ISSR 2018
Serotonin on the Wild Atlantic Way

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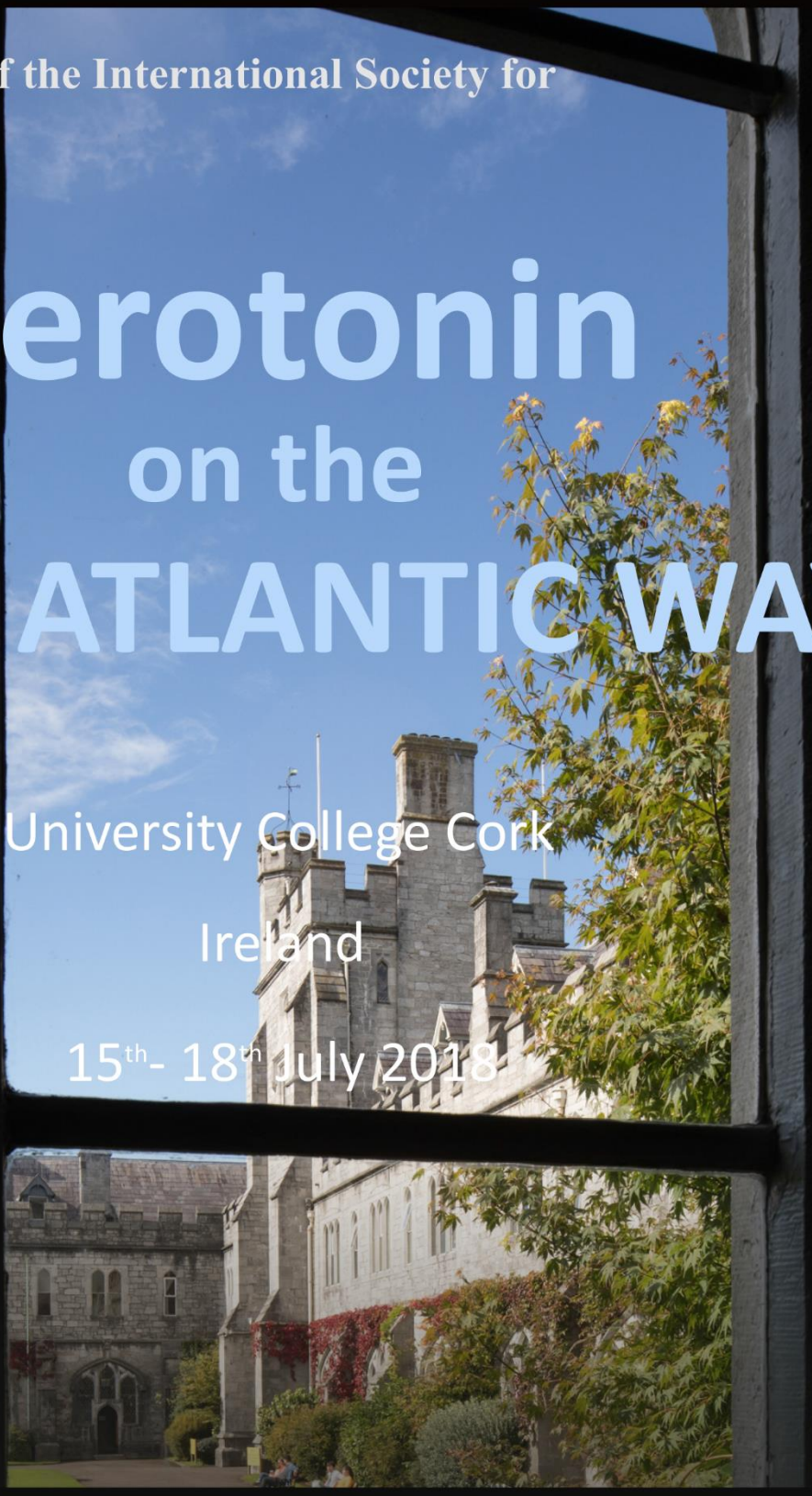
The 19th Meeting of the International Society for Serotonin Research

Serotonin on the 'WILD ATLANTIC WAY'



University College Cork

Ireland

15th- 18th July 2018

BOOK OF ABSTRACTS

19th Meeting of International Society for Serotonin Research

Abstract Book

ISSR 2018

"Serotonin on the Wild Atlantic Way"

University College Cork, Ireland

July 15-18, 2018

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SPECIAL LECTURES

HONORARY IRVINE PAGE PLENARY LECTURE

A sentimental Journey through the serotonin Isles.

Patricia Gaspar
Inserm, Institut du Fer à Moulin, Sorbonnes Universités, Paris

Serotonin research covers a wide territory that includes a multitude of different isles; each with unexpected charms and unsolved mysteries. Once a researcher lands into one of these serotonin isles, it is difficult not to want to explore the next, and then to become fascinated by their complex interconnections, and their secret labyrinths. The talk will tell the story of a personal experience in this exploration. It starts somewhere in the land of dopamine-and Parkinson's disease, and it ends somewhere in the field of development of serotonin systems in the landscape of psychiatric disorders. In the interim there are a few stop overs in the barrel cortex with agressive mice, the visual system and the prefrontal cortex with anxiety-prone mice. Overall this voyage shows that serotonin remains a marvelous platform to start an exploration of neural circuit development and implications in neurological and psychiatric disorders.

Supported by : Agence Nationale de la Recherche, Fondation de la Recherche Médicale, Labex BIOPSY.

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HONORARY PAUL VANHOUTTE LECTURE

Serotonin Matters in Addiction: Strategies for Pharmacotherapeutics

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The misuse of psychoactive drugs, the pervasive trajectory to substance use disorders (**SUDs**), and the limited access to efficacious treatment strategies is a worldwide public health crisis, with significant personal and societal burdens noted in the United States. A hallmark of SUDs is “continued drug use despite adverse consequences,” which aligns with the definition of impulsivity, a predisposition toward rapid unplanned reactions to stimuli without regard to negative consequences. Impulsivity is linked to a myriad of SUDs including cocaine (**CUD**) and opioid use disorder (**OUD**). Impulsivity is interlocked with cue reactivity which is defined as the attentional orientation toward drug-associated stimuli that predict reward in humans and rodents. We report translational studies employing gene-mediated viral vector targeting strategies in rodents and analyses of structural and effective connectivity in fMRI studies in humans that interrogate the neurocircuitry engaged in impulsive action and the response bias toward cocaine-associated cues. Furthermore, we have uncovered that impulsive action and cue reactivity are mechanistically-linked to disrupted serotonin (**5-HT**) signaling through a 5-HT_{2A} receptor (**5-HT_{2A}R**) and **5-HT_{2C}R** interaction within this circuitry. These data are fueling our drug discovery efforts to normalize the balance of 5-HT_{2A}R and 5-HT_{2C}R function to suppress phenotypic behaviors that promote relapse and continued drug use. This presentation will highlight these serotonergic ligands as innovative pro-abstinence, anti-relapse therapeutics for SUDs. Given the importance of cue reactivity and impulsivity in the risk for relapse in SUDs, the outcomes of these studies contribute a greater appreciation of the serotonergic neurocircuitry underlying these constructs and suggest new concepts in the treatment of SUDs.

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HONORARY MAURICE RAPPORT PLENARY LECTURE

Neuropsychedelia: From psychotomimetics to psychotherapeutics

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The study of psychedelic drugs has a long history that is intimately related to the history of serotonin research. Investigations of these so-called “mind-expanding” substances have elucidated mechanisms that are relevant to both the substrates and treatment of multiple psychiatric disorders. We will review the conjoint histories of serotonin and psychedelic research with a focus on cross-species translational experimentation as presented over the years since the formation of the Serotonin Club.

Supported by the National Institute of Mental Health, the National Institute on Drug Abuse, and the Heffter Research Institute.

SYMPOSIA AND SHORT ORAL PRESENTATIONS

MONDAY JULY 16, 2018

Symposium 1

Serotonin Signaling in the BNST Regulates Aversive Learning

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Serotonin, also known as 5-hydroxytryptamine (5HT), can regulate a wide variety of behaviors via activation of discrete receptors in the brain. Work from our group, and others, have found that 5HT can drive enhanced fear and anxiety via engagement of 5HT2C-R in the Bed Nucleus of the Stria Terminalis (BNST). I will discuss our ongoing studies probing the role of other 5HT receptors in the BNST on both fear and anxiety. Specifically, I will show that genetic deletion of 5HT1A-R from the BNST selectively enhances fear, but has no impact on anxiety. This, combined with previously published data suggests a model where 5HT release in the BNST engages multiple systems to regulate aversive learning, and that a balance of inhibitory and excitatory 5HTR signaling plays a key role in determining outcomes.

Ventral hippocampal serotonin signaling in anxiety and antidepressant mechanisms

Anne M. Andrews

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Serotonin modulates the development of anxiety- and mood-related brain circuitry. The ventral hippocampus, in contrast to dorsal hippocampus, is key for processing emotionally salient information [1, 2] and specifically, elevated-plus maze open-arm behavior in rodents [3]. Studies in rodents have identified a postnatal developmental period wherein exposure to serotonin-selective reuptake inhibiting antidepressants (SSRIs) and other manipulations of the serotonin system cause persistent emotion-related behavioral changes. Our recent findings in mice show that exposure to escitalopram (ESC; Lexapro®), one of the most widely prescribed SSRIs, during postnatal development is associated with decreased anxiety-like behavior during adolescence and throughout adulthood [4]. This reduced anxiety phenotype is associated with decreased basal extracellular serotonin in the ventral hippocampus. Moreover, adult serotonin1A autoreceptor function is potentiated in mice exposed to ESC during early postnatal development further implicating reduced serotonin signaling with a reduced anxiety-related phenotype. Developmental changes in mice

exposed to ESC are dissimilar from those associated with early life fluoxetine exposure and constitutive reductions in serotonin transporter expression. Thus, specific SSRIs such as ESC may impart a resilient phenotype in offspring of mothers treated with this drug for depression and anxiety during pregnancy. Ongoing studies are aimed at pinpointing key developmental timeframes and mechanistic underpinnings of developmental SSRI effects on adult serotonin neurotransmission and behavior.

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Serotonin – Regulating Neuronal Powerplants

Sashaina E. Fanibunda[1,2], Sukrita Deb[1], Babukrishna Maniyadath[1], Ashok.D.B.Vaidya[2], Ullas Seetharam-Kolthur[1*], Vidita A.Vaidya[1*]

Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India[1]
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Mitochondrial dysfunction has emerged as an important factor in the pathophysiology of psychiatric disorders such as depression and anxiety. Neurons depend on mitochondria for critical neuronal functions, yet the factors that influence mitochondrial physiology in neurons are poorly elucidated. The relationship between serotonin and mitochondrial physiology in neurons is currently unknown. In cortical cultures, 5-HT increases mitochondrial biogenesis, mtDNA and ATP levels, as well as basal and maximal respiration, and spare respiratory capacity. These effects are mediated via the 5-HT_{2A} receptor, which via an Erk and PLC-mediated pathway, regulates master modulators of mitochondrial biogenesis, Sirt1 and PGC-1α. Sirt1 is required for the effects of 5-HT on both mitochondrial biogenesis and function. In vivo studies indicate that chronic stimulation with the 5-HT_{2A} receptor agonist DOI, increases mtDNA and ATP levels, an effects that is lost in cortex-specific Sirt1 loss of function mice. 5-HT also decreases cellular reactive oxygen species (ROS), and enhances superoxide dismutase 2 and catalase expression. 5-HT exerts strongly neuroprotective roles, buffering against both excitotoxic and oxidative stress in vitro. These findings highlight the importance of 5-HT as an important upstream modulator of mitochondrial function, and motivate future studies to address the importance of these effects of 5-HT on bioenergetics in the context of treatment and pathophysiology of psychiatric disorders.

Supported by TIFR intramural funding to VV and UK

Short oral presentation 1

The anxiolytic and antidepressant effects of fluoxetine are mediated by specific regions along the longitudinal axis of the hippocampus

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Depression is currently the leading cause of disability worldwide and yet antidepressant treatments remain suboptimal. This is due in part to our incomplete understanding of the neurobiology underlying the pathophysiology and successful treatment of depression. However, human neuroimaging studies suggest that the hippocampus area of the brain likely plays a key role. Accumulating studies in rodents suggest that the hippocampus is functionally segregated along its longitudinal axis into a dorsal region (dHi) which is predominantly involved in spatial learning and memory, and a ventral region (vHi) which regulates anxiety, a symptom often co-morbid with depression. Gene expression studies suggest that the area between these areas, the intermediate hippocampus (iHi) might also be functionally independent but few studies have interrogated its function. Similarly, little is known about the roles of these specific hippocampal subregions in the antidepressant response. Thus, the present study investigated the impact of ibotenic acid-induced lesions of the dHi, iHi or vHi on the regulation of anxiety and depressive-like behaviour in the absence or presence of the antidepressant and selective serotonin reuptake inhibitor, fluoxetine. In the absence of fluoxetine, vHi lesions reduced anxiety, while none of the lesions affected depressive-like behaviour under these conditions. On the other hand, only vHi lesions prevented the acute antidepressant effects of fluoxetine in the tail suspension test. Similarly, only vHi lesions prevented its anxiolytic effects in the novelty-induced hypophagia test. Interestingly, only iHi lesions prevented the antidepressant effect of chronic fluoxetine treatment in the forced swim test. dHi lesions did not impact antidepressant- or anxiety-related behaviour either in the absence or presence of fluoxetine. Taken together, the present data demonstrate that the vHi plays a key role in anxiety and its modulation by chronic fluoxetine treatment, and that both the iHi and vHi play distinct roles in fluoxetine-induced antidepressant-like behaviour.

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Symposium 2

Rationale behind the 5-HT_{1F} receptor as an antimigraine target

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Migraine is a neurovascular disorder involving activation of the trigeminovascular system and cranial vasodilation mediated by release of calcitonin gene-related peptide (CGRP). The last decades have witnessed a remarkable progress in acute antimigraine therapy with the development of the triptans, which are tryptamine derivatives with agonist properties at serotonin 5-$HT_{1B/1D/(1F)}$ receptors. Their antimigraine actions are thought to be mediated via activation of: (i) vascular 5-HT_{1B} receptors in cranial blood vessels, resulting in cranial vasoconstriction; (ii) prejunctional 5-$HT_{1D/(1F)}$ receptors on trigeminal fibers, inhibiting trigeminal release of CGRP; and (iii) central 5-$HT_{1B/1D/(1F)}$ receptors involved in (anti)nociceptive modulation. Notwithstanding, in addition to producing medication overuse headache, triptans' therapy: (i) may result in a wide variety of adverse (central and peripheral) side effects; (ii) may induce (in very rare cases) myocardial ischemia and stroke; and (iii) is contraindicated in patients with cardiovascular disease due to their 5-HT_{1B} receptor-mediated vasoconstrictor properties in coronary arteries. Consequently, other novel antimigraine drugs devoid of vascular (side) effects have been explored, including 5-HT_{1D} and 5-HT_{1F} receptor agonists (which inhibit the trigeminovascular system without producing vasoconstriction). Since the 5-HT_{1D} receptor agonist PNU-142633 proved ineffective in the acute treatment of migraine, further attempts have led to the development of the ditans (selective 5-HT_{1F} receptor agonists), including lasmiditan. Several lines of evidence discussed in this symposium will show that lasmiditan: (i) fails to contract human coronary arteries; (ii) inhibits CGRP release from trigeminal nerves via prejunctional 5-HT_{1F} receptors; and (iii) has shown positive results in Phase III clinical trials. These findings represent a major scientific breakthrough, particularly when considering the increased cardiovascular risk of migraine patients. Admittedly, lasmiditan's exact central sites of action remain elusive, although its high lipophilicity suggests a direct action on central descending antinociceptive pathways.

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Preclinical pharmacological investigation of lasmiditan in experimental migraine models

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Lasmiditan is a selective 5-HT_{1F} receptor agonist with proven efficacy in migraine. We have investigated the selectivity of lasmiditan for the human 5-HT_{1F} receptor and studied the vasoconstrictive potential of lasmiditan, as well as its ability to inhibit prejunctional release of CGRP. Results were compared to those obtained with sumatriptan. Shortly, we constructed concentration response curves to sumatriptan and lasmiditan in segments of human isolated

coronary and internal mammary arteries. While sumatriptan induced concentration-dependent contractions, lasmiditan did not induce any contraction. The pharmacological selectivity of lasmiditan for the 5-HT_{1F} receptor was assessed in CHO cells expressing gene constructs for various 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{7}) by a second messenger activity assay. Lasmiditan was demonstrated to be a selective agonist at human 5-HT_{1F} receptors. In addition, vasoconstrictive properties of lasmiditan were investigated *in vivo* in Beagle dogs. While sumatriptan induced significant constriction in both the coronary and carotid arteries at therapeutic plasma concentrations, lasmiditan was devoid of vasoconstrictive effects. Finally, we investigated the ability of lasmiditan to inhibit prejunctional release of CGRP in mice isolated dura mater, trigeminal ganglion and trigeminal caudal nucleus. Both lasmiditan and sumatriptan induced inhibition of CGRP release in all three above-mentioned structures. Taken together, lasmiditan is an effective antimigraine drug that is devoid of vasoconstrictive properties, which seems to be a cardiovascular safety advantage compared to the triptans. Inhibition of CGRP release may contribute to the therapeutic efficacy of lasmiditan in migraine.

Clinical findings on the use of lasmiditan for the treatment of migraine

Kirk W. Johnson

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Migraine is a disease characterized by episodes of headache pain that can last from 4-72 hours, associated with nausea, photophobia and/or phonophobia, and with or without prodromal symptoms and aura. Migraine affects approximately 18% of women and 6% of men in the United States, and has a prevalence greater than heart disease, diabetes and asthma. The most frequently used acute migraine treatments, nonsteroidal anti-inflammatory drugs (NSAIDS) and triptans, have cardiovascular warnings and precautions. In addition, migraine is associated with cardiovascular (CV) disease and increased risk factors for CV disease. Therefore, the selective 5-HT_{1F} agonist lasmiditan has been evaluated in clinical trials for the acute treatment of migraine. Lasmiditan does not vasoconstrict blood vessels as observed with other treatments such as the 5-$HT_{1B/1D/1F}$ agonist sumatriptan. Two phase 3 clinical trials evaluating the efficacy of lasmiditan were conducted. The first study, SAMURAI, evaluated two dose levels of lasmiditan (100 and 200 mg) and placebo in 1856 patients with CV risk factors. The second study, SPARTAN, studied three dose levels of lasmiditan (50, 100 and 200 mg) and placebo in 2156 patients including those with CV disease. The results from both studies show that lasmiditan was significantly more efficacious compared to placebo for the primary endpoint of pain-freedom at 2 hours post-dose, at all doses evaluated. Significant improvement versus placebo for the secondary endpoints of pain relief, freedom of most bothersome symptom (MBS), patient global impression of change and patient-reported disability were achieved. Dizziness, paresthesia and somnolence were the most frequently reported treatment emergent adverse events reported for lasmiditan. However, triptan-like adverse effects such as chest tightness and pressure were not reported. Lasmiditan appears to be an effective and well-tolerated option for acute migraine treatment.

Supported by Eli Lilly and Company

Short oral presentation 2

Genetically encoded photocross-linkers locate the heteromeric interface in a serotonin GPCR heteromer.

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Protein-protein interactions represent a fundamental process involved in many aspects of cell physiology. G protein-coupled receptors (GPCRs) were initially assumed to exist and function as monomeric plasma membrane proteins. Nevertheless, more recent findings suggest that GPCR homodimeric/homomeric assemblies may occur in living cells. Of particular interest is also the observation that GPCRs form heteromeric complexes, which may ultimately affect GPCR trafficking, pharmacology and function.

We previously reported that the family A serotonin 2A (5-HT_{2A}) receptor and the family C metabotropic glutamate 2 (mGlu2) receptor are assembled as a GPCR heteromeric complex in living mammalian cells. Our previous mutagenesis studies reported that three residues (A4.40, A4.44 and A4.48) located at the intracellular end of the transmembrane domain 4 (TM4) of the mGlu2 receptor were necessary to form the 5-HT_{2A}-mGlu2 receptor heterocomplex. Although interesting, these data do not indicate directly whether these three residues interact physically with the 5-HT_{2A} protomer of the 5-HT_{2A}-mGlu2 heteromeric complex. This is particularly relevant when considering that previous crystal structures of mGlu receptor dimers revealed a TM1-TM1 homodimeric interface, whereas findings based on a cysteine crosslinking protocol suggested that the main mGlu2 receptor homodimeric interface is formed by TM4 and TM5.

With the final goal of mapping precisely the mGlu2 receptor residues that interact physically with the 5-HT_{2A} receptor, we applied a relatively novel receptor-based targeted photocross-linking approach. Activation by ultraviolet light induces covalent cross-linking of the interacting proteins, which can be detected by immunoblotting. As compared to the classical cysteine crosslinking approach, the major advantages of photocross-linking are greater specificity of cross-linking owing to the short lifetimes of the excited intermediates, and absence of the need for introduction of mutations at highly-conserved cysteine residues potentially involved in GPCR structure and function. We used amber codon suppression to introduce the photoreactive unnatural amino acid (UAA) *p*-azido-L-phenylalanine (azF) at selected positions in mGlu2 receptors. The mGlu2 variants were expressed in mammalian HEK293 cells and retained their pharmacological ligand binding and functional properties. Notably, we found that mGlu2 variants with azF in certain positions located at the intracellular end of TM4 cross-linked efficiently to the 5-HT_{2A} protomer. These results show for the first time the residues that interact physically at a GPCR heteromeric complex interface, using a targeted photocross-linking strategy with genetically encoded UAAs. This method will be important in future studies that will provide additional functional and structural insights into serotonin GPCR complexes.

Supported by NIH R01 MH084894 and R01 MH111940.

Symposium 3

The molecular mechanisms of psychostimulant action at serotonin and organic cation transporters

Harald H. Sitte, Felix P. Mayer, Julian Maier, Tina Hofmaier, Oliver Kudlacek, Lynette C. Daws

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Serotonin transporters are the clinically relevant target of antidepressant drugs and a number of important psychostimulants; either they inhibit the reuptake of these monoamines by competitively blocking the transporters' action or they act as substrates and induce a reversal of the transport direction, so-called non-exocytotic neurotransmitter release (efflux). Thereby, these compounds enhance the extracellular concentration of serotonin and dopamine, which is relevant for their success, either in terms of clinical reduction of depressive symptoms or wakefulness in the case of psychostimulants. In recent years, a novel class of compounds has been flooding the street markets, novel psychoactive substance, where rogue chemists introduce subtle changes to circumvent the legislation of illicit compound use. In addition to the monoamine transporters which have been defined as “uptake 1”-transporters, there are low affinity and high capacity transporters, organic cation transporters (OCT), which also take part in the removal of monoamines from the extracellular space. Most recently, we have described that psychostimulants also target OCT3, which is the predominant isoform expressed in the central nervous system. Recent advancement in the understanding of the structural and molecular mechanisms of psychostimulant-induced efflux via both serotonin transporters and OCT3 will be discussed, mainly amphetamine- and cathinones. Finally, the important regulatory role of membrane lipids in these processes, phosphoinositides and PIP_2, will also be highlighted.

Supported by the Austrian Science Fund / FWF (grants W1232 and F3506 to H.H.S).

DATs not all it cracked up to be: Organic cation transporters in the actions of psychostimulants

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Organic cation transporters (OCTs) and plasma membrane monoamine transporters (PMAT) are low-affinity, high-capacity (“uptake-2”) transporters for dopamine, norepinephrine, and serotonin. OCT3, in particular, is emerging as an important player in regulation of biogenic amine homeostasis. However, whether psychoactive drugs, including psychotherapeutic and abused drugs, have activity at OCTs remains unclear. Findings from our lab, and others, suggest that activity of OCT3 may underlie, in part, the lack of therapeutic benefit afforded by drugs targeting high-affinity, low-capacity “uptake-1” transporters for dopamine, norepinephrine, and serotonin (DAT, NET, and SERT, respectively). For example, antidepressant-like effects of SERT-targeting drugs are bolstered by concurrent blockade of OCTs. Moreover, “uptake-1” targeting therapeutics for psychostimulant abuse have not been effective, suggesting that additional mechanisms of action of psychoactive drugs are at play. To this end, we investigated a role for OCTs in neurochemical and behavioral effects

of amphetamine, ethanol, and ketamine, three drugs with complex pharmacology, with the goal to uncover novel targets for therapeutic intervention in the treatment of addiction and related psychiatric disorders. A combination of pharmacological and genetic approaches were employed, together with *ex vivo* and *in vivo* neurochemistry, and behavioral readouts. We found that i) amphetamine-induced dopamine release and hyper-locomotion are OCT3-dependent; ii) ethanol inhibits serotonin clearance in hippocampus in a manner that is SERT-independent, but putatively OCT3-dependent; and iii) ketamine inhibits serotonin uptake in a manner indicative of activity at both "uptake-1" and "uptake-2" transporters, and produces antidepressant-like effects that are PMAT-dependent. These studies begin to fill fundamental knowledge gaps about the role of OCT3 in abuse-related effects of amphetamine, which will help to identify novel molecular targets for medications to treat amphetamine addiction that may generalize to more recently emerged amphetamine-type psychostimulants. Likewise, they reveal a mechanistic basis for co-abuse of cocaine and alcohol, one of the most common, and dangerous drug pairings. Finally, our results support a role for PMAT in the actions of ketamine. Taken together, these results support OCTs as potential novel targets for therapeutic intervention in addiction and psychiatric disorders.

