. J Mol Neurosci* **41**, 294-303,(2009); [18]Woods, A. S. *et al. J Mol Neurosci* **26**, 125-132,(2005); [19]Borroto-Escuela, D. O. *et al.* in *Receptor and Ion Channel Detection in the Brain* Vol. 110 *Neuromethods* (eds R. Lujan & F. Ciruela) Ch. 9, 109-126 (Springer, 2016); [20]Borroto-Escuela, D. O. *et al. Methods in cell biology* **117**, 141-164,(2013); [21]Borroto-Escuela, D. O. *et al. Trends Neurosci* **39**, 5-15,(2016); [22]Borroto-Escuela, D. O. *et al. Int J Mol Sci* **22**,(2021); [23]Perez de la Mora, M. *et al. Cells* **11**,(2022); [24]Borroto-Escuela, D. O. *et al. Cells* **10**,(2021); [25]Borroto-Escuela, D. O. *et al. ACS omega* **2**, 4779-4789,(2017); [26]Borroto-Escuela, D. O. *et al. Biol Psychiatry* **71**, 84-91,(2012); [27]Borroto-Escuela, D. O. *et al. Biochem Biophys Res Commun* **452**, 347-353,(2014); [28]Borroto-Escuela, D. O. *et al. Int J Mol Sci* **15**, 8570-8590,(2014); [29]Hedlund, P. B. *et al. Eur J Pharmacol* **224**, 203-205,(1992); [30]Borroto-Escuela, D. O. *et al. Molecules* **23**,(2018); [31]Borroto-Escuela, D. O. *et al. Biochem Biophys Res Commun* **393**, 767-772,(2010); [32]Flores-Burgess, A. *et al. Neuropharmacology* **118**, 233-241,(2017); [33]Borroto-Escuela, D. O. *et al. Frontiers in cellular neuroscience* **11**, 309,(2017); [34]Romero-Fernandez, W. *et al. Molecular psychiatry* **18**, 849-850,(2013); [35]Borroto-Escuela, D. O. *et al. Frontiers in molecular neuroscience* **15**, 1055344,(2022); [36]Chruscicka, B. *et al. ACS chemical neuroscience* **10**, 3225-3240,(2019); [37]Chruscicka, B. *et al. Neuropharmacology* **183**, 108394,(2021); [38]Borroto-Escuela, D. O. *et al. Philos Trans R Soc Lond B Biol Sci* **370**,(2015); [39]Borroto-Escuela, D. O. *et al. Frontiers in molecular neuroscience* **12**, 230,(2019); [40]Borroto-Escuela, D. O. *et al. Pharmacological reports : PR* **70**, 936-950,(2018); [41]Narvaez, M. *et al. Neuropharmacology* **170**, 108070,(2020)

18:00-19:00	**Short Break**
19:00-21:00 Maya 5-8	**Gala Reception/Dinner and Awards**

We look forward to seeing you all at our next meeting in 2025!

Symposia Abstracts

Towards a better understanding of how serotonin modulates impulsivity

While dopamine is generally thought of as the major modulator of impulsivity and risky decision making, a large body of literature now shows that serotonin has an important role in regulating these behaviors. The multi-dimensional aspects of these behavioral systems coupled with the complexities of serotonin signaling make this topic timely especially as new behavioral and neuroscience measures emerge. The goal of this symposium is to discuss the scope and extent of serotonin control over these behavioral and cognitive systems, to highlight advances in our understanding of the neural circuit and genetic mechanisms of this control, and to look to the impact of serotonin signaling throughout the brain.

Chair: Katherine M. Nautiyal, PhD
Assistant Professor
Dartmouth College

Speaker #1
Kelly Hrelja
Graduate Assistant
University of British Columbia
Differential effects of 5-HT2C antagonists across risk-based versus effort-based decision-making tasks in the rat

Speaker #2
Katherine M. Nautiyal, Ph.D.
Assistant Professor
Dartmouth College
Using the GRAB-5-HT sensor to identify what aspects of action and reward are encoded by serotonin
Serotonin signaling throughout the brain is involved in reward processing, motivation, and behavioral control, however the role of serotonin in specific brain regions on a timescale compatible with individual rewards has previously been difficult to understand. Using a fluorescent biosensor (GRAB-5-HT) to measure serotonin release in the dorsal striatum, we find that serotonin levels track reward anticipation, value and the prospective value of an action. Using one photon calcium imaging, we see that dorsal striatum medium spiny neurons have decreases in activity in anticipation of reward that are mediated by serotonin signaling through the 5-HT1B receptor.

Speaker #3
James Bjork, **Ph.D.**
Associate Professor
Virginia Commonwealth University
Serotonergic modulation of semantic drug cue-elicited brain connectivity in addictions
This presentation will feature findings from a series of neuroimaging-focused clinical trials of potential addiction pharmacotherapies. Drug-word cues evoke mechanistically-relevant directional brain connectivities that are in turn perturbed by serotonergic medications, with further moderation of medication effect by functional serotonergic genotype. These findings suggest potential utility of serotonergic medications to blunt cue-evoked cravings in some contexts and individuals, such as abstinent persons in recovery.

Speaker #4 Travel Awardee
Farank Vahid-Ansari, Ph.D.
Assoc Neurosci Researcher
Univ Ottawa
Chronic desipramine induces norepinephrine neuroplasticity and behavioral recovery in a fluoxetine-resistant mouse model of depression

The fruit fly: An important model for the study of serotonin

The fruit fly, *Drosophila melanogaster*, has been an important model system for over 100 years that has been critical for our current understanding of modern human biological and disease processes. Drosophila express five different serotonin receptors throughout the brain and body where they mediate conserved processes to their mammalian counterparts: 5-HT_{1B}Dro and 5-HT_{1B}Dro are homologs to mammalian 5-HT_{1A} receptors, 5-HT_{2A}Dro is a homolog to mammalian 5-HT_2 receptors, 5-HT7Dro is a homolog to the mammalian 5-HT_7 receptor, and the 5-HT_{2B}Dro is a GPCR responsive to serotonin that couples to Gq. Conserved roles of serotonin in the fly include developmental processes, CNS function, behaviors, and physiological processes. The first talk by Dr. Charles Nichols will discuss the role of individual 5-HT receptors in the brain with respect to complex behaviors relevant to psychiatric disorders including social interaction, drug abuse, and depression. The second talk by Dr. Andrew Dacks will explore the consequences of serotonin receptor diversity for odor coding and an instance in which serotonin receptor expression is organized in a stimulus specific, rather than a cell-class specific, manner. The third talk by Dr. David Krantz at UCLA will focus on the effects of serotonin transporter mutants on behavior and transcription in the CNS.

Chair: Andrew M. Dacks, Ph.D.
Associate Professor
West Virginia University

Co-Chair: David Krantz, M.D./Ph.D.
Professor Psychiatry and Biobehavioral Sciences
UCLA

Speaker #1
Charles D. Nichols, Ph.D.
Professor
LSU Health Sciences Center – New Orleans, Department of Pharmacology
The role of serotonin and its receptors in behaviors relevant to human psychiatric disorders in *Drosophila melanogaster*

Speaker #2
Andrew M. Dacks, Ph.D.
Associate Professor
West Virginia University
Stimulus-specific modulation by serotonin in the olfactory system
Neuron types within a network perform specific computations that allow our sensory systems to encode different features of a given stimulus modality. Sensory systems must remain flexible under a variety of contexts and rely on neuromodulators acting through a diverse set of receptors to differentially control individual neuron types. In this manner, a single neuromodulator can have distinct effects on the functions served by each neuron type. My group recently established an atlas of serotonin receptor expression within the first olfactory neuropil (the antennal lobe or AL) of Drosophila melanogaster and for the most part, individual neuron types consistently had uniform expression of one or a couple of receptors. However, there was non-uniform serotonin receptor expression within one population of output neurons, such that all receptor types were expressed but by subsets of neurons. This implied that rather than having a uniform effect, serotonin differentially modulates subsets of
cells based on functional differences within this neuron type. In my seminar I will discuss our anatomical, molecular and physiological findings suggesting that serotonin differentially impacts subsets of output neurons depending on their odor tuning.

Speaker #3
Name: David Krantz, M.D./Ph.D.
Professor Psychiatry and Biobehavioral Sciences
UCLA
The effects of serotonin transporter mutants on behavior and transcription in the CNS

Speaker #4 Travel Awardee

Felix Mayer, Ph.D.
Postdoctoral Fellow
University of Copenhagen, Denmark

Utilizing genetically encoded sensors for serotonin and microdialysis in freely moving mice for the identification of novel serotonin-releasing agents

Preclinical and clinical developments in psychedelic therapy for psychiatric disorders

The resurgence of interest in the field of psychedelics has primarily focused on clinical use for the treatment of depression. Although this is an important area for study, there have also been important advances in the preclinical arena. These include mechanistic studies in cell-based and rodent models, and drug development efforts to identify novel molecules with improved pharmacological profiles over currently used 5-HT2A receptor agonists in the clinic. In this symposium we will span preclinical to clinical, and highlight important recent advances in each area. In the first talk, Dr. Mark Rasenick will present data regarding potential cellular and molecular mechanisms underlying the rapid anti-depressant effects of serotonergic agents like psilocybin. In the second talk, Dr. Kevin Murnane will present his research toward the development of “non psychedelic” psychedelics that retain therapeutic potential but minimize behavioral effects. The final presentation will be from Dr. Matt Johnson, who will discuss recent clinical developments using psilocybin to treat depression and substance use disorder in human patients.

Chair: Charles Nichols, PhD
Professor
LSU Health Sciences Center

Co-Chair: Mark Rasenick, PhD
Professor
University of Illinois Chicago

Speaker #1
Mark Rasenick, PhD
Distinguished Professor
University of Illinois Chicago, Physiology and Biophysics
Cellular Features of Psychedelic Antidepressant Action

Speaker #2
Kevin Murnane, PhD
Associate Professor
Health Sciences Center Shreveport
Development of novel serotonin 2A receptor activators with reduced psychoactivity for mental health substance use disorders

Speaker #3
Matthew W. Johnson, Ph.D.
Professor
Johns Hopkins University, Psychiatry and Behavioral Sciences
Psilocybin in the treatment of depression and substance use disorders

Speaker #4 Travel Awardee
Lindsay Cameron, Ph.D.
Postdoctoral Fellow
University of California, Davis
Development and mechanistic understanding of non-hallucinogenic psychedelic analogs

Mining Serotonin for Precision Medicine for Substance Use Disorders

Substance use disorders (**SUDs**) develop in the context of risk vulnerability and result in clinically significant impairment. While treatment has a clear goal of reducing or eliminating drug intake, risk for continued relapse is promoted by disruption of executive, reward processing and activation of stress circuitry, with its psychopathology marked by craving, impulsivity, and drug cue sensitivity. This complex clinical profile has no single etiology to predict an SUD, its progress or resolution, culminating in profound heterogeneity in SUD clinical trials and poor signal detection, hindering the progress of therapeutic development. 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 ultimately inform refined, personalized pharmacotherapeutic intervention for the treatment of SUDs. The present panel focuses on recent preclinical, translational and clinical research directed to identify serotonergic mechanisms that may be purposed as pharmacodynamic and/or predictive biomarkers in medications development studies.

Chair: Kathryn A. Cunningham, Ph.D.
Chauncey Leake Distinguished Professor of Pharmacology; Director Center for Addiction Sciences and Therapuetics
Center for Addiction Sciences and Therapeutics, Department of Pharmacology and Toxicology, UTMB Galveston, TX, USA

Speaker #1
Joy Schmitz, Ph.D.
Louis A. Faillace, MD, Professor, Department of Psychiatry and Behavioral Sciences, and Director of the Center for Neurobehavioral Research on Addiction,
University of Texas McGovern Medical School, Houston, TX, USA

Adaptive clinical trials to optimize efficacy of the SSRI citalopram for the treatment of cocaine use disorder

Finding an effective medication for the treatment of cocaine use disorder (CUD) has been a longstanding goal, challenge, and priority. Targeting the serotonin system for treating CUD is a viable and well-supported strategy from a neurobiological and neuropharmacological standpoint. Clinically, the evidence has been mixed, with the SSRI citalopram producing the most promising signal to date. This talk will review findings from clinical trials of citalopram, highlighting the use of adaptive methods to identify the optimal therapeutic dose with precision and efficiency. Knowledge derived from these trials has provided information on possible moderators and mediators of citalopram effects on CUD outcomes. Discussion will focus on further exploring the role of decision-making impairments in predicting *who* responds to citalopram treatment and *how*.

Speaker #2
Kathryn A. Cunningham, Ph.D.
Chauncey Leake Distinguished Professor of Pharmacology; Director Center for Addiction Sciences and Therapuetics
Center for Addiction Sciences and Therapeutics, Department of Pharmacology and Toxicology, UTMB Galveston, TX, USA

5-HT_{2A}R and 5-HT_{2C}R dynamics and neurocircuitry in cocaine use disorder: Unmasking a relapse biosignature

Cocaine was involved in nearly 20% of overdose deaths in 2019; in 2020, ~1.4 million people reported current cocaine use disorder (CUD), a debilitating condition evidenced by clinically significant health consequences. There are currently no FDA-approved pharmacotherapies to facilitate recovery from CUD in part due to its complex etiology and enduring relapse vulnerability. This talk will focus on serotonin target-phenotype relationships engaging mPFC circuitry and molecular targets which precipitate CUD risk, particularly engaging 5-HT_{2A}R and 5-HT_{2C}R neurobiology. The ultimate goal is to define circuit-based relapse biosignatures pf CUD to facilitated targeted therapeutics to reduce relapse risk in CUD.

Speaker #3

Jamie Peters, Ph.D.
Associate Professor, Anesthesiology and Pharmacology
University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Preclinical models identify serotonergic interventions to treat opioid use disorder with comorbid alcohol use

Tabernanthalog is a non-hallucinogenic ibogaine-derivative that activates serotonin 5-HT2A receptors and exhibits therapeutic efficacy in preclinical models of depression, alcohol use disorder, and opioid use disorder. In some cases, its effects are long-lasting, on the order of days to weeks, after a single treatment. Because of the high incidence of opioid and alcohol co-use, we were interested in evaluating the efficacy of tabernanthalog, and another more selective (albeit hallucinogenic) 5-HT2A agonist, DOI, in a preclinical model of opioid and alcohol co-self-administration. Preliminary data suggest that tabernanthalog is capable of acutely reducing both heroin and alcohol motivation, measured as break points under progressive ratio tests where the amount of effort (in lever presses) increases exponentially for each subsequent earned reward. By contrast, DOI selectively reduces heroin but not alcohol break points in a similar preclinical model of polydrug (heroin and alcohol) co-use. Future studies will evaluate whether other serotonergic mechanisms of action can account for this distinction between tabernanthalog and DOI on opioid versus alcohol motivation.

Speaker #4 Travel Awardee

Christina Merritt, Ph.D.
Director of Molecular and Translational Therapeutics
Center for Addiction Sciences and Therapeutics, UTMB Galveston, TX, USA

Loss of accumbal 5-HT_{2C}R blunts efficacy of lorcaserin to suppress oxycodone intake

Development and plasticity of serotonergic circuitry

Early alterations in 5-HT signaling have been implicated in neuropsychiatric disorders. 5-HT modulation of neural circuitry is determined, in part, by intrinsic developmental programs and can be fine-tuned by 5-HT-induced plasticity mechanisms. In this symposium, the speakers will present new findings on the development, plasticity, and behavioral impact of serotonergic signaling and connectivity. The presentations will highlight new research advancing our understanding of the development of serotonergic neuron subtypes, the expansive connectivity of serotonergic neurons, and how this connectivity can be selectively modified in response to its own endogenous transmitter and protected transcriptomically.

Chair: Massimo Pasqualetti, Ph.D.
Professor
Department of Biology, Unit of Cell and Developmental Biology, University of Pisa

Co-Chair: Susan Dymecki, M.D./Ph.D.
Professor
Department of Genetics, Harvard Medical School

Speaker #1
Susan Dymecki, M.D./Ph.D.
Professor
Department of Genetics, Harvard Medical School
Embracing differences: heterogeneity in the brain serotonergic neuronal system across lifespan
The adult brain 5-HTergic neuronal system in mice is organized into numerous neuronal subtypes that, while sharing generic 5-HTergic properties, are otherwise distinct molecularly, and in many cases also demonstrated to be distinct functionally and hodologically. A current 5-HTergic cell census (>20 cell subtypes) was arrived at in part through single-cell-resolution transcriptomics (sc-RNA sequencing performed by our lab as well as others) of adult brain 5-HTergic neurons, fate-mapping studies, target-specific along with subtype-targeted perturbations, and projection mapping. As shown by the Deneris Lab [Zhang, Spencer,…Deneris, 2022 eLife], notably fewer molecularly-defined neuron subgroups characterize the embryonic 5-HTergic (*Pet1*-expressing) neuronal system. We sought to bridge these studies by exploring *Pet1*-expressing neurons across postnatal development at a resolution sufficient to identify neuron subtypes and reveal potential molecular pathways and temporal sequences by which the mature subtype organization arises. Towards this goal, we recently generated scRNAseq data for a wide range of early postnatal time points and found several novel features of *Pet1*-neuron postnatal development. Progress around this work will be presented.

Speaker #2
Massimo Pasqualetti, Ph.D.
Professor
Department of Biology, Unit of Cell and Developmental Biology, University of Pisa
Early-life vs adulthood Fluoxetine-induced change of 5-HT homeostasis: distinct impact on serotonergic fiber wiring
The serotonergic neurons provide a profuse innervation to the whole central nervous system. As previously shown, genetically induced abrogation of 5-HT demonstrated that maintaining proper serotonin homeostasis in the adult brain is crucial to preserve the correct serotonergic axonal wiring and showed that 5-HT fibers maintain a high structural plasticity to adulthood being reshaped by fluctuations of 5-HT content. We aimed to investigate whether serotonergic fibers can be remodeled by 5-HT fluctuations within the peri-physiological range. To this aim, we chronically treated early-life or adult Tph2GFP knock-in heterozygous mice with the antidepressant fluoxetine. Combining GFP immunofluorescence with confocal microscope imaging and 3D-reconstruction revealed that chronic fluoxetine exposure dramatically reduces the density of 5-HT fibers innervating the hippocampus, although with a distinct long-term effect for those treated in early-life versus those treated in adulthood.

Speaker #3
W. Clay Spencer, Ph.D.
Research Scientist
Department of Neurosciences, Case Western Reserve University School of Medicine
Safeguarding adult axons and synapses against degeneration
Neurons must function for decades of life, but how these non-dividing cells are preserved is poorly understood. Using mouse serotonin (5-HT) neurons as a model, we report an adult-stage transcriptional program specialized to ensure the preservation of neuronal connectivity. We uncover a switch in Lmx1b and Pet1 transcription factor function from controlling embryonic axonal growth to sustaining a transcriptomic signature of 5-HT connectivity comprising functionally diverse synaptic and axonal genes. Adult-stage deficiency of Lmx1b and Pet1 causes slowly progressing degeneration of 5-HT synapses and axons, increased susceptibility of 5-HT axons to neurotoxic injury, and abnormal stress responses. Axon degeneration occurs in a die back pattern and is accompanied by accumulation of α-synuclein and amyloid precursor protein in spheroids and mitochondrial fragmentation without cell body loss. Our findings suggest that neuronal connectivity is transcriptionally protected by maintenance of connectivity transcriptomes; progressive decay of such transcriptomes may contribute to age-related diseases of brain circuitry.

Speaker #4 Travel Awardee
Name: Joanna Golebiowska
Graduate Assistant
Maj Institute of Pharmacology Polish Academy of Sciences
Comparison of social behaviour in rats with life-long and acute genetic depletion of brain serotonin

Are cerebral functions of serotonin 4 receptors evolutionarily conserved?

The cerebral distribution of serotonin (5-HT, 5-hydroxytryptamine) 4 receptors (5-HT_4Rs) seen in rodents is conserved in humans, with the highest levels in the nucleus accumbens, a critical brain area of the reward system; and, the lowest in the cerebral cortex. 5-HT_4Rs mediate a rare (may be unique) positive feedback effect on the dorsal raphe (DR)-5-HT cells, not from the DR (they are absent) but from the ventral part of the medial prefrontal cortex. In 1975, L. Descarries described that 5-HT binds receptors (5-HTRs), more often located at 100 □m than at 20 nm (synaptic transmission) from the site of 5-HT release, introducing the *volume transmission*. The preponderant 5-HT volume transmission extends the ubiquitous distribution of the serotonergic system, supporting its multiple functions; all physiologically interrelated, from habituation, memory, moving etc., to; protecting survival (antidepressant-like behavior), likely for critically contributing to adaptive and adapted responses to stress (environmental changes). The phylogenetically old serotonergic system then appears as a *continued red line* underlying crucial functions, which appear *sophisticated* to the point of a 5-HT-independent action of some 5-HTRs, including 5-HT_4Rs, to evoke constitutive activity. Consistently, the present symposium highlights the conservation of specific functions (memory, food intake, adaptive response to stress) of 5-HT_4Rs seen in simpler transgenic (or not) animal models and humans. Two decades of our studies suggest that inhibition of eating, abilities to adapt stress, self-preserve (antidepressant-like) and to prevent fear, learn and memorize and, to react to novelty involve 5-HT_4Rs. Compensatory high levels of 5-HT_4Rs and 5-HT depletion were detected in brains in rodents and humans with obesity (high 5-HT_4R constitutive activity reduces food intake), memory impairments and finally with, inabilities to remind affective words in healthy humans; suggesting an evolutionary specific implication of one of the ten (only four in mice) splice variants of *Htr*4 gene in humans, consistent with the implication of 5-HT_4Rs in dendritic spines growth. In the actual scientific context, our studies of 5-HT_4Rs' functions suggest that excessive formation of synapses to the detriment of volume transmission implements repetitive behaviors, while the equilibrium between the volume and the synaptic transmission would favor adaptive (*flexible*) behavior.

Chair: Valérie Compan
Professor
Nimes University
Dpt. Sciences. BRAINS' LABORATORY_LSCO (Brain, Anorexia, Addiction, Innovation in Sciences) Place Gabriel Péri, 30021 NIMES, FRANCE

Speaker #1
Gitte Knudsen
Professor, MD, DMSc
Dept. Neurology and Neurobiology Research Unit, Copenhagen University Hospital, Rigshospitalet, COPENHAGEN, DENMARK
Serotonin 4 receptors in human brains with obesity, depression and memory impairments

Speaker #2
Christine Denny
Assistant Professor
Division of Integrative Neuroscience, Research Foundation for Mental Hygiene, Inc. (RFMH)/New York State Psychiatric Institute (NYSPI), New York, NY, USA and Department of Psychiatry, Columbia University, NYSPI Kolb Research Annex, New York, NY, USA
Stimulation of serotonin 4 receptor prevents fear and depressive-like behavior in animal models

Speaker #3
Evgeni Ponimaskin
Professor
Medical School Hannover Cellular Neurophysiology, HANNOVER, GERMANY
Functional maturation of dendritic spines involves changes in the cytoskeleton through activation of G13/RhoA signaling pathway induced by stimulation of 5-HT_4 receptors

Speaker #4 Travel Awardee

Briana Chen
Postdoctoral Fellow
Division of Systems Neuroscience, Research Foundation for Mental Hygiene, Inc./New York State Psychiatric Institute, Department of Psychiatry, Columbia University Irving Medical Center, New York, NY 10032, USA

Simultaneous targeting of 5-HT4Rs and NMDARs exerts additive effects against stress

Physiology and behavioral functions of raphe serotonin neurons

This symposium will focus on links between the physiology of raphe serotonergic neurons and animal behavior. The speakers use a range of physiological measurements, neuronal manipulations, and behavioral paradigms to study the functions of serotonergic neurons and their synaptic partners. The three talks will span a range of *ex vivo* and *in vivo* electrophysiological measurements, optogenetic and chemogenetic manipulations, and behavioral tasks in rodents. Dr. Kirby will present behavioral pharmacology, chemogenetic and *ex vivo* electrophysiology data showing a critical role for corticotropin releasing-factor-5-HT circuits in the dorsal raphe nucleus in models of both stress-induced negative affect and stress-modulated opioid and alcohol self-administration. Together, these findings support the hypothesis that 5-HT dorsal raphe circuits contribute to both reward potency and negative affective responses that motivate opioid- and alcohol-taking. Dr. Béïque will describe electrophysiological, behavioral and optogenetic experiments used with computational approaches that seeks to study processing features of the raphe. Using the inputs from the prefrontal cortex and lateral habenula as comparative testbeds, he will present data that identifies neural strategies that implements selection and classification of competing inputs and that explores their roles in modulating goal-directed behaviors. Dr. Amilhon will present work using electrophysiology, optogenetic tools and calcium sensors to elucidate neural circuits that contribute to anxiety. She has identified a critical role for ventral hippocampal-projecting raphe 5-HT neurons in the expression of anxiety in female but not male mice and modulation of associated hippocampal oscillations. Dr. Ravenelle will describe electrophysiology, optogenetic and behavioral pharmacology experiments elucidating serotonergic circuits involved in fear learning in male and female mice. She will show data to suggest greater sensitivity of females to serotonin-induced increases in fear learning and involvement of raphe-extended amygdala circuits and 5-HT_{2C} receptors in these sex-specific effects. Taken together, the four talks will provide attendees with an update on the field's understanding of serotonin's multiple functions, from synapses to computation and behavior.

Chair: **Lynn Kirby**
Professor
Lewis Katz School of Medicine at Temple University

Speaker #1
Lynn Kirby
Professor
Lewis Katz School of Medicine at Temple University, Center for Substance Abuse Research

CRF-5-HT interactions and motivation for stress-induced opioid and alcohol self-administration

Substance use disorders are motivated by both drug reward as well as by avoidance of negative affective state. The 5-HT dorsal raphe nucleus (DRN) system is modulated on both short and long timescales by exposure to drugs of abuse and by stressors. Data from our laboratory and others have shown evidence that stressors and the stress neurohormone corticotropin releasing-factor (CRF) can inhibit 5-HT DRN neurotransmission at low/moderate doses of CRF via the CRF-R1 receptor subtype on inhibitory GABA afferents. More recently we have established a causal role for CRF-R1 signaling in the DRN and stress-induced negative affect in rats, as reflected in 22 kHz ultrasonic vocalizations. We are currently employing *ex vivo* electrophysiology and chemogenetic tools to explore 5-HT DRN signaling in basal and compulsive alcohol and heroin self-administration. In the alcohol model, animals are either socially isolated or group housed during adolescence, followed by exposure in adulthood to models of basal and compulsive consumption (footshock-punished self-administration). The heroin self-administration model also includes a footshock-punishment phase to test both basal and compulsive drug-taking. *Ex vivo* electrophysiology is employed to assess the long-term impact of early life stress and drug history on 5-HT DRN excitability. Chemogenetic methods in Tph2-iCre rats are used to examine the behavioral consequence of stimulating 5-HT DRN neuronal activity during basal and compulsive drug self-administration. Adolescent social isolation produces long term hypoactivity of 5-HT DRN neurons as well as elevated basal and compulsive alcohol consumption, particularly in female rats. Chemogenetic activation of 5-HT DRN neurons modulates both alcohol and heroin intake in a manner reflecting a leftward shift of the dose-response curve or a decrease in reward potency. Chemogenetic activation of 5-HT DRN neurons increases compulsive consumption (drug-taking despite punishment) of both alcohol and heroin, potentially by buffering the inhibitory effect of footshock on 5-HT DRN activity and its impact on negative affect. These data suggest that the 5-HT DRN

system can impact drug-taking behaviors across multiple timescales by modulation of both appetitive and aversive factors.