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Activity at serotonin transporters modulates dopaminergic effects of newly-emerging stimulant drugs of abuse

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Therapeutic and abused stimulant drugs exert their effects by interacting with plasma membrane dopamine transporters (DAT), norepinephrine transporters (NET), and 5-HT transporters (SERT) in the central nervous sytem. Transporter ligands can act as either non-transported uptake blockers (e.g., cocaine) or transportable substrates that induce non-exocytotic neurotransmitter efflux by reverse transport (e.g., amphetamine). Historical evidence shows that locomotor and reinforcing effects of amphetamine-type stimulants depend upon substrate activity at DAT, which leads to elevations in extracellular dopamine in mesolimbic reward circuits. In recent years, we have examined the role of DAT and SERT in mediating the pharmacological effects of newly-emerging synthetic cathinones, often known as "bath salts". From a chemical structure perspective, all cathinone compounds are β–keto amphetamines. Our *in vitro* studies show that ring-substituted cathinones like 3,4-methylenedioxymethcathinone (methylone) are non-selective transporter substrates which elevate extracellular dopamine and 5-HT in the brain. By contrast, pyrrolidine-containing cathinones like 3,4-methylenedioxypyrovalerone (MDPV) are DAT-selective transporter blockers which elevate extracellular dopamine. *In vivo* studies examining locomotor activity, intracranial self-stimulation and drug self-administration demonstrate that increasing potency at SERT relative to DAT reduces motor stimulant and reinforcing effects of cathinone-type drugs. These findings have implications for predicting the abuse potential of newly-emerging drugs of abuse and for developing novel medications for ADHD, depression and obesity.

Short oral presentation 3

Aptamer field-effect transistors to monitor serotonin *in vivo*

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Monitoring serotonin flux associated with complex neuropathological processes *in vivo* and in real-time necessitates chemically specific neurotransmitter sensors that approach the spatiotemporal resolution of neuronal signaling while differentiating structurally similar neurochemicals. To address this challenge, we employ rationally designed oligonucleotide sequences, termed aptamers as artificial receptors for molecular recognition. Aptamers can be designed for a range of target detection, signal transduction, response speeds, and *in vivo* stability. Beyond the challenge of discovering recognition elements with high specificity and selectivity for neurotransmitters, translation of electronic biosensing platforms such as field-effect transistors for *in vivo* environments has been hindered by the Debye length, the sensing distance beyond semiconducting channel surfaces wherein changes in local electric fields affect the distributions of channel free-charge carriers to the greatest extent. Structure-switching aptamers undergo large conformational changes upon target capture that involve rearrangement of their highly negatively charged backbones in close proximity to semiconducting channels resulting in measurable changes in channel conductances. Coupling serotonin-specific aptamers to field-effect transistors, we detected unprecedented femtomolar concentrations of serotonin in undiluted buffers (phosphate-buffered saline and artificial cerebrospinal fluid). Serotonin-functionalized sensors show excellent selectivity with the capacity to differentiate precursors (*L*-tryptophan and *L*-5-HTP), metabolites (5-HIAA), and other neurotransmitters at physiological concentrations. We have conducted measurements *ex vivo* in brain tissue homogenates of Tph2 knockout mice that lack brain serotonin to test device responses upon controlled addition of serotonin, while assessing biofouling of devices for acute measurements. With the goal of *in vivo* sensing, we have designed and fabricated miniaturized field-effect transistors on silicon microprobes (120 μm in width). Optimization of these aptamer-field-effect transistor neuroprobes will lead to *in vivo* detection of serotonin in the brain to link serotonin signaling with complex behaviors.

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Symposium 4

The 5-HT_7 serotonin receptor is preassociated with the G protein G_s

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According to classical models of G-protein-coupled receptor (GPCR) signalling, G proteins only interact with activated receptors. However, the 5-HT_7 receptor displays pharmacological properties inconsistent with this model, such as no change in potency of agonists to activate adenylyl cyclase by increasing receptor expression and the finding that partial agonists do not become full agonists even at very high receptor expression levels, indicating a lack of spare receptors [1]. It is also peculiar among GPCRs in that agonist potency to activate adenylyl cyclase is lower than the agonist binding affinity, as opposed to most other receptors. Using the molecular imaging techniques Fluorescence Recovery After Photobleaching (FRAP) and Förster/Fluorescence Resonance Energy Transfer (FRET) we have demonstrated that the human 5-HT_7 receptor, as opposed to the 5-HT_4 receptor, is actually preassociated with G_s [2]. In a series of chimeric 5-HT_7 receptors with intracellular segments from 5-HT_4, we determined preassociated and agonist-induced G_s-receptor interaction by antibody-immobilized FRAP and FRET. We identified the ICL3 and C-tail of the 5-HT_7 receptor to be responsible for the preassociation with G_s and further delineated the TM5 extension in the ICL3 and Helix 8 in the C-tail as the molecular determinants. These chimeric exchanges converted the 5-HT_7 receptor into a collision-coupled receptor that recruited G protein only upon agonist activation, while the reverse exchange converted the 5-HT_4 receptor to a preassociated receptor. Finally, we found that the 5-HT_7 receptor displays a characteristic two-component agonist-induced G_s signalling with a high- and low-potency component. We found that the same segments were involved in both low potency signalling and preassociation and propose that the preassociated state corresponds to low-potency G_s signalling.

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The chronic depressor effect of circulating serotonin is mediated exclusively by the 5-HT$_7$ receptor

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Previous work indicates that *acutely* administered serotonin has complex CV effects mediated via several distinct serotonin receptor subtypes located on vascular smooth muscle, endothelial cells, autonomic nerve endings and the central nervous system. Increased circulating serotonin has been reported in *chronic* diseases that affect the cardiovascular (CV) system, so it is important to understand how *long-term* changes in plasma serotonin influence CV function. We have previously explored the *chronic* CV actions of serotonin using a model of one-week long infusions of serotonin (25 µg/kg/min s.c.) into Sprague-Dawley rats. We found that such infusions caused sustained falls in blood pressure and total peripheral resistance, and increases in cardiac output. Since work by Terron *et al.* suggested that the depressor response to *acute* administration of serotonin is mainly mediated through the 5-HT$_7$ receptor (5-HT$_7$R) subtype, here we sought to determine if the *chronic* hemodynamic effects of circulating serotonin in our model are due to 5-HT$_7$R activation. Hemodynamic data were collected from both conscious and anesthetized rats using radiotelemetry, vascular catheters, Doppler flowmetry and ultrasound imaging. Reversible antagonism of the 5-HT$_7$R was accomplished using the selective antagonist SB269970, whereas permanent deletion of the 5-HT$_7$R was achieved by creation of a genomic knockout rat (5-HT$_7$RKO). Chronic infusion of serotonin caused: 1) decreased arterial blood pressure and total peripheral resistance, and increased heart rate and cardiac output: 2) increased blood flow to skeletal muscle and skin; 3) no effect on flow through the splanchnic circulation but a prominent splanchnic venodilation; 4) increased resistance to flow in the hepatic circulation. All of these changes were reversed by acute administration of SB269970 and when tested were absent in 5-HT$_7$RKO rats. We conclude that the chronic hemodynamic effects of serotonin in our model are mediated exclusively via the 5-HT$_7$R. The 5-HT$_7$RKO rat should be a useful tool to explore the CV effects of circulating serotonin in disease.

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Serotonin 5-HT$_7$ receptor in endothelial cells

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The 5-hydroxytryptamine type 7 receptor (5-HT$_7$R) regulates many physiological processes, including learning and memory, circadian rhythm, and behavior. Its role is also implicated in psychiatric disorders. Little is known about the 5-HT$_7$R function in endothelial cells (ECs). Here, we report that 5-HT$_7$R endogenously expressed in ECs may promote cell migration and adhesion. Using Boyden chamber migration assay and wound healing “scratch” assay we demonstrated that stimulation of the receptor with 5-HT$_7$R agonists 5-CT and AS 19

significantly increased EC migration. In addition, 5-CT and AS 19 treatment increased EC adhesion to extracellular matrix. Downregulation of 5-HT_7R using specific siRNA significantly inhibited baseline and 5-HT-induced EC migration. Additionally, pretreatment of ECs with PKA inhibitor 14-22 amide significantly reduced 5-CT- or AS 19-induced EC migration, suggesting that PKA is involved in the regulation of EC migration mediated by 5-HT7R. Expression of 5-HT_7R-YFP fusion protein in endothelial cells leads to filopodia-like processes formation, which may be essential to 5-HT_7R-dependent cell migration. Our results suggest a prominent role of 5-HT_7R in promoting cell migration and adhesion and identify 5-HT_7R as a potential regulator of physiological and pathophysiological processes involving cell migration and adhesion.

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5-HT7 receptor mediated control of locomotion

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Serotonin has been shown to elicit locomotion in both adult rodents and in isolated spinal cord preparations from neonatal rats and mice. 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors are implicated in this action. We have investigated the role of 5-HT_7 receptors in locomotion in the neonatal rodent, in adult intact and decerebrate as well as paraplegic rodents. Using isolated neonatal rat brainstem-spinal cord preparations, we showed that electrical or chemical stimulation of a specific population of the descending 5-HT neurons of the medulla, particularly in the parapyramidal region (PPR), also known as the lateral paragigantocellular nucleus (LPGi) elicited fictive locomotion in vitro, characterized by rhythmic alternating discharges in the ventral roots of the lumbar enlargement. Interlimb (left-right) and intralimb (flexor-extensor) coordination detected in these recordings was produced by this stimulus, but not by stimuli applied to other 5-HT neuron populations of the medulla. The use of the specific 5-HT_7 receptor antagonist, SB-269970, slowed and interfered with inter- and intralimb coordination, and eventually abolished this locomotor activity, establishing a role for 5-HT_7 receptors in this response. 5-HT was able to elicit rhythmic activity in the isolated spinal cord of 5-HT_7 receptor knockout mice (5-HT_7 $^{-/-}$), but coordinated locomotor-like activity was not produced. In adult mice, direct application of SB-269970 onto the spinal cord disrupted coordinated locomotion. Blockade of spinal 5-HT_7 receptors with intrathecal application interfered with voluntary locomotion in adult intact rats as well as fictive locomotion in paralysed decerebrate rats with no afferent feedback, consistent with a requirement for activation of descending 5-HT neurons acting at spinal 5-HT_7 receptors for production of locomotion. No direct effect of SB-269970 on motoneuron excitability was observed in these experiments. However, the direct control of coordinating interneurons by 5-HT_7 receptors observed in neonatal animals was not found during fictive locomotion in adults, revealing a developmental shift from direct control of locomotor interneurons in neonates to control of afferent input from the moving limb in adults. In attempts to replace the lost function of the descending 5-HT system after spinal cord injury, we grafted 5-HT

neurons into the sublesional spinal cord of paraplegic rats. SB-269970 altered the improved locomotion that was produced by the graft. 5-HT receptor gene expression is altered in spinal motoneurons after spinal cord injury, and the grafts tend to restore this gene expression to normal levels. Recent evidence suggests there may be a role for interactions with other receptors, but whether this is the case for spinal 5-HT_7 receptors requires further investigation.

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Short oral presentation 4

Sleep deprivation rapidly upregulates 5-HT_{2A} receptor expression in the prefrontal cortex via the immediate early gene *Egr3*

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Dysfunction of serotonin 2A receptors (5-HT_{2A}Rs) has been implicated in the symptoms of schizophrenia, and levels of the receptor are reduced in schizophrenia patients' brains. Insufficient 5-HT_{2A}R receptor expression is one potential explanation for these findings. However, the molecular mechanisms that regulate 5-HT_{2A}R expression are unknown. We have previously reported that six hours of sleep deprivation upregulates expression of *Htr2a*, the 5-HT_{2A}R gene, in the mouse cortex. Furthermore, this induction requires the activity-dependent immediate early gene transcription factor, early growth response 3 (*Egr3*). In the current studies, we have used quantitative reverse transcription-PCR to show that sleep deprivation induces *Htr2a* in prefrontal cortex (PFC), but not in more anterior or posterior cortical regions, in wildtype mice, and that this requires *Egr3*. This is paralleled by an increase in 5-HT_{2A}R protein, detectable by autoradiography using the selective ligand [^{3}H]-M100907, after just eight hours of sleep deprivation. Chromatin immunoprecipitation demonstrated that neuronal activity significantly upregulates EGR3 binding to the *Htr2a* promoter in the mouse PFC. Furthermore, *in vitro,* EGR3 induces expression of luciferase reporter constructs driven by the *Htr2a* promoter containing either of two EGR3 binding sites. Finally, viral-mediated expression of *Htr2a* in the frontal cortex of *Egr3*-deficient (-/-) mice, which we have previously shown have reduced 5-HT_{2A}R levels, rescues anxiety-like behavioral abnormalities of these animals. These findings demonstrate that EGR3 can regulate expression of the 5-HT_{2A}R via direct binding to the *Htr2a* promoter, and strongly suggest that EGR3 may directly regulate 5-HT_{2A}R expression in the PFC in response to the

physiologic stimulus of sleep deprivation. In addition, these results suggest that the reduced 5-HT_{2A}R levels in the brains of *Egr3*-/- mice are responsible for at least some of the behavioral abnormalities in these animals. Finally, these findings suggest the possibility that inadequate activation of EGR3 may contribute to the reduced 5-HT_{2A}R levels in schizophrenia patients' brains.

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Symposium 5

Impact of stress on 5-HT neuron translatome.

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The complex relationship between stress and adaptations in the serotonin system have remained elusive. In this study we examined the impact of stress on RNAs actively undergoing translation in mouse serotonin neurons using RNAseq of RiboTag isolated RNA.

Pet1-Cre mice were crossed with floxed RiboTag mice. Male and female mice were subjected to a modified forced swim stress procedure (one 15' swim on day 1, four sequential 6' swims on day 2) that produced escalating immobility across the swim sessions. RiboTag enriched RNA originating from serotonin neurons was collected 4° after the final swim, immunopurified, and compared to total input RNA from midbrain punches as well as RiboTag-negative RNA controls, RNA libraries were prepared, and the resulting libraries were subjected to RNAseq. Bioinformatic analysis was performed and the differentially expressed genes (DEGs) were filtered sequentially allowing stress-regulated DEGs to be considered at different levels of filtering. Low "signal" genes were excluded if they were not significantly different from "noise" in negative controls. Highly enriched genes were identified relative to RNAseq of the input RNA from the same individuals, which reflected the transcriptome of the tissue punch. Stress-sensitive DEGs were analyzed in males, females, and combined sex analyses. Each level of filtering provided informative data regarding the DEGs.

We identified DEGs that differed by sex and by stress exposure but only two DEGs associated with RiboTag-immunoprecipitated RNA survived sequential filtering in stressed animals of both sexes: Myrip and Fkbp5. We confirmed these and other DEGs by RT-qPCR in the same and additional cohorts of mice. We verified that FKBP5 is predominantly expressed by Pet1-positive serotonin neurons and that stress increased FKBP5 mRNA and protein in these neurons histologically. We also found that FKBP5 is elevated in the TpH2-positive neurons of human DRN in a small sample of suicide completers as compared to matched controls. We conclude that FKBP5 is an intriguing, stress-regulated gene in serotonin neurons with profound implications for mental disorders and their treatment.

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Transcriptome-wide gene profiling approaches to identify molecular pH sensors in 5-HT neurons

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We breathe continuously from birth to death with rare exception, and thus the control of ventilation is among the most vital neural motor outputs controlled by the brain. Serotonin (5-HT) neurons are embedded within the complex neurocircuitry controlling lung ventilation, and drive breathing through two major mechanisms: 1) tonic neuromodulatory excitation of respiratory pre-motor and motor neurons, and 2) through an intrinsic pH/CO_2 sensitivity in a minority (30-40%) of brainstem 5-HT neurons. pH sensitivity in 5-HT neurons in the caudal

raphe nuclei is absent at birth in rodents, but is evident around 12 days of age and increases until maturity. This pH-sensing property of caudal 5-HT neurons appears intrinsic, as electrophysiological recordings of cultured neurons, eYFP-labeled neurons in acute brainstem slides, and acutely dissociated 5-HT neurons show reversible increases in firing rate with modest acidification in synaptic blockade (SNB) media. While many experimental paradigms indicate that a sub-population of individual 5-HT neurons have properties of respiratory pH/CO_2 chemoreceptors, we have yet to identify the molecular determinants of this unique neuronal function. We are currently using large-scale transcriptomics in an attempt to test the overall hypothesis that pH sensitivity in chemosensitive 5-HT neurons is a transcriptionally regulated phenotype, perhaps due to differential expression of known pH-sensitive ion channels. To test this, we first employed cell-assisted cell sorting (FACS) to isolate brainstem 5-HT neurons from newborn (P0-4) or mature (>P60) transgenic rats expressing eGFP driven by the enhancer region of the Pet-1 promotor ($SS^{ePet1:eGFP}$ rats). mRNAs were isolated and cDNA libraries generated from $eGFP^+$ and $eGFP^-$ cell pools, and subjected to whole genome RNA-sequencing and bioinformatics data processing. Unbiased overlapping differential expression analyses identified several hundred genes altered with increasing age, including several pH-sensitive ion channel genes (kcnj10 (Kir4.1), kcnj16 (Kir5.1), kcnk1 (TWIK-1), kcnk3 (TASK-1) and kcnk9 (TASK-3). Only kcnj10 and 16 increased with age, and only kcnj16 (Kir5.1) protein was localized to 5-HT neurons whereas kcnj10 (Kir4.1) was localized to neighboring astroglia. While mutation of Kir5.1 in rats ($SS^{Kcnj16-/-}$ rats) led to renal and respiratory dysregulation of pH, suggesting an important functional role for Kir5.1 in the ventilatory pH/CO_2 chemoreflex, this FACS-based approach indicated a need for more specificity. Ongoing studies pairing cellular recording of firing rate before, during and after bath application of high CO_2 solutions in acute brainstem slices followed by isolation of intracellular contents and subsequent single-cell RNA-Sequencing (scRNA-Seq) indicate a high degree of 5-HT-neuron specific gene expression profiles with little astroglial gene esxpression. Transcriptomic comparisons of identified genes from individual 5-HT neurons using an unsupervised cluster analyses indicate common transcriptional profiles in pH-sensitive 5-HT neurons, distinct from pH-insensitive profiles. These pilot experiments suggest that this patch-to-Seq approach is superior, and may yield unique transcriptional profiles in electrophysiologically-identified pH-sensitive 5-HT neurons. Ultimately, these experiments have the potential to identify uniquely regulated genes (or other RNAs) that may underlie this vital and unique neuronal function.

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Regulatory factors controlling 5-HT neuron maturation

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Neurons possessing a 5-HT-type transmitter identity are generated between E10.5 and E12.5 in the mouse. While having acquired the capacity to synthesize and reuptake 5-HT, these newborns neurons exist in an immature state as they have not yet migrated to their adult raphe locations, lack adult firing characteristics and have yet to establish afferent and efferent connectivity. Newly generated 5-HT neurons gradually develop mature molecular, morphological and functional features during the remainder of embryogenesis and into at least the third postnatal week. Our recent RNA-sequencing studies have revealed dramatic changes in 5-HT transcriptomes as these neurons mature. Hundreds of genes associated with development of neuronal morphology and excitability are significantly

upregulated while hundreds of other genes associated with earlier stages of 5-HT neuron development are downregulated.

Transcription factors (TFs), Pet1 and Lmx1b, are crucial regulators of postmitotic 5-HT neuron development. These TFs induce 5-HT-type transmitter identity in postmitotic precursors by activation of 5-HT pathway genes: *Tph2, Gch1, Gchfr*, *Slc6a4*, *Slc22a3*, *Slc18a2, Maoa and Maob.* Robust expression of Pet1 and Lmx1b continues through the maturation stage suggesting these TFs may subsequently act to control the genes needed for development of adult morphological and functional characteristics of 5-HT neurons. Indeed, our published and unpublished findings indicate that ongoing Pet1 and Lmx1b function is required for maturation of 5-HT neuron transcriptomes encoding effectors associated with excitability and axonal growth and pathfinding. Loss of Pet-1 function prevents maturation of 5-HT neuron passive and active membrane properties, excitability, and expression of neurotransmitter receptors needed for various kinds afferent inputs. Current studies are revealing major roles for Lmx1b in the formation of the ascending and descending 5-HT axonal architectures. Our findings indicate that Pet1 and Lmx1b are master regulators of 5-HT neuron maturation through their stage-specific control of serotonergic transcriptomes.

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Short oral presentation 5

Orthosteric and Allosteric Activation Mechanisms of the Serotonin 5-HT$_{2B}$ Receptor

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The serotonin 5-HT$_{2B}$ receptor is an important off-target receptor where chronic activation by former anti-migraine medications (e.g. methysergide) has been linked to cardiac valvulopathy. Here we present four structures of the 5-HT$_{2B}$ receptor in complex with methylergonovine, methysergide, lisuride, and LY266097 and biochemical data that identify and characterize key ligand-receptor interactions in the orthosteric and an allosteric binding site responsible for modulating 5-HT$_{2B}$ serotonin receptor activation. These structures reveal that activation mechanisms can occur via the orthosteric binding pocket by ligand engagement with T3.37 or A5.46 at the TM3/TM5 junction, and also via the extended binding pocket with L7.35 in TM7. Importantly, mutations in these regions reveal divergent effects on β-arrestin recruitment, and provide a potential mechanism of ligand bias occurring via ligand-contact with TM7 in the extended binding pocket. These results illuminate structural determinants essential for the serotonin receptor activation and biased signaling, and shed light on atomic activation mechanisms that ultimately lead to cardiac valvulopathy.

Symposium 6

Role of 5-HT$_{1A}$ receptors in animal models of psychosis

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In addition to the widely studied role of dopamine and glutamate NMDA receptors, several studies have shown a role for serotonin, in particular serotonin-1A (5-HT1A) and serotonin-2A receptors in the symptoms of schizophrenia and the mechanism of action of antipsychotic drugs. Animal models allow to unravel the role of serotonin in psychosis-like behaviour. While it is clear that rats and mice can not display the complex symptomatology of this mental illness, individual aspects and neurotransmitter pathways can be studied in detail. We have focused on locomotor hyperactivity as a model of psychosis, and prepulse inhibition as a model of the sensory information processing deficits characteristic of the illness.

Acute treatment with dopaminergic stimulants induces locomotor hyperactivity in rats and mice. This effects can be significantly enhanced after chronic pretreatment with such stimulants and it has been suggested that this reflects a similar dopaminergic sensitization as is seen in schizophrenia. To assess the involvement of 5-HT1A receptors in this sensitization phenomenon, we treated 5-HT1A receptor knockout mice and wildtype controls mice chronically with methamphetamine using an escalating dose regimen. Several weeks later, all mice pre-treated with methamphetamine showed significantly greater locomotor hyperactivity in response to an acute challenge injection of this drug when compared to non-pretreated mice. 5-HT1A receptor knockouts showed a moderate increase in the acute effect of amphetamine. However, locomotor sensitization was not different between 5-HT1A knockouts and controls, possibly due to compensatory mechanisms induced by the life-long constitutive knockout of these receptors.

People with schizophrenia show reduced ability to filter sensory input resulting in the individual unable to focus on the relevant stimuli and ignore irrelevant information. This filtering mechanism can be studied in humans as well as experimental animals by methods such as prepulse inhibition (PPI) and paired-pulse gating. We have used both methods to study the role of serotonin receptors in sensory gating.

In line with several previous studies, administration of the 5-HT1A receptor agonist, 8-OH-DPAT, decreased PPI and paired-pulse gating ratios in rats. In contrast, depending on the strain, a range of 5-HT1A ligands do not decrease or even increase PPI in mice, potentially because of differences in baseline serotonergic activity. The effect of 8-OH-DPAT on PPI in rats is mediated by downstream dopaminergic activation. We showed that chronic estrogen treatment could prevent 8-OH-DPAT-induced disruption of PPI most likely by an effect on downstream dopamine receptor and transporter density.

These studies show that 5-HT1A receptors are involved in behavioural animal models of aspects of schizophrenia. This involvement is most likely mediated by downstream activation of mesolimbic dopaminergic activity, consistent with the prevailing hyperdopaminergia model of psychosis. These findings would suggest that altered stimulation of 5-HT1A receptors modulates dopaminergic hyperactivity in psychosis, which could explain some of the beneficial effects of antipsychotic drugs with activity at 5-HT1A receptors. However, the results of unaltered methamphetamine sensitization in 5-HT1A receptor knockout mice illustrate the complexity of serotonin-dopamine interaction in psychosis.

These studies were supported by grants from the NHMRC of Australia.

Effect of serotonin receptors and hallucinogens on temporal discrimination in mice

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Timing deficits are observed in patients with schizophrenia. Serotonergic hallucinogens can also alter the subjective experience of time. Characterizing the mechanism through which the serotonergic system regulates timing will increase our understanding of the linkage between serotonin (5-HT) and schizophrenia and will provide insight into the mechanism of action of hallucinogens. We investigated whether interval timing in mice is altered by hallucinogens and other 5-HT_2 receptor ligands. C57BL/6J mice were trained to perform a discrete-trials temporal discrimination task. In the discrete-trials task, mice are presented with two levers after a variable interval. Responding on lever A is reinforced if the interval is <6.5 s and responding on lever B is reinforced if the interval is >6.5 s. A 2-parameter logistic function is fitted to the proportional choice for lever B (%B responding), yielding estimates of the indifference point (T_{50}) and the Weber fraction (WF, a measure of timing precision). The 5-HT_{2A} antagonist M100907 increased T_{50} whereas the 5-HT_{2C} antagonist SB-242,084 reduced T_{50}, indicating that 5-HT_{2A} and 5-HT_{2C} receptors have countervailing effects on internal clock speed. The hallucinogens 2,5-dimethoxy-4-iodoamphetamine (DOI; 3 mg/kg IP), a 5-HT_2 agonist, and psilocin (0.8 mg/kg SC), a non-selective 5-HT receptor agonist, flattened the response curve at long stimulus intervals and shifted it to the right, increasing T_{50} and the WF. The effects of DOI and psilocin were antagonized by M100907 but were unaffected by blockade of other 5-HT receptors. Similar to DOI and psilocin, the selective 5-HT_{2A} agonist 25CN-NBOH (6 mg/kg SC) increased T_{50} and the WF. These results demonstrate that hallucinogens alter temporal perception in mice, effects that are mediated by the 5-HT_{2A} receptor. It appears that 5-HT regulates temporal perception, suggesting that altered serotonergic signaling may contribute to the timing deficits observed in schizophrenia and other psychiatric disorders.

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Gestational poly I:C followed by post-weaning social isolation alters serotonin, cortical cytokines and cognitive function in rats and provides mechanistic insight into the neurodevelopment of psychosis.

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Early-life social adversity and maternal infection are recognised risks for development of schizophrenia that is associated with glutamatergic hypofunction. Social isolation (SI) of rat pups from weaning is an established neurodevelopmental model causing hyperactivity, reduced social interaction and cognitive deficits accompanied by hippocampal and cortical dysfunction reminiscent of core features of schizophrenia. We examined whether maternal immune activation (MIA, poly(IC) or neonatal phencyclidine (PCP) administration (which both produce similar behavioural changes to SI) enhance the long-term impact of SI. For MIA dams received 10mg/kg poly(I:C) on GD15 (or saline control) and male pups were housed in groups (4/cage, GH) or individually (SI) from weaning (post-natal day, PND 22). Separate

cohorts of rat pups received PCP (10mg/kg PND 7, 9, 11) followed by GH or SI. PCP enhanced the social interaction deficit but had lesser effects on hyperactivity and cognitive deficits produced by SI alone and some of these effects were reversed by the antipsychotic, cariprazine, suggesting predictive validity. MIA-SI prevented the novel arena hyperactivity, impairment in conditioned freezing (associative learning deficit) and the elevation in cortical mTOR activity but had no effect on the social interaction deficit that were produced by SI alone. SI rats had elevated frontal cortical cytokines (IL-1β, IL-2, IL-6, IL-10, IL-12 and TNFα) and p-mTOR:mTOR not seen in MIA-SI. In an attentional set shifting task SI rats exhibited selective impairment in reversal 1 accompanied by reduced orbitofrontal (but not prelimbic) 5-HT:SERT ratio. Cytokine and mTOR alterations (2 weeks post SI) did not precede cognitive/serotonergic dysfunction suggesting they may be a consequence rather than a cause of the SI-induced developmental changes. Gestational MIA (that induced maternal sickness behaviour and weight loss) attenuated frontal cortical responses to isolation rearing and might produce resilience rather than susceptibility to a subsequent SI stress. However, neonatal manipulation of glutamatergic function may enhance the impact of subsequent SI on social behaviour suggesting that both the nature and timing of the dual stressor may be vital to determine their combined impact.

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Short oral presentation 6

GABAergic, not serotonergic, alterations appear to underlie reduced efficacy of 5-HT_6 receptor antagonists in a dual-hit neurodevelopmental model for schizophrenia

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Schizophrenia has a complex aetiology involving early-life environmental factors. One approach to improved preclinical modelling (to understand disease and evaluate therapeutics) incorporates dual neurodevelopmental 'hits', like neonatal phencyclidine then post-weaning isolation rearing of rats (PCP-Iso). Hippocampal slices from PCP-Iso show attenuated glutamate responses to the 5-HT_6 receptor antagonist SB-399885 (but not the $mGluR_7$ antagonist MMPIP; King et al., 2015). The current study extends this by cognitive evaluation of SB-399885 in PCP-Iso versus single-hit isolates (Veh-Iso), then immunohistochemistry for hippocampal 5-HT and calbindin-positive GABAergic interneurons (that express 5-HT_6 receptors; Helboe et al., 2015) to investigate reduced activity of SB-399885 in PCP-Iso.

41 male Lister-hooded rats (Charles River UK) received vehicle or PCP (10mg/kg s.c.) on postnatal day (PND) 7, 9 and 11, before housing in groups (Gr) or isolation from weaning (PND21). They underwent novel object discrimination (NOD: King et al., 2018) three times, following vehicle, SB-399885 or MMPIP (10mg/kg i.p.) on separate days (1-2 week intervals; PND57-80). Brains were collected immediately after the final NOD for immunohistochemistry. Data (n=13-14) were analysed by ANOVA with Sidak's/Tukey's post-hoc.

Veh-Gr discriminated the novel object irrespective of acute treatment ($P<0.05$-0.01). Veh-Iso and PCP-Iso-induced impairments ($P>0.05$) were reversed by MMPIP ($P<0.01$-0.001),

but SB-399885 was only effective in Veh-Iso ($P<0.001$), not PCP-Iso ($P>0.05$). PCP-Iso had unaltered hippocampal 5-HT-immunoreactivity, but fewer calbindin-positive cells throughout the dorsal hippocampus ($P<0.0001$), particularly in strata oriens ($P<0.0001$) and radiatum ($P<0.01$) of CA1. Calbindin (but not 5-HT) immunoreactivity correlated with NOD performance following SB-399885 ($P<0.01$).
Reduced cognitive and glutamatergic responses to SB-399885 in PCP-Iso do not appear due to reduced 5-HT tone, but instead loss or dysfunction of the predominant 5-HT_6 expressing hippocampal interneurons. This study highlights the importance of improved understanding for selection of appropriate preclinical models, especially when disease neuropathology impacts upon pharmacological effects of potential therapeutics.

Funded by the University of Nottingham

TUESDAY JULY 17, 2018

Special symposium A

Mucosal Serotonin Signaling in the Gut: Functions, dysfunctions and therapeutic targets

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Serotonin (5-HT) is an important signaling molecule in the gut, where it is synthesized and released by epithelial enterochromaffin (EC) cells, and by a subset of enteric neurons in the myenteric plexus. 5-HT released from EC cells can activate intrinsic and extrinsic gut reflexes, including motility, secretion, vasodilation, pain, nausea, and vagal digestive reflexes. Because of these roles in intestinal functions and sensation, and because gut functions are altered in response to drugs such as SSRIs that affect 5-HT signaling, many investigations have evaluated whether changes in 5-HT signaling are a feature of inflammatory and/or functional GI disorders. Furthermore, drugs that affect 5-HT signaling have been developed to alleviate GI symptoms.

Intestinal 5-HT levels are elevated in animal models of intestinal inflammation, but they are decreased or unchanged in the intestines of humans with inflammatory bowel disease (IBD). A consistent feature of intestinal inflammation in both animals and humans is a decrease in serotonin transporter (SERT) expression. Decreased SERT expression is also a feature of irritable bowel syndrome (IBS). Studies involving a human colonic epithelial cell line have demonstrated that the pro-inflammatory cytokines, IFN-gamma and TNF-alpha likely mediators of decreased SERT expression and function in colitis and in IBS.

Serotonin-related compounds that have been developed for therapeutic treatments of GI symptoms include the biosynthetic enzyme, tryptophan hydroxylase 1 inhibitors (diarrhea), 5-HT3 antagonists (diarrhea, nausea), and 5-HT4 agonists (gastroparesis, constipation). Recent pre-clinical evidence suggests that epithelial 5-HT4 receptors could provide a safe and effective target for the treatment of constipation and IBD.

In summary, 5-HT is an important signaling molecule in the gut and gaining a more thorough understanding of mucosal serotonin signaling is leading to novel therapeutic targets.

Spohn and Mawe (2017) *Nature Reviews Gastroenterology and Hepatology* PMC5672796

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A tale of two amines: microbial mediators of gastrointestinal motility and secretion

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The human gut is a diverse ecosystem harboring trillions of microbial cells and hundreds of microbial species. Like other microbial ecosystems, it responds to changes in environment

including nutrient availability (e.g., diet) and antibiotic use. Gut resident microbes and their interactions with the host can benefit the host, but have also been implicated in diseases such as irritable bowel syndrome (IBS). There is now an increased awareness regarding the role of the gut microbiome in modulating gastrointestinal function. However the complex signaling between microbes and their human hosts, and the resulting impact on normal physiological functions, is still poorly understood. We have described two potential mechanisms by which gut microbiota alter host GI physiology. We found human- and mouse-derived gut microbiota promote serotonin (5-HT) biosynthesis through stimulatory activities of SCFAs on EC cells. We also found gut microbiota can alter host secretory response to 5-HT, in part by an effect on 5-HT_3R expression via acetate production. Our findings provide a potential link between dysfunction of serotonergic system and altered gut microbiota both of which have been implicated in IBS. Microbial conversion of dietary substrates into small bioactive molecules represents another potential regulatory mechanism by which gut microbes can alter intestinal physiology. One such example is tryptamine, a monoamine similar to 5-HT which results from decarboxylation of tryptophan and can bind 5-HT receptors. While production of tryptamine is not as common among human commensal bacteria, we found *Ruminococcus gnavus* and *Clostridium sporogenes* express tryptophan decarboxylase and hence can decarboxylate tryptophan to tryptamine. We found bacterially produced tryptamine can increase intestinal secretion by activating G-protein coupled receptor 5-HT_4R. Together these findings provide mechanisms by which gut microbes and their metabolites interact with the host serotonergic system resulting in a change in GI physiology. This provides new targets for novel microbiota-directed therapies for treatment of diseases like IBS.

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The gut microbiome, brain function and behaviour: Focus on microbial checkpoints for host serotonin and tryptophan metabolism

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The gut microbiome can signal along the gut-brain axis to influence many fundamental aspects of brain function and behaviour. Serotonin functions as a key neurotransmitter at both terminals of this communication network and there is a striking overlap between behaviours influenced by the gut microbiota and those which rely on intact serotonergic neurotransmission. This range of behaviours include those relevant to depression, anxiety and pain as well as host stress physiology. Exposure to stressors can also alter microbiome-gut-brain axis signalling by modulating the serotonergic system. Threats to the optimal assembly and composition of the gut microbiota, which overlap sensitive developmental periods for the host serotonergic system, can result in long-term effects on physiology and behavior, at least in animal models.
Research efforts continue to identify the precise mechanisms underpinning these effects and possible routes of communication include the vagus nerve, the neuroendocrine system and immune factors. There is now an increasing focus on microbial regulation of tryptophan metabolism and the serotonergic system arising from a number of key observations taken from a variety of strategies used to parse the role of microbiota in brain function include germ free animals, antibiotic treatments, dietary manipulations, probiotics and prebiotics.

Microbiota-deficient animals in particular consistently show alterations in the availability of tryptophan, frequently associated with serotonergic alterations in the CNS.
Bacteria within the lumen of the bowel can also influence local serotonin synthesis and release by enterochromaffin cells. Direct or indirect microbial control over metabolism of tryptophan along the kynurenine pathway can also yield neuroactives that may be important in the neurobiological basis of gut-brain axis communication. Prototypical clinical gut-brain axis disorders such as irritable bowel syndrome are associated with aberrations in the serotonergic system and tryptophan metabolism. These translational insights will be critical as we move promising preclinical research towards mechanisms and therapeutic targeting of the gut microbiome in the clinical setting.

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The gut microbiome in pediatric autism spectrum disorder as a model for the interconnectivity of serotonin with changes in microbes, GI symptoms, and behavior.

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It is now well established that the microbiome-gut-brain axis plays a critical role in the manifestation of a variety of symptoms associated with autism spectrum disorder (ASD). Using pediatric ASD as a model, here we will discuss the mucosal microbiome as it relates to both serotonin and neuroimmune profiles, the gut microbiome and metabolome in the largest stool-based study in ASD to date, and a case study detailing parallel changes along the microbiome-gut-brain axis. Comprehensive clinical data, including behavioral and gastrointestinal (GI) phenotypes, have been analyzed in parallel with microbiome and metabolome results to create novel multi-omic profiles.

For the mucosal microbiome study, paired rectal biopsy and blood specimens from ASD children with functional GI disorders (FGID) were compared to typically developing (TD) children with and without abdominal pain. In addition to microbiome characterization, inflammatory cytokines and serotonergic metabolites were quantified and correlated with GI symptoms. For the study of the gut microbiome in >400 children, extensive clinical history was obtained as well as data from several behavioral surveys and a two-week diary detailing diet, stooling pattern, and GI pain. Stool specimens were collected from pediatric subjects with ASD, unaffected siblings, and unrelated healthy TD children. Microbiome characterization and global metabolomics were performed. Multiple bioinformatics and biostatistical approaches were utilized to identify individual organisms and metabolites of interest, with particular emphasis on correlation with the serotonin pathway.

Differences in both bacterial composition and diversity were observed across groups in both the mucosal and stool-based microbiomes. The greatest shifts in the gut microbiome

were associated with GI pain, with distinct differences noted in the ASD group that reported pain. Several of these organisms were previously reported as increased in ASD. These organisms were also associated with a variety metabolites, including metabolic pathways associated with serotonin metabolism. A longitudinal case study of daily stool samples in a child with ASD who experienced a GI episode also yielded evidence of increases in specific organisms and metabolites during a GI exacerbation, with increases in self-injurious behavior noted in parallel. Behavioral patterns and overall severity were also associated with a variety of microbial profiles.

Distinct differences exist in the gut microbiome and metabolome of children with ASD compared to their typically developing peers, with the serotonin pathway consistently playing a role in the differentiation of not just ASD and TD children but in the differentiation of ASD children with and without GI symptoms. Within the spectrum of autism, subgroups can be identified based on complex phenotypes composed of behavioral characteristics, GI symptoms, and microbiome/metabolome profiles, and these subgroup designations will aid in future treatment selection.

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Modulating the Role of Serotonin in Enteric Dysfunction

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ABSTRACT RELEASED AT LATER DATE

5-HTP slow-release as a new therapeutic approach

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The biological factors that determine whether an individual develops depression-related mental illness or responds adequately to pharmacotherapy still remain enigmatic. Converging evidence suggests involvement of deficiency of the brain serotonin (5-HT) system. To evaluate the importance of brain 5-HT levels in determining stress susceptibility and antidepressant responses, we have used a genetic model of 5-HT deficiency with face and construct validity. The tryptophan hydroxylase 2 (R439H) knock-in (Tph2KI) mice express a loss-of-function mutant form of the *Tph2* gene identified in a cohort of elderly depressed humans, and display 60-80% reductions in the levels of brain extracellular 5-HT (5-HT_{Ext}) (Zhang et al., Neuron 2004; Jacobsen et al., *Mol. Psychiatry*, 2012). While this *TPH2* mutation is very rare in humans, the Tph2KI mice arguable represent a naturalistic model of general 5-HT deficiency. Our results show that brain 5-HT deficiency reduces the threshold at which mice display social avoidance behavior following repeated psychosocial stress in the paradigm of social defeat. In addition, we also demonstrate that 5-HT deficiency prevents the ability of chronic fluoxetine administration to elevate 5-HT_{Ext} and to reverse stress-induced behavioral avoidance. Associated with these phenotypes are changes in gene expression in nucleus accumbens, frontal cortex and amygdala.

We also used Tph2KI mice as a model to help validating a novel concept of SSRI-augmentation therapy for treatment resistant depression (TRD) – adjunctive 5-HTP slow release (5-HTP SR). A limited number of older small clinical studies have suggested that the immediate precursor of 5-HT, 5-HTP, augments the efficacy of 5-HTergic antidepressants. But 5-HTP's rapid absorption and short half-life limit 5-HTP as an effective therapy. Using mini-pumps to model 5-HTP SR in mice, we previously showed that 5-HTP SR synergistically augments the 5-HT_{Ext} elevating effects of chronic SSRI treatment, in both WT and Tph2KI mice, with no apparent adverse effects. In fact, when modeling oral 5-HTP SR by 5-HTP via the food, 5-HTP SR elevated 5-HT_{Tissue} in the frontal cortex, hippocampus, and nucleus accumbens, irrespective of fluoxetine co-treatment. Oral 5-HTP SR also elevated 5-HIAA_{Tissue} in all regions indicating effective brain penetration and normal metabolic processing. Further, oral 5-HTP SR did not interfere with vesicular storage of DA. Moreover, adjunctive oral 5-HTP SR caused no adverse effects on motor activity, motor coordination, working memory, social behavioral, and sexual behavioral measures. Likewise, as opposed to normal/acute 5-HTP administration, adjunctive oral 5-HTP SR caused no diarrhea or head twitches. Further, oral 5-HTP SR augmented the effects of SSRI treatment in 5-HT-sensitive behaviors and gene-transcription. 5-HTP had oral bioavailability acceptable for drug therapy.

When seen in light of clinical therapeutic evidence with 5-HTP and in light of 5-HT clinical pharmacology, these mouse data suggest that oral adjunctive 5-HTP SR would be a safe and effective approach to enhance the antidepressant efficacy of SSRIs, when SSRI treatment alone is insufficient. Moreover, 5-HTP SR may have therapeutic potential, in additional CNS indications, and in indications beyond the CNS.

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Disturbances in the 5-HT system in Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a complex gastrointestinal (GI) disorder in which pain and disturbed gut motility are major symptoms. Furthermore, patients often suffer from pain syndromes and psychiatric comorbidity such as anxiety and depression. The pathogenesis of IBS is complex with intrinsic (genetics, microbiota) and extrinsic factors (infection, stress, nutrition, life style) leading in concert to the manifestation of the phenotype. To date, neither a specific diagnostics nor targeted therapy is established owing to the poor understanding of the various pathomechanisms involved. Consequently, patients are often treated on a *trial and error* basis. Diagnostics is purely symptom-based according to Rome IV criteria classifying patients based on their major stooling pattern as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), mixed form (IBS-M) or unclassified (IBS-U).
Serotonin is a key regulator in GI function and involved in the regulation of gut motility, secretion and visceral perception which are known to be disturbed in IBS. Increasing evidence points to impaired serotonin re-uptake and release, metabolic as well as receptor function in IBS. Furthermore, serotonergic drugs have been proven to be beneficial at least in subsets of IBS patients. This talk will wrap up the current knowledge on the role of the 5-HT system in IBS, in particular taking crucial players such as the tryptophan hydroxylase 1 (TPH1), the serotonin transporter SERT and 5-HT_3 and 5-HT_4 receptors and their genetic and epigenetic modulation and interplay with the environment into account. Furthermore, an outlook on clinical relevance of these findings and potential targeted interventions (pharmacogenetic approaches) will be given.

Serotonin initiates intestinal inflammation, defends enteric neurons from it, and contributes to the pathogenesis of gastrointestinal disorders

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Intestinal inflammation is a defensive reaction of the bowel to potentially harmful stimuli. When it is initiated inappropriately or excessive intestinal inflammation causes dysfunction, as in inflammatory bowel disease (IBD) and necrotizing enterocolitis, which is responsible for a great deal of morbidity and mortality in premature infants. Endogenous signaling molecules, including 5-HT from enterochromaffin (EC) cells and enteric neurons, are important regulators of intestinal inflammation. EC cell 5-HT may initiate intestinal inflammation in response to microbial provocation and also increases the severity of the inflammatory response. Enteric neuronal 5-HT exerts an opposing effect, which is both neuroprotective and anti-inflammatory. Because of the importance of 5-HT, the 5-HT transporter (SERT), which in the gut is expressed both in the mucosal epithelium and neurons, plays a critical role in modulating the pro-inflammatory drive that 5-HT provides. In experimental intestinal inflammation and NEC, therefore, inhibition or deletion of SERT amplifies, while genetic gain-of-function mutations in SERT diminish, inflammatory manifestations. Deletion of tryptophan hydroxylase 1 (TPH1) or mucosally restricted TPH inhibition of ameliorates intestinal inflammation and NEC. The importance of 5-HT in intestinal inflammation suggests that mucosally restricted TPH inhibition, which depletes 5-HT only in the intestinal lining may have therapeutic value in IBD and NEC; moreover, effects on the bowel should be considered when using anti-depressants that inhibit SERT.

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The Lead Candidate Drug CSTI-300; a novel 5-HT_3 receptor partial agonist with potential to treat patients with irritable bowel syndrome and carcinoid syndrome

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Background and purpose: The 5-hydroxytryptamine (5-HT; serotonin) 5-HT_3 receptor is an excitatory ligand-gated ion channel expressed in for example the brain and the gastrointestinal tract. It represents a clinically effective target for antagonists, such as alosetron, to deliver symptomatic relief to patients with diarrhoea-predominant irritable bowel syndrome (IBS-d) and carcinoid syndrome, consistent with these pathologies being associated with elevated levels of 5-HT. Unfortunately, this pharmacological strategy can present side effects (e.g. severe constipation, ischemic colitis) that limit the use of 5-HT_3 receptor antagonists in IBS-d whereas 5-HT_3 receptor antagonists are prescribed 'off-label' for symptomatic relief from carcinoid syndrome. The present study investigated the potential of a novel 5-HT_3 receptor partial agonist, CSTI-300, to treat patients with IBS-d, and other conditions with diarrhoea as a symptom (e.g. carcinoid syndrome) that are responsive to 5-HT_3 receptor antagonists, with a predicted reduced side-effect profile compared to antagonists.

Experimental approach: The *in vitro* and *in vivo* pre-clinical pharmacology of the drug CSTI-300 was investigated to explore the potential to treat patients with IBS-d and carcinoid syndrome.

Key results: CSTI-300 displayed selective high affinity as a competitive ligand for the orthosteric site of the human (h) 5-HT_3 receptor (K_i approximately 2.0 nM; no other receptor interaction identified in a Cerep selectivity screen) and acted as a partial agonist (approximately 30-40% intrinsic efficacy relative to the endogenous agonist, 5-HT) *in vitro*. In an *in vivo* model of IBS-d that translates to the clinic, the rat colon distension model, CSTI-300 displayed dose-dependent efficacy with maximal effects at least comparable to a maximal effective dose of alosetron. In addition, oral administration of CSTI-300 to normal, healthy dogs that achieved plasma levels of the drug exceeding the K_i value for the 5-HT_3 receptor failed to either evoke emesis or alter the state of faeces. Pharmacokinetics for CSTI-300 across four species (mouse, rat, dog and mini-pig) identified high levels of oral availability with $t_{1/2}$ values in the range of 2.4-4.4 hours.

Conclusions and implications: The pre-clinical pharmacology of the Lead Candidate Drug, CSTI-300, supports the potential of this novel drug to offer symptomatic relief to patients with IBS and carcinoid syndrome with a rationale for a reduced 'on-target' side-effect profile relative to 5-HT_3 receptor antagonists such as alosetron. CSTI-300 has now entered IND-enabling studies as development moves towards clinical assessment.

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Special symposium B

Novel crosstalk and heterodimerization of the 5-HT2 receptor family with the oxytocin receptor and its potential for social behavior

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The serotonin (5-HT) hypothesis of major depression has been at the center of understanding the neurochemical basis of mood disorders. In addition to the role of 5-HT as a key neurotransmitter in mood, 5-HT has also been shown to be crucial in reciprocal social interactions and social abnormalities, seen across the majority of psychiatric disorders with altered mood and sociability such as anxiety, depression, autism and schizophrenia. The 5-HT2C and 5-HT2A receptors are key serotonergic receptors playing a role in the centrally-mediated pathways of anxiety and mood. The oxytocin receptor (OTR) is another major player in the development of sociability and mood. Interestingly, OTR expression in serotonergic neurons has been shown to exert an anxiolytic effect, while administration of oxytocin results in altered serotonergic innervation. Although both neurotransmitters and their receptors have been shown to affect one another, the exact mechanism of this is unknown, which prompted us to investigate receptor crosstalk between the 5-HT2C/2A and the OTR.

We show original observations demonstrating crosstalk between the OTR and the 5-HT2A and 5-HT2C receptors with remarkable downstream signalling consequences. Physical interaction of the 5-HT2A/OTR and the 5-HT2C/OTR pair is demonstrated by co-localized expression, confocal microscopy, ligand-mediated internalization and flow cytometry based FRET (fcFRET). Calcium mobilisation and IP-1 accumulation reveal a significant decrease in OTR Gq-mediated signalling in HEK293A cells co-expressing both heterodimer pairs. Further investigation demonstrated that attenuated OTR signalling is restored following treatment with 5-HT2C antagonists, SB242084 and RS102221, but not with 5-HT2A ligands.