Speaker #2
Jean-Claude Béïque
Professor
University of Ottawa's Center for Neural Dynamics and Artificial Intelligence
Synaptic and network strategies in the dorsal raphe that supports dynamical classification of long-range inputs
Serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN) receive a diverse constellation of long-range synaptic inputs, yet unifying principles of local circuitry and its dynamics are largely unknown - a crucial component of understanding how 5-HT output controls behavior. Here, we developed a formalism bridging optogenetic, electrophysiological, computational and behavioral strategies to reveal how local DRN dynamics control the expression of reward associations. While examining the effect of activating long-range inputs from the lateral habenula (LHb) to the DRN, we uncovered unsuspected 5-HT1A receptor-mediated local recurrent connections between 5-HT neurons, refuting classical theories of autoinhibition by somatodendritic 5-HT1A receptors. These inhibitory 5-HT connections were slow, stochastic, strongly facilitating, and gated spike output of 5-HT neurons. Targeted physiology and modeling approaches revealed that these functional connectivity features collectively support the emergence of a paradoxical excitation-driven inhibition of 5-HT neurons in response to high frequency LHb activation, and of a winner-take-all computation over protracted timescales. We employed an auditory classical conditioning paradigm in mice in order to test quantitative model predictions in vivo, and found that optogenetic activation of LHb inputs to the DRN at high, but not low, frequency contextually suppressed goal-directed anticipatory licking behavior. We thus suggest that winner-take-all computations in the DRN support a contextual integration of learned associations with acute environmental stimuli to trigger sharp behavioral state transitions.

Speaker #3
Bénédicte Amilhon
Assistant Professor
CHU Sainte-Justine Research Center, Université de Montréal, Département de Neuroscience, Montréal, Canada
Serotonergic modulation of the ventral hippocampus underlies sex-related differences in anxiety
Anxiety disorders are among the most prevalent mental disorders worldwide and affect women twice as often as men. Yet, our understanding of the neural circuits underlying the higher vulnerability of female brains to anxiety disorders is incomplete and needs to be refined. 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, in particular through oscillatory communication with other brain regions. 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 local field potential recordings in the vHP, we show that the raphe-vHP pathway modulates behavior and oscillatory activity differentially in males and females. Optogenetic activation of vHP-projecting 5-HT neurons elevated anxiety levels exclusively in females. Increases in anxiety in response to 5-HT release in the vHP were accompanied by shifts in the frequency and power of delta-range (1-4 Hz) and theta-range (4-12Hz) rhythms, both known to underlie defensive behaviors. 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.

Speaker #4 Travel Awardee
Rebecca Ravenelle
Postdoctoral Fellow
Columbia University Irving Medical Center
Activation of serotonin input to the dorsal BNST leads to sex differences in fear learning

Serotonylation: new vistas of receptor-independent serotonin signaling.

Although serotonin (5-HT) is a molecule with diverse functions, such as acting as a morphogen during development, an autacoid in the periphery, and a neurotransmitter in the central nervous system, for decades most of its actions have been thought to be mediated via its interactions with specific membrane bound receptors. However, receptor-independent actions of serotonin were additionally discovered in 2003 – i.e., the covalent bonding of the amino group of serotonin to glutamine resides in certain cytosolic proteins, a reaction catalyzed by the enzyme Transglutaminase (referred to as serotonylation). Since then, multiple physiological functions for this posttranslational modification have been identified, including roles in hemostasis, insulin secretion and vascular processes. Most recently, it was discovered that nuclear proteins, such as histones, can be serotonylated, providing evidence that serotonin is an important direct regulator of transcriptional activity. In this symposium, we will provide a comprehensive overview of serotonylation functions, focusing on recent discoveries of intranuclear serotonylation, its roles in cellular differentiation and embryonic development and serotonin's role in control of neuroendocrine tissue development. Michael Bader (Berlin, Germany) will give a historical and biochemical overview of the process and highlight roles for serotonylation in the cardiovascular system. Jennifer Chan (New York, USA) will provide an extensive description of recent findings of histone serotonylation and its role in epigenetic regulation, modulation of chromatin structure and transcriptional activity. Igor Adameyko (Stockholm, Sweden) will present recent data about how maternal-to-embryonic serotonin controls the numbers of neuroendocrine chromaffin cells in vertebrate adrenal glands and converts the amount of maternal stress during pregnancy into the behavioral differences in progeny. Rocío Foltran (Buenos Aires, Argentina) will show how hyposerotonergia affects neuronal survival, BDNF levels and behavior in mice.

Chair: **Dr. Natalia Alenina**
Senior scientist
The Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany

Speaker #1
Michael Bader, PhD
Group leader
The Max Delbrück Center for Molecular Medicine (MDC)
Serotonylation in the cardiovascular system
Michael Bader, Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany
In 2003 we discovered that serotonin is not only signalling via its 5-HT receptors but also by covalently binding to glutamine resides of certain cytosolic proteins. This reaction, for which we coined the term serotonylation, is catalyzed by transglutaminases, in particular transglutaminase 2. Multiple physiological functions for this posttranslational modification have been identified, including roles in hemostasis, insulin secretion and vascular processes. The talk will give an introduction in the mechanisms and functions of serotonylation focusing on the cardiovascular system.

Speaker #2
Jennifer Chan, PhD
Postdoctoral Fellow
Department of Neuroscience, Icahn School of Medicine at Mount Sinai
Histone H3 serotonylation: a novel epigenetic mediator of placental and brain regulation
Jennifer Chan, Department of Neuroscience, Icahn School of Medicine at Mount Sinai
Recently, our lab identified an epigenetic role for 5-HT, whereby this important biogenic monoamine can be transamidated to glutamine 5 of histone H3. Deposition of 5-HT at this site stabilizes neighboring H3K4me3, resulting in the combinatorial H3K4me3Q5ser modification (termed histone serotonylation). We found that histone serotonylation positively regulates gene expression associated with serotonergic neuronal differentiation, suggesting an important role for 5-HT in chromatin regulation of neurodevelopment. In this talk, we focus on the involvement of histone serotonylation in the placenta, another serotonergic tissue during embryogenesis that is critically involved in regulating brain development. The placenta serves as an interface between maternal and offspring circulation, with crucial functions including provision of 5-HT to the embryonic forebrain for establishment of serotonergic projection patterns. Using ChIP-sequencing and immunoblotting

techniques, we demonstrate that placental histone serotonylation levels are developmentally regulated and associate with important biological pathways during embryogenesis. To determine how placental serotonylation processes are organized, we examined tissues from several transgenic mouse lines in which known 5-HT transporters (Sert and Oct3) and/or the rate-limiting enzyme of 5-HT synthesis (Tph1) were globally deleted. Alterations in these tissues suggest histone serotonylation may impact placental function, which would subsequently perturb the trajectory of offspring brain development. Our work supports a non-canonical, epigenetic role for 5-HT in modulation of placental biology during critical windows of offspring neurodevelopment that, if disrupted, may contribute to developmental origins of brain disorders.

Speaker #3

Igor Adameyko, PhD
Head of Department of Comparative and Developmental Physiology
Center for Brain Research, Medical University of Vienna, Vienna, Austria; Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

The role of serotonin in controlling neuroendocrine tissue development and transgenerational adaptations

Polina Kameneva and Igor Adameyko, Karolinska Institutet, Stockholm, Sweden

The adrenal glands are vital organs that release catecholamines and regulate our response to stress. The mechanisms that maintain the balance between the production of adrenergic chromaffin cells and protection against neuroblastoma tumours remain mysterious. Our findings have revealed that serotonin (5HT) governs the number of chromaffin cells by acting on their immediate progenitor "bridge" cells through the 5-hydroxytryptamine receptor 3A (HTR3A). Additionally, we have discovered that HTR3Ahigh human neuroblastoma cell lines, which are highly aggressive, decrease proliferation in response to HTR3A-specific agonists. In embryos (in vivo), the natural increase of 5HT results in a lengthening of the cell cycle in "bridge" progenitors, resulting in a smaller chromaffin population and altering the balance of hormones and behavioural patterns in adulthood. These behavioural changes and smaller adrenals were reflected in the offspring of pregnant female mice subjected to experimental stress, indicating a maternal-fetal connection that controls developmental adaptations. Lastly, these findings corresponded to a size-distribution of adrenals found in wild rodents with varying coping strategies.

Speaker #4 Travel Awardee

Rocío B Foltran, PhD
Postdoctoral fellow
Inst. de Biología Celular y Neurociencias (IBCN), Buenos Aires, Argentina.

What happens when mice lack serotonin? Consequences in behavior and in the BDNF pathway in two models of hyposerotonergic mice

SERT and beyond in treatment of depression

The serotonin transporter (SERT), the high-affinity clearance mechanism for serotonin, has been the subject of intense research for many decades due to its important role in regulating the strength and duration of serotonin signaling, and as a target for psychotherapeutic drugs. Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed treatment for a host of psychiatric diseases, primary among these, depression. However, SSRIs leave many patients without symptom relief, underscoring a need to improve upon existing SSRIs. Speakers in this symposium will discuss exciting new advances to this end. Dr. Claus Loland will present the structural basis for the inhibition of SERT by the antidepressant drug vilazodone. Vilazodone binds with low nanomolar affinity to an allosteric site distinct from the canonical substrate binding site. The cryo-EM structure of the SERT:vilazodone complex is substantiated by pharmacological assays showing that vilazodone is a non-competitive inhibitor of serotonin uptake and that mutation of residues in the proposed vilazodone binding site decreases its affinity. Insertion of the fluorescent unnatural amino acid L-Anap was used as a reporter for the SERT conformational changes during vilazodone binding. Dr. Harald Sitte will introduce organic cation transporter 3 (OCT3), a low-affinity, high-capacity transporter for monoamines and its role as a regulator of monoaminergic neurotransmission. He will discuss new data showing how the tertiary and quaternary arrangement of OCT3 is impacted by compounds and constituents of the plasma membrane, which also regulate their functional activity. These new insights may reveal important new possibilities for the development of novel therapeutics. Dr. Parastoo (Parry) Hashemi revisits the monoamine hypothesis of depression by measuring *in vivo* serotonin dynamics with voltammetry in mice. She finds that ambient serotonin levels are robustly lower in animals with behavioral phenotypes of depression, and that this is a consequence of increased extracellular histamine, arising from neuroinflammation, which inhibits serotonin release via inhibitory H3 heteroreceptors on serotonin terminals. Moreover, she finds that agents with antidepressant activity also inhibit histamine uptake, which can explain the variable clinical efficacy of SSRIs, as a consequence of individual levels of inflammation. Her work highlights the merits of the monoamine hypothesis of depression via focus on a lesser-known monoamine, histamine.

Chair: Harald Sitte, Ph.D.
Professor
Medical University of Vienna

Co-Chair: Lyn Daws, Ph.D.
Professor
University of Texas Health San Antonio

Speaker #1
Claus Juul Loland, Ph.D.
Professor
University of Copenhagen

Allosteric binding of the antidepressant vilazodone to the serotonin transporter

Depression is a common mental disorder and one of the main causes of disability worldwide. The standard medical treatment is the selective serotonin reuptake inhibitors (SSRIs). All investigated SSRIs are competitive inhibitors of the serotonin transporter (SERT). A non-competitive inhibitor might possess a different therapeutic profile. Vilazodone is a novel antidepressant with limited knowledge of its molecular properties. Here we use molecular pharmacology and cryo-EM structural elucidation to characterize vilazodone binding to SERT. We find that it has non-competitive inhibition to serotonin uptake and it impeded the dissociation of [3H]imipramine with low nanomolar concentrations. Our cryo-EM structure of SERT with bound imipramine and vilazodone reveals a unique binding pocket for vilazodone expanding the extracellular vestibule. The binding site is substantiated with systematic mutagenesis of interacting residues all with the effect of decreasing the allosteric binding of vilazodone. Our findings underlines the versatility within the potential of SERT allosteric ligands.

Speaker #2
Harald Sitte, Ph.D.

Professor
Medical University of Vienna
The structural basis of organic cation transporter 3 inhibition
Organic cation transporters (OCTs) facilitate the translocation of catecholamines, drugs and xenobiotics across the plasma membrane in various tissues throughout the human body. OCT3 plays a key role in low-affinity, high-capacity uptake of monoamines in most tissues including heart, brain and liver. Its deregulation plays a role in diseases. Despite its importance, the structural basis of OCT3 function and its inhibition has remained enigmatic. In my talk, I will discuss the structure of human OCT3 bound to two prototypical inhibitors, corticosterone and decynium-22. In addition, I will relate the functional characteristics of an extensive collection of human genetic variants to structural features, thereby providing a basis for understanding the impact of OCT3 polymorphisms – and show recent data on how to rescue misfolded variants from the endoplasmic reticulum.

Speaker #3
Parastoo Hashemi, Ph.D.
Professor
Imperial College London
Inflammation mediated histaminergic inhibition of serotonin: Not yet time to give up on the monoamine hypothesis of depression

The monoamine hypothesis of depression has gained and lost popularity for decades. Here, we revisit this hypothesis of depression by measuring in vivo serotonin dynamics with fast voltammetry in mice. We found, in a chronic stress model, that ambient serotonin level robustly marked chronic stress in mice. Where mice had behavioural phenotypes of depression, the serotonin levels correlated with the index of depression. We found that this lowered serotonin was a consequence of increased inflammation-induced histamine, acting on inhibitory H3 hetero-receptors on serotonin terminals. Further, escitalopram also inhibited histamine uptake, making this antidepressant much less capable of restoring serotonin under inflammation. Further evidence for the monoamine hypothesis came from a set of experiments where the effects of antidepressants with different modes of action were evaluated on ambient serotonin. We observed the effects of 2 SSRIs, an SNRI and ketamine. We found all of these agents increased extracellular serotonin and propose mechanisms for this increase. Further we create mathematical models, based on our experimental data and propose mechanisms for clinical phenomena that cannot be adequately tested in mice such as why SSRIs take weeks to have clinical effect (due to SERT over/under expression), and why they have such variable clinical efficacy (inflammation). This highlights the fact that the monoamine hypothesis of depression is still valid, but that physiological and pathophysiological nuances, such as inflammation, need to be considered to refine the hypothesis.

Speaker #4 Travel Awardee
Marco Niello, Ph.D.
Postdoctoral Fellow
Medical University of Vienna
Enantiomer-specific pharmacology of cathinones shapes their potential as a scaffold for novel therapeutic agents

Physiological relevance of serotonin receptor functional crosstalk

Functional crosstalk between G protein coupled receptors (GPCRs) in cells promote pharmacological diversity and tailored cellular responsivity. This symposium will discuss the convergent evidence that functional crosstalk for serotonin (5-HT) receptors exist *in vivo*, including in complexes with receptors responding to other natural ligands than 5-HT. The 5-HT_{2A} receptor (5-HT_{2A}R) and 5-HT_{2C} receptor (5-HT_{2C}R) in the central nervous system are implicated in a range of normal behaviors (e.g., appetite, sleep) and physiological functions (e.g., endocrine secretion) while dysfunctional 5-HT_{2A}R and/or 5-HT_{2C}R are implicated in neuropsychiatric disorders (e.g., addiction, obesity, schizophrenia). Preclinical studies suggest that the 5-HT_{2A}R and 5-HT_{2C}R may act in concert to regulate the neural bases for behavior. Dr. Allen will discuss CRISPR/Cas9 strategies being used to elucidate molecular signaling pathways engaged by the 5-HT_{2A}R and 5-HT_{2C}R. These studies highlight the involvement of β-arrestins as key adaptor proteins that profoundly control the trafficking and duration of 5-HT_2R signaling to agonists, including serotonin and psychedelics. Dr. Noelle Anastasio will discuss the physical interaction between the 5-HT_{2A}R and 5-HT_{2C}R detected in heterologous cellular systems, rat brain with a focus on the receptor interfaces which mediate this interaction and may influence intracellular signaling. The metabotropic glutamate 2 receptor 2 (mGluR2) and the 5-HT_{2A}R are GPCRs that are speculated to play a pivotal role in processes related to cognition, perception, and mood. Unbalanced levels of Gq-coupled serotonin 5-HT2A and of Gi/o-coupled mGluR2receptors are involved in psychosis. Pioneering work by members of the Gonzalez-Maeso lab demonstrated using various approaches that 5-HT_{2A}R and mGluR2 can associate in heteromeric complex in *in vivo* cortical neurons. This receptor heteromer displays a remarkable inverse cross-regulation to likely explain the convergent effects of mGluR2- and 5-HT_{2A}R-targeted antipsychotic compounds (and of pro-psychotics and hallucinogens), which, in summary, regulate the Gi/o-Gq-coupling balance of these heterodimers. Hence, selecting receptor complex-specific responses of specific heterocomplexes may constitute an emerging strategy to improve therapeutic strategies.

Chair: Noelle C. Anastasio, Ph.D.
Associate Professor
Center for Addiction Sciences and Therapeutics, Department of Pharmacology and Toxicology, UTMB
Galveston, TX, USA

Speaker #1

Name: John A. Allen, Ph.D.
Assistant Professor
Center for Addiction Sciences and Therapeutics, Department of Pharmacology and Toxicology, UTMB
Galveston, TX, USA

CRISPR/Cas9 approaches to define signaling mechanisms and crosstalk between 5-HT_2 receptors

We previously determined that 5-HT_{2A}R and 5-HT_{2C}R form a very close interaction in cells and this heteromeric complex may allow unique receptor signal transduction and crosstalk. This presentation will highlight our recent efforts using CRISPR/Cas9 strategies to elucidate G protein and β-arrestin pathway mechanisms engaged by the 5-HT_{2A}R and 5-HT_{2C}R. Wildtype HEK293 parental cells, or cells in which β-arrestins or G proteins were stably knocked out (KO) using CRISPR/Cas9 genome editing were generated and validated. The wildtype and knockout cells expressing human 5-HT_{2A}R or 5 HT_{2C}R were studied using a combination of radioligand binding, cell surface labeling and imaging, and live cell calcium imaging. Our studies highlight that agonist-induced 5-HT_2R endocytosis is highly dependent on β-arrestins, and that β-arrestins rapidly interact with 5-HT_{2A}R receptors to limit both the intensity and duration of Gq/11-mediated calcium signaling. These studies indicate an essential role of β-arrestins in regulating rapid 5-HT_2R trafficking, and therefore the signaling duration to serotonin and psychedelic agonists.

Speaker #2

Noelle C. Anastasio, Ph.D.
Associate Professor
Center for Addiction Sciences and Therapeutics, Department of Pharmacology and Toxicology, UTMB
Galveston, TX, USA

Interrogation of the 5-HT_{2A}R:5-HT_{2C}R protein:protein interface

Multiple lines of evidence indicate that GPCRs participate in protein:protein interactions (PPIs) to influence signaling of other GPCRs through functional crosstalk. Here, we propose an innovative, versatile approach to address this fundamental gap in knowledge of the biophysical and functional interfaces between GPCRs in the native environment, termed iDiMeRA (in-cell Directed Measurement of Receptorsome Allostery). We highlight the Class A GPCR 5-HT_{2A}R:5-HT_{2C}R receptorsome. Our team bridges chemistry, pharmacology, and biochemistry to gain a deeper understanding of the molecular processes controlling the 5-HT_2R receptorsome. How 5-HT_2R PPIs modify their physiological roles and participate in disease-specific deregulations, is of prime importance since they can be differentially affected by various drugs.

Speaker #3

Somdatta Saha, Ph.D.
Postdoctoral Fellow
Virginia Commonwealth University, Richmond, VA, USA

Co-translational assembly of 5-HT_{2A}R and mGluR2 in mammalian cells

We previously reported that 5-HT_{2A}R and mGluR2 are able to interact to form an heteromeric GPCR complex, which also modulates pharmacological, signaling and subcellular localization processes of the two protomers. Additionally, our previous findings suggested a crosstalk mechanism between 5-HT_{2A}R and mGluR2 that affected transcriptional processes. As two examples, 5-HT_{2A}R-KO mice showed reduced cortical expression of *mGluR2* mRNA, and chronic treatment with atypical antipsychotics such as clozapine down-regulated cortical *mGluR2* mRNA via 5-HT_{2A}R. Previous reports by other groups proposed co-translational association of mRNA encoding subunits of heteromeric ion channels, but whether complex assembly of GPCRs occurs during translation remains unknown. Our data *in vitro* in HEK293 cells suggest co-translational modulation of *5-HT_{2A}R* and *mGluR2* mRNAs upon siRNA-mediated knockdown. Additionally, our preliminary data suggest that both transcripts could be copurified with an antibody against the c-Myc tag located at the N-terminus of the 5-HT_{2A}R construct. Based on these findings, we are currently evaluating whether mRNA transcripts could co-immunoprecipitate based solely on the interactions of their nascent polypeptides or alternatively if the transcripts associate even when the nascent 5-HT_{2A}R and mGluR2 polypeptides do not interact. Together, these novel insights will provide mechanistic information about co-translational association of GPCR heteromeric complexes in mammalian cells.

Speaker #4 Travel Awardee

Thomas Flanagan, Ph.D.
Postdoctoral Fellow
LSU Health Science Center New Orleans, New Orleans, LA, USA

5-HT_2 Receptor Activation Differentially Impacts Linker Histone H1.5 Kinetics and Induces the Expression of Factors Relevant for Global Chromatin Architecture

Lipid dynamics and serotonin signalling in the brain: From molecular interactions to behaviour

Serotonergic signaling in the brain is determined by the expression and activity of synthetizing enzymes, transporters, and receptors, which are mainly located within or near lipid membranes. However, the role of the membrane in the function of these proteins has been largely ignored until recently when we have started to engage with the complex interplay between membrane components and membrane protein function to understand the temporal-spatial organisation, which is essentially determined by the lipid landscape. The lipid-shaped synaptic membrane is far from a static and constant environment but emerged as highly dynamic during normal plasticity as well as a source of dysfunction and behavioural pathologies. This has a direct and pronounced effect on serotonergic signaling components and, thus, the neurobiology of all serotonergic systems. In this symposium we discuss how different lipid classes shape the function of key proteins in the serotonergic system as well as effects on whole system activity. The symposium will explore the role of cholesterol, sphingolipids and lipid signaling molecules in serotonergic neurotransmission by bringing together current research using clinically relevant animal models of acute and chronic lipid manipulations and highlight recent advances towards the elucidation of molecular mechanisms underlying the bidirectional interaction of serotonin system proteins with their lipid environment.

Chairs: **Jana Haase and Christian P. Müller**

Speaker #1
Steffen Sinning
Dept. Of Forensic Medicine, Aarhus University, Denmark
The role of a specific cholesterol site for monoamine transporter function, folding and pharmacology
The monoamine transporters are Na+-dependent neurotransmitter transporters involved in mood, depression and addiction. The function of these transporters have been described to be modulated by cholesterol. We identified a specific cholesterol binding site between TM1, 5 and 7. Binding of cholesterol to this site is in a dynamic equilibrium that affects the conformational equilibrium of the transporter, which in turn has profound implications for the transport function and folding of the transporter. We find that cholesterol binding facilitates the conformational transition that is rate-limiting for transport and in this way accelerates transport of monoamine neurotransmitters. The impact of cholesterol on the action of several psychotropic drugs was evaluated and it was found that the hallucinogenic drug, Ibogaine, similar to cholesterol depletion, chaperoned the folding trajectory of folding-deficient serotonin transporter mutants. We propose a mechanism for how cholesterol activates monoamine transporters at their desired site of action and how cholesterol-lowering medication may interact with antidepressants.

Speaker #2
Jana Haase
University College Dublin, Ireland
Linking sphingolipid dynamics to sex specific serotonin transporter regulation
The serotonin transporter (SERT) has been implicated in the molecular mechanisms underlying inflammation-induced depression. Using a clinically relevant animal model of chronic inflammation, i.e. the collagen-induced arthritis (CIA) model of rheumatoid arthritis, we have previously shown TNFα dependent depression-like behaviour and up-regulation of SERT activity specifically in the hippocampus of male mice. However, we also observed substantial sex differences in behavioural symptoms and the regulation of SERT; female CIA mice do not display anhedonia or enhanced serotonin uptake in any brain region tested. To better understand the causes for this sexual dimorphism, we recently conducted quantitative mass spectrometry analysis. Among differentially regulated proteins we identified enzymes of the ceramide/sphingomyelin pathway. Follow-up experiments confirmed sex differences in the activity of key enzymes in this pathway, including sphingomyelinases, ceramidases and sphingomyelin synthase. Together with previously reported findings on the acute effects of bacterial sphingomyelinase on serotonin uptake in synaptosomes as well as the association of SERT with lipid microdomains, our data suggest a role for sphingolipid dynamics in the sex specific regulation of SERT activity.

Speaker #3
Christian P. Müller

University Erlangen, Germany

Sphingolipid control of serotonergic activity – a pathway into depression and back

Sphingolipids (SLs) are major components of cellular membranes. Thereby, ceramides and their precursor SLs, the sphingomyelins, are shaping membrane specialisations and lipid rafts that contain proteins involved in the specific signalling of designated neurotransmitter systems. The SLs of the brain are regulated by a plethora of enzymes, the SL-rheostat. Previous studies showed that the SL environment of a membrane is highly dynamic and, by that way, involved in normal behaviours and their plasticity in learning and memory. Dysfunctions of the SL system and its rheostat, however, may give rise to molecular and behavioural pathologies. Here it is discussed how environmental and genetic challenges can cause stress and depression by altering SL enzyme function and subsequently the SL composition of cellular membranes in specific brain areas. These changes also affect the serotonergic system and the control of brain serotonin (5-HT) levels. Many antidepressant drugs are, next to their SSRI properties, also functional inhibitors (FIASMAs) of the enzyme acid sphingomyelinase (ASM). A genetically induced ASM hyper-expression leads to a ceramide over-production in the hippocampus, where neurogenesis becomes impaired. This or a local treatment with ceramide alone induce depression-like behaviour in mice, which can be inhibited by a FIASMA drug. Parallel studies showed that these depressed mice display an enhanced alcohol self-administration, by which they attenuate the depressed phenotype. This happens by a partial normalisation of brain ASM activity and a downstream normalization of 5-HT tissue levels. Besides the ASM, also other enzymes of the SL rheostat appear to modulate serotonergic signalling by controlling membrane lipid environment of 5-HT signalling proteins.