Further *in vivo* studies exploring the functional nature of the interaction between the OTR and the 5-HT2A and 5-HT2C receptors are now warranted. The existence of novel 5-HT_{2A}/OTR and 5-HT_{2C}/OTR receptor heterodimers is poised to lead to more selective current and future pharmacotherapies for the treatment of mood and social disorders.

FGFR1-5-HT1A Heteroreceptor Complexes in the Raphe-Hippocampal serotonin System and their disturbances in a Genetic Rat Model of Depression

Dasiel Oscar Borroto-Escuela[1], Patricia Ambrogini[2], Maria Lindskog[3], Kjell Fuxe[1]

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The ascending serotonin neurons from the mesencephalic raphe play a significant role in depression by modulating the critical limbic neuronal circuitry. Bioluminescence resonance energy transfer, co-immunoprecipitation, in situ proximity ligation assay, binding assay, in cell western and the forced swim test were used to demonstrate the FGFR1-5-HT1A

heteroreceptor complexes in the raphe-hippocampal neurons in the Sprague-Dawley rat. The combined acute and repeated intracerebroventricular treatment with FGF2 and 8-OH-DPAT was found to produce evidence of highly significant antidepressant actions in the forced swim test.
The findings also indicated that in the Flinders sensitive line rats (FSL) compared with SD rats changes can take place in the ability of 8-OH-DPAT and combined FGFR1 and 5-HT1A agonist treatment to increase the density of FGFR1-5-HT1A heteroreceptor complexes of the dorsal raphe. It was proposed that deficits of this type found in FSL rats may reflect a failure of the combined agonist treatment to uncouple the 5-HT1A autoreceptors from the G protein regulated inwardly rectifying potassium channels. This may contribute to the failure of producing antidepressant-like effects in the FSL rat by combined agonist treatment as seen in the SD control rat in the forced swim test. The antidepressant-like effects seen with the 5-HT1A agonist alone treatment in FSL but not in SD rats may instead involve significant increases in the FGFR1-5-HT1A complexes of the CA2 and CA3 areas of the hippocampus. Thus, disturbances may have developed in the enhancing allosteric FGFR1-5-HT1A receptor-receptor of this receptor complex in the hippocampus in this genetic rat model of depression.

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Evidence for the existence of 5-HT1A-5-HT2A isoreceptor complexes in the brain with antagonistic allosteric receptor-receptor interactions

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5-HT1A and 5-HT2A are two major 5-HT receptor subtypes in the brain with 5-HT1A having inhibitory actions via Gi/o and 5-HT2A excitatory actions via Gq/11. It is therefore of high interest that using *in situ* proximity ligation assay (PLA) it was possible to demonstrate the existence of 5-HT1A-5-HT2A isoreceptor complexes in the dorsal hippocampus and the anterior cingulate cortex. The findings were supported by the demonstration of these complexes in cellular models using a BRET^2 saturation assay. In the dorsal hippocampus of untreated SD rats, a high density of PLA positive clusters was found in the pyramidal cell layer of the CA1-CA3 regions, while only a few were found in the stratum oriens and radiatum of these areas. However, a highly significant and marked reduction of the density of PLA positive clusters was observed in the CA1 and CA2 regions 24h after a forced swim session indicating the dynamics of this 5-HT isoreceptor complex. Antagonistic allosteric receptor-receptor interactions likely exist in this isoreceptor complex since a standard 5-HT2A agonist TCB2 significantly and markedly reduced the affinity of the 5-HT1A agonist ipsapirone for the 5-HT1A agonist binding sites in the frontal lobe using the 5-HT1A radioligand binding assay. This action was blocked by the 5-HT2A antagonist ketanserin. It is proposed that the demonstrated 5-HT1A-5-HT2A heteroreceptor complexes may play a role in depression through integration of 5-HT recognition, signalling and trafficking in the plasma membrane in two major 5-HT receptor subtypes known to be involved in depression.

Maturation and trafficking of inter-family GPCR complexes

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Receptor trafficking, which involves maturation and insertion of newly synthesized G protein-coupled receptors (GPCRs) to the cell membrane as well as processes related to internalization followed by subsequent recycling or degradation, plays a fundamental role in controlling the net density of GPCRs at the cell surface. Family A 5-HT_{2A}R and family C mGluR2 are GPCRs primarily coupled to $G_{q/11}$ and $G_{i/o}$ proteins, respectively. These two receptors participate in brain processes such as perception, cognition and sensorimotor gating, and have been involved in psychiatric conditions such as schizophrenia, depression and alcoholism. Previous findings demonstrate that 5-HT_{2A}R and mGluR2 receptors are able to form heteromeric complexes in living mammalian cells. In unstimulated cells, most of the GPCRs are normally located on the cell's surface at the plasma membrane. However, certain GPCRs, including the 5-HT_{2A}R, have a significant intracellular presence. Thus, visualization of individual living cells shows that, at steady state, the bulk of 5-HT_{2A}Rs is present in punctate, intracellular vesicles. In the present study, we aimed to assess the specific components of the intracellular trafficking pathway followed by 5-HT_{2A}R alone, and complexed with mGluR2 in HEK293 cells. Our results indicate that 5-HT_{2A}R and mGluR2 are assembled as a GPCR heteromeric complex at early stages of the maturation trafficking pathway. We also show that the mGluR2 receptor co-localizes with the 5-HT_{2A}R within the same population of endocytic vesicles. This pattern of colocalization was not observed in living cells co-expressing 5-HT_{2A}R and either mGluR3 or mGluR2ΔTM4N (an mGluR2/mGluR3 chimeric construct that is not able to form the 5-HT_{2A}R-mGluR2 complex). Furthermore, exposure to the 5-HT_{2A}R agonist DOI resulted in increased co-localization of mGluR2 with the endosomal marker Rab5. This effect of 5-HT_{2A}R on mGluR2 receptor trafficking was also observed in mouse cortical neuron cultures. Together, these findings suggest that inter-family GPCR heteromerization affects maturation and trafficking.

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Ligand-induced regulation of serotonin and dopamine receptor homomers

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A wide range of both biochemical and biophysical techniques have been employed to show that many rhodopsin-like, Class A, G protein-coupled receptors are able to form, at least transiently, multimeric complexes. For various serotonin receptor subtypes both homomeric and heteromeric complexes have been detected. Key questions that remain unresolved include the stability of such complexes, if they are regulated by the binding of agonist and antagonist ligands, whether they can be observed in native tissues and how organisational structure beyond monomer affects signalling functionality and ligand efficacy. We have been approaching each of these questions using the serotonin 5-HT_{2c} and the dopamine D3 receptors as exemplars.
By illustrating the power of Spatial Intensity Distribution Analysis (SPIDA)[1] and other techniques that combine molecular brightness analysis and statistical interrogation of fluorescence fluctuations of an appropriately fluorophore-tagged receptor I will assess (a) the steady state proportion of monomers, dimers and oligomers of these receptors, (b) whether

this is regulated by receptor expression level and/or by drug treatments, (c) whether effects of ligands are chemotype-dependent and (d) the molecular basis for receptor-receptor interactions.

1. Pediani et al (2018) Trends Pharmacol Sci. **39,** 175-186.

Biophysical Validation of Serotonin 5-HT_{2A} and 5-HT_{2C} Receptor Interaction

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The serotonin (5-HT) 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. Here, we utilize three distinct biophysical and immunocytochemistry-based approaches to identify and study this receptor complex in cultured cells. Employing a split luciferase complementation assay (LCA), we demonstrated that formation of the 5-HT_{2A}R:5-HT_{2C}R complex exists within 50 nm, increases proportionally to the 5-HT_{2C}R:5-HT_{2A}R protein expression ratio, and is specific to the receptor interaction and not due to random complementation of the luciferase fragments. Using a proximity ligation assay (PLA), we found that cells stably expressing both the 5-HT_{2A}R and 5-HT_{2C}R exhibit 5-HT_{2A}R:5-HT_{2C}R heteroreceptor complexes within 40 nm of each other. Lastly, bioluminescence resonance energy transfer (BRET) analyses indicates the formation of a specific and saturable 5-HT_{2A}R:5-HT_{2C}R interaction, suggesting that the 5-HT_{2A}R and 5-HT_{2C}R form a close interaction within 10 nm of each other in intact live cells. The bioengineered receptors generated for the LCA and the BRET exhibit 5-HT-mediated intracellular calcium signaling as seen for the native receptors. Taken together, this study validates a very close 5-HT_{2A}R:5-HT_{2C}R interaction in cultured cells. Ongoing research will employ these technologies to further investigate the formation and function of the receptor complex.

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Specific subsets of 5-HT2C receptors regulate appetite, body weight and glucose homeostasis

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Obesity has become one of the major health concerns of this century because of its prevalence and resistance to treatment. Specifically, one in four people in the UK are currently clinically obese. Significant progress has been made over the past decade in clarifying brain neurochemicals and regions regulating energy homeostasis. The 5-hydroxytryptamine (5-HT, serotonin) 2C receptor (*Htr2c*; 5-HT_{2C}R) agonist lorcaserin (Arena Pharmaceuticals) is a new medication for obesity that also improves type 2 diabetes.

However, the neural circuits mediating lorcaserin's therapeutic effects remain to be elucidated. We observed that preventing *Pro-opiomelanocortin* (*Pomc)* expression (*Pomc*^{NEO}) within the arcuate nucleus of the hypothalamus (ARC) abolished lorcaserin's anorectic and glucoregulatory effects and that restoration of *Pomc* specifically within a subset of ARC neurons expressing 5-HT$_{2C}$Rs (*Pomc*^{Htr2c}) is sufficient to mediate lorcaserin's therapeutic effects. Using a combination of viral technology and genetics, we reveal a downstream circuit through which lorcaserin influences appetite, but not glycaemic effects. Specifically, lorcaserin suppresses appetite via downstream melanocortin4 receptors (*Mc4r)*. Moreover, brain-derived neurotrophic factor (*Bdnf*) neurons within the ventromedial nucleus of the hypothalamus (*Bdnf*^{VMN}) mediate lorcaserin's anorectic effect in a melanocortin4 receptor (*Mc4r)*-dependent manner. We next investigated the circuit through which lorcaserin influences glucose homeostasis. We observed that lorcaserin improves insulin sensitivity and suppresses hepatic glucose production (HGP) and that lorcaserin requires functional *Mc4rs* to elicit glycaemic effects. On a *Mc4r* null background, the selective restoration of *Mc4r* function within ChAT neurons (*Mc4r*^{ChAT}) is sufficient to mediate lorcaserin's glucoregulatory, but not anorectic effects. Thus, our results reveal a divergence in the neurocircuitry underpinning lorcaserin's anorectic and glycaemic therapeutic effects.

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Serotonergic modulation of aversive memory via 5-HT$_{1A}$ receptor signaling in the BNST

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Serotonin (5-hydroxytryptamine; 5-HT) orchestrates emotional responses to stress through a variety of pre- and postsynaptic receptors distributed across functionally diverse neuronal networks in the central nervous system. Efferent projections from the dorsal raphe to the bed nucleus of the stria terminalis (BNST) are generally thought to enhance anxiety and aversive learning by activating 5-HT$_{2C}$ receptor (5-HT$_{2C}$R) signaling (Ravinder et al., 2012; Marcinkiewcz et al., 2015; Marcinkiewcz et al., 2016; Pelrine et al., 2016), although an opposing role for 5-HT at 5-HT$_{1A}$Rs has recently been suggested (Garcia-Garcia et al., 2017). Furthermore, the precise contribution of pre- and postsynaptic 5-HT$_{1A}$Rs to these behavioral outcomes is still unclear. In the present study, we sought to delineate a role for postsynaptic 5-HT$_{1A}$Rs in the BNST in aversive behaviors using a transgenic mouse line containing a conditional knockout of the 5-HT$_{1A}$R. Site-specific infusion of an adeno-associated viral vector (AAV-cre-GFP) into the BNST selectively ablated 5-HT$_{1A}$Rs from postsynaptic neurons in order to assess their contribution to anxiety and fear-related behavior. Both males and females were tested to tease out sex-specific effects. We found that postsynaptic deletion of 5-HT$_{1A}$Rs in the BNST did not significantly alter anxiety-like behavior under high or low stress conditions in either sex but heightened both cued and contextual fear recall in male mice. Deletion of 5-HT$_{1A}$Rs in the BNST also enhanced basal excitability of BNST neurons after contextual recall in fear conditioned mice, but not in unconditioned mice. These results suggest that postsynaptic 5-HT$_{1A}$R signaling can buffer against aversive memory in a sex-specific manner without affecting anxiety-like behavior. Ongoing studies using whole-mount fos immunolabeling (iDISCO) will elucidate how 5-HT$_{1A}$R signaling in the BNST shapes global brain networks recruited during contextual fear recall. Together, these studies will reveal important insights into serotonergic modulation of

aversive memory, which may lead to new therapeutic targets for psychiatric disorders like PTSD.

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Opposite Control by 5-HT2C Receptors of Generalized and Focal Seizures

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The 5-HT_{2C} receptor (5-HT_{2C}R) is thought to be involved in neuronal excitability and seizure generation. 5-HT_{2C}R knockout mice display spontaneous generalized convulsive seizures which cause a high mortality rate and a reduced threshold for various convulsing stimuli. Conversely, 5-HT_{2C}R activation increased the threshold of generalized convulsive seizures induced by pentylenetetrazole and electroshock in mice.

Here we show the 5-HT_{2C}R also negatively controls non-convulsive generalized seizures, in the polygenic animal model of absence epilepsy, the Genetic Absence Epilepsy Rat from Strasbourg. RO60-0175, lorcaserin and CP809101 were capable of halting absence seizures. Interestingly, as expected, SB242084 blocked the effect of lorcaserin and CP809101, but also showed some anti-absence effects. One possible mechanism by which 5-HT_{2C}R activation exerts antiepileptic effects is via the normalization of the aberrant $GABA_A$R tonic inhibition in the ventrobasal thalamus which has been seen in different animal models of absence epilepsy.

The 5-HT_{2C}R system seems instead devoid of any modulatory role in focal onset seizures, or paradoxically, has a pro-epileptic role in this type of epilepsy. Indeed, we observed that mCPP and lorcaserin, but not RO60-0175, were able to halt hippocampal afterdischarges in a rat model of temporal lobe epilepsy, an effect potentiated and insensitive to SB242084 pretreatment. We confirmed this evidence in the pilocarpine-model of status epilepticus in rats, where the RO60-0175 was devoided of any anti-seizures effect.

In summary, 5-HT_{2C}R agonists may have new therapeutic utility in generalized (both convulsive and non-convulsive) but not in focal epilepsy.

Symposium 7

Brainstem Dysfunction in Neuropsychiatric Disorders – AD/PD/Depression

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Despite the fundamental role of the brainstem in regulating vital functional abilities such as arousal, breathing, autonomic nervous system activity as well as regulating all higher cerebral functions via neurotransmitter projections systems originating in the brainstem, the role of the brainstem has received relatively little attention in most neuropsychiatric disorders. Besides the dorsal and median raphe nuclei complex comprising mainly serotonin-producing neurons, the brainstem also contains noradrenalin, dopamine and histamine-producing nuclei, i.e. resp. the locus coeruleus, the substantia nigra and the mamillary bodies. The brainstem is furthermore the relay station of afferent and efferent projections between the autonomic nervous system in the peripheral body and higher cerebral brain regions. The current presentation aims to review the neuroanatomy of the brainstem as well as the current status on findings, derived from a wide range of studies using molecular, cellular and imaging technologies, of brainstem involvement in neurodevelopmental (i.e. autism, schizophrenia) and neurodegenerative disorders (Alzheimer's and Parkinson's disease).
Over the past decades, the incidence of age-related, neurological and psychiatric disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), but also depression has considerably increased. Mood disorders are strongly related to the exposure to stress. The hippocampus and other forebrain structures are the apex of the stress hormone control mechanism and damage to them may be one way in which stress hormone secretion escapes from inhibitory control in depression. In turn, stress, probably through toxic effects of glucocorticoids, decreases neurogenesis and cell survival while antidepressants enhance these processes in experimental animals. Therefore, since treatment strategies are not yet available, primary prevention in these age-related and stress related neurological disorders is of importance. As mentioned before most of the focus on neurobiological questions on above mentioned disease are related to forebrain structures since they are often associated with cognitive dysfunction. The brainstem is a highly neglected brain area in neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's (PD) disease and frontotemporal lobar degeneration. Likewise, despite a long-standing recognition of brainstem involvement, relatively few studies have addressed the exact mechanisms that underlie brainstem autonomic dysfunction. Improved insight in the cellular and molecular characteristics of brainstem function is pivotal to study the developmental origins. As brainstem dysfunction also poses health issues in several other, neurodegenerative, disorders (like AD and PD), progress in these neurological fields will benefit from scientific advancement in the current proposal as well. In the area of depression, several observations have been made in relation to changes in one particular brain structure: the Dorsal Raphe Nucleus (DRN). In addition dysfunction of the cerebellum is also observed in AD and associated with pulmonary deregulation. The DRN is also related in the circuit of stress regulated processes and cognitive events. In order to gain more information about the

underlying mechanisms that may govern the neurodegeneration, e.g. amyloid plaques, neurofibrillary tangles, and impaired synaptic transmission in AD, a rat dissociation culture model was established that allows mimicking certain aspects of our autopsy findings. We observed a similar phenomenon in brains from patients suffering from neurodegenerative disease since this also related to changes in BDNF levels. The ascending projections and multitransmitter nature of the DRN in particular and the brainstem in general stress its role as a key target for AD/PD research and autonomic dysfunction. It also points towards the increased importance and focus of the brainstem as key area in various neurodevelopmental and age-related diseases.

Serotonergic neurons signal reward rate during dynamic decision making

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Decisions take place in dynamic environments. The nervous system must continually learn the best actions to obtain rewards. In the theoretical framework of optimal control and reinforcement learning, policies (the probability of performing an action given a state of the environment) are updated by feedback arising from errors in the predicted reward. Whereas these reward prediction errors have been mapped to dopaminergic neurons in the midbrain, how the decision variables that generate policies themselves are represented and modulated is unclear. Here, we trained mice on a dynamic foraging task, in which they freely chose between two alternatives that delivered reward with changing probabilities. We found that corticostriatal neurons, in the medial prefrontal cortex (mPFC), maintained persistent changes in firing rates that represented relative and total action values over long timescales. These are consistent with control signals used to drive flexible behavior.

We next recorded from serotonergic neurons in the dorsal raphe in the same task, to determine whether their firing rates tracked ongoing variables that could be used to modulate the decision variables in mPFC. We found that serotonergic neurons represented reward rate over long timescales (tens of seconds to minutes). These signals are consistent with modulatory signals used to regulate the robustness of ongoing decision variables.

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Dual processing in the primate dorsal raphe neurons for decision making under different mood

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Serotonin, like dopamine, is a monoamine essential for normal brain function. However, the nature of the function of serotonin system has been still mysterious. We therefore studied single neurons' activity of the dorsal raphe nucleus (DRN), a major source of serotonin, while monkeys performed cognitive tasks.

In a Pavlovian procedure with an appetitive or aversive context where a reward or an airpuff was delivered probabilistically, we found that more than half of the neurons

discriminated between appetitive and aversive contexts by tonic modulation in activity. In the appetitive context, they then kept track of moment-to-moment changing expected reward value indicated by the conditioned and unconditioned stimuli, phasically and tonically. In the aversive context, the same neurons maintained tonic modulation in their activity throughout the block, with much less responses to the cues or outcomes. These results suggest that single DRN neurons encode both appetitive and aversive information, but with different time scales: a tonic one to discriminate emotional contexts, and a phasic one to encode rewarding events.

Such temporally distinct DRN neuronal activities may provide the neural basis of alternation of decision making under different emotional contexts. To test this hypothesis, we developed a reversal choice task in which the monkey chose one of two different objects related to reward or no-reward. Because the object-reward contingency switched occasionally, flexible switching of the choice was required. By presenting appetitive or aversive- conditioned cues between trials, the monkeys performed this task under different emotion, measured by pupil diameters. We found that a group of DRN neurons showed tonic activity associated with specific emotional context, phasic responses to appetitive or aversive events, and their significant interaction. These results indicate the integrative representation of emotional and cognitive signals on single DRN neurons with specific temporal dynamics.

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Multiscale computational modeling of serotonergic function

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The complex structure and function of the serotonergic system present formidable challenges toward obtaining a full understanding of its mechanisms. Recent studies with more precise measurements are beginning to uncover some of these mechanisms albeit revealing another layer of complexity, particularly the heterogeneous nature and multifunctional properties of the serotonergic system. Hence, an integrative account of its functions and principles remains elusive. Computational modeling approach can provide a new quantitative and conceptual understanding of the observed experimental data. In particular, biologically based computational models can allow the synthesis of separate experimental data-sets across multiple scales to provide a more unified view of serotonergic function while offering testable predictions. In this talk, I will present some of these computational modeling approaches my collaborators and I have used to elucidate the dynamics and functions of the serotonergic and related systems.

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Symposium 8

Hot and Bothered: Inflammatory Cytokine Signaling, SERT and the Serotonergic Modulation of Behavior

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The presynaptic, antidepressant-sensitive serotonin (5-HT) transporter (SERT) exerts powerful control over the ability of 5-HT to modulate brain circuits that support fundamental behavioral traits, including appetite, satiety, libido, fear, social behavior and reward, to name just a few. We previously demonstrated that the inflammatory cytokines IL-1β and TNFα can rapidly elevate SERT activity in cell lines and brain preparations, with evidence supporting Il-1β acting through an IL-1R/p38α MAPK signaling pathway. Moreover, activation of the peripheral innate immune system via lipopolysaccharide (LPS) or poly I:C can rapidly activate CNS SERT in an IL-1R dependent manner and enhance 5-HT clearance, with uptake and behavioral effects dependent on 5-HT neuron p38α MAPK signaling. These and other findings suggest that serotonergic neurons may be important transducers of peripheral and CNS immune system activation, resulting at times in a healthy elaboration of homeostatic responses, but at other times, an elaboration of pathological responses that elevate risk for neuropsychiatric disorders. In my lecture, I will discuss our group's efforts to elucidate molecular pathways that support inflammatory cytokine signaling to SERT and serotonin neurons, to develop novel mouse models that can link cytokine signaling to behavioral actions, and to translate findings into novel therapeutic strategies.

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STAT3 controls IL6-dependent regulation of Serotonin Transporter function and depression-like behavior

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Abstract. Experimental evidence suggests a role for the immune system in the pathophysiology of depression. A specific involvement of the proinflammatory cytokine interleukin 6 (IL6) in both, patients suffering from the disease and pertinent animal models, has been proposed. However, it is not clear how IL6 impinges on neurotransmission and thus contributes to depression. Here we tested the hypothesis that IL6-induced modulation of serotonergic neurotransmission through the STAT3 signaling pathway contributes to the role of IL6 in depression. Addition of IL6 to JAR cells, endogenously expressing SERT, reduced SERT activity and downregulated SERT mRNA and protein levels. Similarly, SERT

expression was reduced upon IL6 treatment in the mouse hippocampus. Conversely, hippocampal tissue of IL6-KO mice contained elevated levels of SERT and IL6-KO mice displayed a reduction in depression-like behavior and blunted response to acute antidepressant treatment. STAT3 IL6-dependently associated with the SERT promoter and inhibition of STAT3 blocked the effect of IL6 in-vitro and modulated depression-like behavior in-vivo. These observations demonstrate that IL6 directly controls SERT levels and consequently serotonin reuptake and identify STAT3-dependent regulation of SERT as conceivable neurobiological substrate for the involvement of IL6 in depression.

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TNFα-dependent upregulation of hippocampal SERT activity and depression-like behaviour in a mouse model of collagen-induced arthritis

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The serotonin transporter (SERT) facilitates high affinity reuptake of 5-HT from the extracellular fluid and dysregulation of SERT function has been implicated in a range of mood disorders including depression. Recent studies have linked immune system activation to depression as well as to altered serotonin transporter activity. Advancing previous studies which focussed on acute effects of immune system activation, we have used collagen-induced arthritis (CIA) in mice as a model of chronic inflammatory disease, to investigate the effect of prolonged systemic inflammation on brain SERT function and behaviour. We found that CIA mice display anhedonia, a core depression-like behaviour. Furthermore, behavioural symptoms are temporally correlated with a brain region-specific upregulation of SERT activity in the hippocampus which occurs at a post-translational level and is independent of SERT trafficking. Kinetic analysis of 5-HT uptake revealed that the elevation of transporter activity is due to an increase in 5-HT transport capacity (V_{max}) with no change in apparent K_m values, suggesting that different regulatory mechanisms govern SERT modulation under chronic versus acute inflammatory conditions. Expression levels of tumour necrosis factor α (TNFα) and its receptor, TNFR1, were increased in the hippocampus of CIA mice, indicating altered TNFα signalling specifically in this brain region. Anti-TNFα treatment using etanercept not only diminished joint inflammation, but also prevented the development of depression-like behaviour and the upregulation of SERT activity in the hippocampus, suggesting a key role for TNFα signalling in brain function regulation in this disease model. Our study provides novel insight into serotonergic mechanisms underlying comorbid depression in chronic inflammatory diseases.