Speaker #4 Travel Awardee

Lluis Miquel-Rio

Instituto de Investigaciones Biomédicas de Barcelona, Spanish National Research Council (CSIC), IDIBAPS, Institut d'Investigacions August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Humanized mice overexpressing α-synuclein in serotonin neurons evoke a depressive phenotype: Reversal by conjugated antisense therapy

Novel classes of 5-HT$_{2C}$ agonists with therapeutic promise and novel signaling mechanisms

This panel will provide a survey of the 5-HT$_{2C}$-selective agonist landscape (McCorvy), a novel selective 5-HT$_{2C}$ agonist currently in clinical trials (Vasilkevich), and detail non-canonical G protein 5-HT$_{2C}$ signaling mechanisms that may be important toward clinical efficacy (Bonniwell).

Chair: John D. McCorvy, Ph.D.
Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States

Speaker #1

John D. McCorvy, Ph.D.
Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Surveying the 5-HT$_{2C}$-selective agonist landscape: What's selective and what is not?

John D. McCorvy, Joseph J. Hennessey, Emma M Bonniwell
Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

The 5-HT$_{2C}$ receptor has been a target for a variety of neuropsychiatric disorders, including schizophrenia, major depression, anxiety, obsessive compulsive disorder, appetite, and obesity. Lorcaserin, a 5-HT$_{2C}$ agonist, was briefly approved for the latter purpose but was withdrawn after post-marketing surveillance indicated increased risk for cancer. Therefore, viable 5-HT$_{2C}$-selective agonists for therapeutic purposes are severely lacking. We set out to profile and determine 5-HT$_{2C}$ selectivity using a combination of bioluminescence resonance energy transfer (BRET) and second messenger assays designed to assess the degree of 5-HT$_{2C}$ selectivity compared across all serotonin subtypes, including rodent receptor isoforms. We reveal that a range of diverse pharmacological profiles exist for purported 5-HT$_{2C}$-selective agonists (e.g. biased agonism and lack of selectivity), which may explain the clinical failure and drawbacks for these compound classes. Furthermore, the potential for the design of biased agonists for the 5-HT$_{2C}$ is discussed as it pertains to emerging clinical compounds and future clinical indications.

Support: Bright Minds Biosciences Sponsored Research Agreement, Medical College of Wisconsin Research Affairs Counsel Pilot grant, and National Institutes of Health General Medical Sciences grant (NIGMS R35GM13342).

Speaker #2

Alex Vasilkevich, Ph.D.
Bright Minds Biosciences, New York, NY, USA

BMB-101: A selective 5-HT$_{2C}$ agonist in clinical trials with therapeutic utility

Alex Vasilkevich[1], Jianmin Duan[1], Mark Smith[1], Jan Pedersen[1], David G. Morgan[2], Christina Merritt[3], Kathryn Cunningham[3], Andrew B. Cao[4], Hailey A. Bock[4], Joseph J. Hennessey[4], John D. McCorvy[4], Alan P. Kozikowski[1]
[1]Bright Minds Biosciences, New York, NY; [2]Michigan State University College of Human Medicine, Grand Rapids, MI; [3]University of Texas Medical Branch, Galveston, TX; [4]Medical College of Wisconsin, Milwaukee, WI.

BMB-101 is a novel highly selective 5-HT$_{2C}$ receptor agonist that shows minimal 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor activity linked with the psychedelic and cardiotoxic effects, respectively. The detailed pharmacological profiling of BMB-101 highlights the role of understanding receptor pharmacology in pre-clinical species relative to humans in order to establish successful translation to human studies. BMB-101 has demonstrated efficacy in a range of animal models including in seizure models (Scn1Lab zebrafish and 6Hz psychomotor stimulation in mice), addiction (rat model of fentanyl-self administration) and symptoms of Alzheimer's Disease (reduced agitation in APP+PS1 mice). In rat SmartCube™ model, the predominant class for BMB-101 demonstrated an antipsychotic drug signature. Currently, we are conducting a randomized, double-blind, placebo-controlled, study of BMB-101 in healthy human subjects at a single center in Australia. This study consists of 3 parts: a single ascending dose (SAD), a food effect, and multiple ascending dose (MAD). All doses in the SAD study were generally well tolerated, with mainly mild adverse effects (AEs) reported. The most common AEs were nausea, oral paresthesia and headache. No serious or severe AEs were reported.

Plasma half-life is estimated at 4.8-5.7 hrs, with exposure as targeted. Therefore, in the ongoing MAD study, BMB-101 is being administered twice a day as originally planned. Based on preclinical evidence from animal models, as well as the developing safety and tolerability profile in humans, we believe that BMB-101 has a potential to be a "best in class" 5-HT_{2C} agonist for treatment of seizures, addictions, psychosis and other disorders.
Support: Bright Minds Biosciences Sponsored Research Agreement

Speaker #3
Emma M. Bonniwell
Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA
Investigating 5-HT_{2C} Non-Canonical Signaling Profiles
Emma M. Bonniwell[1], Joseph J. Hennessey[1], John D. McCorvy[1]
[1]Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI.
The serotonin 5-HT_{2C} is a G protein-coupled receptor (GPCR) widely expressed in the central nervous system and has been designated as a potential therapeutic drug target for multiple neuropsychiatric disorders. 5-HT_{2C} is canonically $G_{q/11}$-coupled; however, recent research has revealed that the 5-HT_{2C} receptor readily activates multiple G protein subtypes ($G_{i/o}$, $G_{12/13}$, $G_{q/11}$). Unfortunately, the significance of promiscuous G protein signaling toward the therapeutic potential of 5-HT_{2C} agonists is not well-studied. We have utilized bioluminescence resonance energy transfer (BRET) assays to identify signaling profiles induced by various 5-HT_{2C} agonists (selective and non-selective), including for psychedelics. This work aims to identify patterns and structure-activity relationships for existing 5-HT_{2C} agonists. Overall, our goal is to develop novel biased ligands as tools to dissect therapeutic effects from side-effects.
Support: Medical College of Wisconsin Research Affairs Counsel Pilot grant and National Institutes of Health General Medical Sciences grant (NIGMS R35GM13342)

Speaker #4 Travel Awardee
Justin Saunders
Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA
Glucocorticoid receptor dysregulation underlies 5-HT2AR-dependent synaptic and behavioral deficits in a mouse neurodevelopmental disorder model

Recent advances in molecular neuroimaging of the serotonin system

Neuroimaging with positron emission tomography (PET) is one of the most powerful tools for capturing 5-HT transmission in the living human brain and studying its role in the pathophysiology and treatment of psychiatric disorder. Currently PET radioligands are available to detect many of the 5-HT receptor subtypes in human brain, as well as the 5-HT transporter and 5-HT synthesis, but progress continues to exploit the utility of the technology and improve spatiotemporal resolution. This symposium brings together international experts in the field to discuss recent advances in the development of PET radioligands and PET technology, and their application in psychiatry especially in relation to major depressive disorder (MDD). Prof. Rupert Lanzenberger will present clinical studies performed with ultrafast functional PET and multimodal imaging in psychiatric patients and healthy controls. Results are ranging from new approaches to quantify the serotonin transporter occupancy by SSRIs in association with efflux transporters to imaging-genetics and transcriptomics for precision pharmacotherapy in psychiatry. Prof. Gitte Moos Knudsen will discuss the current status regarding the utility of PET to measure release of 5-HT, using examples of studies which follow the displacement of PET radioligands by 5-HT releasing agents in healthy human volunteers and patients with MDD. Finally, Dr. Hanne D. Hansen will showcase preclinical and clinical experiments that demonstrate how hybrid neuroimaging approaches based on the combination of PET and magnetic resonance imaging, opens up possibilities to test the mechanism of novel drugs by evaluating their blood-brain barrier passage, regional 5-HT receptor occupancy and functionality. Overall, this symposium will overview both preclinical and clinical studies to provide a perspective on the current state and future application of PET technology to understand the complex role of the 5-HT system in normal and dysfunctional brain function.

Chair: Hanne Demant Hansen, Ph.D.
Senior Researcher
A.A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, USA

Co-Chair: Trevor Sharp, Ph.D.
Professor of Neuropharmacology
University of Oxford, UK

Speaker #1
Rupert Lanzenberger, M.D./Ph.D.
Professor of Clinical Neuroscience and Head of the Neuroimaging Labs, Department of Psychiatry
Department of Psychiatry and Psychotherapy, Medical University Vienna, Austria
Functional PET, imaging (epi)genetics and transcriptomics

Speaker #2
Gitte Moos Knudsen, Ph.D.
Professor of Neurobiology, Chairman of the Neurobiology Research Unit
Neurology and Neurobiology Research Unit, Copenhagen Univy Hospital, Rigshospitalet, Denmark
Measuring 5-HT release with PET in healthy volunteers and patients with major depressive disorder

Speaker #3
Hanne Demant Hansen, **Ph.D.**
Senior Researcher
A.A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, USA
Functional characterization of drugs targeting the serotonin system using simultaneous PET/MR imaging

Speaker #4 Travel Awardee
Nako Nakatsuka, Ph.D.
Senior Scientist
Institute for Biomedical Engineering, ETH Zürich
***Ex Vivo* Nanoscale Serotonin Mapping with Electrophysiology**

Travel Awardee, Short Oral Talks, and Poster Abstracts

(In alphabetical order)

Molecular mode of action of new *ecstasy* analogues: methylenedioxyphenyl group bioisosteric replacement

Ana Sofia Alberto-Silva[1], Nina Kastner[1], Letícia Alves da Silva[1], Kathrin Jäntsch[1], Marco Niello[1], Oliver Kudlacek[1], Thomas Stockner[1], Simon Brandt[2], Pierce Kavanagh[3], Harald H. Sitte[1,4]

[1]Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna, Austria; [2]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, United Kingdom; [3]Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St James Hospital, Dublin, Ireland; [4]Center for Addiction Research and Science, Medical University of Vienna, Austria

3,4-methylenedioxymethamphetamine (MDMA, commonly known as *ecstasy*) is one of the most widely used recreational psychostimulant drugs in the world, with adverse events dependent on dose, individual susceptibility, and circumstances in which the drug is taken. Despite its current illegal status in most countries, MDMA has been re-emerging in clinical settings as a candidate for the treatment of specific psychiatric disorders (e.g. post-traumatic stress disorder (PTSD)) in combination with psychotherapy. Chemically, MDMA is a synthetic ring-substituted amphetamine derivative, containing a methylenedioxyphenyl group.
In this study, we explore the impact of three bioisosteric replacements of the methylenedioxyphenyl group in MDMA. We explore their impact at several targets, including the monoamine transporters (MATs), organic cation transporters (OCTs), plasma membrane monoamine transporter (PMAT) and serotonin 2A, 2B and 2C ($5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, and $5\text{-}HT_{2C}$) receptors. The *in vitro* methods performed used human embryonic kidney (HEK) 293 cells and included radiotracer assays (uptake inhibition and release assays), transporter electrophysiology (whole-cell patch-clamp) and fluorescence-based assays (using a GCaMP calcium sensor). The *in silico* methods included molecular docking (GOLD software, version 2022.2.0). Altogether, our results show overlapping pharmacological activity of the MDMA bioisosteres at MATs, OCTs and PMAT, but a slightly decreased activity at $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, and $5\text{-}HT_{2C}$ receptors, compared with MDMA. Additionally, we show a more detailed analysis on the substrate profile of these compounds at serotonin and dopamine transporters (SERT and DAT, respectively), suggesting that all compounds behave as full substrates at SERT but as partial substrates at DAT.
Considering MDMA-induced adverse events, it is advantageous to explore MDMA-related congeners which can keep the same therapeutical potential but decrease off-target effects. At the moment, we are exploring the pharmacological profile of these bioisosteres on each mentioned serotonin receptor and their overall toxic potential compared with MDMA.

This work has received funding from European Union H2020-MSCA-ITN-2019, grant agreement N° 860954. Additionally, this work was supported by the Austrian Science Fund/FWF, grant numbers P33955 and P35589 (to HHS).

Medial Prefrontal Serotonergic Input Regulates Cognitive Flexibility Behavior in Mice

Ashlea A. Morgan[1,2*], Nuno D. Alves[1,2*], Gregory S. Stevens[1,2], Tamanna T. Yeasmin[3], Alexandra Mackay[2], Saige Power[4], Derya Sargin[5], Carla Hanna[3], Arwa L. Adib[3], Annette Ziolkowski-Blake[1,2], Evelyn K. Lambe[4] & Mark S. Ansorge[1,2]
[1]Dept. of Neurobiology & Behavior, Columbia University;
[2]New York State Psychiatric Institute (NYSPI), New York, NY 10032;
[3]Dept. of Neuroscience & Behavior, Barnard College;
[4]Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada;
[5]Department of Psychology, University of Calgary, Calgary, Northwest Territories T2N 1N4, Canada.

The medial prefrontal cortex (mPFC) regulates cognitive flexibility and emotional behavior. Furthermore, neurons that release serotonin (5-HT) project to the mPFC, and drugs targeting the 5-HT system influence emotional regulation and cognitive flexibility. Yet, the specific role of endogenous 5-HT release in the mPFC on neurophysiology and behavior is unknown. Here we selectively mapped, monitored, and manipulated 5-HT input into the mPFC to gain insight into the functional roles of this pathway. Using *in vitro* optogenetics paired with whole-cell slice electrophysiology we observed strong and dominant 5-HT_{1A} receptor-mediated inhibition of mPFC pyramidal neurons. *In vivo* fiber photometry recordings revealed task-specific activity signatures in 5-HTergic neurons projecting from the in dorsal raphe to the mPFC during a cognitive flexibility task but not in the open field test. Furthermore, *in vivo* optogenetic activation of the 5-HTergic dorsal raphe-to-mPFC pathway selectively improved extradimensional rule shift performance while inhibition impaired it, demonstrating sufficiency and necessity for mPFC 5-HT release in cognitive flexibility. Locomotor activity or anxiety-like behavior in the open field test was not affected by either optogenetic manipulation. Collectively, our data reveal a powerful and specific modulatory role of endogenous 5-HT release from dorsal raphe-to-mPFC projecting neurons in cognitive flexibility, independently of effects on locomotor and anxiety-like behavior.

Funding: Sackler Innovation Award (NDA), NSF GRFP (DGE 16-44869, AAM) & NIMH (R01 MH099118-01A1, 2R01MH080116-06A1, MSA), NSERC Discovery Grant (EKL), CIHR Canada Graduate Scholarship Doctoral Award (DS).

Investigating the free energy profile of the substrate-induced occlusion of the human serotonin transporter

Letícia Alves da Silva[1], Erika Lazzarin[1], Ralph Gradisch[1], Amy Clarke[1], Thomas Stockner[1]

[1]Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna, Waehringerstr. 13A, 1090 Vienna, Vienna

The serotonin transporter (SERT) is a protein that is responsible for the reuptake of serotonin, a neurotransmitter that plays a crucial role in regulating mood and other neuronal activities. SERT is located on the membrane of nerve cells, specifically on the presynaptic side of the synapse. It works by binding to serotonin molecules that have been released into the synapse, and then transporting them back into the presynaptic neuron, where they can be reused or recycled. This process is important for maintaining the appropriate levels of serotonin in the brain, and for regulating the activity of the serotonergic system. Dysfunction of SERT has been linked to several psychiatric and neurological disorders, including depression, anxiety, and obsessive-compulsive disorder. Nonetheless, available medications have limited efficacy and severe side effects, owing to a lack of understanding of their mechanism of action. Molecular dynamics (MD) simulations have made significant progress in understanding the conformational changes that cause SERT occlusion, but transitions between conformations are still necessary and must be accompanied by a quantification of the dynamics of the transporter and the associated free energy. Here, we developed Markov State Models (MSMs) based on MD simulations to investigate the SERT occlusion's free energy landscape. We used a total of 12μs MD simulations of SERT bound to serotonin (5HT) and the reaction coordinates of the process of occlusion were successfully obtained using time-lagged Independent Component Analysis (tICA). Our MSM analysis revealed that SERT occlusion involves multiple conformational substates, indicating the presence of an obligatory intermediate state before the transporter is fully occluded. Furthermore, structural and force distribution analysis revealed the key occlusion residues, including residues at position 98, 335, 334, 548 and 127 in SERT. Taken together, our findings provide an in-depth description of the role of key residues previously identified by experiments as critical for substrate transport. Because some of these residues, such as 98 and 335, are highly conserved among members of the monoamine transporter subfamily, this research sheds light on new hypotheses that may be important in understanding similarities and differences in family members' transport mechanisms.

Funding sources
This project has received funding from European Union H2020-MSCA-ITN2019, grant agreement No 860954.

Impact of 5HT2c agonists on behavioral anomalies of mice expressing a human Tph2 loss of function variant.

Jean Martin Beaulieu

Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto, Toronto, Canada.

Tryptophan hydroxylase 2 (Tph2) is the rate limiting enzyme for serotonin synthesis in the adult brain. rs120074175 is a rare coding variant of human *TPH2* which minor allele results into a R441H amino acid substitution and major reduction in enzymatic activity. Frequency of the minor allele has been evaluated to 0.003% out of 32056 samples. Mice engineered to express this variant (Tph2-KI) display 80% reduction of brain 5HT levels. These mice were used to investigate behavioral consequences of *TPH2* loss of function and pharmacological treatments. Tph2-KI mice display increased anxiety-related behaviors, altered social behaviors, disruption of reversal learning and preference to ethanol under aversive conditions. Interestingly administration of serotonin 2C receptor (5HT2c) agonists restored cognitive and social behavior in these mice. This is pointing to a role of 5HT2c receptor signaling in mediating behavioral outcomes of adult brain 5HT deficits.

Novel fluorescent probes for imaging the serotonin transporter

Oliver John Belleza,[1,4] Iakovos Saridakis,[2] Miran Lemmerer,[2] Margaux Riomet,[2] Nadja Singer,[3] Pedro A. Sánchez Murcia,[3] Nina Kastner,[1] Yi Xiao,[2] Sergio Armentia Matheu,[2] Saad Shaaban,[2] Xavier Westergaard,[4] David Sulzer,[4] Leticia González,[3] Nuno Maulide,[2] Harald H. Sitte[1]

[1] Institute of Pharmacology, Medical University of Vienna, 1090 Vienna, Austria
[2] Institute of Organic Chemistry, University of Vienna, 1090 Vienna, Austria
[3] Institute of Theoretical Chemistry, University of Vienna, 1090 Vienna, Austria
[4] Department of Psychiatry, Columbia University, New York, New York 10032, United States

The serotonin transporter (SERT) belongs to the solute carrier 6 family (SLC6) of transmembrane proteins which are expressed in the nervous system. These proteins facilitate the cellular transport of biogenic monoamine neurotransmitters. More specifically, SERT is primarily responsible for the clearance of serotonin from the synaptic cleft, therefore regulating its neuromodulatory effects. SERT, along with the dopamine transporter (DAT) and norepinephrine transporter (NET), are also important drug targets for the treatment of mental and behavioral disorders such as depression and attention-deficit hyperactivity disorder. Therefore, compounds that can serve as pharmacological tools to investigate the activity of these transporters are clinically relevant. One such tool is a fluorescently labeled ligand which can bind to a protein target and provide visual information of its expression, binding events, and cellular trafficking/localization. In this project, we explore a recently developed class of fluorescent compounds called PyrAtes which offer interesting chemical properties, photostability, and large Stokes shifts. Herein, we designed two compounds each with a PyrAte fluorophore attached to (*S*)-citalopram, a selective serotonin reuptake inhibitor (SSRI), via either a six-carbon chain linker (MILE753) or a three-carbon chain linker (IASA554). The activity of these resulting compounds was observed in HEK293 cells overexpressing SERT using cell-based radioligand uptake assays, electrophysiology, and confocal microscopy experiments. Additionally, their utility in imaging endogenously expressed transporters was explored *ex vivo* in acute mouse brain slices using two-photon microscopy. The inhibitory activity of (*S*)-citalopram was reduced by the fluorescent modification by around 10- to 20-fold, with IASA554 and MILE753 possessing IC_{50} values of 0.40 and 0.83 µM, respectively. These compounds, however, were nevertheless effective and specific in the fluorescent labelling of SERT. We have shown for the first time a fluorescent (*S*)-citalopram conjugate that binds specifically to endogenously expressed SERT. Our results not only confirm the utility of such compounds in the fluorescence imaging of transporters, but also provide some insights into ways of improving the design of PyrAte fluorophores and their fluorescent drug conjugates in the future.

Funding information:

This work is supported by the Austrian Science Fund - DK Molecular Drug Targets (FWF W1232).

Existence of 5-HT1AR-FGFR1 heteroreceptor complexes in hippocampal astrocytes. Putative link to 5-HT and FGF2 modulation of hippocampal gamma oscillations

Borroto-Escuela DO[1, 2], Corrales-Ferro F.[2], Ochoa-Torres V.[2], Gozalez-Cristo E[2], Lopez-Salas A.[2], Alvarez-Contino, J.E.[2], and Fuxe K[1]

[1]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden and [2]Receptomics and Brain disorders lab, Department of Human Physiology, Faculty of Medicine, University of Malaga, Málaga, Spain.

The serotonin 1 A receptor-fibroblast growth factor receptor 1 (5-HT1AR-FGFR1) heterocomplexes are involved in neuroplasticity in the rat hippocampus and in the mesencephalic raphe 5-HT nerve cells. Disturbances in these heterocomplexes in the raphe-hippocampal 5-HT system were found in a genetic rat model of depression. The majority of 5-HT1AR-FGFR1heterocomplexes in the hippocampus appeared to be located mainly in the neuronal networks and a relevant target for antidepressants. Through a neurochemical and electrophysiological analysis, it was therefore tested in the current study if astrocytic 5-HT1AR-FGFR1 heterocomplexes also exist in hippocampus. They may modulate the structure and function of astroglia in the hippocampus leading to possible changes in the gamma oscillations. Localization of hippocampal 5-HT1AR-FGFR1 heterocomplexes in astrocytes was found using in situ proximity ligation assay combined with immunohistochemistry using GFAP immunoreactivity as a marker for astroglia. Acute i.c.v. treatment with 8-OH-DPAT alone or together with FGF2 significantly increased 5-HT1AR-FGFR1 heterocomplexes in the GFAP positive cells, especially in the polymorphic layer of the dentate gyrus (PoDG) but also in the CA3 area. Also, structural plasticity changes were observed in the astrocytes, especially in the PoDG region, upon these pharmacological treatments. They may also be of relevance for enhancing the astroglial volume transmission with increased modulation of the neuronal networks. The effects of combined agonist treatments on gamma oscillations point to a significant antagonistic interaction in astroglial 5-HT1AR-FGFR1 heterocomplexes that may contribute to counteraction of the 5-HT1AR-mediated decrease of gamma oscillations.

Serotonergic brain signatures of peripheral *SLC6A4* DNA methylation

S.E.P. Bruzzone[1,2], B. Ozenne[1,3], P. M. Fisher[1,2], G. M. Knudsen[1,2], K.P. Lesch[4,5], V. G. Frokjaer[1,2,6]

Affiliations:
[1] Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark
[2] Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3] Department of Public Health, Section of Biostatistics, University of Copenhagen, Copenhagen, Denmark
[4]Division of Molecular Psychiatry, Center of Mental Health, University Hospital Würzburg, Würzburg, Germany
[5]Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs), Maastricht University, Maastricht, The Netherlands
[6] Psychiatric Centre Copenhagen, Copenhagen, Denmark

Abstract
The serotonin transporter (5-HTT) is a key regulator of serotonergic neurotransmission and alterations in its activity have been linked to several psychiatric disorders. Alterations in peripheral DNA methylation in the transcriptional control region of 5-HTT gene (*SLC6A4*) have previously been linked to depressive symptoms, impaired antidepressant treatment outcome, recent stressful life events and childhood trauma. However, it is not clear whether peripheral blood *SLC6A4* methylation is associated with brain serotonergic targets or it only reflects peripheral 5-HTT activity. In this study, we investigated the relation between peripheral *SLC6A4* DNA methylation and in vivo brain serotonin markers in 239 healthy adults and in 71 patients with major depressive disorder (MDD). We used bisulfite conversion and pyrosequencing on DNA extracted from whole blood nucleated blood cells to estimate DNA methylation levels at four CpG sites that have been previously linked to clinical phenotypes. We imaged brain 5-HTT and serotonin receptor 4 (5-HT_4) binding levels using positron emission tomography (PET) and the radioligands [^{11}C]DASB and [^{11}C]SB207145, respectively. We applied linear latent variable models to estimate the associations between DNA methylation and 5-HTT in healthy controls, 5-HT_4 in healthy controls, and 5-HT_4 in individuals with MDD. We did not find evidence for any significant associations between peripheral DNA methylation at *SLC6A4* and brain 5-HTT or 5-HT_4 levels in any of the cohorts (all $p>0.05$). Although we only evaluated a small portion of the CpG island within *SLC6A4*, these results suggest that peripheral DNA methylation at these sites does not map onto relevant brain serotonergic targets, neither in healthy controls nor individuals with MDD. Peripheral *SLC6A4* DNA methylation may thus be considered as a peripheral marker of inflammation or serotonin-mediated immune function rather than a marker for brain serotonergic activity.

Funding support:
This study was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 953327 (Serotonin & Beyond) and No. 728018 (Eat2beNICE), the Lundbeck Foundation (BrainDrugs grant: R279-2018-1145), the Ivan Nielsen Foundation, the Doctor Sofus Carl Emil Friis and Wife Olga Doris Friis' foundation, the Innovation Fund Denmark (grant ID 4108-00004B), the Research Council of Rigshospitalet (grant ID A6594) and the Lundbeck Foundation supporting the Cimbi database (10.1016/j.neuroimage.2015.04.025).

Development and mechanistic understanding of non-hallucinogenic psychedelic analogs

Lindsay P. Cameron[1], Seona D. Patel, Maxemiliano V. Vargas, Eden V. Barragan, Shekib Jami, Hunter T. Warren, Winston L. Chow, Shane B. Johnson, Isak Aarrestad, Arabo A. Avanes, Conor Liston, John A. Gray, David E. Olson.

AFFILIATIONS
[1]Neuroscience Graduate Program, University of California, Davis, Davis, CA 95618, USA

ABSTRACT
Psychedelic compounds have displayed antidepressant potential in both humans and rodents. Despite their promise, psychedelics can induce undesired effects that pose safety concerns and limit their clinical scalability. One question that scientists have debated over is whether experiencing the hallucinations associated with these compounds is necessary for achieving the therapeutic effects. Here, I will discuss the systematic design of non-hallucinogenic psychedelic analogs, and present a mechanistic understanding of how these molecules produce therapeutic effects. While the hallucinogenic properties of psychedelics are generally attributed to activation of serotonin 2A receptors (5-HT2ARs), it is currently unclear if these receptors also mediate their therapeutic effects. Here, we use a combination of pharmacological and genetic tools to demonstrate that activation of 5-HT2A receptors is essential for tryptamine-based psychedelics to produce antidepressant-like effects in rodents. Our results suggest that psychedelic and non-hallucinogenic psychedelic tryptamines can induce hallucinogenic and therapeutic effects through activation of the same receptor.

SUPPORT
This work was supported by funds from the National Institutes of Health (NIH) (R01GM128997 and R01DA056365 to DEO), three NIH training grants (T32GM113770 to CL; T32GM099608 to MV and EVB; T32MH082174 IA; and T32MH112507 to LPC, AAA), the Camille and Henry Dreyfus Foundation (DEO), a Hellman Fellowship (DEO), the Boone Family Foundation (DEO and JAG), a sponsored research agreement with Delix Therapeutics (DEO), and a UC Davis Provost's Undergraduate Fellowship (SDP).

Simultaneous targeting of 5-HT$_4$Rs and NMDARs exerts additive effects against stress

Briana K. Chen[1,2], Holly C. Hunsberger[1,2,3], Indira Mendez-David[4], Denis J. David[4], Alain M. Gardier[4], and Christine A. Denny[1,2]
Affiliations: [1]Division of Systems Neuroscience, Research Foundation for Mental Hygiene, Inc. (RFMH) / New York State Psychiatric Institute (NYSPI), New York, NY 10032, USA, [2]Department of Psychiatry, Columbia University Irving Medical Center (CUIMC), New York, NY 10032, USA, [3]Center for Neurodegenerative Diseases and Therapeutics, Rosalind Franklin University of Medicine and Science / The Chicago Medical School, North Chicago, IL 60064, [4]CESP/UMRS 1178, Univ Paris-Sud, Fac Pharmacie, INSERM, Université Paris-Saclay, Châtenay-Malabry, France 92290

Background: Serotonin (5-HT) receptors and *N*-methyl-D-aspartate receptors (NMDARs) have both been implicated in the pathophysiology of affective and anxiety disorders. In particular, 5-HT type 4 receptor (5-HT$_4$R) agonists and NMDAR antagonists have been suggested to act as rapid-acting antidepressants and resilience-enhancing prophylactic drugs. Here, we evaluated whether targeting both receptors through combined dosing of (*R*,*S*)-ketamine and prucalopride, a 5-HT$_4$R agonist would have additive effects, resulting in reductions in stress-induced fear, behavioral despair, and hyponeophagia.
Methods: A single injection of saline, (*R*,*S*)-ketamine, prucalopride, or a combined dose of (*R*,*S*)-ketamine + prucalopride was administered before or after contextual fear conditioning (CFC) stress in male and female 129S6/SvEv mice. Drug efficacy was assayed using a variety of behavioral tests, including the forced swim test (FST), elevated plus maze (EPM), open field (OF), marble burying (MB), novelty-suppressed feeding (NSF), and contextual fear discrimination (CFD). All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the New York Psychiatric Institute (NYSPI). c-fos and parvalbumin (PV) expression in the hippocampus (HPC) and medial prefrontal cortex (mPFC) was assayed using immunohistochemistry. Generally, the effect of Drug was analyzed using an analysis of variance (ANOVA), using repeated measures where appropriate. Post-hoc Dunnett, Sidak, or Tukey tests were used where appropriate.
Results: A single dose combination of prophylactic (*R,S*)-ketamine + prucalopride (10 + 3 mg/kg) attenuated learned fear in male mice (n = 6-10 male mice per group; ANOVA; $p = 0.0035$) and decreased behavioral despair in both sexes (n = 6-10 male mice per group; ANOVA; $p < 0.0001$; n = 6-12 female mice per group; ANOVA, $p < 0.0001$). Combined administration of (*R,S*)-ketamine + prucalopride exerted an additive effect in preventing stress-induced hyponeophagia in both male and female mice, but not when administered separately (n = 6-10 male mice per group; ANOVA; $p = 0.0004$; n = 6-12 female mice per group; ANOVA, $p = 0.0467$). Prophylactic (*R,S*)-ketamine + prucalopride also exerted an additive effect of enhancing and facilitating contextual fear discrimination in male mice (n = 6-7 mice per group; RMANOVA; $p < 0.0001$). Combined, but not separate, administration of (*R,S*)-ketamine + prucalopride significantly increased neural activity and PV expression, as well as overlap, in ventral CA3 of the HPC and infralimbic area of the mPFC (n = 3-8 mice per group; ANOVAs; $p = 0.0001$, $p = 0.0197$, $p = 0.0178$, $p = 0.0498$, $p = 0.0122$, respectively).
Conclusion: Our results indicate that simultaneously targeting NMDARs and 5-HT$_4$Rs using a drug combination of (*R,S*)-ketamine + prucalopride exerts additional and distinct neural and behavioral effects in reducing a wide variety of stress-induced fear, behavioral despair, and hyponeophagia behaviors in both male and female mouse models of stress. Simultaneously targeting NMDARs and 5-HT4Rs is sufficient to enhance both excitatory and inhibitory signaling in specific subregions of the HPC and mPFC, brain regions that are critically involved in stress processing and psychiatric disorders. Together, our findings demonstrate the potential of leveraging combinatorial pharmacological treatment to advance targeted therapies for stress-induced psychiatric disorders.

Supported by: BKC was supported by an NIMH F31 MH121023. HCH was supported by an NIA K99 AG059953 and an NIA R24 AG061421. IM-D was supported by a National Alliance for Research on Schizophrenia and Depression (NARSAD) 2017 Young Investigator Award from the Brain & Behavior Research Foundation and by the Deniker Foundation. CAD was supported by an NICHD R01 HD101402, an NIA R21 AG064774, and an NINDS R21 NS114870, and a gift from the For the Love of Travis Foundation.

The Tripnogram: AI assisted morphological, electroencephalographic, and behavioral signatures of diverse psychedelics

April Contreras, Haley N. Strong, Rochelle M. Hines, & Dustin J. Hines

Psychological & Brain Sciences, and Interdisciplinary Neuroscience, University of Nevada Las Vegas, Las Vegas, NV, USA

It is well-established that serotonergic psychedelics exert their hallucinogenic effects by activation of receptors in the serotonin type 2 (5-HT_2) family, with serotonin 5-HT_{2A} receptor (5-HT_{2A}R) activation considered central to their mechanism of action. Hallmarks of 5-HT_{2A}R activation, such as the head-twitch response, behavioral arrest, and distinct waveform activity in the cortical EEG of mice have been used to characterize the response to psychedelics, however, we currently lack direct and deep comparative characterization of diverse psychedelic ligands. Further, while a major downstream mechanism proposed for psychedelic therapies is induction of synaptic plasticity, the relative abilities of psychedelics to trigger spine morphology changes remains uncharacterized. In the present study we comprehensively compared psychedelics from distinct classes including psilocybin, LSD, mescaline, and 25I NBOH, each at differing doses. While all psychedelics were capable of inducing plasticity of dendritic spines, they varied in the extent of spinogenesis and the morphology of resulting spines. We also found that all psychedelics induce common hallmarks in the cortical EEG but can be distinguished by the pattern and density of these waveforms over time. Finally, by examining a rich repertoire of behavioral indices we identified signatures in addition to the head-twitch response that allow further delineation of unique psychedelic features that scale with dose and map onto human subjective experience. This work provides a platform for insight into the activity of diverse psychedelic substances, including novel chemical entities being developed for potential therapeutic use.

Neuronal correlates of declarative memory impairments in a condition of chronic serotonin deficiency

R.C. Coray[1,2], J. Zimmermann[1,2], A. Haugg[2,3], E. Seifritz[4], A. Stock[5], C. Beste[5], D.M. Cole[1], B.B. Quednow[1,2]

[1]Experimental and Clinical Pharmacopsychology, Department of Psychiatry, Psychotherapy, and Psychosomatics, Psychiatric University Hospital Zurich, University of Zurich, Zurich Switzerland, [2]Neuroscience Center Zurich, ETH Zurich and University of Zurich, Zurich, Switzerland, [3]Department of Child and Adolescent Psychiatry and Psychotherapy, Psychiatric University Hospital Zurich, University of Zurich, Zurich, Switzerland, [4]Department of Psychiatry, Psychotherapy and Psychosomatics, Psychiatric Hospital, University of Zurich, Zurich, Switzerland, [5]Cognitive Neurophysiology, Department of Child and Adolescent Psychiatry, Faculty of Medicine, TU Dresden, Germany

Abstract

The acute pharmacological depletion of serotonin (5-HT) is consistently associated with impairments in declarative memory in humans and animals. However, the neural underpinnings of 5-HT deficiency-related memory alterations remain largely unknown to date. The current study thus examined the functional resting-state whole-brain connectome in relation to memory performance in a group of 3,4-methylenedioxymethamphetamine (MDMA, “Ecstasy”) users in the context of a human serotonergic lesion model. MDMA strongly affects 5-HTergic neurotransmission and it has been shown that its repeated use results in both chronic and selective central 5-HT deficiency as well as declarative memory impairments.

We examined a group of 44 chronic MDMA users and 41 demographically matched controls. Declarative memory performance was assessed by Rey Auditory Verbal Learning Test and a visuo-associative learning test. To uncover alterations in the whole brain connectome between groups, we employed a data-driven multi-voxel pattern analysis (MVPA) approach on participants resting-state functional magnetic resonance imaging data. MDMA use was confirmed by hair analyses.

MDMA users showed strong impairments in delayed recall across tasks, which was particularly evident in the verbal memory domain. MVPA revealed a large cluster located in the left postcentral gyrus of global connectivity differences between MDMA users and controls. Post-hoc seed-based connectivity analyses with this cluster unravelled hypoconnectivity to temporal areas belonging to the auditory network and hyperconnectivity to dorsal parietal regions belonging to the frontoparietal network in MDMA users. Seed-based connectivity strength was associated with verbal memory performance in the whole sample as well as with MDMA intake patterns in the user group.

Our findings suggest altered patterns of multimodal sensory integration as consequence of MDMA-associated central 5-HT hypofunction between auditory processing regions and a functional heteromodal connector hub, the left postcentral gyrus. This might be a consequence of diminished cortical synaptic plasticity in sensory areas participating in mnemonic circuits. In addition, hyperconnectivity in regions of a cognitive control network might indicate compensation for degraded sensory processing. Considering previous research on the role of 5-HT in learning and plasticity, our finding revealing primary functional connectivity changes in regions of lower- and higher-level language and verbal memory processing is conclusive. Together, our results suggest a role for 5-HT in humans to verbal memory encoding and consolidation at its earliest stages already.

Funding sources: RC was supported by grants of Novartis Foundation for Medical-Biological Research (#19C167) and Swiss National Science Foundation (Grant 320030L_179450)

Adult hippocampal neurogenesis is required for vortioxetine -induced prevention of anxiety/depression relapse phenotype.

Indira Mendez-David[1], Jean-Philippe Guilloux[1], Abhishek Shah[2], Laurent Tritschler[1], Céline Defaix[1], Philippe Fossati[3], Romain Colle[4], René Hen[5,6,7], Christine A. Denny[5,6], Alain M. Gardier[1], Emmanuelle Corruble[4], Denis J. David[1]

[1]CESP/UMRS 1178, Univ Paris-Sud, Fac Pharmacie, INSERM, Université Paris-Saclay, Châtenay-Malabry, France;
[2]Columbia College, Columbia University, New York, NY ;
[3]Inserm, CNRS, institut du cerveau et de la moelle, hôpital Pitié-Salpêtrière, Sorbonne universités, UPMC université Paris 06, AP-HP, boulevard de l'Hôpital, 75013 Paris, France.
[4]CESP/UMRS 1178, Univ Paris-Sud, Faculté de Médecine Paris-Sud, Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris Sud, Service Hospitalo-Universitaire de Psychiatrie, Hôpital de Bicêtre, Le Kremlin Bicêtre, France;
[5]Department of Psychiatry, Columbia University, New York, NY 10032, USA
[6]Division of Systems Neuroscience, Research Foundation for Mental Hygiene, Inc (RFMH) / New York State Psychiatric Institute (NYSPI), New York, NY 10032, USA
[7]Department of Neuroscience, Columbia University, New York, NY 10032, USA

Previously, we showed that vortioxetine (VORT) [a serotonin reuptake inhibitor combined with actions at serotonin receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_3, 5-HT_7)] protected against stress reinstatement induced anxiety/depression-like phenotype and decrease in adult hippocampal neurogenesis (AHN). In a mouse model of genetic ablation of AHN [glial fibrillary acidic protein (GFAP)-positive neural progenitor cells mouse line], we assessed whether AHN is required for VORT -induced prevention of anxiety/depression. Four weeks before the start of the corticosterone (CORT) treatment to induce depression-like behavior and until the end of the protocol, male GFAP-TK positive mice (TK+) and their littermates (TK-) were administered with valganciclovir in the chow to arrest AHN. After 4 weeks of chronic vehicle (VEH) or CORT, TK+ and TK- mice were administered with saline or VORT (10 mg/kg/day, i.p) treatment for 4 weeks. Behavioral assays (Elevated Plus Maze, EPM; the Novelty Suppressed Feeding, NSF; the Splash Test, ST) were chosen to assay anxiolytic-antidepressant-like activity during VORT treatment and 3 weeks after withdrawal. We confirmed that chronic VORT induced anxiolytic/antidepressant-like effects and protected against CORT reinstatement–induced anxiety/depression-like phenotype in both genotypes. In the EPM and the ST, ablation of AHN in TK+ mice did not alter anxiolytic/antidepressant-like response induced by VORT and prevention of stress reinstatement. However, in the NSF, chronic VORT treatment-induced decrease in latency to feed and prophylactic effects against stress reinstatement in TK- mice, is arrested in TK+ mice. In conclusion, AHN is required not only for VORT -induced antidepressant effects but also for the prevention of relapse.

The Impact of Early Life Stress on Serotonin Circuits

Moriah Edge-Partington[1,4,5*], Raksha Ramkumar[1,4,5*], Yi Ren[2,3,4], Dylan Terstege[2,3,4], Kabarat Adigun[2,3,4], Naila Jamani[1,4,5], Mio Tsutsui[1,4,5], Jonathan Epp[2,3], and Derya Sargin[1,4,5]

Department of Psychology[1], Department of Cell Biology and Anatomy[2], Cumming School of Medicine[3], Hotchkiss Brain Institute[4], Alberta Children's Hospital Research Institute[5], University of Calgary, Canada
*Denotes co-first author

Chronic childhood stress is a prominent risk factor for developing mood disorders, yet mechanisms underlying this association remain unclear. Serotonin plays a crucial role in neurodevelopment and vulnerability to mood disorders. Exposure to early life stress (ELS) has important implications for the serotonin system, as the highest serotonergic activity occurs during brain development. During early development, serotonin regulates cell survival, growth and differentiation, and is important for shaping the maturation of neural circuits. Developmental manipulations that alter the serotonin system, such as stress, physical abuse, and lack of parental care, have been associated with chronic behavioral deficits in rodents and primates. Yet, we don't fully understand the long-term impact of ELS on serotonin connectivity. Using a mouse model of chronic developmental stress, we sought to determine how ELS impacts brain-wide serotonin activity and behavior in adulthood. To induce ELS, the limited bedding and nesting (LBN) model was implemented during the first postnatal week of life (postnatal days 2-9). When mice reached adulthood, we assessed behavioral changes under low- and high-threat conditions. *In vivo* calcium imaging in the dorsal raphe nucleus (DRN) revealed that ELS disrupts the serotonin response to high-threat environments. Using FosTRAP mice, we identified ELS-induced disruptions in the functional connectivity of the raphe nucleus. Next, we performed fiber photometry to determine ELS-induced changes in serotonin release in selected circuits during behavior. Overall, our 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.

Funding:
Canadian Institutes of Health Research (CIHR)
Canada Foundation for Innovation
Alberta Children's Hospital Research Institute (ACHRI)

Functional impact of PIP_2 on the Serotonin Transporter (SERT)

Cristina Fenollar-Ferrer[1-3], Dino Lüthi[4,5], Tina Hofmaier[4], Gerhard J. Schütz[5], Thomas Stockner[4] and Harald Sitte[4].

[1]Stiles-Nicholson Brain Institute at Florida Atlantic University
[2]Laboratory of Molecular and Cellular Neurobiology, National Institute of Mental Health (NIH)
[3]Laboratory of Molecular Genetics, National Institute on Deafness and other Communication Disorders (NIH)
[4]Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna
[5]Institute of Applied Physics, Vienna University of Technology

The serotonin transporter (SERT) clears the serotonin from the synaptic cleft by transporting the neurotransmitter towards inside the presynaptic cell and thus terminating the serotonergic signal transduction between neurons. SERT is a transmembrane protein that belongs to the SCL6 family that also comprises the dopamine (DA) and norepinephrine (NE) transporters (DAT and NET) and is the target of SSRIs and psychostimulants like cocaine, amphetamine or ibogaine. Previous studies revealed that amphetamine-induced 5-HT reverse transport requires the SERT N terminus, which acts as a lever. Additionally, the action of substrate-type amphetamines depends on binding of the phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) to SERT. However, it currently remains unclear whether, like DAT, PIP_2 binding to SERT N terminus is a prerequisite for the action of amphetamines on SERT. PIP_2 binding to SERT plays an important role on SERT oligomerization; in fact, PIP_2 kinetically traps SERT in oligomers and its depletion or mutation of the PIP_2 binding site (SERT-R144 and SERT-K352) causes its dissociation. In this work we combined biochemical experimental data with computational techniques to gain insight, from a structural point of view, into the role of PIP_2 and the N terminus in amphetamine-induced 5-HT efflux and SERT oligomerization. The results indicate that while the N terminus alone does not interact with PIP_2, it does take part into PIP_2 binding by cooperating with the transmembrane (TM) domain to form a cavity with positive electrostatic potential where PIP_2 binds. Furthermore, neutralization of the positively charged residues in the N terminus to alanines reduces amphetamine-induced 5-HT efflux in a similar manner to the Ala substitutions of the PIP_2 binding residues R144, K352 located at the TM domain. Our structural model of SERT also indicates that the positively charged cleft, formed by the TM and N terminal domains, where PIP_2 binds is only formed when SERT is in an outward-facing and not in an inward-facing conformation. This suggests that PIP_2 binding is conformationally selective and therefore that SERT oligomerization might also be conformation dependent. Indeed, our results show that substrate-like compounds dissociate SERT oligomers, while inhibitors like cocaine that trap SERT in an outward-facing conformation do not affect SERT's oligomeric state.

5-HT_2 Receptor Activation Differentially Impacts Linker Histone H1.5 Kinetics and Induces the Expression of Factors Relevant for Global Chromatin Architecture

Thomas W. Flanagan[1], Gerald B. Billac[1], and Charles D. Nichols[1]

[1]Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA USA 70112

Eukaryotic cells have evolved complex, tightly coordinated genomic maintenance and immune defense strategies to respond to inflammation-inducing stimuli. Accordingly, remodeling of local and global chromatin structure is necessary to ensure the appropriate immune response is activated following an inflammatory event. Our laboratory has discovered that across several allergic asthma models, activation of the 5-HT_2 receptor with the highly selective agonist (*R*)-2,5-dimethoxy-4-iodoamphetamine [(*R*)-DOI] inhibits the expression of some, but not all, components of the acute inflammatory response, suggesting a dynamic regulation of nucleosome structure and chromatin accessibility. To evaluate this possibility, we first employed fluorescence activity after photobleaching (FRAP) to determine how a variety of 5-HT_{2A} structural ligands affect linker histone mobility, a proxy for global chromatin compaction, in human embryonic kidney (HEK) cells stably expressing the 5-HT_{2A} receptor. We see here that 5-HT_{2A} structural agonists such as (*R*)-2,5-dimethoxy-4-iodoamphetamine [(*R*)-DOI] significantly slow the kinetics of the linker histone H1c. Interestingly, this alteration to linker histone kinetics and overall chromatin compaction state is counter to the effects we observe when evaluating overall histone deacetylase HDAC activity, as 5-HT_2 compounds with potent anti-inflammatory activity significantly reduce HDAC activity. These results indicate a novel mechanism for the regulation of chromatin structure and epigenetic modification following 5-HT_{2A} receptor activation.

Acknowledgements and Disclosure
Support from this work was provided in part by the Eleusis Benefit Corporation and the LSU Foundation. Dr. C.D. Nichols had a former sponsored research contract with the Eleusis Benefit Corporation.

What happens when mice lack serotonin? Consequences in behavior and in the BDNF pathway in two models of hyposerotonergic mice

Rocío B Foltran (1), Karen Stefani (1), Sophie Scotto-Lomassese (2), Catherine Béchade (2), Luc Maroteaux (2), Silvina L Diaz (1)

Author Affiliations *
(1) Inst. de Biología Celular y Neurociencias. CONICET-UBA. Buenos Aires, Argentina;
(2) Institut du Fer à Moulin. Sorbonne University, INSERM U1270. Paris, France

Abstract body *
Modulation of serotonergic neurotransmission has revealed as an exciting tool to study the process of neurogenesis in the adult hippocampus (HC). Chronic inhibition of tryptophan hydroxylase by para-chlorophenylalanine (PCPA) in mice induces a decrease of around 70% serotonin (5-HT) levels, whereas most of serotonergic neurons do not differentiate in Pet1$^{-/-}$ mice, leading to an 80 % depletion of 5-HT. Interestingly, both mice models show enhanced survival of newborn neurons in the HC, and we thus wonder, if these supernumerary neurons modulate certain behaviors.Young adult male mice from both hyposerotonergic models, i.e. PCPA-treated and Pet1$^{-/-}$ mice, were studied. Compared to their respective control groups, both models showed a tendency to an increased compulsive behaviour in the Nestlet® shredding test, but no effect was seen in the Marble Burying test. To study the role of the new neurons in the HC, we also conducted the Object Pattern Separation (OPS), a test that allows finding subtle differences compared to classical tests. In this case, both mice models behaved differently in the test, showing an enhance (Pet1$^{-/-}$) or no change (PCPA) in the discrimination index. As the brain derived neurotrophin factor (BDNF) signaling pathway is linked to neuron survival, BDNF isoforms and their receptors were analyzed in the HC by Western blot and RT-qPCR. Proteins and RNA were extracted from hippocampi and levels of BDNF, TrkB, p75, and proBDNF were quantified. In both models, changes in the BDNF pathway were observed, compared to their respective controls. When analyzing the expression of the different transcripts of the BDNF gene, significant changes were also seen in serotonin depleted mice, with each mice model altering a different BDNF pathway. Our results show that both hyposerotonergic mice models are similar in their compulsive behaviour, but not in their ability for pattern separation. On the other hand, although the BDNF pathway is clearly involved in both models, its regulation seems different. This could be due to the fact that the establishment of the 5-HT depletion in PCPA treated mice takes place in young adult animals whereas it is constitutive, i.e. throughout all their lifetime, in the Pet1$^{-/-}$ mice.

Support
CONICET, Agencia Nacional de Promoción Científica y Tecnológica (Argentina), Boehringer Ingelheim Fonds (Germany), the French Ministry of Research (Agence Nationale pour la Recherche ANR-17-CE16-0008 and the Investissements d'Avenir programme ANR-11-IDEX-0004-02)

Intranasal (*R, S*)-ketamine delivery induces sustained antidepressant effects associated with changes in cortical balance of excitatory/inhibitory synaptic activity

Thi Mai Loan Nguyen[1], Céline Defaix[1], Indira Mendez-David[1], Laurent Tritschler[1], Isabelle Etting[2], Jean-Claude Alvarez[2], Walid Choucha[3,4], Romain Colle[3,4], Emmanuelle Corruble[3,4], Denis J. David[1], Alain M. Gardier[1§]

[1] Université Paris-Saclay, Faculté de Pharmacie, UMR 1018, CESP-Inserm, MOODS Team, Chatenay-Malabry, 92290, France.

[2] Lab. Pharmacologie-Toxicologie, Centre Hospitalier Universitaire Raymond Poincaré, AP-HP, Garches, France.

[3] Université Paris-Saclay, Faculté de Médecine, UMR 1018, CESP MOODS team, Bicêtre Hospital, Université Paris-Saclay, Le Kremin-Bicêtre, 94270, France

[4] Service Hospitalo-Universitaire de Psychiatrie de Bicêtre, Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris-Saclay, Hôpital de Bicêtre, Le Kremlin Bicêtre, F-94275, France.

§ Corresponding author:
Pr. Alain M. GARDIER
Laboratoire de Neuropharmacologie
Université Paris-Saclay, CESP-Inserm, Faculté de Pharmacie, 5 Rue J-B Clement, Tour D1, 2e étage, F-92296 Chatenay Malabry cedex. France
Tel: (33) 1 46 83 54 16
E-mail: alain.gardier@universite-paris-saclay.fr

ABSTRACT

In 2019, an intranasal (IN) spray of esketamine SPRAVATO® was approved as a fast-acting antidepressant by drug Agencies US FDA and European EMA. At sub-anesthetic doses, (±)-ketamine, a non-competitive glutamate N-methyl-d-aspartate (NMDA) receptor antagonist, increases the overall excitability of the medial prefrontal cortex (mPFC), an effect being essential for its rapid antidepressant activity. We wondered if this effect of ketamine could come from changes in the balance between neuronal excitation and inhibition (E/I balance) in the mPFC. Here, we performed a preclinical approach to study neurochemical and behavioral responses to a single IN ketamine dose in BALB/cJ mice, a strain more sensitive to stress. By using *in vivo* microdialysis, we measured cortical 5-HT release and the E/I balance as the ratio between glutamate to GABA extracellular levels 24h post-ketamine. We found, for the first time, that E/I balance was shifted in favor of excitation rather than inhibition in the mPFC but more robustly with IN KET than with a single intraperitoneal (IP) dose. Increases in plasma and brain ketamine, norketamine and HNKs levels suggest different metabolic profiles of IP and IN ketamine 30 min post-dose. It may be linked to the greater magnitude in E/I ratio following IN delivery relative to IP at t24h. This study suggests that both IP and IN are effective brain delivery methods inducing similar sustained antidepressant efficacy of KET, but the way they induced–neurotransmitter changes is slightly different.