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Short oral presentation 7

Garcinia mangostana Linn displays antidepressant, antipsychotic and pro-cognitive effects in translational models of depression and schizophrenia: Role of serotonin and immune-inflammatory cascades

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The indonesian fruit, *garcinia mangostana Linn* (GML) or mangosteen, contains over 85 polyphenols, the best characterized of which are α-, β-, and γ-mangostin. Raw and purified extracts of GML have diverse anti-inflammatory, antioxidant and neuroprotective actions, while α- and γ-mangostin inhibit cyclic adenosine monophosphate phosphodiesterase and serotonin 5-HT_{2A} receptors, respectively. Disorganized redox balance and inflammation characterize major depression and schizophrenia, with antioxidant/anti-inflammatory agents representing novel treatments for these illnesses. We studied the therapeutic properties of chronic raw GML extract in two translational animal models, the Flinders Sensitive Line (FSL) rat model of depression and the maternal immune-activation (MIA) model of schizophrenia. Behaviour, as well as cortico-hippocampal monoamines, lipid peroxidation and plasma pro- and anti-inflammatory cytokines (interleukin-1, IL-1; tumour necrosis factor-α, TNF-α) were studied. We considered GML alone vs. imipramine or haloperidol, and as adjunctive treatment with haloperidol. Chromatographic fingerprinting of GML revealed the presence of α-mangostin (117 mg/g) and γ-mangostin (11 mg/g). FSL rats showed significant cognitive deficits and depressive-like behaviour, disordered cortico-hippocampal monoamines and elevated hippocampal malondialdehyde levels. GML (50 mg/kg/day x 14d) displayed antidepressant and pro-cognitive effects equal to imipramine (20 mg/kg x 14d) and reversed hippocampal lipid peroxidation. Behavioral and monoamine assessments suggest a serotonergic action for GML. Haloperidol (2 mg/kg x 14d) and GML (50 mg/kg x 14d) were equally effective in reversing MIA-induced deficits in sensorimotor gating and depressive behaviour, with haloperidol+GML more effective than either alone. MIA-induced elevated IL-6 and TNF-α levels and cortico-striatal lipid peroxidation were reversed by haloperidol, GML and haloperidol+GML. Prenatal MIA-induced sensorimotor gating deficits and depressive manifestations were reversed by haloperidol or GML, with depressive manifestations more responsive to GML. Again, haloperidol+GML were augmentative. GML presents with anti-inflammatory and antioxidant actions, having comparable antidepressant and antipsychotic efficacy vs. reference agents while bolstering the actions of the latter.

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WEDNESDAY JULY 18, 2018

Symposium 9

Dendritic spine plasticity depends on spatial dynamics of RhoA controlled by serotonin 5-HT4 receptors

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Learning and memory formation in the brain involve adaptive, multi-level nerve cell morphogenesis pertinent to changes in neural network connectivity. Although serotonin receptors provide an important signaling pathways for such morphogenesis, the underlying molecular mechanisms remain poorly understood.

Here, we found that activation of serotonin receptor 5-HT4 (5-HT4R) stimulated G13-mediated cofilin phosphorylation, resulting in an increased F/G-actin ratio and the rounding of neuroblastoma cells. Using a FRET-based approach, we documented spatiotemporal dynamics of 5-HT4R-dependent RhoA activation, at sub-micron resolution, in living cells, revealing the sequence of morphogenic steps in neuroblastoma cells. Cultured hippocampal neurons also displayed 5-HT4R-dependent cofilin phosphorylation. Acute treatment of 5-HT4R with the receptor agonist BIMU8 triggered a prominent increase in the head width of dendritic spines, while prolonged stimulation increased the number of mushroom spines. Time-lapse FRET monitoring of individual spines revealed 5-HT4R-activated dynamic patterns of RhoA, which lead to the accumulation of filamentous actin followed by spine enlargement. The 5-HT4R-mediated boost in dendritic spine maturation enhanced spontaneous neuronal activity and increased the LTP expression level while reducing the amplitude of evoked multi-synaptic currents which might reflect homeostatic changes in the circuitry. The dynamic reorganization of spine morphology mediated by the 5-HT4R/G13/RhoA signaling emerges therefore as a novel molecular pathway underpinning memory trace formation in the brain.

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Gsα-coupled GPCRs have a biphasic relationship with tubulin/microtubules to alter neurite outgrowth and constrain GPCR signaling: a universal mechanism for antidepressant action?

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Tubulin binds specifically to the G proteins Gsα, Giα1,2, Goα, but not to Gqα or Gtα (Kd ca. 100 nM). Only Gsα is internalized after activation by GPCR, and internalized Gsα associates with the plus end of microtubules, increasing their dynamicity. This results in increased neurite outgrowth, independent of cAMP, PKA or Epac. A dominant negative protein, based on a Gsα/Gtα chimera, blocks both Gsα-induced microtubule dynamicity and neurite outgrowth, suggesting that it is a direct result of Gsα association with tubulin. Tubulin dimers (or other tubulin structures that are not microtubules) populate lipid rafts and anchor Gsα within those structures. Acetylation of tubulin by tubulin-acetyltransferase (TAT), a process that contributes to microtubule stability, renders tubulin unable to bind Gsα. This allows Gsα to exit lipid rafts, where it binds more effectively and fruitfully to adenylyl cyclase 6 (AC6), increasing cellular cAMP. Histone Deacetylase 6 (HDAC6) inhibitors increase the extent of acetylated tubulin and HDAC6 inhibitors have been reported to show antidepressant activity in rodents. Consistent with this, HDAC6 knockout mice are resistant to stress paradigms. This is reinforced by an examination of human post mortem tissue, revealing that the proportion of acetylated tubulin in lipid rafts from cerebral cortex of depressed subjects (either suicides or other causes) is much lower than that seen in non-depressed controls. This applies only to membrane tubulin, as total tubulin acetylation, as well as total tubulin, was identical in all groups. This is consistent with the increased lipid raft:non-raft ratio of Gsα seen in depression vs control samples. Curiously, while HDAC 6 inhibitors increase acetylated tubulin, other antidepressants did not change the acetylation status of tubulin even though they elicited exodus of Gsα from lipid rafts and reduced, profoundly, the extent to which Gsα was complexed with tubulin. Thus, it appears that HDAC6 inhibitors have a biosignature similar to all antidepressants examines thus far, but diverge from other drugs in the mechanism by which they abrogate the anchoring of Gsα to tubulin in lipid rafts.

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Functional consequences of 5-HT$_{2A}$ receptor mediated synaptic plasticity: A single dose of psilocybin has long lasting and sustained antidepressant and anxiolytic effects in rats.

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Activation of serotonin 5-HT$_{2A}$ receptors with psychedelics has been demonstrated to increase synaptic plasticity, density, and complexity. We have measured these changes in glutamatergic pyramidal neurons of the rat mPFC, and in glutamatergic neuropil within the Elipsoid Body of the central complex of Drosophila brain. Others

have recently demonstrated this in primary cultures of cortical neurons and Drosophila body wall sensory neurons. These synaptic changes induced by psychedelic drug activation of 5-HT_{2A} receptors are mediated by the mTOR pathway, similar to the synaptic effects of ketamine. Both ketamine and the psychedelic drug psilocybin have demonstrated profound efficacy for the treatment of major depression. Whereas the antidepressant effects of ketamine wear off after only a few weeks, the effects of psilocybin appear to be long lasting and persist for at least several months in human patients. Although the antidepressant effects of both ketamine and psilocybin are hypothesized to involve increases in synaptic densities and complexities at the cellular level resulting from mTOR pathway activation, the reason for the long lasting effects of psilocybin compared to ketamine is not known. We have investigated the potential of psilocybin to treat depression-like behaviors in the rat towards developing a model system to utilize to elucidate the unique underlying cellular and molecular mechanisms of the anti-depressant effects of psilocybin observed in human patients. We have found that a single treatment with psilocybin (1.0 mg/kg; i.p.) has long lasting stable antidepressant and anxiolytic effects. In normal Sprague Dawley rats, a single dose of psilocybin produces a large anxiolytic effect measured at 42 days on the elevated plus maze (EPM). In an endogenously depressed rat strain, WKY, a single dose of psilocybin produces a significant and sustained antidepressant effect over 35 days of weekly testing in the forced swim test (FST). Remarkably, in WKY rats administered a single dose of psilocybin and not tested again for 42 days there is a very large antidepressant effect in the FST that is greater than that typically seen after chronic dosing with an SSRI. Biological samples from these rats have been gathered and are currently being analyzed for molecular and cellular changes between treatment groups that may inform on the long lasting anti-depressant and anxiolytic effects observed in the treatment of depressed patients with psilocybin and to further develop the use of psychedelics as a therapeutic strategy to treat anxiety and depression.

Short oral presentation 8

Lack of brain serotonin affects feeding and differentiation of newborn cells in the adult hypothalamus

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Serotonin is a crucial signal in the neurogenic niche microenvironment. Deregulation of the serotonin system leads to neurogenic decline and mood disorders but also to changes in appetite and metabolic rate. Tryptophan hydroxylase (*Tph*)2-deficient (*Tph2*$^{-/-}$) mice depleted of brain serotonin display alterations in these parameters, e.g., increased food consumption, modest impairment of sleep and respiration accompanied by a less anxious and highly active phenotype. The newly discovered neural stem cell niche of the adult hypothalamus has potential implications for homeostatic functions. Positioned between the third ventricle and the median eminence, specialized radial glial tanycytes are thought to regulate the hypothalamic in- and output of circulating hormones and nutrients to maintain body homeostasis. Cell genesis in the adult hypothalamus may have an important role in feeding and reproduction control, in mediating stress responses, and in energy metabolism. Serotonin impacts a variety of these functions. In a direct

approach, we use *Tph2*$^{-/-}$ mice to elucidate the role of serotonin in feeding and cell genesis in the adult hypothalamus. Specifically, we examine precursor cell proliferation and survival in *Tph2*$^{-/-}$ mice at baseline and following six weeks of high cholesterol diet (HCD). Our main finding is increased food intake of *Tph2*$^{-/-}$ mice independent of the diet without affecting body weight. Wild type mice under dietary challenge increase body weight accompanied by a decline in proliferation and survival of newly generated cells in the hypothalamic niche. In contrast, increased food consumption of *Tph2*$^{-/-}$ mice does not come along with decreased cell numbers. However, lack of brain serotonin results in a shift of precursor cell phenotypes that was abolished under HCD. We show that precursor cells in the hypothalamus retain fate plasticity and respond to changes in the environment. A novel link between serotonin signaling and cell genesis in the hypothalamus could be exploited as therapeutic target in metabolic disease.

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Symposium 10

Early-life serotonin and cortical interneuron subtype development

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Cortical microcircuit function relies on the activity of a large variety of GABAergic interneurons (INs). The diversity of cortical interneuron subtypes arises during development and their integration in cortical microcircuits is thought to require coordinated and sequential genetic programs. The specific molecular mechanisms that control the emergence of cortical interneuron subtype diversity and the rules that govern their circuit integration remain largely unknown. Furthermore, the role of early-activity in constraining the maturation of molecularly distinct subgroups of INs remains to be determined. In recent years, we have focused our interest on the molecular mechanisms regulating the migration and early integration of cortical interneuron subtypes which specifically express the serotonin 3a receptor (Htr3a) and mainly originate from the caudal ganglionic eminences (CGE). Using fluorescent-assisted cell sorting combined to transcriptomic analysis at early perinatal time-points, we have found that HTR3A is required for the migration and proper positioning of CGE-derived INs and have identified novel HTR3A-dependent down-stream effectors. Using single-cell RNA sequencing, we have mapped the emergence of molecularly distinct subtypes of Htr3a+ INs and have identified transcriptional networks operating in main cardinal subclasses during early steps of circuit assembly. Current work aims at studying the influence of HTR3A-dependent calcium activity on transcriptomic modules controlling the maturation of Htr3a+ interneuron subtypes at the single-cell level. Overall, our investigations are providing evidence that Htr3a-expressing interneuron subtypes are highly diverse and represent relevant cellular targets for the action of early-life serotonin.

Mapping cellular and molecular heterogeneity within the *Pet1*+ dorsal and media raphe to selective behavioral and efferent outputs

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Cellular heterogeneity within the brain serotonergic system is proving substantial and likely parallels the diversity of effects modulated by serotonin - from sensory processing to cognition and motivated behaviors to autonomic responses. Anatomical topography has been used to subdivide groups of serotonin neurons. In this talk, genetic tools and findings will be presented that refine the serotonergic structure-function map, superimposing onto topography new serotonin cell-subtype information, from molecular expression to efferent bouton locations to modulated organismal behavior. Specifically, this mapping platform plots (1) serotonergic developmental cell lineage, (2) cell groups based on unbiased clustering analyses of global gene expression profiles, (3) cellular electrophysiological properties registered within the context of lineage and transcriptomic information, (4) efferent projections of the

serotonergic neuron molecular subtypes including terminal and en passant bouton locations as a first step in connectivity mapping, and (5) specific organismal behaviors and physiological processes altered upon in vivo, neuron-subtype-specific perturbation. Highlighted here will be molecular and cellular subtypes identified within the *Pet1*$^{+}$ dorsal and median raphe and their relationship to specific efferent organization and behavioral outputs ranging from social dominance and aggression to pro-survival startle reflexes to modulation of cocaine memory strength relevant to addiction vulnerability.

Serotonin modulates maturation of afferent projections to the amygdala

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It is well established that serotonin (5-HT) signaling modulates anxiety and depression-related behaviors and cognitive function. However, the developmental regulation and the circuit specific mechanistic understanding of 5-HTergic modulation of behavior remains superficial. Here, we first present data on the role of 5-HT projections to the basolateral amygdala (BLA) and report a selective effect on fear conditioning-related behaviors. Specifically, optogenetically triggered neurotransmitter release from 5-HTergic axon terminals in the BLA during training reduces fear expression during retrieval, both in contextual and cued fear conditioning paradigms. Furthermore, we find that the maturation of 5-HT projections to the BLA is sensitive to 5-HT signaling during development. Mice that experienced 5-HT transporter blockade from P2-11 display reduced anatomical connectivity between the raphe and the BLA, and exhibit increased behavioral fear responsivity, as well as increased amygdala reactivity in adulthood.

In a second set of experiments, we investigated the consequence of developmental 5-HT transporter blockade in cortical glutamatergic neurons and find impaired fear extinction, a phenotype that mimics the effect of global developmental 5-HT transporter blockade. We hypothesize that this effect is driven by alterations in connectivity or function of mPFC-to-BLA projections. Lastly, we present data demonstrating evolutionary conversation of 5-HT transporter gene expression in cortical neurons, providing biological basis for translatability of our findings.

Together our data reveal a powerful role of developmental 5-HT signaling on the maturation of afferent projections to the BLA, with long-lasting consequences on fear behavior, and thereby aid our understanding of the developmental origins of neuropsychiatric disorders.

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Short oral presentation 9

Sphingolipid control of serotonin balance in depression

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Depression is a major psychiatric disorder that frequently emerges comorbid with alcohol abuse. Pathogenesis as well as pharmacological treatment approaches classically focused on protein dysfunctions. Thereby, the molecular components of the serotonergic system have proven to be essential. Together with cholesterol and glycerophospholipids, sphingolipids are the most common lipids in brain membranes. Sphingolipids form lipid rafts and signaling platforms, which are membrane compartments enriched in G-protein-coupled receptors. Acid sphingomyelinase (ASM) hydrolyses sphingomyelin to ceramide and phosphorylcholine and, thus, represents a major regulator of sphingolipid metabolism. We found that overexpression of ASM in mice (tgASM) reduces serotonin (5-HT) tissue levels in many brain areas, reduces hippocampal neurogenesis and induces depression-like behaviour. tgASM mice were found to drink significantly more alcohol and escalate consumption after withdrawal than WT mice. Free-choice alcohol drinking, but not forced alcohol exposure, normalized 5-HT levels and reduced depression-like behaviour selectively in depressed animals by normalization of ASM activity. However, extracellular 5-HT levels were preserved in naïve tgASM mice as measured by in-vivo microdialysis, but the acute 5-HT response to an alcohol challenge or a preferred food was attenuated in the hippocampus. This evidence suggests a strong control of 5-HT activity by the sphingolipid rheostat in the brain that may, when dysfunctional, lead to emotional disorders and enhanced preference for drugs that interfere with them.

Symposium 11

Synaptic and mitochondrial plasticity of rat hippocampus follows treatment with Vortioxetine

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Previous studies showed that a single dose of vortioxetine increased plasticity-related gene expression and 1-week vortioxetine treatment induced changes in spine number and density and dendritic morphology, whereas an equivalent dose of fluoxetine had no effects. Recovery of synaptic connections and synaptic remodeling plays a critical role for the clinical efficacy of a rapid antidepressant response. Here, we investigate the impact of vortioxetine on synaptogenesis and remodeling of synapses in the rat hippocampus. Furthermore, we also assess the role of mitochondria and microvessels in synaptic plasticity at the mRNA and protein levels after vortioxetine treatment. Rats were dosed for 1 week with vortioxetine and fluoxetine at doses relevant for antidepressant activity. Stereological principles were employed to quantify number of synapses and mitochondria, and the length of microvessels in the hippocampus. The BDNF and VEGF protein levels were visualized with immunohistochemistry. mRNA levels were measured by real-time quantitative polymerase chain reaction (qPCR) and protein expression levels were quantified by Western blots. The number of synapses and mitochondria, and length of microvessels significantly increased in the vortioxetine group compared to the vehicle and fluoxetine groups. BDNF and VEGF protein levels in hipppocampus were significantly higher in vortioxetine treated rats compared to vehicle and fluoxetine treated rats. qPCR analysis revealed that only Rac 1 in the vortioxetine treated group and Homer1 in the fluoxetine treated group was significantly increased compared to the vehicle group. The level of Rac-1 protein in hippocampal CA1 was significantly higher in the vortioxetine group compared with the vehicle group. However, the levels of Homer-1 and BDNF proteins were significantly down-regulated in fluoxetine group compared with vehicle group. Our results indicate that vortioxetine rapidly induced synaptic plasticity and elevation of BDNF and VEGF levels suggesting increased mitochondrial and vascular support. Moreover, the changes in mitochondrial morphology and number are a consistent feature of neuroplasticity. Therefore, synaptic plasticity may rely on enhanced metabolic activity by increased number of mitochondria and length of microvessels.

Synaptic remodeling depends on unusual signaling between serotonin receptors and the extracellular matrix

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Rewiring of synaptic circuitry pertinent to memory formation has been associated with morphological changes in dendritic spines and with extracellular matrix (ECM) remodeling. Here, we mechanistically link these processes by uncovering a novel signaling pathway involving the serotonin 5-HT7 receptor (5-HT7R), the matrix metalloproteinase-9 (MMP-9), the hyaluronan receptor CD44, and the small GTPase Cdc42. We highlight a physical interaction between 5-HT7R and CD44 (identified as a MMP 9 substrate in neurons), and find that 5-HT7R stimulation increases local MMP 9 activity, triggering dendritic spine remodeling, synaptic pruning and impairment of long-term potentiation (LTP). The underlying molecular machinery involves 5-HT7R-mediated activation of MMP-9, which leads to CD44 cleavage followed by Cdc42 activation. One important physiological consequence of such interaction includes an increase in neuronal outgrowth and elongation of dendritic spines, which might have a positive impact on complex neuronal processes (e.g. reversal learning and neuronal regeneration).

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Developmental and Functional Diversity of Serotonergic Fibers in the Post-Natal Mouse Brain

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Serotonergic (5-HT) neurons, confined in the nuclei B1-B9 of the brain stem, provide a profuse innervation of the whole central nervous system. Despite sharing the same neurochemical identity, 5-HT neurons are characterized by heterogeneity at different levels, such as developmental origin, projection pattern, molecular and electrophysiological properties. Unravelling the complexity of 5-HT system is critical to better understand the plethora of physiological functions regulated by 5-HT is involved. To this aim we used different approaches to investigate the morphological and functional diversity of 5-HT fibers during postnatal development and in adult mice.

Our analysis highlighted region-specific developmental patterns of serotonergic fiber density, ranging from a linear and progressive colonization of the target to a transient increase in fiber density occurring in a time- and region-specific manner. Moreover, taking advantage of the peculiar features of the recombinant rabies virus labelling system, we mapped the serotonergic system wiring transmission and we built a correlation map between specific raphe subfields and each distinct target brain area. Finally, we established causation between serotonin release and regional functional activity combining chemogenetics and functional magnetic resonance imaging.

Short oral presentation 10

Sustained activation of postsynaptic 5-HT_{2A} receptors gates long-term depression in prefrontal cortex

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Abstract
The prefrontal cortex (PFC) plays a key role in many high-level cognitive processes. It is densely innervated by serotonergic neurons originating from the dorsal and median raphe nuclei, which profoundly influence PFC activity. Among the 5-HT receptors abundantly expressed in PFC, 5-HT_{2A} receptors located in dendrites of layer V pyramidal neurons control neuronal excitability and mediate the psychotropic effects of psychedelic hallucinogens, but their impact on glutamatergic transmission and synaptic plasticity remains poorly characterized. We previously demonstrated that the activation of presynaptic 5-HT_{2A} receptors located at thalamocortical synapses enhances NMDA transmission and gates the induction of spike timing-dependent long-term depression (t-LTD) mediated by presynaptic NMDA receptors, which might underlie their influence on specific cognitive tasks such as associative memory retrieval (Barre et al. Proc Natl Acad Sci U S A. 2016 Mar 8;113(10):E1382-91). Here, we show that a prolonged (20 min) exposure of mouse PFC slices to serotonin or the 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) produces a long-lasting depression of evoked AMPA excitatory postsynaptic currents (EPSCs) in layer V pyramidal neurons. DOI-elicited long-term depression of synaptic transmission is absent in slices from 5-HT_{2A} receptor-deficient mice, is rescued by viral expression of 5-HT_{2A} receptor in pyramidal neurons and occludes classical electrically-induced LTD. Furthermore, 5-HT_{2A} receptor activation promotes phosphorylation of GluA2 AMPA receptor subunit at Ser^{880} and AMPA receptor internalization, indicating common mechanisms with electrically-induced LTD. Mimicking the effect of prolonged exposure of PFC slices to 5-HT_{2A} receptor agonists, sub-chronic administration of fluoxetine to mice, which results in a sustained increase in extracellular concentration of serotonin and, consequently, a prolonged activation of 5-HT_{2A} receptors, also occludes electrically-induced LTD. Collectively, these findings provide one of the first examples of LTD gating under the control of a G protein-coupled receptor that might lead to imbalanced synaptic plasticity and memory impairment following a non-physiological elevation of extracellular serotonin that occurs after the onset of SSRI treatment in depressed patients.

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Symposium 12

The effect of excessive serotonin during prefrontal cortex development

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 # equal contribution

With respect to biological underpinnings, studying neurodevelopment of the prefrontal cortex (PFC) is of major importance. The PFC controls key cognitive functions like executive functioning, attention and social interactions and is important for our emotional well-being. Development of the basic neuronal anatomy of the PFC involves a sequence of events, such as areal specification, progenitor proliferation, neuronal differentiation, radial neuronal migration and interneuron integration. Correct assembly of neuronal PFC circuits furthermore relies on integration of long-range inputs from various brain areas including modulatory projections from the catecholaminegic and serotonergic system. It is unclear however how these systems influence each other during development. I will present data describing the parallel development of the catecholaminergic and serotonergic prefrontal projection systems in rat and demonstrate a close engagement of both systems in the proximity of Cajal Retzius cells. In the absence of the 5-HT transporter (5-HTT), not only the developing serotonergic but also the catecholaminergic system, including their projections towards the mPFC, is affected. In addition, the layer identity of the mPFC neurons and reelin-positive interneuron number and integration are altered in the absence of the 5-HTT. Together, these data suggest a functional interplay between the developing catecholaminergic and serotonergic systems that target the mPFC. This calls for a holistic approach in studying neurotransmitter systems-specific developmental consequences for adult behavior, to eventually allow the design of better treatment strategies for neuropsychiatric disorders.

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Influence of the serotonergic system on CDC42 signaling pathway.

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The neurotransmitter serotonin is implicated in several physiological and behavioral functions lifelong. We have previously demonstrated that the rats with a total deletion of the serotonin transporter ($SERT^{-/-}$) showed a reduction of GABAergic markers in the prefrontal cortex and hippocampus and reduced inhibitory control over excitatory neurons in the cortex. Here we investigated whether altered inhibitory tone in $SERT^{-/-}$ rats is paralleled by

modifications of spine formation by measuring glutamatergic markers in the prefrontal cortex of SERT$^{-/-}$ rats and to what extent these defects are associated with modification of the CDC42 pathways. Our results showed that the inactivation of SERT reduced the expression of fundamental markers of the postsynaptic density, such as GluN1 and PSD95, an effect that was already present during the first week of life. Moreover, we found a decreased expression of CDC42 and SEPT7, which may destabilize the formation of normal dendritic spines.
We suggest that these molecular abnormalities may contribute to the behavioral alterations observed in SERT$^{-/-}$ rats, pointing to the need of novel pharmacological intervention able to normalize such alterations in order to ameliorate functional defects in psychiatric disorders that are associated with synaptic dysfunctions.