Comparison of social behaviour in rats with life-long and acute genetic depletion of brain serotonin

Joanna Golebiowska[1], Malgorzata Holuj[1], Piotr Popik[1], Natalia Alenina[2], Michael Bader[2], Agnieszka Nikiforuk[1]

1 Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland
2 Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany

Serotonin (5-HT) is a monoamine, which appears early during neurodevelopment. Brain serotonin level manipulations are associated with central nervous system processes, including social behaviour. Tryptophan hydroxylase 2 (TPH2) is a rate-limiting enzyme of serotonin synthesis. Therefore, downregulation of this enzyme results in central serotonin depletion. The goal of the study was to examine two groups of genetically modified rats, the TPH2-knockout rats (TPH2-KO) and TPH2-inducible shRNA knockdown rats (TPH2-KD) in the social interaction test. The TPH2-KO rats carry an 11 nucleotides deletion in exon 7 of the rat Tph2 gene, resulting in a frame shift mutation and a premature stop in translation. The resulting homozygous animals are completely lacking serotonin in the brain from birth throughout the whole life. TPH2-KD rats carry a transgene that allows inducible expression of small hairpin RNAs (shRNAs) to downregulate TPH2. Doxycycline (DOX) administration in these rats results in a drop in TPH2 expression leading to a significant decrease in brain serotonin levels. In contrast to TPH2-KO rats, TPH2-KD animals have normal serotonin levels in the brain until the onset of DOX administration.

The comparison of these two rat strains with their wild-type (WT) controls allows to study the differences between the effects of life-long (TPH2-KO) and acute (TPH2-KD) serotonin depletion on social behaviour. Two unfamiliar male rats of matched genotype and body weight (± 5 g) were placed in the open field arena, and their social behaviour was recorded for 10 min a using camera connected to the Noldus MPEG recorder. The social interaction time was measured for each rat separately with the use of Noldus MPEG Observer program. The following active social behaviours were scored: sniffing (the rat sniffs the body of the conspecific), anogenital sniffing (the rat sniffs the anogenital region of the conspecific), social grooming (the rat licks and chews the fur of the conspecific), following behaviour (the rat moves toward and follows the other rat), climbing (the rat climbs over the back of the conspecific), as well as sexual activity and fighting with the conspecific.

We report that TPH2-KO and TPH2-KD rats demonstrated disturbed patterns of social behaviour. TPH2-KO rats spent significantly more time on sniffing the conspecific and less time on climbing and following as compared to the WT controls. In contrast, TPH2-KD rats spent less time on sniffing and climbing only. Of note, TPH2-KO, but not TPH2-KD rats demonstrated copulatory-like behaviour directed toward their male partners.

The present study confirms the role of central serotonin in the regulation of social behaviour and points to sexual hyperactivity due to life-long serotonin depletion.
This study was supported by the grant ERA-NET Neuron II JTC 2015 Respond and by the Statutory Activity of the Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

Looking from the other side – What converts a full substrate to a partial substrate, releaser or even a blocker at the human serotonin transporter?

Ralph Gradisch[1], Katharina Schlögl[2], Erika Lazzarin[1], Marco Niello[1], Julian Maier[1], Leticia Alves da Silva[1], Felix P Mayer[3], Randy D Blakely[3], Harald H Sitte[1], Marko Mihovilovic[2], Thomas Stockner[1]

[1] Medical University of Vienna, Institute of Physiology and Pharmacology, Weahringer Straße 13A, 1090 Vienna, Austria
[2]Technical University of Vienna, Institute of Applied Synthetic Chemistry, Getreidemarkt 9/163, 1060 Vienna, Austria
[3]Florida Atlantic University, Charles E. Schmidt College of Medicine, Department of Biomedical Science, Jupiter, FL, 33458, USA

Associated with a variety of neuropsychiatric diseases, members within the solute carrier (SLC) superfamily, more precise within the SLC6 family, represent high valuable pharmacological targets. In particular, the serotonin transporter (SERT, SLC6A4) depicting a presynaptic transmembrane protein that retrieves previously released serotonin (5HT) back into the synapse. Thus, ensuring serotoninergic neurotransmitter homeostasis as well as shaping post synaptic signalling frequency and amplitude. Clinically approved as well as illicit drugs of abuse targeting SERT can be classified as releasers or inhibitors. Releasers act like substrates by initiating the transport cycle but additionally change the transporter's direction from a forward-mode to an exchange-mode, thereby leading to substrate efflux. Inhibitors most likely lock the transporter in a particular conformation, thus preventing 5HT reuptake. Both alterations result in a rise of extracellular neurotransmitter concentration. Unbiased molecular dynamics simulations, chemical synthesis and a variety of in-vitro as well as ex-vivo approaches were conducted to probe the impact of the length of a homologous series of 5HT molecules on the transport cycle. We found that 5HT as the cognate substrate is optimal to initiate the transport cycle by flawlessly interacting with a highly conserved gating residue. Truncation as well as elongations of the ethylamine moiety, while maintaining the chemical properties of the indole-moiety, renders the substrate a partial substrate or even a blocker by impairing this interaction involved in the detain-or-pull mechanism of SERT occlusion. Finally, we want to propose a pharmacological concept of physico-chemical requirements that thermodynamically, mechanistically and kinetically decipher a compound for being a substrate, releaser or blocker at SERT.

Funding source: Austrian Science Fund (FWF) stand-alone grant P32017

5-HT-glutamate co-releasing neurons are activated by acute stress and may be involved in stress coping

L. Sophie Gullino[1], Cara Fuller[1], Stéphanie Daumas[2], Salah El Mestikawy[2,3], David Bannerman[4], Trevor Sharp[1]

[1]Department of Pharmacology, University of Oxford, UK [2]Sorbonne Université, Paris, France, [3]Department of Psychiatry, McGill University, Canada, [4]Department of Experimental Psychology, University of Oxford, UK.

Background
5-hydroxytryptamine (5-HT) plays a critical role in emotional modulation, stress sensitivity and coping behaviour, and is a target for antidepressant and anxiolytic drug therapies, including selective serotonin reuptake inhibitors (SSRIs). Recent data show that many 5-HT neurons express the vesicular glutamate transporter 3 (VGLUT3) and co-release 5-HT and glutamate. The function of this co-released glutamate is still poorly understood.

Aims & Objective
Given the strong links between 5-HT and stress, we hypothesised that glutamate co-releasing 5-HT neurons would be sensitive to stress and potentially be involved in stress coping. Here we used c-Fos immunocytochemistry to examine the effect of acute stress on 5-HT-glutamate co-releasing neurons. We then investigated whether conditional deletion of VGLUT3 in 5-HT neurons altered the behavioural response to the stressor.

Methods
Adult C57BL/*6J* mice (6-7/group) were either home-cage controls, or injected with saline or fluoxetine (10 mg/kg i.p.) 30 min prior to a 6-min swim stress (water-filled 12 cm i.d. cylinder) during which immobility, swimming and climbing (an active coping strategy) were scored. After transcardial perfusion 90 min later, brain tissue was collected and processed for immunocytochemistry. Colocalization of c-Fos, TPH2 and VGLUT3 identified activated 5-HT-glutamate neurons. Mice with VGLUT3 deficient 5-HT neurons (SERT-Cre::VGLUT3$^{LoxP/LoxP}$, C57BL/*6J;* 15-20/ group) and their wildtype littermates (SERT$^{+/+}$::VGLUT3$^{LoxP/LoxP}$) were also exposed to acute swim stress. Data were analysed by ANOVA and post-hoc Tukey's tests, or Mann Whitney U tests for non-parametric data. $P<0.05$ was considered statistically significant.

Results
Compared to non-stress controls, swim stress increased c-Fos expression in the ventral region of the dorsal raphe nucleus (DRN) but not in the lateral wings or median raphe nucleus. This effect was notable in neurons triple-labelled for c-Fos, TPH2 and VGLUT3 ($F_{(2,17)}= 4.896$, $p=0.021$, post-hoc $p=0.036$). Fluoxetine reduced the latter effect (Tukey's test, $p=0.042$), and increased climbing time during swim stress (Mann-Whitney U, $p=0.016$). Interestingly, in the stress paradigm mice with VGLUT3 deficient 5-HT neurons spent more time climbing versus littermate controls (Mann-Whitney U, $p=0.0428$). Fluoxetine did not add further to this effect.

Discussion & Conclusion
The current immunocytochemical data provide new evidence that 5-HT-glutamate co-releasing neurons in the DRN are activated by stress in an antidepressant-sensitive manner. Moreover, the finding that mice with 5-HT neurons lacking VGLUT3 showed increased active coping behaviour when exposed to stress supports the hypothesis that co-released glutamate is involved in stress sensitivity.

Identification of 5-HT$_{2A}$ Signaling Pathways Responsible for Psychedelic Potential

Halberstadt, Adam[1,2]; Cao, Andrew[3]; Calkins, Maggie[3]; Heim, Andrew[4]; Lanham, Janelle[3]; Bock, Hailey[3]; Bonniwell, Emma[3], Hennessey, Joseph[3]; McNally, Lisa[3]; Anderson, Emilie[3]; Morris, Hamilton[4]; de Klein, Robbin[1]; Klein, Adam[1]; Cuccurazzu, Bruna[1]; Gamrat, James[4]; Fananna, Tilka[4]; Zauhar, Randy[5]; Wallach, Jason[4]; McCorvy, John[3]

[1]Department of Psychiatry, University of California San Diego, La Jolla, California 92093, United States; [2]Research Service, VA San Diego Healthcare System, San Diego, California 92161, United States; [3]Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States; [4]Department of Chemistry, Saint Joseph's University. Philadelphia, Pennsylvania 19104, United States; [5]Department of Pharmaceutical Sciences. Saint Joseph's University. Philadelphia, Pennsylvania 19104, United States

Abstract

Serotonergic psychedelic drugs have shown therapeutic potential in depression, inflammatory disease, and addiction. Although serotonin 5-HT$_{2A}$ receptor activation appears to mediate psychedelic effects, 5-HT$_{2A}$ couples to multiple signaling pathways. To determine the 5-HT$_{2A}$ signaling profile for classical psychedelics, we used a bioluminescence resonance energy transfer (BRET) approach, which provides a proximity measure of intracellular effector engagement. Using BRET, we found that 5-HT$_{2A}$ primarily couples to Gq/11 and β-arrestin2 and that prototypical psychedelics do not show a preference for either effector, making it unclear which 5-HT$_{2A}$ signaling pathway is responsible for psychedelic potential. As most classical psychedelics are not selective for the 5-HT$_{2A}$ receptor, we developed a series of 5-HT$_{2A}$-selective ligands with various efficacies for Gq-mediated signaling, including several β-arrestin-biased ligands. Studies with these ligands revealed that 5-HT$_{2A}$ Gq signaling but not β-arrestin2 recruitment efficacy predicts psychedelic potential, as measured by the magnitude of the head-twitch response (HTR) in male C57BL/6J mice. We further show that disruption of the Gq-PLC pathway attenuates the HTR *in vivo* and that weak partial 5-HT$_{2A}$ agonists below a specific threshold of Gq activation are devoid of psychedelic-like behavioral effects. These results advance our understanding of the neurobiology of serotoninergic psychedelics and establish 5-HT$_{2A}$ Gq activation efficacy as serving a key role in psychedelic-like behavioral effects, paving the way for rational development of non-psychedelic 5-HT$_{2A}$ agonists. Finally, we found that 5-HT$_{2A}$ β-arrestin-biased compounds show therapeutic utility with rapid induction of tolerance *in vivo* and 5-HT$_{2A}$ downregulation *in vitro*, and have an antipsychotic-like behavioral profile. Overall, these studies show that 5-HT$_{2A}$ signaling can be fine-tuned to exhibit unique pharmacological activities with therapeutic properties that are distinct from those of classical 5-HT$_{2A}$ psychedelics.

Support: Medical College of Wisconsin Research Affairs Counsel Pilot grant, NIGMS (R35GM133421), NIDA (R01 DA041336), and the Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center.

Organic cation transporter 3 contributes to serotonin clearance in basolateral amygdala and sex-dependently modulates fear-related behaviors

†Lauren E. Honan[1], †Nikki J. Clauss[1], W. Anthony Owens[1], Rebecca E. Horton[1], Glenn M. Toney[1], & Lynette C. Daws[1,2]

Departments of [1]Cellular & Integrative Physiology and [2]Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX

Converging evidence implicates dysregulation of serotonergic neurotransmission in fear-related affective disorders. Leading pharmacotherapies for these disorders target the high-affinity, low-capacity serotonin (5-HT) transporter, but demonstrate variable clinical efficacy, suggesting yet to be elucidated mechanisms contribute to affective disorders. Recent evidence suggests the low-affinity, high-capacity organic cation transporter 3 (OCT3) plays a significant role in maintaining serotonergic homeostasis. Considering its rich expression in emotion-regulating circuitry and critical role in 5-HT neurotransmission, we hypothesized that OCT3 strongly influences serotonergic tone in emotion-regulating circuitry and attendant affective behaviors. To probe this hypothesis, we used multiple gene depletion strategies to diminish OCT3 function *in vivo*. We used adult offspring of OCT3 floxed and ePet-cre mice in which OCT3 is depleted from 5-HT neurons during embryogenesis (ePet-Cre x $OCT3^{fl/fl}$ mice) or adult Nestin-cre/ERT2 mice in which OCT3 is depleted from neurons and glia (Nes-Cre/ERT2 x $OCT3^{fl/fl}$ mice) following systemic tamoxifen administration. To evaluate more specifically the contributions of OCT3 in basolateral amygdala (BLA, a region essential to processing and consolidation of fear memory), we bilaterally injected AAV5-EF1a-mCherry-IRES-WGA-Cre, or AAV5-EF1a-mCherry as a control, into BLA of adult OCT3 floxed mice. We characterized 5-HT clearance in BLA using *in vivo* high-speed chronoamperometry. We found 5-HT clearance to be prolonged in both sexes of ePet-Cre x $OCT3^{fl/fl}$ mice, consistent with OCT3 playing a key role in restraining extracellular 5-HT and limiting synaptic 5-HT neurotransmission. Preliminary results in Nes-Cre/ERT2 x $OCT3^{fl//fl}$ mice show similar trends. However, viral depletion of postsynaptic, and possibly glial, OCT3 in BLA was not sufficient to prolong 5-HT clearance, suggesting presynaptic OCT3 in BLA is essential to this effect. Constitutive depletion of OCT3 from serotonin neurons (ePet-Cre x $OCT3^{fl/fl}$ mice) had no effect on fear learning and recent cued and contextual fear retrieval tested 48 and 72 hours after training, respectively. However, preliminary data in mice with tamoxifen-induced depletion of OCT3 from neurons and glia (Nes-Cre/ERT2 x $OCT3^{fl/fl}$ mice) and virally induced depletion of OCT3 from BLA show marked, sex-dependent differences in fear learning and memory. Tamoxifen-induced depletion of OCT3 from neurons and glia resulted in slower fear learning but enhanced contextual fear memory in females, and no effect on fear learning or memory in males. Viral depletion of OCT3 from BLA resulted in more rapid fear learning and enhanced cued and contextual fear memory in females. In males, acquisition of fear was unaffected and cued and contextual fear memory was reduced. Lack of effects in mice with constitutive OCT3 depletion from 5-HT neurons suggest compensation, or that depletion of OCT3 from other neuronal subtypes and glia are necessary to elicit these behavioral effects. Altogether, these data suggest that OCT3 contributes to 5-HT clearance in BLA, and may play a critical, sex-dependent role in fear memory, although the underlying mechanisms remain unclear. Our finding that presynaptic OCT3 in BLA may play an important functional role raises the possibility that, if localized within the synaptic cleft, OCT3 could limit both direct 5-HT synaptic transmission and volume transmission to nearby receptor elements while also participating in 5-HT recycling, possibly preserving 5-HT release in BLA under high-release conditions, including emotionally arousing states such as fear.

Supported by R01 MH093320 to LCD and GMT.

Targeting Intestinal Mucosal Serotonin Alone Is Critical For Modulation Of Mood

Lin Y. Hung[1] Andrew Del Colle[1], Nuno Alves[2], Yan Tong[1], Zixing Huang[1], Sarah Najjar[1], Narek Israelyan[3], Melissa Medina[5], Ray Rahim[1], Roey Ringel[6], Michael Gershon[7], Mark Ansorge[2], Kara Gross Margolis[1,8]

[1]Department of Molecular Pathobiology, College of Dentistry, New York University, New York, New York, USA; [2]New York State Psychiatric Institute, Columbia University, [3]Vagelos College of Physicians and Surgeons, [4]Fu Foundational School of Engineering & Applied Science, Columbia University, [5]Institute of Human Nutrition, Columbia University Irving Medical Center, [6]Columbia College, Columbia University, [7]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, [8]Department of Pediatrics, NYU Grossman School of Medicine and Langone Medical Center, New York, New York, USA

Anxiety and depression are common mood disorders affecting up to 8% of people in the U.S, with percentages tripling during the COVID pandemic. Selective serotonin reuptake inhibitors (SSRIs) are the first line treatment for mood disorders. SSRIs inhibit the serotonin transporter (SERT) and thus impede serotonin reuptake into cells, which is the primary mechanism of serotonin inactivation. SSRIs thus enhance and prolong serotonergic transmission, which is critical for their anxiolytic and anti-depressive effects. SSRI efficacy, however, is severely limited by their unintended effects, including dysmotility and anxiety and anhedonia. SSRIs are systemically absorbed and thus also induce SERT blockade in the gut mucosa, the enteric (ENS) and central nervous systems. We tested the hypothesis that mucosal 5-HT signals to the CNS to beneficially affect mood. If so, then targeted ablation of gut mucosal SERT would enhance mood with minimal impact on GI and ENS function. To test this idea, we created two mouse models in which gut mucosal SERT was selectively ablated. In *Villin^{Cre}::SERT$^{fl/fl}$* mice, gut mucosal SERT is eliminated throughout development and life. In *Villin^{CreERT2}::SERT$^{fl/fl}$* mice, gut mucosal SERT is eliminated only after tamoxifen administration, which was given during adulthood (3 months old). *Villin^{Cre}::SERT$^{fl/fl}$* and *Villin$^{Cre-ERT2}$::SERT$^{fl/fl}$* mice and their wildtype (WT) littermates, were examined for anxiety- and depression-related phenotypes (elevated plus maze, open field, novelty suppressed feeding and tail suspension), *in vivo* GI motility (total GI, colonic, and small intestinal transit and gastric emptying), *ex vivo* colonic motility (measurement of colonic migrating motor complexes (CMMCs) to evaluate ENS function) as well as ENS and CNS morphology (immunocytochemistry). Anxiety and depressive-like behaviors were decreased in *Villin^{Cre}::SERT$^{fl/fl}$* mice without accompanying abnormalities in *in-vivo* GI motility CMMC frequency, total enteric neurons, distribution of neuronal subtypes within the ENS or CNS morphology in brain regions with high levels of SERT (e.g hippocampus). Like Villin^{Cre}::SERT$^{fl/f}$ mice, adult tamoxifen-treated *Villin^{CreERT2}::SERT$^{fl/fl}$* mice displayed anti-anxiety phenotypes as well as no significant changes in *in vivo* or *ex vivo* GI motility or altered ENS and CNS morphology (compared to WT littermates treated with tamoxifen). Together, these findings demonstrate that targeted SERT ablation in the gut mucosa may be a novel and effective way to treat anxiety and depression wthout the adverse effects associated with systemic SSRI use.

Funding: Department of Defense PR160365 and NASPGHAN Foundation/Takeda Pharmaceutical Products Inc. Research Innovation Award

Investigation of the biased agonist signalling properties of psychedelic drugs at the human 5-HT_{2A} receptor

Aurelija Ippolito, [1]Shaun Hurley, [1]Gary Gilmour, [1]Frederick Westhorpe, Sri Vasudevan, Grant Churchill and Trevor Sharp

University Department of Pharmacology, Mansfield Road, Oxford, U.K. and [1]COMPASS Pathways Plc, London, UK

Introduction: Serotonergic psychedelics such as psilocybin are being investigated as treatments for a diverse range of psychiatric disorders, and particularly treatment-resistant depression. Currently, a key outstanding question is whether the hallucinogenic effect is necessary for the therapeutic effects of these drugs. A potential route for separating these effects is biased agonism, whereby an agonist binds to a receptor but preferentially elicits signalling via one downstream pathway versus another. Since the 5-HT_{2A} receptor is critical to psychedelic drug actions, a biased agonist at this receptor offers the potential of an antidepressant that is not a hallucinogen. The 5-HT_{2A} receptor evokes both canonical (G_q-protein coupled) and non-canonical (β-arrestin2 - mediated) signalling but the biased agonist actions of psychedelic drugs are little investigated.

Aims: Here the biased signalling properties of a variety of 5-HT_{2A} agonists, including psilocin and other hallucinogenic agents as well as the reported non-hallucinogen lisuride, were characterised using a human neuroblastoma cell line expressing the human 5-HT_{2A} receptor.

Methods: SH-SY5Y cells transfected with the human 5-HT_{2A} receptor were used to investigate G_q- and β-arrestin2-signalling. Eight 5-HT_{2A} agonists were tested; 5-HT, 5-MeO-DMT, DOI, mescaline, psilocin, LSD, 25B-NBOMe and lisuride. Initially calcium increases were probed with the intracellular calcium indicator, Fluo-4. Then, phosphoinositide signalling was measured via competitive ELISA measurement of IP_1 (Cisbio IP_1 HTRF assay kit). Finally, β-arrestin2 recruitment and signalling was examined by transfection of the Montana Molecular Borealis Arrestin sensor. The role of the 5-HT_{2A} receptor in all responses was isolated using the selective 5-HT_{2A} antagonist, MDL-100,907. Bias plots were constructed for each agonist, comparing two signalling outputs using ΔΔlog(Emax/EC50) values derived from dose-response curves from each signalling assay, with 5-HT as the reference ligand. 95% confidence intervals of bias measurements were used to ascertain significance of bias.

Results: All agonists exhibited dose-related responses in all assays, with most drugs displaying partial agonist properties compared to 5-HT. Also, the rank order of agonist potency and efficacy varied between assays. In the calcium and β-arrestin2 assays, MDL-100,907 abolished responses evoked by all agonists. In the IP_1 assay, responses to most agonists were blocked by MDL-100,907 except lisuride, LSD and 25B-NBOMe, which retained some activity. Psilocin showed no preference for IP_1 versus calcium signalling whereas all other agonists had an IP_1 bias with the largest bias shown by lisuride and LSD. Interestingly, the data suggested lisuride displayed the greatest bias towards IP_1 versus β-arrestin2 signalling.

Conclusions: Overall the current study demonstrated that all agonists tested elicited both canonical (G_q - calcium, IP_1) and non-canonical (β-arrestin2) 5-HT_{2A} receptor-mediated signalling responses in a human neuroblastoma cell line. Most agonists showed bias towards the IP_1 versus calcium signalling, perhaps due to variation in agonist association rates and residency times. Interestingly, lisuride had the strongest bias of any agonist tested towards G_q versus β-arrestin2 signalling. Future studies are needed to understand whether this signalling bias of lisuride links to the drug's reported non-hallucinogenic properties.

Experimental and Theoretical Insights into the Self-organization of the Brain Serotonergic Matrix

Skirmantas Janušonis[1*], Kasie C. Mays[1], Melissa Hingorani[1] Ralf Metzler[2], Thomas Vojta[3]

[1]Department of Psychological and Brain Sciences, University of California, Santa Barbara, Santa Barbara, CA 93106, USA
[2]Institute of Physics and Astronomy, University of Potsdam, D-14476 Potsdam-Golm, Germany
[3]Department of Physics, Missouri University of Science and Technology, Rolla, MO 65409, USA

In vertebrate brains, neural networks operate inside a dense, three-dimensional matrix of serotonergic axons (fibers). At the macroscopic level, this matrix allows “deterministic” descriptions: it varies in density across brain regions, but these regional densities are strongly consistent across individuals. In contrast, the trajectories of individual serotonergic fibers are strongly “stochastic” at the microscopic level: in many spatial locations, fibers show no directional preference and can be highly tortuous. They may remain dynamic in the adult brain because they can be routinely interrupted by mobile elements of neural tissue and are known to have nearly unique regenerative capabilities. Bridging these two descriptions (microscopic/stochastic and macroscopic/deterministic) is essential for the understanding of the self-organization and plasticity of the serotonergic matrix.
Our interdisciplinary program uses a number of experimental and theoretical tools to investigate the dynamics of single serotonergic fibers, which include transgenic mouse lines, brainstem neuronal cell cultures, holotomography, super-resolution microscopy, stochastic modeling, and supercomputing simulations. We have successfully tagged individual serotonergic fibers with a random combination of three fluorophores (using Brainbow AAVs) and are investigating their paths, branching, and interaction (including highly dense regions). In vitro studies and super-resolution microscopy in embryonic brains have shown that advancing serotonergic fibers can be flattened (ribbon-like), produce transient corkscrew-like profiles, and travel along non-serotonergic neurites, thus reflecting the stochastic microarchitecture of the given brain region (normal or diseased). We also have shown that serotonergic fibers can be modeled as paths of fractional Brownian motion, an anomalous diffusion process, and that this process can predict some regional fiber densities, previously reported only by descriptive neuroanatomical studies. Some of this work has been recently published, including our observation that serotonergic fibers suggest novel artificial neural network architectures (*Front. Comput. Neurosci.* 14:56; *Front. Neurosci.* 16: 994735; *Front. Neurosci.* 16: 949934). This presentation focuses on the current developments in this expanding research program.

Supported by: NSF-BMBF CRCNS grant # 2112862/STAXS and a California NanoSystems Institute Challenge-Program Development grant.

Serotonin transporter regulation by Gα proteins: evidence for coupling of serotonin transport and the G protein cycle

A.K.C., Jones[1], S-A., Spreng[1], E., Braun[1], C.J., Mc Veigh[1], A., Inoue[2], G., Ahnert-Hilger[3] and J., Haase[1]

[1] UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Ireland
[2] Graduate School of Pharmaceutical Sciences, Tohoku University, Japan
[3] Institute of Integrative Neuroanatomy, Charité University Medicine Berlin and Max-Planck-Institute for Biophysical Chemistry Göttingen, Germany

Abstract

The serotonin transporter (SERT) mediates Na^+-dependent, high-affinity uptake of serotonin (5-hydroxytryptamine, 5HT) into serotonergic neurons and plays a key role in fine-tuning serotonin-dependent signalling in the brain. Among the proteins we have identified to interact with the transporter and potentially regulate its activity are subunits of heterotrimeric G proteins, including Gαq, Gαi1, and Gαi2. In an initial study, we have recently shown that in the absence of Gαq, SERT-mediated uptake of 5HT was enhanced in midbrain and frontal cortex synaptosomes, specifically in female Gαq knockout mice. This sex-specific modulation was paralleled by changes in tissue 5HT levels and Gαi protein expression (Haase et al, 2021). Here, we present evidence for a novel and previously unknown mechanism by which SERT activity is regulated by Gα proteins. Corroborating our findings from Gαq knockout mice, we demonstrate that in CRISPR Gα knockout HEK cells the transporter turnover rate is substantially enhanced, an effect that can be reversed by co-expression of Gαq. Our findings suggest that SERT interacts with WT Gαq, but also with the "empty pocket" mutant D277N and the constitutively active mutant Q209L. While WT Gαq overexpression has no significant effect on SERT activity, co-expression of either mutant causes a significant inhibition of SERT-mediated transport, suggesting that the "dead-end" Gαq mutants trap the transporter in a distinct conformational state, and thus, prevent the completion of the transport cycle. Gαq inhibitors, YM-254890 and BIM-46187, mimic the effect of Gαq knockout and the nucleotide-free mutant D277N, respectively. Taken together, we are proposing a novel model for SERT regulation by Gα proteins, at the centre of which we hypothesise that the serotonin transport cycle is coupled to the G protein cycle, specifically by reducing the overall turnover rate of the transporter. G protein-coupled regulation of SERT activity will likely profoundly impact our understanding of diseases associated with serotonin signalling, such as anxiety and depression, as well as their therapeutic interventions.

References

- Haase, J., A.K.C. Jones, C.J. Mc Veigh, E. Brown, G. Clarke, and G. Ahnert-Hilger, Sex and brain region-specific regulation of serotonin transporter activity in synaptosomes in guanine nucleotide-binding protein G(q) alpha knockout mice. J Neurochem, 2021. 159(1): p. 156-171.

Funding Sources

- UCD Advance PhD Programme (R19433) to JH
- Health Research Board Ireland (HRA/2009/303) to JH
- Deutsche Forschungsgemeinschaft (GAH 67-7/1,2) to GAH

Characterization of a novel MDMA derivative 1,3-Benzodioxolylbutanamine (BDB) and its structural analogs.

Nina Kastner[1], Julian Maier[1], Marion Holy[1], Fatemeh Kooti[1], Bruce E Blough[2], Joseph Wilson[2], Kevin Murnane[3], Harald H. Sitte[1,4]

[1]Medical University of Vienna, Center for Physiology and Pharmacology, Institute of Pharmacology, Währingerstraße 13A, 1090, Vienna, Austria
[2]Research Triangle Institute, Center for Drug Discovery, Research Triangle Park, NC, USA
[3]Louisiana State University Health Sciences Center, Shreveport, Dept. of Pharmacology Toxicology & Neuroscience and Louisiana Addiction Research Center, Shreveport, LA, USA
[4]AddRess Centre for Addiction Research and Science, Medical University of Vienna, Währingerstraße 13A, 1090, Vienna, Austria

3,4-Methylenedioxymethamphetamine (MDMA) and its structural analog 3,4-methylenedioxyamphetamine (MDA) are commonly abused for recreational purposes due to their psychostimulatory effects. These synthetic drugs received increased attention because of their popularity as party drugs, where they are known under their synonyms “Ecstasy”, “Molly” and “Sally”. Pharmacologically, they are characterized by acting as substrates and releasers at the monoamine transporters for dopamine (DAT), norepinephrine (NET) and serotonin transporter (SERT). With new illicit derivatives of these substances appearing on the street markets, it is vital to elucidate their interactions with the monoamine transporters and potential abuse liability. In this study, we characterized a novel MDMA derivative 1,3-Benzodioxolylbutanamine (BDB) and its structural analog N-Methyl-1,3-Benzodioxolylbutanamine (MBDB) and compared them to the well-established MDA and MDMA. The compounds differ in the addition of a methyl substituent on the terminal amine group and/or increased carbon chain length the α carbon. To evaluate interactions with monoamine transporters, we performed *in vitro* radiotracer uptake inhibition experiments in human embryonic kidney 293 (HEK293) cells stably expressing the human isoform of the respective monoamine transporter. BDB showed potent inhibition in the micro-molar range at SERT, DAT and NET. BDB inhibited NET less strongly by a factor of two. Further, BDB, MBDB and MDA showed a higher selectivity for DAT compared to SERT, while MDMA was more selective for SERT. In conclusion, we showed that that the interaction profile of the newly explored derivative BDB compares very well to MBDB and MDA at SERT and DAT, but slightly differs at NET. Additionally, due to the higher selectivity for DAT than SERT of BDB, MBDB and MDA, they may be associated with higher abuse liability than MDMA. To further characterize the substances, we will continue uptake inhibition assays at low-affinity monoamine transporters such as the human organic cation transporters (OCT) 1–3 and the human plasma membrane monoamine transporter (PMAT) to uncover a possible drug-drug interaction potential. Finally, we plan to investigate hypothesized releasing properties at monoamine transporter. In addition, we are also interested to look into the potential interactions with serotonin (5-HT) receptors, which are known to have a contributing factor in the stimulant effects of MDA and MDMA.

Funding Sources: Austrian Science Funds (FWF) grant number: P 33955-8, FWF-funded Doctoral Program "Molecular Drug Targets" grant number: W 1232

Evaluation of Novel Derivatives of Known Psychedelic Substances at the Serotonin 5HT2A Receptor

David Eunhyun Kim[1], Nicola Janz[1], Asher Brandt[1,2], Hye-Ji Jay Kim[1], Ayat Zagzoog[1], Robert Laprairie[1]*

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Canada; [2]Department of Chemistry, University of Saint Joseph, USA

Introduction: Despite the growing interest in the psychotherapeutic potential and recreational use of psychedelic compounds, the current understanding of their pharmacology is limited. Novel psychoactive substances (NPS) that are chemical derivatives of psychedelic substances such as lysergic acid diethylamide (LSD) and psilocin may act as agonists of the type 2a serotonin receptor (5HT2A). These NPSs have been introduced to global grey markets. The purpose of this study was to assess the pharmacology of novel NPSs using *in silico* and *in vitro* techniques.

Material and Methods:
Eighteen NPSs were for assessed *in silico* study using Spartan™ and Schrodinger (Maestro™) software; and *in vitro* using [^{3}H]ketanserin radioligand binding; 5HT2A-G$\alpha_{q/11}$-depdendent Ca^{2+} release, and 5HT2A-dependent β-arrestin2 recruitment in CHO-K1 cells expressing human 5HT2a.

Results and Discussion:
In silico data indicated all NPSs bound to the orthosteric site of 5HT2A similar to LSD or psilocin. Most NPSs were biased towards increase in 5HT2A-G$\alpha_{q/11}$-depdendent Ca^{2+} release versus β-arrestin2 recruitment, while 6-allyl-6-nor-LSD (AL-LAD) showed no bias but high efficacy in both assays.

Conclusion: This assessment of NPSs furthers our understanding on the importance of moiety variations of psychedelics on 5HT2A pharmacology, which may aid our understanding of their downstream physiological effects, safety profiles of other similar psychedelics and their psychotherapeutic potentials.

Support: Funding for this project was provided by a research contract from Health Canada to RBL. NJ's stipend was provided by the Branch Out Neurological Foundation. HJJK and AZ are supported by funding from the College of Pharmacy and Nutrition, University of Saskatchewan.

The pro-longevity gene flavin-containing monooxygenase 2 modulates serotonin metabolism to regulate exploratory behavior in *C. elegans.*

Elizabeth S Kitto[1], Scott F Leiser[2]

1.Molecular & Integrative Physiology Department, University of Michigan, Ann Arbor, MI
2. Department of Internal Medicine, University of Michigan, Ann Arbor, MI

Funding Source: NSF GRFP

The field of biogerontology has identified interventions that can extend lifespan across model organisms. These interventions often involve exposure to mild environmental stressors that activate stress-response pathways to promote longevity. However, exposure to environmental stressors can also change an animal's behavior and mental state. To determine whether longevity interventions that mimic exposure to stressful conditions can also affect mental state, we examined the behavior of *C. elegans* mutants with altered expression of the longevity-promoting gene, *fmo-2.* We found that *fmo-2* knockout (KO) and *fmo-2* overexpressing (OE) animals exhibit altered sensory perception and decision-making relative to wild-type (WT) controls. In particular, both the *fmo-2* KO and OE explored novel environments less than WT worms. Similar changes in exploratory behavior have been previously reported in multiple strains with dysregulated serotonin signaling. By comparing the behavior of *fmo-2* mutants to the behavior of other *C. elegans* FMO mutants with similar substrate profiles, we found that altered *fmo-2* expression likely decreases exploration by altering flux through tryptophan metabolism. Specifically, knocking out *fmo-2* may decrease exploration by pushing tryptophan towards serotonin production, while the *fmo-2* OE may decrease exploration by increasing flux of tryptophan towards kynurenine-derived metabolites. Together, these results suggest that longevity-promoting metabolic interventions may also change behavior by altering the availability of multiple neuromodulators. Further examination of the mechanism behind *fmo-2* mediated behavioral change could determine whether the longevity and behavioral effects of *fmo-2* overexpression are separable and improve our understanding of how metabolic perturbation can regulate serotonin signaling in the nervous system.

Buspirone decreases oxycodone self-administration in a drug vs milk "choice" procedure in nonhuman primates

Nora Monahan, Yasaman Razavi, Stephen J Kohut

Behavioral Neuroimaging Laboratory, McLean Hospital/Harvard Medical School, Belmont, MA, USA

Intravenous drug self-administration procedures in which subjects have concurrent access to drug and non-drug reinforcers – e.g., drug vs. food "choice" procedures - are thought to be highly translational for human drug use. Buspirone, a partial agonist at $5HT_{1A}$ receptor subtypes and dopamine $D_{3/4}$ receptor antagonist, has previously been evaluated as a candidate medication for substance use disorder. However, few studies have examined its effects on self-administration of opioids. Here we evaluate the efficacy of acute buspirone pretreatment on the reinforcing effects of oxycodone in nonhuman primates. Three adult female squirrel monkeys with a history of opioid self-administration responded under concurrent second-order FR3(FR5:S);TO45s schedules of reinforcement for intravenous oxycodone (0.0032-0.1mg/kg/inj) or saline on one lever and sweetened condensed milk on the other during daily 1-hour sessions. When responding was stable, buspirone (0.03-0.1mg/kg) was administered intramuscularly 10-min prior to self-administration sessions. Results show that, under control conditions, subjects responded exclusively on the milk lever when saline or a low dose of oxycodone (0.0032 mg/kg) was available (>60 milk deliveries and 0 injections) and exclusively on the drug lever when doses greater than 0.01 mg/kg oxycodone were available (i.e., 0.01-0.1 mg/kg). Oxycodone injections followed a prototypical inverted-U shaped dose-effect curve with peak injections (~30 injections) occurring at 0.01 mg/kg oxycodone. Buspirone pretreatment produced a dose-dependent decrease in oxycodone self-administration while concomitantly increasing milk deliveries at the highest dose tested. Specifically, 0.1 mg/kg buspirone flattened the oxycodone dose-effect curve and abolished oxycodone self-administration at 0.01 mg/kg; preference for oxycodone across the full dose range was shifted more than 10-fold compared to control conditions. These data demonstrate that buspirone can reduce oxycodone intake while producing reallocation of behavior from drug to non-drug reinforcers and supports further research into buspirone as a potential treatment for opioid use disorder.

This work was supported by NIH grant R01DA047130

Serotonin 2A- or 2C-like discriminative stimulus effects of novel 4-phenyl-2-dimethylaminotetralins (4-PATs) in nonhuman primates.

Stephen J. Kohut[1,2,3], Marissa Costa[1], and Raymond G. Booth[3]

[1]Behavioral Neuroimaging Laboratory, McLean Hospital, Belmont, MA; [2]Department of Psychiatry, Harvard Medical School, Boston, MA; [3]Center for Drug Discovery, Departments of Pharmaceutical Sciences and Chemistry and Chemical Biology, Northeastern University, Boston, MA.

Identification of drug candidates that selectively activate the serotonin (5-HT) 2C receptor subtype is a key step in the development of medications for several psychiatric disorders. The 4-phenyl-2-dimethylaminotetralin (4-PAT) chemotype can yield selective 5-HT2C receptor agonism with competitive inverse agonism and antagonism at 5-HT2A and 5-HT2B receptors *in vitro*. Here we evaluate the *in vivo* behavioral pharmacology of novel 4-PAT analogs in nonhuman primates. Separate groups of male squirrel monkeys (n=4/group) were trained to discriminate intramuscular injections of either the 5-HT2C agonist WAY163,909 (0.56 mg/kg) or the 5-HT2A agonist (-)2,5-dimethoxy-4-iodoamphetamine (0.06 mg/kg; R-DOI) from saline. Substitution tests were conducted in WAY163,909-trained subjects with the (-)-trans-4-PATs, MBP and MCP (high efficacy - 80% and 60% efficacy vs 5-HT [respectively] - 5-HT2C partial agonists and 5-HT2A inverse agonists) and MFP (5-HT2A/2C inverse agonist). Antagonism tests were conducted in R-DOI-trained subjects with 4-PATs administered in combination with R-DOI. Both training drugs produced dose-dependent effects in their respective discriminations. R-DOI did not elicit drug-like responding in the WAY163,909-trained group, and WAY163,909 did not produce drug-like responding in the R-DOI-trained group. In WAY163,909-trained subjects, MBP and MCP produced dose-dependent substitution for the WAY163,909 discriminative stimulus with maximal drug-lever responding at ~60% for MBP and ~30% for MCP. The 5-HT2A/2C inverse agonist MFP did not produce WAY163,909-like responding up to doses that reduced response rate. In antagonism tests, pretreatment with either MBP or MCP produced approximately 3-10-fold rightward shifts in the R-DOI dose effect curve while MFP produced a modest rightward shift. These findings suggest that the two training conditions elicit discriminative stimulus effects selective for 5-HT2A (R-DOI-trained) and 5-HT2C (WAY163,909-trained) agonist activity. Further, the 4-PATs MBP and MCP showed *in vivo* behavioral pharmacology consistent with agonist effects at 5-HT2C receptors and inverse agonist/antagonist activity at 5-HT2A receptors, whereas MFP behaved as a 5-HT2A/2C inverse agonist.

This work was supported by NIH grant R01DA047130

Investigating the dynamics of the human serotonin transporter with respect to substrates and inhibitors

Erika Lazzarin, Ralph Gradisch, Leticia Alves da Silva, Thomas Stockner.

Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna, Waehringerstr. 13A, 1090 Vienna, Austria

The human serotonin transporter (hSERT) is a membrane protein that mediates the reuptake of the neurotransmitter serotonin (5HT) from the synaptic cleft into the presynaptic neurons. Three main conformations describe the transport cycle of the protein: outward-open, occluded and inward-open. Their structural rearrangement leads to the exposure of substrates to the extracellular or intracellular environment, allowing for transport across the biological membrane. Transporters are complex, highly dynamic proteins that undergo multiple changes during the transport process, such as binding and releasing substrates, and transitioning between different conformations. These processes involve intricate underlying mechanisms. The probability distribution and transition rates between states are governed by thermodynamic and kinetic laws reflecting the conformational landscape. Dysregulations in this ensemble of motions can alter the transport cycle, affecting serotoninergic homeostasis and thereby playing a crucial role in behavioral and neuropsychiatric disorders. Understanding the mechanistic details of the transport cycle becomes clear as it can provide us with the knowledge and tools to intervene and regulate it, potentially developing better treatments for these disorders.

Here, we present novel insights into how inhibitors, blockers, and substrates can differently affect substrate-induced occlusion, the first step in the transport cycle. Unbiased molecular dynamics simulations and dimensionality reduction approaches allowed for identifying the principal modes of motion and shed light on communicative pathways within the transporter. To gain sufficient sampling and statistics of the occlusion process, multiple repeats of SERT bound to the different compounds were simulated, reaching a total length of 100 µs. Collectively, our results provide a framework for explaining how SERT dynamics during occlusion are affected by the cognate substrate 5HT in comparison to other compounds, and how this translates into the exerted pharmacological effects.

Acknowledgments:
This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 860954 to A.B., E.B., T.S., and C.Z.

Delineating A Serotonin Receptor Pathway for Weight-loss Therapy

Li Li[1], Baijie Xu[1], Jong-Woo Sohn[2], Katherine Nautiyal[3], René Hen[4], Chen Liu[1]

[1]Dept. of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA
[2]Dept. of Biological Sciences, KAIST, Daejeon, Korea
[3]Department of Psychological and Brain Sciences, Dartmouth College, Hanover, NH, USA.
[4]Department of Psychiatry, Columbia University and Research Foundation for Mental Hygiene, New York State Psychiatric Institute, New York, USA

The brain serotonin (5-HT) system is a critical target for multiple weight loss therapies. Notably, lorcaserin, a specific agonist for 5-HT 2C receptors (*Htr2c*), has been an anti-obesity medication until its recent withdrawal due to unexpected cancer risks. In search of new 5-HT based weight-loss therapies, we find that agonists for 5-HT 1B receptors (*Htr1b*) dose-dependently reduce food intake in mice. These include several triptans—a class of commonly prescribed anti-migraine drugs. We show that the anorectic potency of Htr1b agonists is stronger than that of lorcaserin. Furthermore, such an effect is independent of *Htr2c* but requires endogenous *Htr1b.*

By ablating *Htr1b* in four different brain regions, we demonstrate that *Htr1b* engages in spatiotemporally segregated neural pathways to regulate postnatal growth and the anorectic response to 5-HT agents. Moreover, these studies reveal AgRP neurons in the arcuate nucleus of the hypothalamus (ARH) as one critical site that mediates the hypophagic effects of Htr1b agonists.

To further probe the neural basis of the anorexigenic *Htr1b* circuit, we have generated and characterized *Htr1b-Cre* mice. We find that ARH *Htr1b* neurons activate in response to food deprivation. Moreover, chemogenetic activation of these neurons promotes food intake whereas their inhibition—mimicking the effect of activation of the Gαi-coupled Htr1b—suppresses hunger. Furthermore, single-nucleus RNA sequencing analyses reveal that *Htr1b* marks a subset of AgRP neurons lacking leptin receptor expression. We next use an intersectional genetic approach to specifically target the subset of AgRP neurons expressing *Htr1b* ($Htr1b^{AgRP}$ neurons). We find that the cell bodies of these neurons are uniquely positioned at the mediobasal part of the ARH. Furthermore, $Htr1b^{AgRP}$ neurons preferentially innervate the paraventricular nucleus of the hypothalamus (PVH). Consistent with these findings, we show that $Htr1b^{AgRP}$ neurons directly regulate food intake through a $Htr1b^{AgRP} \rightarrow$ PVH circuit.

A loss of appetite has been noted in patients taking triptans. By illustrating a 5-HT receptor pathway for appetite suppression, our findings highlight the therapeutic potential for *Htr1b* agonists as a novel weight loss therapy.

This project is supported by U.S. NIH grants R01 DK114036, DK130892 to C.L.

Utilizing genetically encoded sensors for serotonin and microdialysis in freely moving mice for the identification of novel serotonin-releasing agents

Felix P. Mayer[1,2,3], Marco Niello[3], Hideki Iwamoto[2], Marko D. Mihovilovic[4], Michael H. Baumann[5], Harald H. Sitte[3,6], Randy D. Blakely[1,2]

[1]Department of Biomedical Science, Florida Atlantic University, Jupiter, FL, USA
[2]Stiles-Nicholson Brain Institute, Florida Atlantic University, Jupiter, FL 33458, USA
[3]Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna, Vienna, Austria
[4]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria.
[5]Designer Drug Research Unit, Intramural Research Program, National Institute on Drug Abuse, NIH, Baltimore, MD, USA
[6]AddRess, Center for Addiction Research and Science, Medical University of Vienna, Vienna, Austria

Elevation of extracellular serotonin (5-HT) has proven beneficial for the treatment of various neuropsychiatric disorders, including depression and anxiety-related disorders. Extracellular levels of 5-HT are shaped by vesicular release and subsequent transporter-mediated clearance. The presynaptic 5-HT transporter (SERT) mediates the reuptake of previously released 5-HT, rendering SERT a chief regulator of extracellular 5-HT levels. Consequently, SERT serves as a target for clinically relevant agents, such as selective serotonin reuptake inhibitors (SSRIs), which represent first line treatments for major depressive disorder and post-traumatic stress disorder (PTSD). Recent studies uncovered the potential of the 5-HT releasing agents 3,4-methylenedioxymethamphetamine (MDMA) and fenfluramine for the treatment of PTSD and Dravet's syndrome, respectively. In contrast to SSRIs, which inhibit SERT-mediated reuptake, 5-HT releasers promote reverse transport of 5-HT via SERT, which occurs independent of ongoing vesicular release. However, current 5-HT releasers under clinical investigation may be associated with abuse liability and adverse side effects, including neurotoxicity and/or valvular heart disease.
Using genetically encoded fluorescent sensors for 5-HT and microdialysis in freely moving mice, we identified novel 5-HT releasing agents that promote the release of 5-HT via SERT in vivo. Importantly, the compounds identified in this study did not substantially elevate extracellular dopamine, a feature that is typically associated with abuse liability. Moreover, in vitro assays revealed that the compounds under scrutiny did not interact with 5-HT_{2B} receptors and vesicular monoamine transporters, indicative of reduced potential for cardiovascular side effects and neurotoxicity, respectively.
Consequently, the compounds identified in our study may serve as lead compounds for the development of 5-HT releasing agents with improved pharmacological properties for the treatment of disorders that are linked reduced levels of extracellular 5-HT. Further, this study highlights the utility of fiber photometry and genetically encoded sensors for neurotransmitters for the comparison of various drugs in vivo.

Funding:
Max Kade Fellowship of the Austrian Academy of Sciences (FPM)
NIH award MH094527 (RDB)
Austrian Science Fund (FWF) grants W1232 (MolTag) and FWF project P34670 (HHS)

Serotonin transporter (SERT) Ala276 mouse: Novel model to assess the biochemical, physiological, and behavioral impact of SERT Thr276 phosphorylation *in vivo*

Carina Meinke[1,2], Adele Stewart[2], Felix P. Mayer[2], Sammanda Ramamoorthy[3], and Randy D. Blakely[2,4]
[1]International Max Planck Research School for Synapses and Circuits, Max Planck Florida Institute for Neuroscience, Jupiter, FL USA, [2]Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Jupiter, FL USA, [3]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA USA, and [4]Florida Atlantic University Stiles-Nicholson Brain Institute, Jupiter, FL USA.

Serotonin (5-HT) is as an essential neuromodulator of several fundamental processes, including mood, cognition, and social behavior. The presynaptic 5-HT transporter (SERT) mediates the clearance of extracellular 5-HT, tightly regulating the availability of 5-HT for synaptic and extra synaptic signaling. Consequently, alterations in SERT function have been proposed to contribute to the etiology of 5-HT associated disorders such as depression and anxiety for which the 5-HT selective reuptake inhibitors (SSRI) are widely prescribed. Ample evidence has demonstrated that SERT function is amenable to regulation via kinase-mediated phosphorylation. *In vitro* studies revealed that SERT Ala276 is a key site targeted by PKG and that phosphorylation at this residue biases SERT conformation, leading to changes in activity and drug responses. Using SERT Ala276 knock-in (KI) mice, we are investigating the requirement for SERT Thr276 phosphorylation for 5-HT neurotransmission as well as basal and drug modulated behaviors. The SERT Thr276Ala substitution revealed changes basal SERT activity in synaptosomes with significant reductions in 5-HT uptake in males and females. Furthermore, the SERT Ala276 mutation significantly impacted the potency of Paroxetine, but not Citalopram, to inhibit SERT mediated 5-HT uptake in male and female mice. Since total protein levels were found to be normal, these data indicate conformation changes of the SERT Ala276 transporter. Preliminary data from surface biotinylation studies in striatum brain slices of adult male mice revealed an increase in SERT surface expression after treatment with the PKG activator 8-Br-cGMP, consistent with prior cell culture studies, whereas this effect is lost in Ala276 mice. Recently, we reported that SERT Ala276 homozygotes displayed sex-dependent alterations in repetitive and social behavior. SERT Ala276 females exhibit a decrease in marble burying whereas males exhibit a decrease in social dominance in the tube test. Interestingly, conducting the three-chamber social preference test revealed that females, but not males, display a decrease in social preference compared to WT controls. We hypothesize that males may require SERT Thr276 phosphorylation to sustain social interactions whereas females require SERT Thr276 phosphorylation to motivate social engagement. The results of our studies will allow us to understand how SERT regulation contributes to synaptic 5-HT homeostasis and behavior in normal and pathological states and thereby provide insights into mechanisms underlying neuropsychiatric disorders.

Support: NIH award MH094527 to Randy D. Blakely

Loss of Accumbal 5-HT$_{2C}$R Blunts Efficacy of Lorcaserin to Suppress Oxycodone Intake

Merritt CR[1], Fox RG[1], Anastasio NC[1], and Cunningham KA[1]

Center for Addiction Sciences and Therapeutics, John Sealy School of Medicine University of Texas Medical Branch, Galveston, TX, USA

Background: The misuse of prescription opioids [e.g., oxycodone (OxyContin®)] can evolve into opioid use disorder (OUD), an acquired brain disorder. Current FDA-approved treatment options for OUD are effective in suppressing opioid intake, however individuals report severe adverse side effects and there is a critical need for novel treatment options. We previously demonstrated that acute pretreatment with the 5-HT$_{2C}$ receptor (5-HT$_{2C}$R) agonist lorcaserin (Belviq®) dose-dependently decreased oxycodone intake in male rats trained on an intravenous oxycodone self-administration (SA) assay. In the present study, we extended these observations to test the hypothesis that the efficacy of lorcaserin to suppress oxycodone SA is sustained upon 10 days of lorcaserin treatment. Given that lorcaserin is DEA-controlled as a Schedule IV substance and is in several clinical studies in OUD and other substance use disorders in humans, we also assessed the abuse liability of lorcaserin in rats trained in oxycodone SA. The second aim of this project sought to define key neuronal mechanisms involved in 5-HT-mediated regulation of the rewarding effects of prescription opioids. The impetus to pursue and consume opioids initially involves nodes within the mesocorticolimbic pathway including the nucleus accumbens (NAc) and which receives prominent 5-HT innervation from midbrain and contains a rich population of the G protein-coupled 5-HT$_{2C}$R. Taken together with previous data in mind, we postulate that the NAc is the primary site of action for the effect of lorcaserin and hypothesize that rats lacking accumbal 5-HT$_{2C}$R will be resistant to the suppressive effects of 5-HT$_{2C}$R agonist treatment during oxycodone SA.