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Serotonin and development: the role of peripheral sources of serotonin in mammals

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Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine working as an autacoid in the periphery and as a neurotransmitter in the central nervous system. Tryptophan hydroxylase (TPH) is the rate limiting enzyme of serotonin synthesis. It converts tryptophan (Trp) to 5-hydroxytryptophan (5-HTP) and belongs to the family of pterin-dependent hydroxylases, that also comprises tyrosine and phenylalanine hydroxylases (PAH). In mammals TPH has 2 isoforms: TPH1, responsible for serotonin synthesis in periphery, and TPH2, which is restricted to serotonergic neurons in the raphe nuclei in the brain and in the enteric nervous system. Since in adult mammals serotonin cannot cross the blood-brain barrier, these two enzymes define two serotonin systems with independent regulation and different functions. In the early phases of embryonic and postnatal life, serotonin is also a trophic factor that modulates cell proliferation, migration and differentiation in the brain and in peripheral tissues. During development, besides its own production by TPH1 starting embryonic day E14, and TPH2 starting E11, there are other sources of serotonin, including maternal 5-HT, which is actively transported through the placenta via the serotonin transporter (SERT).
In my talk I will give an overview of possible sources of serotonin during prenatal development, based on the data obtained in mice lacking different components of serotonergic system such as SERT, TPH1, and TPH2. Moreover, I will show our recent data providing evidence that PAH represents an alternative enzyme for serotonin synthesis and is the major source of residual serotonin in animals lacking TPH enzymes.

Short oral presentation 11

Increased maternal extracellular serotonin levels beneficially influences offspring's anxiety- and anhedonia-like behaviour

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Serotonin is a critical player in brain development whereby serotonin neurotrophic actions can be regulated through maternal-foetal interactions. Hence, maternal rather than offspring's serotonergic genotype may determine variation in serotonin levels in the early foetal brain, which might result in downstream effects on the development of the brain and potentially influencing behaviour. Indeed, serotonin has been shown to be involved in psychiatric disorders such as autism, anxiety, and depression, but the nature of its etiology so far is unclear.

In our first study, we investigated whether changes in extracellular serotonin levels due to serotonin transporter (SERT) availability (SERT rat model) in the mother influenced the maternal care. Maternal care is a major constituent of early life environment and seems to be related to offspring's behaviour and serotonin levels. We observed that one of the most prominent forms, licking-grooming their offspring, is significantly less often performed by SERT knockout (KO) dams then SERT wildtype (WT) dams. Thus, variation in licking-grooming behaviour seems to be determined by maternal serotonergic genotype.

To delineate whether maternal serotonergic genotype influences offspring's development through changes in foetal serotonin levels and/or through changes in licking-grooming behaviour, we set up a breeding such that both these two questions could be answered. In this study, the offspring was subjected to several behavioural assessments. Our data showed that potential alterations in foetal serotonin levels (KO mother) and a decrease in licking-grooming behaviour (KO care) synergistically strengthen their impact on behaviour. More specifically, we observed diminished anxiety (elevated plus maze test) and diminished anhedonia (sucrose consumption test) in adult offspring from SERT KO mothers which received SERT KO care.

These findings indicate that genetically-induced increases in maternal extracellular serotonin levels has a beneficial effect on offspring's behaviour due to both potential alterations in foetal serotonin levels and decreased maternal licking-grooming behaviour. For this reason, maternal SERT genotype seems to be involved in the development of psychiatric disorders.
To understand in which direction the maternal SERT genotype alters foetal serotonin levels we are currently investigating serotonin metabolism in the placenta, and foetal forebrain and hindbrain, by high performance liquid chromatography.

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Symposium 13

The role of transcriptional deregulation of 5-HT1A autoreceptors in adult behavior and antidepressant response.

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Altered activity of the serotonin (5-HT) system has been implicated in both anxiety and depression, and 5-HT1A autoreceptors are thought to inhibit 5-HT neuronal activity. Thus understanding transcriptional regulation of the 5-HT1A receptor may be relevant to mental illness. For example, the human rs6295 5-HT1A polymorphism blocks its repression by Deaf1, and is associated with depression and related psychopathology, an increase in 5-HT1A autoreceptors in depressed patients, and resistance to serotonin-selective reuptake inhibitors (SSRIs); however these associations are not always robust. We addressed whether there is a mechanistic link between altered 5-HT1A autoreceptor gene regulation and anxiety or depression.

To model rs6295 and assess functional outcomes, we have used mice with conditional adulthood knockout of Freud-1 (cF1KO mice), a key endogenous repressor of the 5-HT1A gene in 5-HT neurons. We examined 5-HT1A receptor levels, 5-HT1A autoreceptor function (DPAT-induced hypothermia, electrophysiology), raphe activity (FosB), 5-HT levels (HPLC), behavior (EPM, OF, NSF, TST, FST) and fluoxetine response.

The cF1KO mice showed increased 5-HT1A autoreceptor binding and function, reduced activity and decreased raphe 5-HT levels, indicating reduced 5-HT tone. The cF1KO mice showed increased anxiety-depression-like behaviors that were reversed upon knockout of 5-HT1A autoreceptors, which alone did not affect these behaviors. In contrast to normal littermates, the cF1KO mice were resistant to chronic fluoxetine, but responded to chronic desipramine, which targets norepinephrine.

These mouse models suggest increased 5-HT1A autoreceptors in adulthood may provide a biomarker for human SSRI-resistant anxiety or depression. Resetting 5-HT1A transcription or targeting other neurotransmitters may prove more effective in patients with 5-HT1A de-regulation to enhance treatment response.

Serotonin signaling through Prefrontal Cortex 5-HT1A receptors during adolescence can bi-directionally determine baseline Mood-related behaviors.

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Life-long homeostatic setpoints for mood-related behaviors emerge during adolescence. Serotonin (5-HT) plays an important role in refining the formation of brain circuits during sensitive developmental periods. We have found that serotonin signaling through 5-HT_{1A} receptors during adolescence can alter these homeostatic setpoints bi-directionally. Suppression of heteroreceptor expression early in life results in a broad depression-like behavioral phenotype including decreased sucrose preference, increased immobility in the forced swim test and increased latency to eat in novelty suppressed feeding. This behavioral phenotype is accompanied by physiological and cellular changes within mPFC-DRN circuitry in adult animals. Specifically, in response to a forced swim stress, we find that fewer mPFC pyramidal neurons express c-fos in mice that lacked heteroreceptors during adolescence. We further find that the change in c-fos expressing neurons can be accounted for almost entirely by mPFC neurons that project to the raphe. Interestingly, we find the opposite result in the raphe, with more serotonergic neurons expressing c-fos in response to a swim stressor in animals that lack heteroreceptors. Combined, these results suggest an altered feedback loop. Remarkably, suppression heteroreceptors in the PFC alone at/during adolescence is sufficient to recapitulate this depression-like behavioral syndrome, Finally, selective stimulation of heteroreceptors during adolescence with a biased agonist that targets mPFC receptors results in the opposite behavioral phenotype (decreased immobility in the forced swim test and decrease latency to eat in the novelty suppressed feeding test) in adult animals. Our results suggest that targeting mPFC 5-HT_{1A} heteroreceptors during adolescence in humans may have life-long ramifications for depression and its treatment.

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A lack of serotonin 1B autoreceptors results in decreased anxiety and depression-related behaviours

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The effects of serotonin (5-HT) on anxiety and depression are mediated by a number of 5-HT receptors, including autoreceptors which act to inhibit 5-HT release. While the 5-HT_{1A} receptor has been a large focus of anxiety and depression-related research, the 5-HT_{1B}

receptor has a lesser known role in modulating emotional behavior. 5-HT_{1B} receptors are inhibitory GPCRs located on the presynaptic terminal of both serotonin and non-serotonin neurons, where they act to inhibit neurotransmitter release. The autoreceptor population located on the axon terminals of 5-HT neurons is a difficult population to study due to their diffuse localization throughout the brain that overlaps with 5-HT_{1B} heteroreceptors (receptors located on non-serotonergic neurons). In order to study the contribution of 5-HT_{1B} autoreceptors to anxiety and depression-related behaviors, we developed a genetic mouse model that allows for selective ablation of 5-HT_{1B} autoreceptors. Mice lacking 5-HT_{1B} autoreceptors displayed the expected increases in extracellular serotonin levels in the ventral hippocampus following administration of a selective serotonin reuptake inhibitor. In behavioral studies, they displayed decreased anxiety-like behavior in the open field and antidepressant-like effects in the forced swim and sucrose preference tests. Current work is using miniature endoscopes for in vivo calcium imaging during freely moving behavior in order to identify the role of 5-HT_{1B} autoreceptors in cellular activity during behavioral assays of anxiety and depressive behavior. Overall our studies point to a potential strategy aimed at blocking 5-HT_{1B} autoreceptors for the treatment of anxiety and depression.

Short oral presentation 12

Effects of perinatal fluoxetine exposure on circadian rhythmicity, 5HT-1A receptor sensitivity and affective behavior in female rats

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Depressive symptoms occur frequently during pregnancy and in some women lead to a major depression, making antidepressant treatment unavoidable. Serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants during pregnancy and are known to cross the placenta and reach the developing fetus. Serotonin plays an important role during early neural development, and SSRIs are known to affect both brain and behavior of the developing child. A likely contributor to neurodevelopmental changes may be the 5-HT_{1A} receptor. Furthermore, SSRIs are able to modulate aspects of circadian rhythmicity, with disturbances of circadian rhythms being a key symptom of mood and anxiety disorders.
In this study, our aim was to investigate whether perinatal treatment with the SSRI fluoxetine alters affective behavior, 5-HT_{1A} receptor sensitivity, circadian rhythmicity and phase shifts to non-photic or high-dose 5-HT_{1A} receptor stimuli in female rat offspring. Pregnant dams were treated daily with either 10 mg/kg fluoxetine or vehicle from gestational day 1 until postnatal day 21. When adult, female offspring was tested for home cage activity, and in the elevated plus maze, home cage emergence and forced swim test. In addition, circadian rhythmicity of activity and temperature was observed under normal and reversed day/night rhythm. Afterwards, phase shifts in response to total darkness followed by a high dose of the 5-HT_{1A} receptor agonist 8-OH-DPAT were tested. After stabilizing the circadian rhythm to a normal 12/12h rhythm, a dose-response of the 5-HT_{1A} receptor agonist F13714 was tested on

hypothermia. Afterwards, a dose-response of the 5-HT_{1A} receptor antagonist WAY100635 was tested. Finally, a combination of F13714 and WAY100635 was given.
Rats exposed to fluoxetine early in development showed increased home cage activity. No differences were observed in affective behavior or in the sensitivity to the 5-HT_{1A} receptor agonist F13714, 5-HT_{1A} receptor antagonist WAY100635 or their combination in hypothermia. Interestingly, after 5 mg/kg 8-OH-DPAT treatment a decreased length of the circadian period (tau) was observed in fluoxetine exposed rats compared to vehicle treated rats, indicating that perinatal fluoxetine leads to alterations in the circadian system. The mechanisms underlying alterations in the circadian system are currently under investigation.

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Symposium 14

Maternal serotonin levels are associated with cognitive ability and core symptoms in autism spectrum disorder

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Background: Biomarker and neuroimaging findings implicate the serotonin (5-HT) system in autism spectrum disorder (ASD). Recent findings in mice indicate that the maternal 5-HT system influences embryonic neurodevelopment. Methods: Whole blood serotonin (WB5-HT) levels were obtained from 181 children diagnosed with Autism Spectrum Disorder, 99 of their fathers and 119 of their mothers. Standardized assessments were used to evaluate cognitive, behavioral, and language phenotypes. Results: Linear regression demonstrated a significant positive relationship between maternal WB5-HT and nonverbal IQ ($F_{1,115} = 3.977$, $p = 0.049$), and a significant negative relationship with ADI-R Domain B- Nonverbal Communication Algorithm scores, ($F_{1,117} = 7.762$, $p = 0.006$), indicating that higher maternal WB5-HT levels tended to be associated with less deficit in these domains. After correcting for proband age, there was also a significant relationship between maternal WB5-HT and overall adaptive function on the Vineland Adaptive Behavior Scales-II ($F_{2,112} = 6.536$, $p = 0.002$). Latent class analysis identified a three-class structure in the assessment data, describing children with low, intermediate, and high severity across measures of behavior, cognition, and adaptive function. Mean maternal WB5-HT differed across classes with the lowest maternal WB5-HT levels seen in the highest severity group, (Welch's $F(2, 46.048) = 17.394$, $p < 0.00001$). Conclusion: These findings suggest that the maternal serotonin system may affect neurodevelopment in humans, as it does in mice. Further studies in animal models may be able to reveal the mechanisms underlying these findings.

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5-HT_{1A} Receptor Stimulation Modifies Repetitive Behaviors and Striatal Glutamate Efflux in SHANK3 $^{+/-}$ mice

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Restricted interests and repetitive behaviors (RRBs) represent a core diagnostic category of autism spectrum disorder (ASD). RRBs encompass a range of behaviors from motor stereotypy to cognitive rigidity. Several cases of ASD have been linked to deletion or mutation in the SHANK3 gene. Haploinsufficiency of the SHANK3 gene contributes to Phelan-McDermid syndrome (PMS), a neurodevelopmental disorder which often presents an ASD behavioral phenotype and a moderate to severe intellectual disability. Deletion of the SHANK3 gene in mice results in elevated excitation of cortical pyramidal neurons that alters

output to other cortical and subcortical areas. Serotonin 1A receptors ($5HT_{1A}$Rs) are highly expressed on layer 2 cortical neurons and are known to have inhibitory actions. Treatment with a $5HT_{1A}$R agonist in PMS may restore excitatory and inhibitory balance to cortico-basal ganglia-thalamo-cortical circuits that alleviate RRBs and learning deficits. To begin exploring this possibility, the present experiments determined whether acute treatment with the partial $5HT_{1A}$R agonist, tandospirone in Shank3B$^{+/-}$ mice attenuated a probabilistic learning deficit and elevated self-grooming. Systemic treatment with tandospirone, at 0.06 mg/kg, alleviated a probabilistic learning impairment in Shank3B$^{+/-}$ mice on a spatial discrimination task using 80/20 probabilistic reinforcement. Systemic treatment with tandospirone, at 0.06 mg/kg dose, also attenuated elevated self-grooming behavior in Shank3B$^{+/-}$ mice without affecting locomotor and rearing behavior. Measurement of dorsal striatal glutamate efflux during grooming behavior revealed that there was greater bidirectional variation in striatal glutamate efflux in Shank3B$^{+/-}$ vs. wildtype mice. Tandospirone, 0.06 mg/kg, concomitantly reduced grooming behavior and bidirectional variation in striatal glutamate efflux in Shank3B$^{+/-}$ mice. Together, these results indicate the potential for tandospirone as a treatment for behavioral symptoms characteristic of PMS and ASD.

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LP-211 as a useful tool to study the effects of 5-HT7 receptor activation in mouse models of Fragile X syndrome and Rett syndrome

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Serotonin 5-HT7 receptor has been the subject of intense research efforts because of its presence in behaviorally-relevant brain areas such as the hippocampus, hypothalamus, and cortex. Preclinical data link the function of 5-HT7 receptor to a number of central nervous system processes including the regulation of circadian rhythms, mood, cognition, pain processing, and mechanisms of addiction. 5-HT7 receptor has been recently shown to modulate neuronal morphology, excitability, and plasticity, thus contributing to shape brain networks during development and to remodel neuronal wiring in the mature brain. Therefore, the activation of 5-HT7 receptor has been proposed as a therapeutic approach for neurodevelopmental and neuropsychiatric disorders associated with abnormal neuronal connectivity.
Over the years, medicinal chemistry efforts have led to the identification of the gold standard selective 5-HT7 receptor antagonist SB-269970 as well as of various selective agonists, including the arylpiperazine derivative LP-211. LP-211 is a high affinity 5-HT7 receptor agonist developed in our laboratory, endowed with selectivity over 5-HT1A, 5-HT1D, 5-HT2A, 5-HT6 receptors and the dopamine D2 receptor. The agonist properties of LP-211 were assessed in guinea pig ileum where it induces relaxation on substance P-stimulated contracture and in neuroblastoma N1E-115 cell expressing 5-HT7 receptor where it increases the intracellular levels of cAMP. A biodistribution study showed that, after intraperitoneal administration in the mouse, LP-211 rapidly reaches the systemic circulation,

achieving the maximal concentration in the brain in 30 min, with quantifiable levels consistently evident for up to 2 h (elimination half-life = 65 min).
LP-211 has been successfully used to study in vitro and in vivo the effects of 5-HT7 receptor stimulation in mouse models of Fragile X syndrome and Rett syndrome, the most common known single gene cause of autism spectrum disorder.

Short oral presentation 13

Characterization of a specialized serotonergic neuron subtype responsive to dopamine and central to social behavior

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Serotonergic (5-HT) neurons modulate diverse behavioral and physiological functions. Increasingly, 5-HT neurons are described as distinct subpopulations that are specialized to regulate distinct biological processes and functions. One such subpopulation that modulates aggression and hyperactivity in mice is distinguished by expression of the type-II dopamine receptor (Drd2) and the pan serotonergic transcription factor Pet1. We refer to these as Drd2-Pet1 5-HT neurons. *In vivo* silencing of Drd2-Pet1 5-HT neurons drives heightened aggression and increased activity. While brain slice electrophysiology demonstrates that their excitability is inhibited cell-autonomously via Drd2 signaling, the requirement for Drd2 receptor activity in these serotonergic neurons for behavior modulation is unknown. To query the functional requirement for Drd2 receptor in Drd2-Pet1 5-HT neurons, we generated mice with serotonin specific deletion of *Drd2* (Drd2-CKO) and administered a panel of behavioral assays. We find that Drd2-CKO males exhibit altered aggressive and social dominance behavior. Further, Drd2-CKO females display altered acoustic startle responses compared to control littermates. These findings suggest an additional role for Drd2-Pet1 5-HT neurons in the modulation of auditory processing and/or sensorimotor gating. Interestingly, Drd2-Pet1 5-HT neurons have axonal projections to many brain regions involved in auditory processing. To further probe the circuitry involving Drd2-Pet1 5-HT neurons, we have generated novel viral vectors for intersectional transsynaptic tracing. The identification of their pre- and post-synaptic partners is ongoing. These experiments will inform upon the molecular, cellular, and circuit pathways that underlie these behavioral phenotypes while generating and testing novel viral-genetic tools for studying neuronal connectivity.

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POSTERS OF TRAVEL AWARDEES (oral and posters)

POSTER #A-1

Investigating organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT) as targets for development of new antidepressant treatments for juveniles and adolescents

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Depression is a psychiatric illness that affects individuals of all ages, yet only two antidepressants are approved to treat depression in children. Both are selective serotonin reuptake inhibitors (SSRI). Moreover, children are less effectively treated by SSRIs than adults. SSRIs block reuptake of serotonin via the high-affinity, low-capacity serotonin transporter (SERT). The resulting increase in extracellular serotonin is thought to initiate a cascade of downstream effects, which underlie the therapeutic utility of SSRIs. However, other transporters also clear serotonin from extracellular fluid, including the low-affinity, high-capacity organic cation transporters (OCTs) and plasma membrane monoamine transporter (PMAT). Our lab has shown that, in adults, decynium-22 (D22), an inhibitor of OCT1-3 and PMAT, produces antidepressant-like effects when SERT function is either genetically or pharmacologically impaired. However, whether OCTs or PMAT may be useful targets for therapeutic intervention in juveniles remains unknown. Our preliminary studies show that D22 has antidepressant-like effects in juvenile (postnatal day 21 (P21)) SERT+/+ mice, suggesting that OCTs and/or PMAT may be functionally upregulated in juvenile mice. Consistent with this notion, we found that [^{3}H]D22 binding in hippocampal homogenates was greater in P21 mice relative to adults (P90). Western blot analyses showed that this increase in [^{3}H]D22 binding is likely driven by increased expression of PMAT protein, and not OCT3. In vivo chronoamperometry studies show that, unlike adults, serotonin clearance rate is the same in juvenile SERT+/+ and SERT-/- mice suggesting that PMAT might be capable of significant serotonin clearance in juveniles compared to adults. In ongoing studies, we are using *in vivo* chronoamperometry to determine the contribution of PMAT to serotonin clearance in hippocampus of PMAT+/+ and PMAT-/- juvenile and adult mice. These studies will expand our understanding of differences between the juvenile and adult brain, and will aid in discovery of novel targets for the development of antidepressants with improved therapeutic efficacy for children suffering from depression.

POSTER #A-2

D-lysergic acid diethylamide (LSD) reverses depressive-like behavior and serotonergic (5-HT) neurotransmission impairments in a murine model of chronic stress.

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Introduction: Depression is a disease involving dysfunctions of serotonergic neuronal activity in the Dorsal Raphe Nucleus (DRN). D- lysergic diethylamide acid (LSD) is a hallucinogen that has recently gained popularity based on clinical evidences reporting mood-enhancing properties. Our previous work has demonstrated that low doses of LSD (5-20 µg/kg) decreased the firing activity of serotonin (5-HT) neurons in DRN while at higher doses (60-120 µg/kg) it induced a cessation of the activity of DA neurons in Ventral Tegmental Area (VTA), suggesting an exclusively low doses LSD effect on 5-HT, without any DA psychotic side-effects. Thus, employing a chronic stress (CS) model of depression, our hypothesis is that short-term administration of low doses LSD could reverse depressive and anxiety-like symptoms and increase the 5-HT firing activity in the DRN, that is a common feature of antidepressant-like activity **Methods:** The CS paradigm was performed: 8-week old male C57BL/6J mice were individually placed in restrainers for 2 hours per day, over 14 days. Control mice (CTL) remained undisturbed in their cages. From 7th to 14th day of stress, CTL and CS mice received subcutaneous LSD (30 µg/kg/day, s.c.) or vehicle (veh); on 15th day after the CS, mice were tested. *In vivo* single unit extracellular recordings of 5-HT DRN neurons and behavioral tests such as Open Field (OF), Forced Swim (FS) and Novelty Suppressed Feeding (NSF) were employed. **Results**: CS mice showed a decreased firing activity of 5-HT DRN neurons compared to CTL. LSD restored rates to CTL levels. In OF, CS mice showed decreased time in the center and frequency of enters. CS group showed increased immobility time in FS and increased latency to feed in NSF, compared to CTL. LSD normalized these parameters.
Discussion: This study reports that treatment with LSD modulates mood through serotonergic neurotransmission.

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POSTER #A-3

Intrinsic regulatory factors governing growth of serotonin neuron axonal architecture

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Of the estimated 86 billion neurons in the human brain only about 400,000 make and use serotonin (5-HT) as a neurotransmitter. Yet, serotonin appears to modulate nearly all neural circuitry in the vertebrate CNS. Pervasive 5-HT signaling is made possible by the expansive axonal architecture issuing from this small group of neurons. 5-HT neurons generate two highly ramified topographically organized axonal subsystems, ascending and descending, that delivers the transmitter throughout the brain and spinal cord, respectively, to influence numerous behavioral and physiological processes. In contrast to our extensive knowledge of the intrinsic regulatory programs that govern the specification and differentiation of these 5-HT neurons, nearly nothing is known about the intrinsic regulators that enable the exuberant axonal growth of developing 5-HT neurons.

The LIM-homeodomain (LIM HD) transcription factor (TF), Lmx1b, is a key intrinsic regulator of 5-HT neuron terminal differentiation through its activation of genes encoding 5-HT synthesis (Tph2), reuptake (Sert), and vesicular monoamine transport (VMAT2). Lmx1b deficient mice lack nearly all brain 5-HT. Notably, Lmx1b expression continues in neurons of the ascending and descending 5-HT subsystems through a fetal to early postnatal maturation stage during which 5-HT neurons build their axonal architectures. Its ongoing expression led us to hypothesize that Lmx1b regulates other genes responsible for ascending and descending 5-HT axonogenesis. To investigate this idea, we generated conditionally targeted mice (*Lmx1b*$^{fl/fl;ePet\text{-}Cre;Ai9}$; *Lmx1b*^{CKO}) by crossing *Lmx1b*$^{flox/flox}$ mice with transgenic mice expressing Cre recombinase specifically in newborn 5-HT neurons and mice carrying the Ai9 (Rosa-CAG-LSLtdTomato-WPRE) reporter to enable marking of ascending and descending 5-HT axons with Td-Tomato. The pattern of Td-Tomato^{+} fibers corresponds to the pattern of 5-HT immunopositive fibers in serotonergic terminal fields of the forebrain and spinal cord of control mice indicating accurate marking of 5-HT axons with Ai9-derived Td-Tomato. In striking contrast, despite normal numbers of 5-HT cell bodies, the spinal cord of *Lmx1b*^{CKO} mice was nearly devoid of Td-Tomato^{+} axons from cervical to lumbar levels. Moreover, investigation of 5-HT terminal fields in the forebrain of *Lmx1b*^{CKO} mice, likewise, revealed normal numbers of Td-Tomato^{+} mutant 5-HT cell bodies but nearly complete absence of Td-Tomato^{+} axons in the hippocampus, cortex, olfactory bulbs, and hypothalamus. Further analyses suggest that 5-HT axons are able to initiate primary growth through the medial forebrain bundle between E12-E15 and reach the thalamus but then abruptly stop and fail to rout to various forebrain structures. To define the Lmx1bregulated axonal transcriptome that governs serotonergic axonogenesis we are performing RNA-seq analyses with flow sorted *Lmx1b*^{CKO} vs. *Lmx1b*^{CON} ascending and descending 5-HT neurons. These studies will solve a major long-standing gap in understanding how the 5-HT transmitter system is generated.