Methods: Male Sprague-Dawley rats (n=12) were trained to SA oxycodone (0.1 mg/kg/infusion) to stability and then received saline or 1 mg/kg of lorcaserin (2x/day for 10 days). After the morning lorcaserin injection, rats underwent their daily oxycodone SA session; the second daily lorcaserin injection occurred in the afternoon. The abuse liability of lorcaserin (0.1 or 0.5 mg/kg/infusion) was assessed in a separate group of rats (n=4) trained on oxycodone SA; saline or lorcaserin was substituted for oxycodone infusions on test days. In a separate cohort, rats received bilateral infusions of a non-silencing control AAV or 5-HT$_{2C}$R shRNA AAV (n=12/genetic manipulation) into the NAc and were trained to SA oxycodone (0.1 mg/kg/infusion). Upon meeting stability criteria, rats were challenged with the 5-HT$_{2C}$R agonist lorcaserin and/or 5-HT$_{2C}$R antagonist SB242084.

Results: Repeated, intermittent lorcaserin attenuated oxycodone intake relative to saline-treated rats over the course of 10 days [$F_{(1,10)}$=5.719, p<0.05]. Neither dose of lorcaserin nor saline supported self-administration. In the second cohort, a mixed model ANOVA reveals a significant effect of genetic manipulation [$F_{(1,22)}$ = 4.33, p < 0.05] and treatment [$F_{(5,110)}$ = 6.423, p < 0.05]. Oxycodone intake was suppressed by 5-HT$_{2C}$R agonist treatment (1 mg/kg; p < 0.05) in rats with intact NAc 5-HT$_{2C}$R. This effect was reversed by SB242084. Treatment with lorcaserin or SB242084 failed to alter oxycodone SA in rats lacking accumbal 5-HT$_{2C}$R.

Conclusions: Repeated lorcaserin treatment consistently suppressed oxycodone intake without evidence of tolerance. Further, NAc 5-HT$_{2C}$R is required for lorcaserin-induced suppression of oxycodone SA. This study provides mechanistic evidence for the 5-HT$_{2C}$R as a viable therapeutic target for the treatment of OUD.

Funding: NIDA P50 DA033935 and U54 DA038999.

Humanized mice overexpressing α-synuclein in serotonin neurons evoke a depressive phenotype. Reversal by conjugated antisense therapy

L. Miquel-Rio[1,2], D. Alarcón-Arís[1,2*], R. Pavía-Collado[1,2], V. Cóppola[4], E. Ruiz-Bronchal[1-3], F. Artigas[1-3], A. Bortolozzi[1-3].

[1]Instituto de Investigaciones Biomédicas de Barcelona (IIBB), Spanish National Research Council (CSIC), IDIBAPS, Barcelona, Spain
[2]Institut d'Investigacions August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[3]Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), ISCIII, Madrid, Spain
[4]Laboratory of Neurobiology and Redox Pathology, Department of Basic Pathology, Federal University of Paraná (UFPR), 80060-000 Curitiba, Brazil

Depression affects 40% of patients with Parkinson disease (PD), often preceding the onset of motor symptoms, and reducing health-related quality of life. However, the mechanisms of depression in PD are not known in detail. Dysfunction of the serotonin (5-HT) system, which regulates mood and emotional pathways, occurs during the prodromal phase of PD and contributes to a variety of non-motor symptoms. This study was designed to establish a mouse model of α-synucleinopathy in raphe 5-HT neurons that could replicate the early histopathological, neurochemical and neuropsychiatric features of human PD neuropathology. We demonstrated that AAV5 vector-induced overexpression of human wild-type α-synuclein (h-α-Syn) *in vivo* in raphe nuclei induced h-α-Syn protein levels 3-fold higher than the murine phenotype, resulting in progressive α-Syn phosphorylation, accumulation of oligomeric α-Syn forms, and axonal degeneration in connected brain regions over an 8-week period. In parallel, synaptic SV2A protein was accumulated in 5-HT fibers in several projection brain areas. Mice overexpressing h-α-Syn showed reduced extracellular 5-HT concentration in caudate putamen and medial prefrontal cortex and decreased brain derived neurotrophic factor (BDNF) expression in the hippocampus, evoking a depressive state in tail suspension and forced swim tests. Intracerebroventricular administration of an indatraline-conjugated antisense oligonucleotide targeting h-α-Syn (IND-1337-ASO, 100 µg/day) for 4 weeks reduced h-α-Syn production, improved 5-HT neurotransmission, increased BDNF levels, and reversed the depressive phenotype. Our findings indicate that α-synucleinopathy in 5-HT neurons and their projections is enough to impair those brain regions that control mood and emotion, and that treatment with conjugated ASO may relieve the PD depressive symptomatology by reducing α-Syn expression, which restores 5-HT neurotransmission throughout the brain.

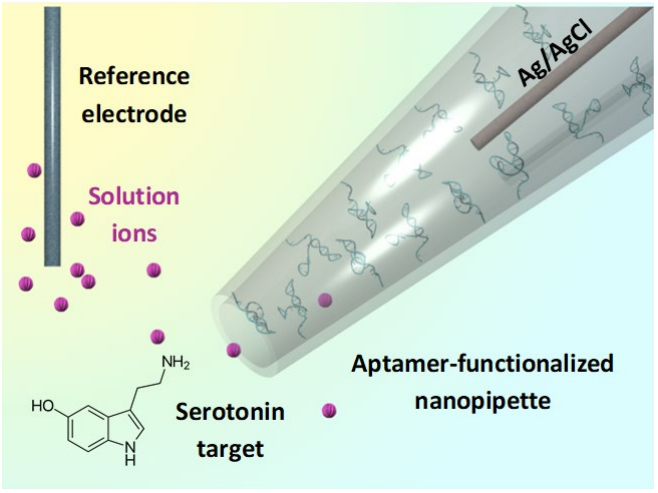
Grants: This research was funded by grants Nº PID2019-105136RB-100, Spanish Ministry of Economy and Competitiveness (MINECO) and European Regional Development Fund (ERDF).
International Society for Serotonin Research 2023, Cancún, Mexico

Ex Vivo Nanoscale Serotonin Mapping with Electrophysiology

Anne Quesnel-Hellmann,[1] Blaise Yvert,[1] Annina Stuber,[2] Nako Nakatsuka,[2]

[1]*Univ Grenoble Alpes, Inserm, Grenoble Institute of Neuroscience U1216, Grenoble, France*
[2]*Laboratory of Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETH Zürich, Switzerland*

ABSTRACT: Advancing our understanding of brain (dys)function necessitates novel nanotools that can monitor chemical signaling with high spatial resolutions. While advanced methods to record electrical signaling from neurons are prevalent (*e.g.,* microelectrode arrays, MEAs), tools to monitor chemical signaling have been limited. We have tackled this challenge by coupling the inherent selectivity of DNA-based recognition elements termed aptamers, with nanoscale pipettes with openings of *ca.* 10 nm. Aptamers are systematically designed oligonucleotide receptors that exhibit highly specific and selective recognition of targets. Aptamers that recognize small-molecule neurotransmitters, including serotonin and dopamine, have recently been isolated [1]. Upon reversible target binding, aptamers undergo a rearrangement of the negatively charged backbone, and these dynamic structural changes can be transduced as measurable changes in current through the nanoscale orifice of the sensors [2]. Nanoscale confinement of the sensor surface results in single-molecule sensitivity while simultaneously reducing biofouling for long-term recordings in complex environments, overcoming a critical bottleneck for clinical biosensors [3]. We have demonstrated the capacity to detect physiologically relevant differences in neurotransmitter amounts released by live neurons in complex media with unprecedented sensitivity [4]. We are currently monitoring serotonin while recording electrical responses in acute mouse embryonic hindbrain–spinal cord preparation isolated on MEAs [5]. Our goal is to correlate electrical activity initiated in the hindbrain that propagates down the spinal cord to localized serotonin release. Thus, we demonstrate the translatability of these sensors to other neuroscience groups and the possibility to conduct continuous in-tissue recordings in localized regions with nanoscale resolution *ex vivo.*



REFERENCES:

1. Nakatsuka *et al.* Aptamer-field-effect transistors overcome Debye length limitations for small-molecule sensing. *Science,* 362(6412):319-324, 2018.
2. Nakatsuka et al. Aptamer conformational change enables serotonin biosensing with nanopipettes. *Analytical Chemistry* 93: 4033-4041, 2021.
3. Frutiger *et al.* Nonspecific binding – fundamental concepts and consequences for biosensing applications. *Chemical Reviews* 121: 8095-8160, 2021.
4. Vadodaria *et al.* Generation of functional human serotonergic neurons from fibroblasts. *Molecular Psychiatry*, 21(1):49-61, 2015.
5. Yvert *et al.* Artificial CSF motion ensures rhythmic activity in the developing CNS *ex vivo*: A mechanical source of rhythmogenesis? *J. Neurosci,* 31(24):8832-8840, 2011.

ACKNOWLEDGEMENTS: This research was supported by ETH Zürich and the Swiss National Science Foundation.

Positive inotropic effects of hallucinogenic drugs in isolated human atrial preparations

Jacob H[1], Gergs U[1], Dimov K[1], Dietrich T[1], Höhm C[1], Hofmann B[2], Kirchhefer U[3], Neumann J[1]

[1]Institute for Pharmacology and Toxicology, Medical Faculty, Halle (Saale), Germany
[2]Department of Cardiac Surgery, Mid-German Heart Center, University Hospital Halle, Halle (Saale), Germany
[3] Institute of Pharmacology and Toxicology, University Hospital Münster, Germany

Bufotenin, lysergic acid diethylamide (LSD), 5-methoxy-dimethyltryptamine and psilocin are hallucinogenic drugs. Their hallucinogenic actions are mediated by 5-HT_{2a}-receptors. We hypothesized that they might also act on cardiac serotonin receptors which are of the 5-HT_4 type in human hearts. To this end, we measured their effects (0.1 – 10 µM, cumulatively applied) on force of contraction in isolated electrically stimulated (1 Hz) right atrial preparations obtained from patients during cardiac surgery. We noted that LSD, bufotenin, 5-methoxy-dimethyltryptamine and psilocin increased force of contraction in a concentration- and time-dependent fashion. LSD, bufotenin, 5-methoxy-dimethyltryptamine and psilocin hastened time of relaxation, increased the rate of tension development and the rate of relaxation (each n=5, $p<0.05$). The maximum effects were obtained at 10 µM of each compound. The contractile effects of the drugs were increased by pre-incubation of the cardiac preparations with 1 µM cilostamide, a phosphodiesterase III-inhibitor. The contractile effects of psilocin, 5-methoxy dimethyltryptamine and bufotenin in human atrial preparations were antagonized by tropisetron, a 5-HT_4-serotonin receptor antagonist. In contrast, the contractile effects of LSD in human atrial preparations could be antagonized by 10 µM cimetidine, an antagonist at the H_2-histamine receptor. In atrial preparations from transgenic mice overexpressing human 5-HT_4-serotonin receptors, we noted that bufotenin, LSD, 5-methoxy-dimethyltryptamine and psilocin exerted positive inotropic and chronotropic effects and increased the phosphorylation state of phospholamban. LSD also increased force of contraction in left atria of transgenic mice overexpressing human H2-histamine receptors. We conclude that in the human atrium, bufotenin, 5-methoxy-dimethyltryptamine and psilocin act via 5-HT_4-serotonin receptors, whereas LSD acts in the human atrium via H_2-histamine receptors. (Supported by the DFG).

Enantiomer-specific pharmacology of cathinones shapes their potential as a scaffold for novel therapeutic agents

Felix P. Mayer[1,2] *, Marco Niello [1,*], Daniela Cintulova[3], Spyridon Sideromenos[4], Julian Maier [1], Yang Li[1,9], Simon Bulling[1], Oliver Kudlacek[1], Klaus Schicker[1], Hideki Iwamoto[5], Fei Deng[6], Jinxia Wan[6], Marion Holy[1], Rania Katamish[2], Walter Sandtner[1], Yulong Li [6], Daniela D. Pollak [4], Randy D. Blakely [2,5], Marko D. Mihovilovic[3], Michael H. Baumann[7] and Harald H. Sitte[1,8]

Affiliation:
[1]Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna, Waehringer Strasse 13a, 1090 Vienna, Austria.
[2]Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Jupiter, FL 33458, USA.
[3]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria.
[4]Department of Neurophysiology and Neuropharmacology, Medical University of Vienna, Vienna, Austria.
[5]Stiles-Nicholson Brain Institute and Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Jupiter, FL 33458, USA.
[6]IDG McGovern Institute for Brain Research, Peking University, 100871 Beijing, China. [7]Designer Drug Research Unit, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA.
[8]AddRess, Center for Addiction Research and Science, Medical University of Vienna, Vienna, Austria.
[9]Present address: Institutes of Brain Science, Fudan University, Shanghai 200032, China.

* These authors contributed equally: Felix P. Mayer, Marco Niello.

Antidepressants are currently one of the most prescribed drugs all over the world. Their use spans from depressive disorders to several neuropsychiatric disorders including anxiety disorders, bipolar disorders, obsessive-compulsive disorders, eating disorders and post-traumatic stress disorders. Most of the currently approved antidepressants target the serotonin (5-HT) transporter (SERT) by acting as non-transported inhibitors. However, the onset of the therapeutic action requires between 3 to 4 weeks. Presumably, this time lag arises from the tonic activation of inhibitory 5-HT_{1A} autoreceptors which dampen the vesicular-mediated 5-HT release. We have recently shown that the illicit drug Mephedrone and its analogs 4-methylcathinone (4-MC) and 4-trifluoromethylmethcathinone (4-TFMMC) act as releasers of the serotonin transporter. Releasers are substrates of the transporter that, once inside the cells, they reversed the transporter determining the efflux of the endogenous neurotransmitter. Since the efflux is independent of vesicular release, we have hypothesized that Mephedrone, 4-MC and 4-TFMMC could act as antidepressants with a faster onset than commonly prescribed antidepressants. Since each of the tested cathinones exists as two enantiomers, *S*- and *R*-, we have first evaluated their *in vitro* potency to target SERT. *S*-mephedrone, *S*-4-MC, and *S*-4-TFMMC were several fold more potent than the corresponding *R*-enantiomers, while their stereochemistry did not affect the pharmacology at DAT, which in general is associated with high abuse liability. Moreover, all the S- enantiomers act as releasers at SERT and increase the extracellular monoamine levels independently from vesicular release. Consistently, when tested in behavioral experiments in mice, all the S-, but not the R-enantiomers or the antidepressants fluoxetine, significantly reduced the immobility time in a forced swim test following acute administration. Importantly, the effective doses did not elicit any increase the locomotor activity. Taken together, our findings suggest that the stereochemistry of synthetic cathinones can shape their potential as scaffold for treating neuropsychiatric disorders.

Molecular and functional architecture of axonal serotonin release machinery

Özge Demet Özçete[1], Pascal Kaeser[1]

[1]Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA

Serotonin controls brain functions including mood, affection, and reward. Modulating serotonergic activity is used to treat anxiety, depression, and other disorders. While serotonin is thought to act as a volume transmitter, the spatial and temporal scales of serotonin transmission are not well understood. At classical synapses, position, timing and extent of neurotransmitter release are controlled by the active zone, a specialized protein complex. Here, we hypothesize that serotonin release relies on sophisticated machinery for fast and synchronous release. To identify the secretory protein clusters that embody release hotspots, we utilized 3-D structured illumination super-resolution microscopy. We found that the active zone scaffolding protein Bassoon was absent in most striatal serotonin axons, while the active zone protein Munc13 was found in ~30% of the serotonin varicosities. To assess the functional spatiotemporal dynamics of serotonin release, we utilized the recently developed genetically encoded fluorescent serotonin sensors. Upon paired stimulation of the striatal slices, we found that serotonin release showed strong short-term depression, suggesting a high vesicular release probability. To determine whether the proteins that mediate spatial precision and rapid release dynamics at synapses are needed for serotonin release, we probed the role of the scaffolding protein RIM. We found that serotonin release upon single stimulus was only mildly affected in varicosities lacking the RIM protein. In summary, our results show that serotonin release hotspots might occur with a high release probability at secretory sites that might contain Munc13 but not Bassoon or RIM, likely differing from classical synaptic and dopaminergic release hotspots.

Funding resources
This work is supported by William Randolph Hearst Postdoctoral Research Grant (to ODO), Human Frontier Science Program Long-term Postdoctoral Fellowship (to ODO), EMBO Postdoctoral Fellowship (to ODO).

Modulation of ultra-slow calcium oscillation in the dentate gyrus during Non-REM sleep

Gergely F. Turi[1,2*], Xinyue Chen[3,4], Sasa Teng[3,5], Fenghua Zhen[3,5], Yueqing Peng[3,5*]

[1]New York State Psychiatric Institute, Division of Systems Neuroscience New York, NY 10032, USA
[2]Department of Psychiatry, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA
[3]Institute for Genomic Medicine, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA
[4]Department of Neuroscience, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA
[5]Department of Pathology and Cell Biology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA
[*]Correspondence to: gt2253@cumc.columbia.edu, yp2249@cumc.columbia.edu

Keywords:
Dentate gyrus, 5-HT, calcium oscillation, sleep, memory

SUMMARY:
The function of sleep in memory consolidation has been well established. The current working hypothesis postulates that episodic memory traces captured during waking hours are replayed in the hippocampal CA1-CA3 areas and transferred to the cortex for long-term storage during sleep. Even though the sensory and spatial information from higher order cortical areas such as the entorhinal cortex primarily enters the hippocampus via the dentate gyrus (DG), this structure has always been considered as the “silent partner” in memory consolidation. Whether and how the captured memory traces are transferred from the DG toward the downstream hippocampal areas is less known. Here, we used optical imaging tools to examine the activity of DG during sleep. Strikingly, we found that DG cells are even more active during sleep than wakefulness and the calcium activity in the DG slowly oscillates during non-REM (NREM) sleep epochs. Furthermore, we found that the cycles of this activity coinciding with microarousals are tightly locked to brief serotonin (5-HT) release during NREM sleep. Pharmacological blockade of 5-HT1a receptors abolished the calcium oscillations in the DG. Furthermore, genetic knockdown of 5-HT1a receptors in the DG leads to memory impairment in spatial and contextual memory tasks. Together, our results indicate that ultra-slow calcium oscillations in the DG during NREM sleep are driven by serotonin fluctuations and they are required for memory consolidation.

Activation of Serotonin Input to the Dorsal BNST Leads to Sex Differences in Fear Learning

Rebecca Ravenelle[1,2], Carolina Fernandes-Henriques[2], Jinah Lee[4], Jia Liu[6], Ekaterina Likhtik[2,3] & Nesha S. Burghardt[4,5]

1. Department of Psychiatry, Columbia University Irving Medical Center, Division of Systems Neuroscience, Research Foundation for Mental Hygiene, Inc., New York State Psychiatric Institute, New York, NY
2. Biology Program, The Graduate Center, City University of New York, New York, NY
3. Department of Biological Sciences, Hunter College, City University of New York, New York, NY
4. Psychology Program, The Graduate Center, City University of New York, New York, NY
5. Department of Psychology, Hunter College, City University of New York, New York, NY
6. Neuroscience Initiative, Advanced Science Research Center, The Graduate Center, City University of New York, New York, NY

Post-traumatic stress disorder (PTSD) is a trauma-related disorder characterized by intense fearful memory formation. Women are twice as likely as men to develop PTSD, indicating that there are sex differences in the underlying circuits. Given that serotonin is implicated in PTSD and modulates fear learning, we investigated whether serotonin plays a role in this sex difference. Using auditory fear conditioning to model fear memory formation, we tested the effects of the selective serotonin reuptake inhibitor (SSRI) citalopram on fear learning in male and female mice. We found higher sensitivity to serotonin-induced increases in fear learning in females than males, which was associated with greater activity in the dorsal bed nucleus of the stria terminalis (dBNST) and the central nucleus of the amygdala (CeA). Using the same auditory fear conditioning protocol, we optogenetically activated serotonergic inputs to the dBNST during training and found that it led to stronger fear memory in females only. An analysis of sex differences in serotonin receptor expression in the dBNST revealed greater transcript levels of the serotonin-2C receptor (*Htr2c*) in females than males. Blocking this receptor with a local infusion of a 5-HT_{2C} antagonist into the dBNST prior to conditioning blocked the serotonin-induced increase in fear learning found in females, indicating a necessary role of the 5-HT_{2C} receptor in mediating sex differences in fear learning. To better understand how increases in serotonin during learning affect extended amygdala circuits, we recorded local field potentials in the dBNST and the CeA during recall. In females, we found that increasing serotonin in the dBNST during learning led to more high gamma power in the dBNST and CeA and increased high gamma dBNST-CeA coherence during recall, indicating a strengthening of CeA-dBNST communication. Notably, these effects were absent in males. Our findings demonstrate sex differences in the raphe-BNST-CeA circuit that may underlie sex differences in risk of developing PTSD. This work also highlights the importance of including both sexes in preclinical research.

FUNDING: Project supported by NIMH R21MH114182 (NB & EL), National Institute on Minority Health & Health Disparities of NIH G12MD007599 (NB) and PSC-CUNY Awards (NB & EL).

Serotonin modifies the profile of genes controlling cellular lipid and cholesterol metabolism in human monocyte-derived macrophages through 5-HT$_7$

Israel Rios, Concha Nieto, Miguel A. Vega, Ángel L. Corbí

Myeloid Cell laboratory, Department of Molecular and Cellular Biology
Centro de Investigaciones Biológicas, Spanish National Research Council (CSIC)

As a first line of defense against pathogens and harmful stimuli, macrophages display a wide functional versatility, what allows them to coordinate innate and adaptive immune responses, initiate and resolve inflammatory processes, maintain tissue homeostasis, orchestrate tissue repair and angiogenesis, and promote or inhibit tumor progression. The functional plasticity of tissue-resident macrophages depends on their developmental origin and surrounding extracellular cues, including serotonin (5-HT). We and others have demonstrated that serotonin receptors 5-HT$_{2B}$ and 5-HT$_7$ are restricted to anti-inflammatory human monocyte-derived macrophages, that 5-HT$_7$ engagement primes macrophages for reduced IFN type I production and the acquisition of a TGFβ1-mediated pro-fibrotic gene signature, and that 5-HT$_{2B}$ activates the Aryl Hydrocarbon Receptor (AhR) and regulates mononuclear phagocyte degeneration and survival.

We have now determined the transcriptome of anti-inflammatory human monocyte-derived macrophages exposed to 5-HT (10 or 100 μM) (GEO accession GSE161774) and determined that 5-HT dose-dependently regulates the expression of MAF-, AhR-, and IFN-regulated genes. Besides, we have found that 5-HT oppositely modulates the expression of genes that control cellular lipid and cholesterol metabolism and are direct targets of the transcription factors Liver X Receptor (LXR) and Sterol Regulatory Element-Binding Proteins (SREBP). These finding is of particular relevance, because independent studies have demonstrated that activation of LXR directly promotes macrophage pro-inflammatory activation and training ability, whereas SREBP is involved in limiting the wound-healing and reparative activity of macrophages. Besides, the effect of 5-HT on LXR- and SREBP-dependent genes could be specifically abrogated by the 5-HT$_7$ antagonist SB269970 but not by the 5-HT$_{2B}$ antagonist SB204741, suggesting that 5-HT affects the expression of the genes controlling lipid metabolism (LXR and SREBP) primarily through 5-HT$_7$.

We will present the transcriptional and functional evidence of the involvement of the link 5-HT-LXR/SREBP in macrophage inflammatory activities.

This work was supported by grant PID2020-114323RB-I00 from Ministerio de Ciencia e Innovación to ALC, Dirección General de Innovación e Investigación Tecnológica de la Comunidad de Madrid (RETARACOVID, P2022/BMD-7274) to ALC , and Red de Enfermedades Inflamatorias (RICORS RD21/0002/0034) from Instituto de Salud Carlos III and co-financed by the European Regional Development Fund “A way to achieve Europe” (ERDF).

Sertraline does not affect veracity judgment but increases behavioral engagement with true and fake news

M. Piksa[1], K. Noworyta[1], Jan Piasecki[2], Aleksander Gundersen[3], Jonas Kunst[3], Mikolaj Morzy[4], and R. Rygula[1*]

[1]Maj Institute of Pharmacology Polish Academy of Sciences, Department of Pharmacology, Affective Cognitive Neuroscience Laboratory, 12 Smetna Street, 31-343 Krakow, Poland
[2]Jagiellonian University Medical College, Faculty of Health Sciences, Department of Philosophy and Bioethics, Kopernika 40, 31-501 Krakow, Poland.
[3]University of Oslo, Department of Psychology, Postboks 1094 Blindern, 0317 Oslo, Norway
[4]Poznan University of Technology, Faculty of Computing and Telecommunications, Piotrowo 2, 60-965 Poznan, Poland

Fake news affects our view of the world and our discussions with other people. It is a major issue in politics, media, national security, and public health. Recent research from our laboratory revealed that several psycho-cognitive processes, such as insensitivity to positive and negative feedback, cognitive rigidity, pessimistic judgment bias, and anxiety, are involved in susceptibility to fake news. All of these processes have been associated with depressive disorder and are sensitive to serotoninergic manipulations; therefore, in the current study, we tested the effects of chronic treatment with the selective serotonin reuptake inhibitor (SSRI) sertraline on susceptibility to true and fake news. Herein, a sample of 1162 Britons was recruited for an online study. Half of the sample reported taking sertraline (Zoloft) for at least eight weeks, and the other half confirmed not taking any psychiatric medication. The sertraline group was further divided according to their daily dosage (50, 100, 150, and 200 mg/day). All participants completed a susceptibility to misinformation scale wherein they were asked to determine the veracity of the presented true and fake news and their willingness to behaviorally engage with the news. The results were compared between the sertraline groups and the non-sertraline control group. The results showed that chronic treatment with sertraline had no significant effects on the assessment of the truthfulness of information or the ability to discern the truth. However, sertraline significantly increased the likelihood of behavioral engagement with the information, and this effect was observed for both true and fake news. Based on the present results, we postulate that in addition to general susceptibility to information, 5-HT plays a role in susceptibility to fake news.