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POSTER #A-4

Evaluating the role of 5-HT_{1B}R agonists on cocaine reinforcement and their potential as a treatment for psychostimulant use disorders.

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Several experiments have demonstrated that systemic administration of serotonin 1B receptor (5-HT_{1B}R) agonists potentiate cocaine self-administration (SA). However, we previously found that the selective 5-HT_{1B}R agonist, CP94,253, enhances cocaine SA initially but attenuates SA after 3 weeks of abstinence from cocaine. Additionally, we found that the FDA-approved 5-$HT_{1B/1D}$R agonist, zolmitriptan, decreases methamphetamine SA regardless of whether rats undergo abstinence. Here we examined if zolmitriptan produces similar attenuating effects on cocaine and sucrose SA; additionally, since little is known about the neural pathways that underlie these 5-HT_{1B}R agonist effects, we also investigated if intracranial infusion of CP94,253, in the ventral tegmental area (VTA) modulated cocaine SA similar to the systemic effects previously observed.

Sprague-Dawley male and free-cycling female rats were tested for the effects of zolmitriptan (0, 3.0, 5.6, & 10 mg/kg, SC) on cocaine (0.075 & 0.75 mg/kg, IV) and sucrose (45 mg pellets) SA while a separate group of male rats were tested for the effects of bilateral VTA infusion of either vehicle (0.3 µL) or CP94,253 (1 µg/0.3 µL) on cocaine (0.075 mg/kg) SA.

We found that in male rats, 5.6 mg/kg of zolmitriptan decreased intake of 0.075 mg/kg cocaine and all doses of zolmitriptan decreased intake of 0.75 mg/kg cocaine. In female rats, 5.6 and 10 mg/kg of zolmitriptan decreased intake of 0.075 mg/kg cocaine and 10 mg/kg of zolmitriptan decreased intake for 0.75 mg/kg cocaine. These same doses of zolmitriptan did not affect sucrose reinforcement rates. Additionally, we found that intracranial VTA infusion of CP94,253 increased cocaine intake and lever responding like the effects of systemic CP94,253 administration. The cocaine doses are on the ascending and descending limbs of the dose-effect curve, suggesting that zolmitriptan decreases cocaine reinforcement in both male and female rats and that the VTA plays a role in modulating the 5-HT_{1B}R agonist effects on cocaine SA in male rats. These findings suggest that 5-HT_{1B}R agonists may have clinical efficacy as treatments for psychostimulant use disorders.

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POSTER #B-1

Probing the contribution of plasma membrane monoamine transporter function to depressive behaviors and poor antidepressant efficacy

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The poor efficacy of antidepressants is hypothesized to be attributable, in part, to high volume transporters with low selectivity (i.e., "uptake-2" mechanisms) that undermine antidepressant blockade of highly selective, low volume transporters (i.e., "uptake-1" transporters) such as the serotonin transporter. Little is currently known about the behavioral or neurochemical consequences of selectively blocking different uptake-2 transporters, such as the plasma membrane monoamine transporter (PMAT). Compared to other high volume organic cation transporters in brain, PMAT preferentially transports serotonin and dopamine, both heavily implicated in the pathophysiology of depression. Because a selective pharmacologic inhibitor of PMAT has yet to be identified, genetic knockout of PMAT is currently the best available method for investigating PMAT's functional role in behavior and neurotransmission. Using a mouse line recently developed in the lab of Dr. Joanne Wang, we compared male and female wildtype (+/+) controls against mice with reduced (+/-) or completely ablated (-/-) PMAT function to evaluate how PMAT deficiency affects depressive-like and other neuropsychiatric-relevant behaviors (e.g., anxiety-like, social, and compulsive behaviors). Remarkably, mice with reduced or ablated PMAT function did not exhibit any substantial perturbations in depressive-like, anxiety-like, social, or compulsive behaviors. Though unexpected, these findings fit with the current understanding of uptake-2 transporters as primarily supplemental, engaging when uptake-1 function is saturated or otherwise reduced. Ongoing experiments are currently evaluating the behavioral and neurochemical responses of PMAT-deficient mice to doses of serotonin transporter (fluvoxamine)- or dopamine transporter (bupropion)-targeting antidepressants that are ineffective in wildtype controls. These experiments will reveal how the presence or absence of functional PMAT impacts serotonin and dopamine clearance to elicit behavioral changes, with particular focus on depressive-like behaviors. Considering PMAT knockout has not been behaviorally evaluated before, these findings will substantially advance understanding of how uptake of serotonin and dopamine by PMAT contributes to behavioral changes - or a lack thereof - following antidepressant administration.

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POSTER #B-2

PRE-NEURONAL SEROTONIN: A NEW OLD FUNCTION OF ANCIENT MOLECULE

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Serotonin and dopamine are widely known as neurotransmitters and humoral regulators of different processes in developing embryo and adult body. At the same time, it's much less known that these substances seem to appear as a signaling molecule at the very early steps of evolution hundreds of millions of years prior to first neurons evolved. Serotonin occurs in prokaryotes, plants, protists and expressed in any of the multicellular animals ever studied. Our data suggests importance of serotonin in the regulation of locomotion and feeding of the most ancient and enigmatic animal - placozoan Trichoplax adhaerens which is lack of nervous system and neurons at all. On the other hand, in higher animals including human monoamines are expressed in the embryos on all stages including male and female gametes, zygote and very early stages of development. However, the role of monoamines in early pre-neuronal development is merely understood.

It is worth mentioning that early developmental stages are especially crucial and are the key for implementation of parental effects. Many embryonic features that will influence the resulting fitness, behavioral modality and future reproductive success of the progeny are initiated during early development. Generally, non-genetic transfer of information to the progeny is widely spread in nature and varies in terms of molecular mechanisms.

Using freshwater gastropods as a model recently we have discovered an entirely new phenomenon – direct transmission of signal about the physiological condition of the mother's body to a progeny through the local serotonergic system in the female's reproductive organs. Such a transmission occurs by means of serotonin content in zygote and early embryo. The entire system includes serotonin receptors, enzymes of serotonin metabolism and transport. Moreover, it recruits the process of monoaminylation (transglutaminase-mediated covalent binding of serotonin to glutamines residues in proteins). We continued our study in this field and our recent research performed on the early embryos of sea urchins and zebrafish revealed some prospective targets for monoaminylation. We hypothesized that both discovered molecular mechanism and principle itself are conservative and vastly distributed in the animals' phyla. In particular, in sea urchins we have described competitive monoaminylation of proteins in blastomeres with serotonin and dopamine as a direct determinant of primary cilia length at blastula stage. In zebrafish we found that serotonin content in the blastomeres of an early embryo can retain in differentiating cells and directly affect further neural development. Our most recent data suggests that modulation of serotonin level in the pre-implantation embryos of mouse affects behavioral patterns in the age of at least up to 3 months. And thus, our concept may be applicable for human.

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POSTER #B-3

Chiral selectivity towards the serotonin transporter is a hallmark of cathinones

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Background
Global drug markets are flooded with new psychoactive substances (NPS). Synthetic cathinone derivatives ("bath salts") dominate the market of stimulant type NPS and abuse thereof has been associated with adverse effects. Many cathinone derivatives act in an amphetamine-like fashion and induce carrier-mediated release of monoamines via the high-affinity transporters for dopamine (DAT), serotonin (SERT) and norepinephrine (NET). Most cathinones possess at least one chiral carbon-atom and exist as stereoisomers. Mounting evidence suggests that stereochemistry essentially impedes on the effects of cathinones on neurochemistry and behavior. We sought to unravel the activity-profile of a series of cathinones in their enantiopure-form at monoamine transporters.

Methods
Radiotracer-flux experiments were applied to determine the effects of test-drugs on MATs. Single-cell FRET measurements were performed to monitor conformational changes in SERT in real-time. Whole-cell patch clamp recordings were performed to monitor the effect of test drugs on the transport cycle of human SERT.

Results
Each of the tested cathinones inhibited uptake mediated by DAT, NET and SERT. No stereoselectivity was observed at DAT and NET, whereas the *S*- and *R*-enantiomers markedly differed in their potencies at SERT. Moreover, the *R*-enantiomers failed to induce full-fledged release via SERT and were less potent than the corresponding *S*-enantiomers. Whole-cell patch clamp recordings revealed that enantiomers substantially differed in their effects on the forward-mode of the transport cycle of SERT.

Conclusions
The tested cathinones exhibited a pronounced stereoselective profile at SERT, but not at DAT and NET. Remarkably, the potencies at SERT followed a regular pattern, i.e. *S*>*R*. Interestingly, we observed that the *R*-enantiomers behaved as "bad substrates" of SERT. These findings suggest that the recently discovered stereoselective anti-addictive and anxiolytic effects of a subset of cathinones arises from their different interaction with SERT. Further study is warranted to develop compounds with optimized stereoselective pharmacology towards monoamine transporters.

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POSTER #B-4

Time-Dependent Biased Signaling Kinetics of Psychedelics at the 5-HT_{2A} Receptor

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Serotonin 5-HT_{2A} receptors have long been known for the role they play in mood, consciousness, and cognition, and are targets for many successful antidepressant and antipsychotic pharmaceutical drugs. Interestingly, serotonin 5-HT_{2A} receptors are also the principal G protein-coupled receptor (GPCR) targets for prototypical psychedelic drugs, but the downstream signaling profiles of such drugs have not been extensively profiled. It has also been shown in animal models that the non-canonical GPCR interacting protein, β-arrestin2, may play a key role in desensitization and internalization of GPCRs. In this study, time-dependent β-arrestin2 recruitment as well as Gq dissociation at the 5-HT_{2A} receptor were measured by bioluminescent resonance energy transfer (BRET) to generate time signatures of arrestin recruitment and G protein activity by psychedelics and compared to non-psychedelic drugs. The results suggest that psychedelic effects display unique β-arrestin2 and G protein (Gq) signaling pathways at the 5-HT_{2A} receptor over time. Progress in this field may help elucidate novel GPCR signaling pathways relevant for mood-related therapeutic development.

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POSTER # C-1

Serotonergic Signaling in Astrocytes

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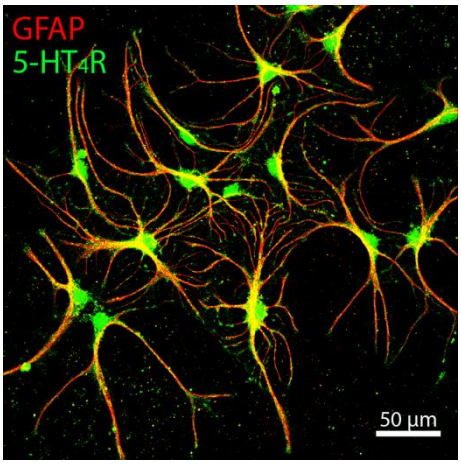
Serotonin is an important neurotransmitter regulating various brain functions via activation of specific serotonin receptors (5-HTRs), which are interestingly also expressed by astrocytes. These glial cells possess a unique morphology allowing single astrocytes to modulate thousands of synapses over distinct anatomical regions. It is also known that astrocytes' Ca^{2+} signaling is implicated in these functions. Properties and propagation of Ca^{2+} signals depend on diffusion and therefore on astrocyte morphology, which is dynamic itself. Therefore, it is important to understand which signaling cascades are involved in controlling astrocyte morphology. In neurons, 5-HTRs can modulate multiple signaling pathways including activation of small GTPases of the Rho family, which determine cell morphology. We investigate molecular mechanisms by which 5-HTRs regulate small GTPases of the Rho family to control astrocyte morphology and astrocyte Ca^{2+} signaling.

We show that astrocytes express the 5-HT_4R *in vivo* and in an *in vitro*-model of primary mouse hippocampal astrocyte cultures. Using FRET-based biosensors, we demonstrate that 5-HT_4R activation results in increased RhoA activity as well as elevated cAMP levels, indicating a functional coupling to Gα13 and GαS, respectively. Furthermore, transient expression of constitutively active variants of the small GTPase RhoA results in drastic morphological changes with decreased size and perimeter of the astrocytes. Sholl analysis also reveals an impact of RhoA on the arborization of mouse hippocampal astrocytes. 5-HT_4R stimulation leads to a reorganization of the actin cytoskeleton, presumably via the Gα13-RhoA signaling pathway, therewith influencing astrocyte morphology and function.



Moreover, our data suggest that astrocyte morphology correlates with their Ca^{2+} dynamics. Together, these data indicate that 5-HTRs are critically involved in regulation of astrocyte morphology and Ca^{2+} signaling and thus 5-HTR activation in astrocytes can substantially affect neuronal network transmission.

This study is supported by the DFG and Rebirth - Cluster of Excellence.

POSTER # C-2

Title: A novel 5-HT_7 receptor antagonist, MC-RG19, decreases cue-induced reinstatement of cocaine seeking behavior.

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Abstract: The serotonin 7 receptor (5-HT_7R) has been implicated in preclinical models of psychiatric conditions, including depression, anxiety, and psychosis, as well as in cognitive functioning and circadian rhythms. Moreover, a single nucleotide polymorphism at the 5-HT_7R gene in human post-mortem brain tissue has been identified as a risk factor for alcohol dependence. 5-HT_7Rs have also been shown to regulate amphetamine-induced dopamine release in the ventral tegmental area and to mediate the anti-nociceptive properties of morphine in the spinal cord. Although it has been suggested that the receptor may be involved in addiction processes, no studies to our knowledge have investigated its role in psychostimulant-related behaviors. In this series of experiments, we tested a novel, highly selective 5-HT_7R antagonist, MC-RG19, for effects on spontaneous locomotor activity and several cocaine-induced and cocaine-conditioned behaviors. We found that MC-RG19 (3, 5.6, and 10 mg/kg, i.p.) decreased spontaneous locomotion only at the 10 mg/kg dose and produced a trend toward a decrease in cocaine-induced (10 and 15 mg/kg, i.p.) locomotion at this dose as well. We observed no effects of MC-RG19 pretreatment (3, 5.6, and 10 mg/kg) on operant responding for sucrose or cocaine on a multiple VI60s schedule of reinforcement, however MC-RG19 did alter cue- and cocaine-induced reinstatement. Reinstatement effects were demonstrated in rats that were trained to self-administer cocaine (0.75 mg/kg, i.v.), which was paired with light and tone cues, and subsequently underwent extinction sessions until cocaine-seeking behavior was extinguished. Rats pretreated with MC-RG19 (5.6 and 10 mg/kg) prior to reinstatement tests showed robust reductions in cue-induced reinstatement of cocaine-seeking behavior, whereas only the high dose of MC-RG19 (10 mg/kg) produced a trend toward a decrease in cocaine-primed (15 mg/kg, i.p.) reinstatement. Collectively, these findings suggest a role for the 5-HT_7R in cocaine-related behaviors, and introduce a novel target for future medication development for cocaine dependence. In addition, our laboratory is currently exploring how 5-HT_7R's may be involved in opioid self-administration and seeking behavior with this novel 5-HT_7R antagonist.

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POSTER #C-3

In vivo impact of the conformational regulatory SERT phosphorylation site Thr276

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In vitro and *ex vivo* studies indicate that serotonin (5-HT) clearance by the CNS 5-HT transporter (SERT) is dynamically regulated through activation of PKG and p38 MAPK pathways. Disruptions in these pathways that mediate SERT activity, either through environmental perturbations or disease associated variants, has been linked to major depressive disorder (MDD) and autism spectrum disorder (ASD). Previously, we found that activation of the innate immune system through a p38 MAPK signaling pathway shifts the transporter to a high affinity state (SERT*) that leads to enhanced 5-HT clearance. This SERT* state appears to be constitutively induced by the ASD-associated SERT coding variant Ala56 that is insensitive to p38 MAPK and PKG signaling. Accumulating evidences implicates phosphorylation at Thr276 as a key step in the conformational dynamics that drive 5-HT transport as well as regulation of SERT by PKG and p38 MAPK. In an effort to understand the role played by phosphorylation at Thr276 *in vivo,* we generated SERT Ala276 knock-in mice utilizing the CRISPR/Cas9 approach. Initial characterization of these mice reveals no gross abnormalities assessed by body weight and reproduction. Additionally, midbrain and hippocampal synaptosomes prepared from SERT Ala276 mice exhibit normal levels of 5-HT transport activity, although a small but significant reduction in SERT mRNA and [^{3}H] citalopram binding in the Ala276 was detected versus WT littermates. Interestingly, western blot analysis of Ala276 synaptosomes reveals a gene dosage dependent loss of immunoreactivity for SERT N-terminal epitopes, suggesting either proteolysis or covalent modification in this region that blocks antibody recognition. Behaviorally, Ala276 mice appear normal in measures of anxiety and depression. However, male Ala276 mice show reduced social interactions as measured in the tube test, whereas female Ala276 show decreased marble burying activity. Future studies aim to further characterize the impact of the Ala276 mutation on SERT phosphorylation and regulation by p38 MAPK/PKG and immune stimulation as well as the consequences of altered regulation on behavior and drug responses. These studies will offer opportunities to assess the contribution of SERT conformational modulation *in vivo* and its physiological significance.

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POSTER # C-4

Early life stress and antidepressant treatment impact the gut microbial and metabolic signatures during pregnancy and lactation in SERT$^{+/-}$ rats

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Symptoms of maternal depression occur in an estimated 20% of all pregnancies and up to 10% of women take selective serotonin reuptake inhibitor (SSRI) antidepressants during the perinatal period. Dysregulation of the neurotransmitter serotonin, which is associated with depression and targeted by SSRI medication, does not only affect the brain but also the gut. In particular, it has become clear that serotonin signaling can affect the bacterial communities within the gut. However, it is unknown whether alterations to serotonin homeostasis during pregnancy and lactation disrupt the structure and functional output of the maternal gut microbiome. This could have long-lasting effects on offspring, since maternal gut microbes produce nutrients essential for optimal offspring development. Thus, we examined the contribution of SSRI antidepressant administration on maternal microbial community dynamics using a rat model that mimics a gene-by-environment interaction associated with depression. In this model, heterozygous serotonin transporter knockout rats (SERT$^{+/-}$) are exposed to early life stress by means of maternal separation (MS). In adulthood, female MS and control SERT$^{+/-}$ rats were treated daily throughout pregnancy and lactation with fluoxetine (FLX), a commonly used SSRI, or vehicle. High-resolution 16S rRNA marker gene sequencing and targeted metabolomic analysis were used to assess the bacterial composition and function of fecal samples across pregnancy and lactation. Our results confirm that the signatures of the maternal gut microbiome and metabolome are vastly different between pregnancy and lactation. Interestingly, MS and FLX enhanced some features of the “normal” transition between pregnancy and lactation, such as a higher relative abundance of *Prevotella*, while masking others. Overall, FLX had a stronger disruptive effect on the gut microbiome and metabolome during pregnancy and lactation than MS. For instance, FLX treated females had lower fecal concentrations of the amino acids aspartate and glutamate than vehicle treated females. In short, we showed for the first time that a risk factor for depression and antidepressant treatment during the perinatal period affect the maternal gut microbiome and its functional capacity. We speculate that this alters the mother’s ability to optimally supply the developing offspring with nutrients.

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POSTER # D-1

5-HT neurons regulate fear learning by modulating the basal amygdala

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The neurotransmitter serotonin (5-HT) is targeted by drugs (e.g. selective serotonin reuptake inhibitors) widely used to treat affective disorders such as anxiety and depression, but mechanisms of 5-HT involvement still remain unclear. Fear learning, which is important for avoiding environmental threats and crucial for survival, can become maladaptive in affective disorders. Dysfunctional neural fear circuits may contribute to emotional dysregulation in the aetiology of such disorders. Lesion studies demonstrate that intact 5-HT fibres in the amygdala are necessary for normal fear behaviour. The basal nucleus of the amygdala (BA) is an important locus of fear learning and receives dense 5-HT input from the dorsal raphe nucleus (DRN). To understand the involvement of 5-HT signalling and to optimise the efficacy of 5-HT-targeting drugs, characterising the mechanisms of 5-HT modulation during fear behaviour is essential. Here, the regulation of BA circuits by 5-HT projections was investigated during fear learning. Light-gated opsins, or a control inert protein, were virally delivered in mice to selectively control 5-HT inputs to the BA. First, 5-HT fibre activity was manipulated during a fear learning task to establish the role of 5-HT projections to the BA. Evidence for the bidirectional involvement of 5-HT was provided by showing that 5-HT fibre photoexcitation or photoinhibition enhanced or reduced fearful behaviour, respectively. *In vivo* electrophysiology recordings suggested that 5-HT fibre photoexcitation during fear learning altered the responsiveness of BA neurons to salient stimuli. Through the combination of retrograde tracing and immunohistochemistry, it was found that the majority of DRN neurons projecting to the BA co-express 5-HT and glutamate markers. C-fos staining indicated that the co-expressing neurons were preferentially recruited in fear-learned mice compared to context-control mice, raising the possibility that glutamate co-release from 5-HT fibres could be involved in the regulation of fear. However, local infusion of 5-HT_{1A} and 5-HT_{2A} receptor antagonists into the BA demonstrated that the photoexcitation-induced enhancement of fear learning was mediated predominantly by 5-HT-dependent mechanisms. Collectively, this study provides new insight into the serotonergic neuromodulation of amygdala function during emotional behaviours that are aberrant in affective disorders.

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POSTER #D-2

Differential 5-HT1A autoreceptor sensitivity to fluoxetine within raphe of a novel treatment-resistant depression/anxiety model

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In clinical studies, deficits in serotonergic transmission and increased serotonin-1A (5-HT1A) autoreceptors have been associated with major depression and impaired response to antidepressants. To address the role of increased 5-HT1A autoreceptor expression in behavior, we generated mice with conditional knockout of a key transcriptional repressor (Freud-/CC2D1A) in adult 5-HT neurons (cF1ko), which display antidepressant-resistant anxiety and depression (Vahid-Ansari et al., J. Neuroscience 2017). In cF1ko mice, levels of 5-HT1A autoreceptor protein, binding and hypothermia responses were increased, with reduced 5-HT content and neuronal activity in the dorsal raphe. To examine changes in chronic neuronal activity, FosB+ cells were quantified in cF1ko vs. wild-type littermates (WT), with significant increases in corticolimbic areas that are highly innervated by raphe 5-HT projections including: medial prefrontal cortex (128±15.2 vs. 50±10.8); hippocampal dentate gyrus (42±8.6 vs. 20±4.2); amygdala (136±7.2 vs. 56±10.6) and septum (46±5.6 vs. 25±6.8). To address actions of fluoxetine (FLX), we quantified the levels of 5-HT and 5-HIAA content and ^{125}I-MPPI (5-HT1A) binding sites in dorsal (DR) and median raphe (MR) after 3-week FLX treatment. DR 5-HT and 5-HIAA levels were reduced in cFko vs. WT mice. Chronic FLX significantly increased 5-HT levels in cF1ko DR while it reduced 5-HT in WT DR. DR and MR 5-HT1A binding was increased in cF1ko vs. wild-type, but only in the MR did FLX reduce binding to WT levels, with no effect in DR. No changes in 5-HT1A binding and 5-HT content were observed in the hippocampus or cortex in both groups. These studies suggest a possible role of differential 5-HT1A autoreceptor sensitivity in dorsal vs. median raphe in cF1ko to blunt the response to a successful treatment. Therefore, augmenting Freud-1 might re-establish the transcriptional regulation of 5-HT1A autoreceptors and provide more robust and sustained antidepressant response.

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POSTER BIOCHEM SOC TRAVEL AWARDEE

POSTER # D-3

Neural circuit modelling of serotonin-dopamine interactions in Ventral Tegmental Area and Dorsal Raphe Nucleus

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Abstract:
The endogenous neurochemicals serotonin and dopamine play a vital role in modulating brain functions and regulating emotion related behavior. Pharmacological drugs for the treatment of various psychiatric and mood disorders have targeted the serotonergic systems, which can lead to severe side effects partly due to the complexities of the system and lack of proper understanding of its functionality. The system is complex because serotonin innervates several brain regions simultaneously, acting on many of its receptor subtypes, while also mutually influencing other important neurochemicals like dopamine. The firing activities of neurons in the ventral tegmental area (VTA) and dorsal raphe nucleus (DRN) have been known to encode various rewarding and/or aversive signals. For instance, tonic serotonin (5-HT) neuronal activity in the DRN encodes reward and punishment state values over much longer timescale. Tonic 5-HT also encodes reward waiting. This is further complicated by findings that indicate other local non-DA/5-HT neuronal types to also play important roles. For example, GABAergic neurons in the VTA seem to signal expected reward, while GABAergic neurons in the DRN encode aversive stimuli but inhibited with rewarding stimuli. Further, phasic/transient activation of DRN's glutamatergic neurons can produce strong reinforcement signals. Given the known reciprocal projections between the DRN and VTA, and that both can receive similar monosynaptic inputs, it may be possible that some signals may be shared and distributed among the neural sub-populations in the DRN-VTA circuit. To understand how the circuit's possible connectivity can contribute to the observed distributed signals, we develop a DRN-VTA neural circuit computational model informed by known neurobiology and experimental findings using Optogenetics to identify plausible neural circuit architectures.