The research leading to these results has received funding from the EEA Mechanism 2014 -2021. Project: 2019/35/J/HS6/03498.

Glucocorticoid receptor dysregulation underlies 5-HT$_{2A}$R-dependent synaptic and behavioral deficits in a mouse neurodevelopmental disorder model

Justin M Saunders[1], Salvador Sierra[1], Patrick M Bearsley[2] and Javier González-Maeso[1]

1. Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA
2. Department of Pharmacology and Toxicology, Virginia Commonwealth University School of Medicine, Richmond, VA

Neuropsychiatric disorders such as schizophrenia and autism have complex etiologies involving both genetic and environmental factors. Among those environmental factors is maternal infection during pregnancy, and a variety of infectious agents are a replicated risk factor for these disorders. Following from this, animal models of maternal immune activation (MIA) have been developed and shown to recapitulate disorder-relevant phenotypes. Notably, the serotonin 5-HT$_{2A}$ receptor (5-HT$_{2A}$R), an important target of atypical antipsychotics and psychedelic drugs, has been found to exhibit increased receptor density in both postmortem prefrontal cortex samples from antipsychotic-free schizophrenia subjects relative to healthy controls and in MIA offspring mouse frontal cortex. However, the underlying cause of 5-HT$_{2A}$R dysregulation within schizophrenia and MIA models, as well as the degree to which this dysregulation contributes to relevant phenotypes, remains unknown. To address these questions, we induced MIA in pregnant mice using the viral mimetic poly-(I:C) and evaluated phenotypes in adult offspring. Given the association of stress and the glucocorticoid system with both the pathophysiology of schizophrenia and 5-HT$_{2A}$R expression, we hypothesized that alterations in glucocorticoid signaling underlie 5-HT$_{2A}$R dysregulation within this model, and that, in turn, 5-HT$_{2A}$R dysregulation underlies a subset of MIA-induced disorder-related phenotypes. Following maternal poly-(I:C) treatment, we observed both increased frontal cortex *5-HT$_{2A}$R* expression by FISH and sensorimotor gating behavioral deficits in adult offspring. To evaluate a potential synaptic correlate for these MIA-induced effects, virally (AAV)-mediated eYFP expression was used to assess frontal cortex dendritic spine density in WT and 5-HT$_{2A}$R KO offspring of poly-(I:C)-treated mothers and controls. MIA produced a decrease in mushroom dendritic spine density on layer 5 frontal cortex pyramidal neurons in WT offspring mice relative to controls, a difference not seen between 5-HT$_{2A}$R KO MIA and control offspring. To explore a mechanism by which serotonin 5-HT$_{2A}$R dysregulation could be produced in MIA offspring mice, immunoblot assays for the glucocorticoid receptor (GR) in mouse frontal cortex were conducted and revealed decreased expression in the nuclear compartment in MIA offspring. Alongside this, chromatin immunoprecipitation using an anti-GR antibody revealed decreased GR enrichment at a predicted binding site on the *5-HT$_{2A}$R* promoter in offspring of poly-(I:C)-treated mothers relative to control offspring, suggesting a negative regulatory relationship between the GR and 5-HT$_{2A}$R expression. To further investigate this relationship, a constitutively translocating GR construct, ΔGR, was packaged into an AAV vector and injected into mouse frontal cortex. Following AAV-ΔGR injection in otherwise untreated mice, *5-HT$_{2A}$R* expression in frontal cortex was decreased relative to AAV-eYFP-injected controls and sensorimotor gating improvement was observed in wild type, but not 5-HT$_{2A}$R KO, mice. Overall, these data provide novel insights into a potential mechanism by which 5-HT$_{2A}$R dysregulation can occur within models of prenatal insults during pregnancy, as well as suggest that alterations in 5-HT$_{2A}$R expression underlie at least a subset of synaptic structural changes observed within these models.

Funding provided by: F30 MH116550, R01 MH084894

Serotonergic modulation of the ventral hippocampus underlies sex-related differences in anxiety

Anne-Sophie Simard[1,2*], van der Veldt Suzanne[1,2*], Fiona Henderson[1,2], Félix Perreault[1,2], Alice Gravel-Chouinard[1,2], Guillaume Ducharme[1] and Bénédicte Amilhon[1,2]

* = co-first authors
1. CHU Sainte-Justine Research Center, Montréal, Canada
2. Université de Montréal, Département de Neuroscience, Montréal, Canada

Abstract:
Anxiety disorders are among the most prevalent mental disorders worldwide and affect women twice as often as men. Yet, our understanding of the neural circuits underlying the higher vulnerability of female brains to anxiety disorders is incomplete and needs to be refined. 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, in particular through oscillatory communication with other brain regions. 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 local field potential recordings in the vHP, we show that the raphe-vHP pathway modulates behavior and oscillatory activity differentially in males and females. Optogenetic activation of vHP-projecting 5-HT neurons elevated anxiety levels exclusively in females. Increases in anxiety in response to 5-HT release in the vHP were accompanied by shifts in the frequency and power of delta-range (1-4 Hz) and theta-range (4-12Hz) rhythms, both known to underlie defensive behaviors. 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.

Selective refinement of glutamate and GABA synapses on dorsal raphe 5-HT neurons during postnatal life

Carla V., Arganãraz*, Tamara S., Adjimann*, Paula P., Perissinotti, Mariano, Soiza-Reilly

Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires (C1428EGA), Argentina.

Serotonin (5-hydroxytryptamine, 5-HT) neurons are implicated in the etiology and therapeutics of anxiety and depression. Critical periods of vulnerability during brain development enable maladaptive mechanisms to produce detrimental consequences on adult mood and emotional responses. 5-HT plays a crucial role in these mechanisms; however, little is known about how synaptic inputs and modulatory systems that shape the activity of early 5-HT networks mature during postnatal development. We investigated in mice the postnatal trajectory of glutamate and GABA synaptic inputs to dorsal raphe nucleus (DRN) 5-HT neurons, the main source of forebrain 5-HT. High-resolution quantitative analyses with array tomography and ex vivo electrophysiology indicate that cortical glutamate and subcortical GABA synapses undergo a profound refinement process after the third postnatal week, whereas subcortical glutamate inputs do not. This refinement of DRN inputs is not accompanied by changes in 5-HT1A receptor-mediated inhibition over 5-HT neurons. Our study reveals a precise developmental pattern of synaptic refinement of DRN excitatory and inhibitory afferents, when 5-HT-related inhibitory mechanisms are in place. These findings contribute to the understanding of neurodevelopmental vulnerability to psychiatric disorders.

*equal contribution

Support:
- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 2021-2023).
- Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (PICT 2019-00807 to M.S.-R. and PICT 2019-02040 to P.P.P.).
- International Brain Research Organization (Return home).
- International Society for Neurochemistry – CAEN (Return home).

Psilocybin increases peri-infarct plasticity following photothrombotic stroke

Haley N. Strong; April Contreras; Rochelle M. Hines; Dustin J. Hines

Psychological & Brain Sciences, and Interdisciplinary Neuroscience, University of Nevada Las Vegas, Las Vegas, NV, USA

Narrow time windows of intervention after stroke onset limit recovery and contribute largely to long term disability. Endogenous mechanisms of recovery contribute to restricting boundaries of damaged tissue and regulating cell death and survival. Glial cells modulate tissue survival and successful formation of synaptic connections regulating peri-infarct plasticity. Identifying therapeutics capable of enhancing recovery of damaged tissue through increasing or strengthening synaptic connections of peri-infarct tissue would widen the time window of treatment. Persistent synaptic plasticity enabled by psilocybin indicates its potential for therapeutic use to enhance endogenous mechanisms of recovery after stroke. The capacity of psilocybin to incite plasticity synergistic with endogenous peri-infarct recovery after stroke remains unknown. In this study, we examine the ability of psilocybin to alter progression of stroke recovery by comparing peri-infarct plasticity and behavioral sparing after photothrombotic motor cortex stroke. Administering psilocybin after stroke led to increased dendritic arborization and spine density regardless of infarct volume. To identify if dendritic recovery was accompanied by behavioral sparing we then tested paw preference in the cylinder task and saw increased use of the affected forelimb across lesion sizes. These results show psilocybin improves post-stroke motor recovery through increasing dendritic plasticity without exacerbating endogenous recovery mechanisms. Enhancing endogenous mechanisms of peri-infarct recovery through psychedelics provides evidence of a synergistic relationship between glial cells and psychedelics after stroke. Ongoing work is examining the mechanisms of psilocybin plasticity in relation to glial cells after stroke.

Early-Life Environmental Factors Regulating Serotonergic-Dopaminergic Interaction and Adult Behavior

Edênia C. Menezes[1,2], Fabiula Abreu[1,2], Cátia M. Teixeira[1,2]

[1]Emotional Brain Institute, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY 10962, USA.
[2]Department of Child and Adolescent Psychiatry, New York University Grossman School of Medicine, New York, NY 10016, USA.

Many neuropsychiatric disorders have developmental origins in which susceptibility to disease is restricted to narrow developmental windows; the perinatal period is a highly plastic time in which environmental factors can derail the normal development of the brain. Serotonin and dopamine are two key regulators of mood, reward-seeking and motivated behavior; several environmental factors have been shown to alter serotonin levels during development and lead to behavioral deficits in the adult. In this talk we will discuss results from our laboratory showing how changes in serotonin levels during development affect the dopaminergic system and lead to deficits in motivation later in life. We further show that these deficits can be rescued by modulating the dopaminergic system.

Funding: R01 HD095966

Contextual valence-dependent effect of psilocybin on Arc immediate early gene expression and anxiety behavior in mice.

Isla Weber[1,2], Carla Dias[1,2], Sean Lim[1,2], Jack Berry[1,2], Christine Ann Denny[1,2], Rene Hen[1,2], Gergely Turi[1,2]

New York State Psychiatric Institute, Systems Neuroscience[1]; Columbia University, Department of Psychiatry[2]

Psychedelics are being shown by medical and scientific methods to be helpful as a mental health therapy across many common diagnostic categories, including depression, PTSD, addiction, and more. As psychedelics may soon be readily available for both recreational and clinical use, systematic and controlled animal research could reveal unknown interactions between psychedelics and various administration contexts, but this research has not yet been done. We hypothesize that the valence of the context in which these compounds are administered may have different effect on circuit activation and behavior. Using mice, we test the hypothesis that psilocybin alters neuronal activation captured by the immediate early gene ARC within regions involved in anxiety and cognition, including the hippocampus, prefrontal cortex, and amygdala, and has a greater anxiolytic effect in a follow-up behavioral battery when administered in a positive context as opposed to a negative, innately fearful context. We used the ArcCreERT2 x EYFP mouse line to tag neuronal populations acutely activated by psilocybin and used the Open Field, Elevated Plus Maze, and Novelty Suppressed Feeding behavioral tasks to assess anxiety-like behavior following treatment. We observed a reduction of the anxiety-like phenotype after psilocybin in the positive context but not after psilocybin in the negative context in the Open Field Task in one experiment, providing some support for our hypothesis. Additionally, that activation of the ventral CA1 of the hippocampus, an emotional processing center, decreased during psilocybin treatment in the negative context but not in the positive context. We found additional context-dependent alterations in activation of the PFC, with psilocybin-induced decreases in Arc expression only in a negative context. These results provide preliminary evidence that psilocybin administration in contexts with positive versus negative valance may drive neural and behavioral signatures of psilocybin's effects and outcomes in mice. This newly developed protocol can be used to further our understanding of the effect of psilocybin administration on neural signatures of varying external stimuli, such as innate and learned fearful cues.

Chronic desipramine induces norepinephrine neuroplasticity and behavioral recovery in a fluoxetine-resistant mouse model of depression

Faranak Vahid-Ansari, Amin Zahrai, Mireille Daigle, Paul R. Albert
OHRI (Neuroscience), University of Ottawa Brain and Mind Research Institute, Ottawa, ON, CA

Introduction: Alterations in serotonin (5-HT) have been implicated in major depressive disorder, for which 5-HT reuptake inhibitors (SSRIs) are the first-line treatment. However, many patients fail to respond and are switched to augmentation or other antidepressants like tricyclic-antidepressants (TCAs) on a trial-and-error basis. To model SSRI resistance, we generated the cF1ko mice, in which the 5-HT1A repressor, Freud-1, is deleted in adult 5-HT cells (Vahid-Ansari et al., 2017). The cF1ko mice have increased 5-HT1A autoreceptor levels and function, leading to reduced 5-HT activity and a fluoxetine-resistant anxiety/depression phenotype. We hypothesized that the TCA desipramine, by targeting both 5-HT and norepinephrine (NE) systems, could overcome the 5-HT1A autoreceptor-induced block of the 5-HT system.

Methods: Wild-type and cF1ko mice were treated chronically with desipramine and examined using validated behavioral tests including elevated-plus maze, open-field, novelty-suppressed feeding, forced-swim and tail-suspension. Immunofluorescence for NE and 5-HT transporters was used to detect brain-wide projections (axons and varicosities) of these systems. Co-immunofluorescence with synaptophysin (presynaptic), and gephyrin and PSD-95 identified synapses to GABAergic or glutamatergic contacts.

Results: Compared to wild-type, cF1ko mice displayed significant alterations in 5-HT projections and these changes were not altered by chronic fluoxetine treatment. In contrast to fluoxetine, chronic desipramine treatment reversed the depression/anxiety phenotypes of cF1ko mice. Compared to WT, cF1ko mice displayed reduced NE axons in the amygdala (BLA), and reduced NE varicosities in several brain regions including BLA and medial prefrontal cortex and desipramine reversed these changes. Chronic desipramine also selectively rescued NE contacts with GABAergic cells in the cF1ko mPFC, hippocampus and BLA.

Conclusion: The cF1ko model may be particularly relevant to patients with SSRI-resistance due to reduced activity of the 5-HT system. Consistent with their fluoxetine-resistant behaviors, fluoxetine did not restore 5-HT projections in cF1ko mice. By contrast, chronic desipramine treatment did improve behavior and restored NE projections to selectively target GABA interneurons in mPFC, hippocampus and BLA of cF1ko mice. These results suggest that desipramine may bypass the SSRI resistant phenotype by targeting the NE system. In summary, these results provide a rational basis for switching SSRI-resistant patients to treatments that target the NE system and could suggest a novel role of 5-HT and NE neuroplasticity to regulate interneurons in the action of monoamine-targeting antidepressants.

* This research was supported by grants from the Canadian Institutes for Health Research.

Pharmacological characterization of quipazine analogs represents a new structural class of psychedelic 5-HT_{2A} receptor agonists.

Jason Younkin[1,4], Archana Paymode[2], Alaina M Jaster[1,3], Richard A. Glennon[2], Małgorzata Dukat[2], Javier González-Maeso[1]

[1]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine.
[2]Department of Medicinal Chemistry, Virginia Commonwealth University School of Pharmacy
[3]Department of Pharmacology and Toxicology, Virginia Commonwealth University School of Medicine
[4]Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University

Psychedelics are currently hot topics in molecular psychiatry and medicinal chemistry research. As more psychedelics are shown to produce desirable therapeutic effects in individuals with psychiatric conditions such as depression and substance use disorder, the clinical potential of these drugs becomes increasingly relevant, despite their negative reputation from the 1960s and 70s. It is accepted that psychedelics produce mind-altering effects such as hallucinations and expansion of consciousness acting mainly via the 5-HT_{2A} receptor (5-HT_{2A}R) – a G protein-coupled receptor involved in processes related to cognition, perception and mood. Structurally, classical psychedelics generally fall into two main categories: phenethylamines such as mescaline and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), and tryptamines which can be subdivided into simple tryptamines such as psilocybin and ergolines such as lysergic acid diethylamide (LSD). We recently reported that quipazine might represent a new group of psychedelics since it lacks a phenethylamine or tryptamine scaffold embedded in its structure. However, the clinical use of quipazine is limited due to its emetic effects via off-targets that include the 5-HT_3R – a pentameric ion channel. Here we synthesized a series of quipazine analogs with the goal of maintaining psychedelic properties via 5-HT_{2A}R while eliminating activity at the 5-HT_3R. Using [^{3}H]ketanserin binding displacement and Ca^{2+} mobilization assays in HEK293 cells stably expressing 5-HT_{2A}R, our data suggest that compounds VCU-1012 and VCU-1021 exhibit affinities and potencies in the micromolar range, with an efficacy ~45% as compared to 5-HT. Volinanserin, a highly specific 5-HT_{2A}R antagonist, blocked intracellular Ca^{2+} mobilization induced by both quipazine analogs. In vivo, both VCU-1012 and VCU-1021 induced a dose-dependent effect on head-twitch behavior in C57BL/6 male mice, with VCU-1012 being more efficacious. Current studies are being conducted to analyze VCU-1012 and VCU-1021 for functionality at the 5-HT_3R using whole-cell patch clamp in transfected HEK293 cells. These findings may lead to the identification of quipazine analogs not only as a new structural class of classical 5-HT_{2A}R-dependent psychedelics, but also as potential fast-acting therapeutics similar to those in clinical trials.

Funded by R01-MH084894 (J.G.M.), T32-MH020030 (J.Y.), and VCU Breakthroughs Fund (J.G.M. and M.D.).

Perinatal SSRI exposure Increases Innate Fear and Fear Circuit Activation in Mice and Humans

Giulia Zanni [1,2], Milenna Van Dijk [3,4], Martha Caffrey Cagliostro[1,2,5], Gregory S. Stevens [1,2], Nicolò Pini [1,2], Ariel L. Rose [1,2], Alexander L. Kesin [1,2], Claudia Lugo-Candelas [6], Priscila Dib Goncalves[7], Alexandra S. MacKay [1,2], Praveen Kulkarni[5], Craig F. Ferris[5], Myrna M. Weissman [3,4,7], Jay Gingrich [1,2], Ardesheer Talati [3] & Mark S. Ansorge [1,2.]

[1] Division of Developmental Neuroscience, Department of Psychiatry, Columbia University, New York- also cite NYSPI
[2] Sackler Institute for Developmental Psychobiology, Columbia University and the New York State Psychiatric Institute, New York
[3] Department of Psychiatry, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY
[4] Division of Translational Epidemiology, New York State Psychiatric Institute, New York, NY
[5] Center for Translational NeuroImaging, Northeastern University, Boston Massachusetts
[6] Department of Child and Adolescent Psychiatry, New York State Psychiatric Institute, New York, NY
[7] Department of Epidemiology, Mailman School of Public Health, Columbia University, New York. NY

Abstract
Serotonin shapes brain structure and function during early development across phylogenetically diverse species. In mice and humans, gestational SSRI exposure produces brain alterations and increased anxiety/depression-related behaviors in the offspring. It remains unclear whether similar brain circuit changes underlie the behavioral impact of perinatal SSRIs across species. We examine how developmental SSRI-exposure in mice and humans changes fear-related brain activation and behavior. SSRI-treated mice showed increased defense responses to a predator odor that was associated with stronger fMRI-based fear circuit activation when compared to saline controls. Similarly, human adolescents exposed to SSRIs *in utero* showed greater activation of fear brain structures and exhibited higher anxiety and depressive symptoms than unexposed adolescents. Perinatal SSRI enhances innate fear-related responses and fear brain circuit activation that are phylogenetically conserved across species.

Keywords: sensitive period, anxiety and depression, fMRI, predator odor, SSRI exposure, DeepLabCut, translational science

Attendees

Igor	Adameyko	Medical University of Vienna, Austria
Ana Sofia	Alberto e Silva	Medical University of Vienna, Austria
Natalia	Alenina	Max Delbrück Center, Germany
John	Allen	University of Texas Medical Branch, USA
Nuno	Alves	Columbia University, USA
Leticia	Alves Da Silva	Medical University of Vienna, Austria
Bénédicte	Amilhon	Université de Montréal - CHU Sainte-Justine Research Center, Canada
Noelle	Anastasio	University of Texas Medical Branch, USA
Mark	Ansorge	Columbia University, USA
Michael	Bader	Max Delbrück Center, Germany
Martin	Beaulieu	University of Toronto, Canada
Jean-Claude	Béïque	University of Ottawa, Canada
Oliver John	Belleza	Medical University of Vienna, Austria
Kelly	Berg	University of Texas Health San Antonio, USA
Safa	Beydoun	University of Michigan, USA
James	Bjork	Virginia Commonwealth University, USA
Randy	Blakely	Florida Atlantic University, USA
Bruce	Blough	RTI International, USA
Emma	Bonniwell	Medical College of Wisconsin, USA
Dasiel	Borroto Escuela	Karolinska Institute, Sweden
Silvia	Bruzzone	Copenhagen University Hospital – Rigshospitalet, Denmark
Lindsay	Cameron	University of California Davis, USA
Jennifer	Chan	Icahn Mount Sinai, USA
Briana	Chen	Columbia University Irving Medical Center, USA
William	Clarke	University of Texas Health San Antonio, USA
Valerie	Compan	Nimes University, France
April	Contreras	UNLV, USA
Rebecca	Coray	University of Zurich, Switzerland
Kathryn	Cunningham	University of Texas Medical Branch, USA
Andrew	Dacks	West Virginia University, USA
Denis	David	Universite Paris-Saclay, France
Indira	David	Universite Paris-Saclay, France
Lynette	Daws	University of Texas Health San Antonio, USA
Christine	Denny	Columbia University, USA
Susan	Dymecki	Harvard University, USA
Moriah	Edge-Partington	University of Calgary, Canada
David	Erritzoe	Imperial College London, UK
Udoka	Ezeaka	University of Saskatchewan, Canada
Maria Cristina	Fenollar Ferrer	Florida Atlantic University, USA

Thomas	Flanagan	LSU Health Sciences Center New Orleans, USA
Rocio	Foltran	Inst. de Biología Celular y Neurociencias. CONICET-UBA, Argentina
Kjell	Fuxe	Karolinska Institute, Sweden
Alain	Gardier	Université Paris-Saclay, France
Joanna	Golebiowska	Maj Institute of Pharmacology Polish Academy of Sciences, Poland
Ralph	Gradisch	Medical University of Vienna, Austria
Luisa (Sophie)	Gullino	Univeristy of Oxford, UK
Jana	Haase	University College Dublin, Ireland
Adam	Halberstadt	University of California San Diego, USA
Doris	Handl	Medical University of Vienna, Austria
Hanne	Hansen	Copenhagen University Hospital – Rigshospitalet, Denmark
Parastoo	Hashemi	Imperial College London, UK
Rene	Hen	Columbia University, USA
Meghan	Hibicke	LSU Health New Orleans, USA
Rochelle	Hines	UNLV, USA
Dustin	Hines	UNLV, USA
Lauren	Honan	University of Texas Health San Antonio, USA
Kelly	Hrelja	University of British Columbia, Canada
Lin	Hung	New York University, USA
Aurelija	Ippolito	University of Oxford, UK
Balaganesh	Janakiraman	Tata Institute of Fundamental Research, India
Skirmantas	Janusonis	University of California Santa Barbara, USA
Matthew	Johnson	Johns Hopkins University, USA
Aimee	Jones	University College Dublin, Ireland
Nina	Kastner	Medical University of Vienna, Austria
Eunhyun (David)	Kim	University of Saskatchewan, Canada
Lynn	Kirby	Lewis Katz School of Medicine at Temple University, USA
Elizabeth	Kitto	University of Michigan, USA
Gitte	Knudsen	Copenhagen University Hospital – Rigshospitalet, Denmark
Stephen	Kohut	McLean Hospital/Harvard Medical School, USA
David	Krantz	UCLA, USA
Rupert	Lanzenberger	Medical University of Vienna, Austria
Erika	Lazzarin	Medical University of Vienna, Austria
Chen	Liu	UT Southwestern Medical Center, USA
Claus	Loland	University of Copenhagen, Denmark
Felix	Mayer	University of Copenhagen, Denmark
John	McCorvy	Medical College of Wisconsin, USA
Carina	Meinke	Florida Atlantic University, USA
Christina	Merritt	University of Texas Medical Branch, USA

Lluis	Miquel-Rio	Spanish National Research Council (CSIC), IDIBAPS, Spain
Christian	Müller	Friedrich-Alexander-University Erlangen-Nuremberg, Germany
Kevin	Murnane	LSU Health Sciences Center Shreveport, USA
Nako	Nakatsuka	Institute for Biomedical Engineering ETH Zürich, Switzerland
Kate	Nautiyal	Dartmouth College, USA
John	Neumaier	University of Washington, USA
Joachim	Neumann	University Hospital, Germany
Charles	Nichols	LSU Health Sciences Center New Orleans, USA
Marco	Niello	Medical University of Vienna, Austria
Ozge Demet	Ozcete	Harvard Medical School, USA
Massimo	Pasqualetti	University of Pisa, Italy
Yueqing	Peng	Columbia University, USA
Jamie	Peters	University of Colorado Anschutz, USA
Evgeni	Ponimaskin	Cellulöar Neurophysiology, Hannover Medical School, Germany
Raksha	Ramkumar	University of Calgary, Canada
Mark	Rasenick	University of Illinois at Chicago, USA
Rebecca	Ravenelle	Columbia University Irving Medical Center, USA
Israel	Rios	Spanish National Research Council (CSIC), Spain
Ethan	Rohrbach	UCLA, USA
Rafal	Rygula	Maj Institute of Pharmacology Polish Academy of Sciences, Poland
Somdatta	Saha	Virginia Commonwealth University School of Medicine, USA
Derya	Sargin	University of Calgary, Canada
Justin	Saunders	Virginia Commonwealth University School of Medicine, USA
Joy	Schmitz	University of Texas Health Science Center at Houston, USA
Trevor	Sharp	University of Oxford, UK
Anne-Sophie	Simard	Université de Montréal - CHU Sainte-Justine Research Center, Canada
Steffen	Sinning	Aarhus University, Denmark
Harald	Sitte	Medical University of Vienna, Austria
Mariano	Soiza-Reilly	CONICET - University of Buenos Aires, Argentina
Jun Ho	Song	Stanford University, USA
William	Spencer	Case Western Reserve University, USA
Haley	Strong	UNLV, USA
Catia	Teixeira	NKI-NYU, USA
Gergely	Turi	New York State Psychiatric Institute, USA
Faranak	Vahid-Ansari	University of Ottawa Brain and Mind Research Institute, Canada
Suzanne	van der Veldt	Université de Montréal - CHU Sainte-Justine Research Center, Canada
Alex	Vasilkevich	Bright Minds Biosciences, USA

Jeremy	Veenstra-VanderWeele	Columbia University and New York State Psychiatric Institute, USA
Deborah	Wagner	University of Washington, USA
Jason	Younkin	Virginia Commonwealth University, USA
Giulia	Zanni	Columbia University, USA