We first investigate the consequences of direct 5-HT-DA connectivity, between the DRN and VTA, with only DA and 5-HT neural populations in the model. These direct connections can effectively be positive or negative, due to different receptor types. We study how these neural populations respond when they receive phasic inputs, mimicking the effects of external rewarding/aversive stimuli. We then include non-DA/5-HT neural populations, namely GABAergic/glutamatergic neural populations, in the model. The neural circuit model now includes several indirect pathways, and we investigate how they can influence the system's overall behaviour. Among several simulated results, we found that direct connections between 5-HT and VTA's DA neural population are weak or not necessary. The model predicts a connection from 5-HT neurons to VTA GABAergic neurons. The model also suggests that VTA's GABAergic activity may be due to a reflection of expected reward from 5-HT activity; alignment of the peak of the VTA's GABAergic activity to outcome may be caused by the delay of 5-HT induced influence. Finally, across-trial baseline activity modulation affects 5-HT but not DA neurons even if such excitatory signal acts on both

neuronal types; due to effective inhibition from 5-HT to VTA's GABAergic neurons, which in turn cancels such excitatory signal at DA neurons. Overall, the network model helps to constrain the possible neural circuit connectivity configurations to reconcile with recent experimental findings. Further, mathematical stability analysis is being conducted to study the network behavior with different parameter values and with different other circuits replicating such diverse experimental findings.

ALL OTHER POSTERS

POSTER # D-4

Delineation of signaling interactomes of hallucinogens and non-hallucinogens at the 5-HT2A receptor

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Serotonin (5-hydroxytryptamine, 5-HT) is an evolutionarily conserved neurotransmitter, with pleiotrophic behavioural effects influencing cognition, anxiety, depression, psychoses, and sensory processing. Amongst the fourteen 5-HT receptor subtypes, 5-HT2A receptor is the primary target for hallucinatory drugs acting via 5-HT neurotransmission. The 5-HT2A receptor is a G-protein coupled receptor (GPCR) that is abundantly expressed in the prefrontal cortex and is the target for hallucinogens (HCs) like 2,5-dimethoxy-4-iodo-amphetamine (DOI). Currently, a mechanistic understanding of how different 5-HT2A receptor agonists mediate hallucinogenic (HC) versus non-hallucinogenic (NHC) consequences remains poorly understood. 5-HT2A receptor via Gq signaling activates phosphokinase C (PKC), inositol phosphate (IP), diacylglycerol (DAG), phospho extracellular signal regulated kinase (P-ERK) and calcium release from internal sources. It also couples to beta-arrestin and regulates P-ERK signaling. A biased agonism could result in stabilizing different receptor conformations, preferentially select for particular downstream signaling cascades, have different post-translational modifications, distinct transcriptome fingerprints, lipid signaling mediators and altered heterocomplex formation. The primary focus of this study is to use an *in vitro* system to identify the distinct signaling signatures associated with HC and NHC 5HT2A receptor ligands. HEK293 cells stably overexpressing the human/rat 5-HT2A receptor fused to GFP were induced with DOI (HC) and lisuride (NHC). Western blotting results revealed distinct phospho ERK, phospho PKC and phospho CREB induction dynamics across the two agonists in dose response and time course experiments. Similarly, phosphoprotein signaling readouts obtained from primary cortical neuron cultures treated with DOI and lisuride corroborated the findings. Inositol phosphate induction measurements showed a similar dose response across the two ligands, however, the levels were slightly lesser for lisuride mediated induction. These preliminary observations necessitate a more detailed characterization of HC versus NHC interactome which is being carried out.

Supported by Tata Institute of Fundamental Research, Mumbai, India

POSTER # E-1

Mapping the serotonergic system wiring transmission

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Serotonergic neurons are part of one of the most widely distributed projection systems of the mammalian brain. Serotonergic fibers have varicosities where the transmitter is synthesized, stored and released in a “volume transmission” mode. Although to a lesser extent, they also present synapse-like specializations where synaptic contacts are established with specific neuronal partners acting in a conventional “wiring transmission” mode. Experimental strategies used to map serotonergic projections so far where not selective for either volume or wiring transmitting terminals. Therefore, the organization and distribution of wiring transmission serotonergic ascending projections remain to be drawn. Taking advantage of the properties of the recombinant rabies virus, whose envelope can drive the infection of neurons exclusively through presynaptic terminals, we have used a retrograde labeling approach to map selectively the serotonergic wiring transmitting system. We investigated 19 brain district and results revealed that each of them receives wiring transmission from a relatively small and region-specific number of serotonergic neurons. It was also possible to build a correlation map between specific raphe nuclei and distinct target brain areas. Altogether, this study sheds new light on the serotonergic system organization and its anatomical properties, paving the way to understanding the selective role of serotonergic wiring transmission.

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POSTER #E-2

The vascular reactivity to serotonin of human chorionic plate arteries in gestational diabetes and preeclampsia

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Context. Recent studies revealed an important role of serotonin in gestational diabetes (GDM) and preeclampsia (PE). Serotonin might play a crucial role not only in the development of these conditions but also in several consequences as it has receptors and effects in different systems and organs including heart, brain, vessels, carbohydrate metabolism. An increased plasma concentration of 5-HT (5-hydroxytryptamine) has been shown in GDM, PE pregnancies. Moreover, studies suggest an important role of downregulated serotonin transporters in GDM. However the vascular reactivity to 5-HT has not been studied comparing considered pregnancy complications.

Objective. To investigate the response to serotonin in isolated human chorionic plate arteries (HCPA) from PE, GDM, PE+GDM and normal pregnancies.

Methods and patients. The contractile responses of 16 HCPA were evaluated using wire myography (DMT A/S, Denmark) in four groups of age-matched women: with GDM+PE, PE, GDM, controls. Contractile response in HCPA was investigated to raising serotonin concentrations: 0,001-10 μM. Data are presented as mean±SEM. Statistical analysis was performed using Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

Main outcome measures. Dose-response curve to serotonin raising concentrations, Emax (% KCl) – response to maximum serotonin concentration (10μM)

Results. Serotonin caused HCPA contraction in a concentration-dependent manner in all investigated groups, at 1-5 μM agonist concentration range contraction in all investigated groups were similar ($p>0,05$). The agonist effect significantly differs at 5μM and higher concentrations ($p<0,01$). Emax (%KCl) for serotonin was significantly higher in GDM and GDM+PE arteries comparing to controls ($p < 0.05$). The mean Emax (%KCl) for controls was 53,68 (±9,77) whilst GDM arteries showed a mean Emax of 163,12 (±42,28) and GDM+PE – 90,52 (±17,21). The mean Emax (%KCl) for PE arteries was 102,38 (±9,80).

Conclusions. This study demonstrated for the first time an augmented response to serotonin in GDM chorionic plate arteries comparing to similar vessel response in PE, GDM+PE and normal pregnancies. Serotoninergic system might be involved in the pathogenesis of studied pregnancy disorder as well as explain the association of GDM with PE, cardiovascular disorders in mother and offspring, fetal heart defects, psychiatric disorders and other long-term consequences of GDM.

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POSTER # E-3

Serotonin deficiency in the central nervous system alters neuroplastic mechanisms in basal condition and influences the response to an acute stress.

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Dysregulation of central serotonergic system may contribute to the pathophysiology of mood disorders. Rats deficient in tryptophan hydroxylase 2 (Tph2), the rate limiting enzyme of serotonin synthesis in the central nervous system, were recently generated using zinc-finger nuclease technology. We took advantage of this animal model, lacking serotonin specifically in the brain, to investigate whether a vulnerable genotype can be associated with alterations of neuronal plasticity from the early stage of maturation of the brain until adulthood.
We found a significant increase of the neurotrophin Brain-Derived Neurotrophic Factor (Bdnf) in the prefrontal cortex of adult Tph2-deficient ($Tph2^{-/-}$) male and female rats in comparison to wild type ($Tph2^{+/+}$) counterpart. Interestingly, a different pattern was observed during early postnatal life, with a decrease in total Bdnf mRNA levels in $Tph2^{-/-}$ compared to $Tph2^{+/+}$ rats at post-natal day (pnd)1 and with an improvement at pnd30 both in male and female $Tph2^{-/-}$ rats. Moreover, to establish if the lack of serotonin may influence the response to a challenging situation, we exposed adult rats to an acute stress. We observed differences in neuronal activation in the prefrontal cortex of $Tph2^{+/+}$ and $Tph2^{-/-}$ rats, measured as gene expression of the immediate early genes Arc and cFos. Interestingly upregulation of Bdnf mRNA levels after stress was more pronounced in $Tph2^{+/+}$ compared to $Tph2^{-/-}$ rats.
In summary, our results demonstrated that serotonin deficiency affects neuroplastic mechanisms following a specific temporal pattern, and influences the response to an acute stress suggesting an impairment in the coping strategies set in motion by a challenging condition.

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POSTER # E-4

Serotonin 2C Receptor-mediated Attenuation of Oxytocin Receptor Signalling

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The serotonin 2C (5-HT_{2C}) receptor, a seven transmembrane G-protein-coupled receptor (GPCR), plays a key modulatory role in many centrally-mediated pathways. Intervention in serotoninergic neurotransmission through the 5-HT_{2C} receptor has been proposed for the treatment of many psychiatric disorders with altered mood and sociability such as anxiety, depression, and schizophrenia. The oxytocin receptor (OTR) is another key GPCR that plays a major role in promoting social behaviours. Interestingly, the respective endogenous neurotransmitters, 5-HT and OT, have been described to be reciprocally regulated following receptor modulation, which prompted us to investigate OT and 5-HT_{2C} receptors crosstalk.

Physical interaction between OT and 5-HT_{2C} receptors was explored with the use of Flow Cytometry based FRET (fcFRET) and confocal microscopy. Ligand-mediated internalization of both receptors was monitored with the use of a fluorescent automatic cell imaging system. Alterations in Gq-dependent signalling were investigated using calcium mobilization and IP-1 accumulation assays. All experiments were performed in HEK293A cells stably expressing 5-HT_{2C} receptor tagged with eGFP, and transiently transduced with lentiviral plasmid encoding the OTR tagged with RFP. Physical interaction between OT and 5-HT_{2C} receptors was demonstrated by a 16% fcFRET signal, and confirmed by a clear co-localization of the OTR/5-HTR_{2C} pair on the cell membrane using confocal microscopy. Calcium mobilisation, IP-1 accumulation, and internalization results revealed a significant attenuation in OTR-mediated signalling in HEK293A cells co-expressing the OTR/5-HTR_{2C} pair. Further investigation demonstrated that attenuated OTR signalling was restored following treatment with 5-HT_{2C} receptor antagonists; SB242084 and RS102221.

Above results, indicate that 5-HT_{2C} receptor-mediated attenuation of OTR signalling is driven by formation of OTR/5-HTR_{2C} heterodimers on the cell membrane. Further *in vivo* studies exploring the functional nature of an OTR/5-HTR_{2C} interaction are now warranted. The existence of a novel 5-HT_{2C}/OT receptors heterodimer is poised to lead to more selective strategies for the treatment of social disorders.

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POSTER # F-1

Endogenous serotonergic tone as a modulator of metabolic homeostasis and response to metabolic challenge

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A remarkable extent to which central and peripheral serotonin (5HT) signaling impacts the regulation of energy balance and, consequently, the obesity and other metabolic disorders, has been recognized recently. Many of the molecular mechanisms underlying serotonin action in the body weight regulation have been identified, but for comprehensive understanding of serotonin-mediated control of energy homeostasis, studies from a whole-body perspective are essential.
In search for the interplay between endogenous serotonin tone and metabolic homeostasis, we use a genetic model of rats (Wistar-Zagreb 5HT rats) with constitutionally altered whole body serotonin system (hyper/hyposerotonergic). The two sublines (high-/low-5HT subline) of the model were developed by selective breeding of rats toward extremes of peripheral (platelet) 5HT transporter activity. Animals from the high-5HT subline show an increased body weight and elevated visceral adiposity as compared to the low-5HT animals, prompting us to investigate metabolic differences between sublines in both, physiological conditions and in response to specific metabolic challenge (high-fat diet).
We have compared 5HT sublines for biochemical parameters (glucose, lipid profile, adipocytokines levels) in blood plasma, expression of genes for relevant metabolic players (cytokines, transcription factors) in white adipose tissue as well as their functional responses to glucose and insulin loading.
Result show that, in addition to increased body weight and adiposity, high 5HT tone of animals was associated with increased blood glucose and lipids, while adipocytokine (leptin) levels differed only in the fed state of animals. Functionally, high-5HT rats were glucose intolerant and insulin resistant in comparison to the low-5HT subline. On the other hand, the low 5HT animals showed more pronounced functional consequences of high-fat diet, specifically, the body weight accumulation, increase in plasma glucose level and the response in glucose tolerance and insulin tolerance tests.
In conclusion, results show a complex interrelation between endogenous 5HT activity and metabolic (dys)regulation. In addition, our Wistar-Zagreb 5HT rats could probably be a usefull animal model to study the (integrative) serotonergic mechanisms in dysmetabolic states such as obesity and insulin resistance.

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POSTER # F-2

OCT3, a putative mechanism contributing to the sedative/hypnotic effects of ethanol in mice

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Pharmacological agents that enhance the aversive effects of ethanol can be effective for the treatment of alcohol use disorder (AUD) by mitigating ethanol's rewarding effects and limiting ethanol consumption. However, the complex mechanisms by which ethanol produces these effects remain to be fully elucidated. Acute administration of ethanol has been shown to increase extracellular serotonin (5HT) in limbic brain regions, which is at least in part due to inhibition of 5HT uptake. Using *in vivo* chronoamperometry, we found that ethanol inhibits 5HT clearance independently of the high-affinity serotonin transporter (SERT), and that this effect was potentiated in SERT knockout (-/-) mice. Complementing these neurochemical findings, SERT-/- mice were more sensitive to the sedative/hypnotic effects of ethanol, relative to wild-type (+/+) control mice. Our lab also showed that organic cation transporter 3 (OCT3), a low-affinity, high-capacity ("uptake 2") transporter, is upregulated in SERT-/- and heterozygous (+/-) mice hippocampi, and preliminary data suggest that ethanol is able to significantly inhibit OCT3-mediated uptake activity at physiologically relevant concentrations. These data raise the possibility that OCT3 could have a role in ethanol's effects on 5HT clearance. Here, we hypothesize that ethanol inhibition of OCT3-mediated serotonin uptake contributes to the sedative/hypnotic effects of ethanol in mice. To explore this, we tested the sensitivity of OCT3+/+, OCT3-/-, SERT+/+, SERT +/-, and SERT -/- male, adult mice to ethanol-induced loss of righting reflex (LORR) and locomotor ataxia. Duration of LORR was significantly shorter in OCT3 -/- relative to OCT3 +/+ mice, although OCT3 -/- mice still exhibited significant locomotor ataxia following ethanol administration. Replicating earlier findings, SERT -/- mice experienced a longer LORR duration relative to both SERT +/+ and SERT +/- mice. Together, these results may point to OCT3 as a putative target for pharmacological therapies treating AUD.

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POSTER # F-3

Common neural underpinnings between anorexia, memory and addiction

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In neurons of the nucleus accumbens (NAc), activation of a cAMP signaling is a means of transforming an immediate reduction of drugs' rewarding effect into a durable dependence, mimicking a form of learning. After recruiting cAMP-response element binding protein (CREB)-binding protein, the resultant phosphorylated CREB (pCREB) favors the expression of some genes (FosB, ΔFosB, and CART: cocaine- and amphetamine-regulated transcript) to the detriment of others (methyltransferase G9a of histone), from where come changes in neuron morphology. Serotonin (5-HT, 5-hydroxytryptamine) volume transmission trough many receptors act on cAMP signaling and thus modulate the activity of the reward neural pathways. Our previous studies show that stimulation of Gs-coupled serotonin 4 receptors (5-HT_4Rs) triggers activation of cAMP/PKA/CART/FosB/ΔFosB signaling pathway, which serve to induce anorexia-like behavior. Here, we examine how cAMP in the NAc impacts food intake. We found that elevated levels in cAMP induced by local infusion of BIMU8, a 5-HT_4Rs agonist, into the NAc were more prominent when BIMU8 was co-infused with St-Ht31 peptide that blocks AKAP (A-kinase anchoring protein) / PKA binding. Results includes that the levels of CART peptide, known to promote anorexia and addiction, were more elevated following St-Ht31/BIMU8 co-treatment than in mice infused only with BIMU8 into the NAc. Finally, mice with highest increased levels of cAMP and CART induced by the blockade of AKAP/PKA binding in the NAc display the highest restrictive food intake following food deprivation, supporting the view that anorexia becomes persistent through similar mechanisms underlying habituation towards learning and memory.
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POSTER #F-4

Psychotropic Drugs and Microbiota-Gut-Brain Axis Function

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Several compounds of the psychotropic class, such as antipsychotics and antidepressants, work through modulation of serotonin levels in the brain. Despite substantial scientific focus on the role of gut microbiota in drug metabolism, there remains a critical lack of understanding of how psychotropic medications might affect the gut microbiota. Unravelling this aspect will provide new insight into the mechanisms of action of these medications and will possibly identify the gut microbiota as plausible target for the treatment of psychiatric disorders.

The aim of this study was to investigate the impact of 4-week treatment with antidepressants and other psychotropic drugs on intestinal parameters and microbiota composition in rats. To this end, seven experimental groups (vehicle, escitalopram, venlafaxine, fluoxetine, lithium, valproate and aripiprazole) received a chronic treatment followed by assessment of locomotor activity, intestinal permeability and 16S bacterial rRNA sequencing of the caecum content. In addition, the antimicrobial activity of these drugs against *Lactobacillus rhamnosus* and *Escherichia coli,* two bacterial strains commonly resident in the human gut, was assessed.

None of the treatments influenced locomotor activity. Fluoxetine, lithium, valproate and aripiprazole increased the richness and diversity of gut microbiota species when compared to the vehicle-treated group. Animals treated with escitalopram, venlafaxine, fluoxetine and aripiprazole exhibited an increased permeability in the ileum. *In vitro*, fluoxetine and escitalopram showed a dose-dependent antimicrobial activity.

These data show that psychotropic medications differentially influence the composition of gut microbiota *in vivo* and that the serotonin-reuptake inhibitors fluoxetine and escitalopram, have specific antimicrobial activity *in vitro*. Interestingly, drugs that significantly altered gut microbial composition did not increase intestinal permeability, suggesting that the two factors are not causally linked. Overall, unravelling the impact of psychotropics on gastrointestinal and microbial parameters offers the potential to provide critical insight into the mechanism of action and side effects of these medications.

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POSTER # G-1

The effects of gestational exposure to SSRI antidepressants on maternal and neonatal parameters in the rat

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Introduction The most commonly prescribed psychotropic drugs in pregnancy are selective serotonin reuptake inhibitor (SSRI) antidepressants[1]. SSRIs alter synaptic availability of serotonin, an important growth factor in embryogenesis. When SSRIs are prescribed during pregnancy, clinical gestational exposure is associated with lower birth weights, preterm birth and developmental delays as SSRIs cross the placental barrier[2]. Observing maternal and neonatal outcomes, this study examined the effects of the four commonly used SSRIs, paroxetine (PRX), sertraline (SERT), citalopram (CIT), and fluoxetine (FLX), *in utero* using a clinically relevant approach developed in our laboratory[3].

Methods Female Sprague-Dawley rats (approx. 4 months old) were mated and singly housed. From gestation day 7 until littering, dams received either vehicle, 1.25, 2.5, or 5 mg/kg PRX, 2.5, 5 or 10 mg/kg SERT or CIT, or 2.5 mg/kg FLX via oral gavage (n=9-13/group). Maternal body weights and food intake were recorded, and pup characteristics such as litter size, sex ratio and mortality were recorded following littering. Depending on the nature of the data, they were analysed using one or two-way ANOVA, Kruskal-Wallis or Chi-Squared test; $p<0.05$ was deemed statistically significant.

Results During gestation, the only drug effect observed was with SERT (10 mg/kg) which significantly reduced maternal weight gain and food consumption. At birth there were no differences in litter size or sex ratio. However, there was a significant increase in pup mortality within the week following littering for PRX (2.5, 5 mg/kg) and SERT (10 mg/kg) exposed dams.

Conclusions Overall, the data demonstrate that at pharmacological doses, SERT decreased maternal weight gain and food consumption. Furthermore, both PRX and SERT have profound effects on neonatal mortality in the rat, whilst CIT at the doses studied had minimal consequences. Such findings in an animal model could have important implications for prescribing SSRI antidepressants during pregnancy.

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POSTER # G-2

Genetic reductions of the serotonin transporter and the transgenerational effects of ethanol

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Alcoholism remains the most severe addiction and the costs for the patient, family and society are horrendous. While around 85% of the general population consume alcohol, only 15 to 20% show hazardous drinking patterns. Genetic studies in animals and humans have identified (among others) the serotonin transporter (SERT) as a risk factor for drug abuse. In addition to the acute effects of alcohol on the individual, there are increasing indications that excessive consumption of alcohol may affect the sperm cells and, by extension, the next generation.

In the present study, we aimed to investigate whether a genetic reduction in SERT activity moderates the acute reinforcing effects of alcohol and/or its transgenerational effects. To address this, $SERT^{+/+}$, $SERT^{+/-}$ and $SERT^{-/-}$ male and female rats were allowed to consume a 20% ethanol solution in a two-bottle intermittent choice paradigm for eight weeks. Six weeks after the last consumption day, the male $SERT^{+/+}$ and $SERT^{-/-}$ rats with the highest levels of ethanol intake were mated with naïve $SERT^{+/-}$ females. The offspring was subsequently tested for ethanol-induced locomotor activity (LMA) and ethanol intake.

The results showed that female $SERT^{-/-}$ rats consumed significantly more ethanol than female $SERT^{+/+}$ rats, while no genotype difference was found in the males. In addition, we found that the male offspring of high ethanol drinking fathers consumed significantly more ethanol than the offspring of non-drinking fathers. This was not observed in females. With respect to LMA, while offspring of control rats showed ethanol-induced hyperactivity, this was not observed in the offspring of ethanol-drinking fathers. However, these transgenerational effects were not moderated by the genotype of the father, nor of the offspring.

In summary, we found that the SERT moderates the initial reinforcing effects of ethanol in females. We also found clear evidence for an enhanced propensity to consume ethanol in offspring of parents that had consumed large quantities of ethanol. However, this latter effect was not dependent on the genotype of the fathers.

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POSTER # G-3

Characterization of 8-OH-DPAT-disrupted spontaneous alternation behaviour as a mouse model of compulsivity.

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Rationale: Serotonin (5-hydroxytrypamine; 5-HT) has long been of interest in the treatment of compulsive disorders. Pharmacological treatments elevating 5-HT levels are first-line treatments for obsessive-compulsive disorder. Rodents exhibit natural exploratory behaviours when faced with novel environments. The spontaneous alteration behaviour (SAB) test can measure such behaviours, and perseverance in this test induced by the 5-HT1AR agonist, 8-OH-DPAT, resembles compulsive behaviours observed in humans.

Objective: This study characterized whether the mechanism of action of 8-OH-DPAT in reducing SAB in adult male C57BL/6 mice is mediated by the 5-HT1A receptor. It was also investigated whether the 5-HT reuptake inhibitor, citalopram, and releasing agent, 3,4-methylenedioxymethamphetamine (MDMA), attenuated the effects of 8-OH-DPAT in order to deepen understanding of 5-HT-related mechanisms.

Methods: Perseverance was induced by administration of 8-OH-DPAT using a three-armed Y-maze. Animals were considered to have performed a correct alternation when they visited three consecutive different arms during a 10 min testing period. Alternation rate was calculated based on the number of correct alternations out of the maximum possible amount of correct alternations and served as a measure of SAB. All drugs were given i.p. with at least 7 days washout between each test. Animals that performed less than 12 arm visits in the SAB test (including the arm they were placed in) were excluded from the dataset because it was not possible to calculate a reliable alternation rate. Alternation rate and total number of arm visits in the SAB test were analysed by single measures analysis of variance (ANOVA) with drug treatments as independent factors.

Results and discussion: Reduction in SAB in was induced by administration of 8-OH-DPAT (1 mg/kg, $p<0.05$), and its 5-HT1A receptor mechanism was confirmed by co-administration of WAY100635 (2 mg/kg, $p<0.05$). Citalopram or MDMA failed to attenuate the robust 5-HT1AR-mediated effects of 8-OH-DPAT on SAB. These findings confirm the importance of 5-HT in regulating perseverative behaviour. Future investigations are required to determine the validity of the 8-OH-DPAT-disrupted SAB as a simple model of compulsive-like behaviours.

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POSTER # G-4

Differential Long-Lasting Behavioural and Molecular Effects of Perturbations of the Microbiome During the Adolescent Period in Mice

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The microbiota-gut-brain axis concerns the ability of the microbiota to influence gastrointestinal tract (GI) and central servosystem (CNS) functions and thereby modulate brain development and behaviour [1]. Although it is well-recognized that the gut microbiota regulates the host gastrointestinal serotoninergic system, it is unknown how changes in the composition of the gut microbiota due to diet or antibiotic treatment may affect host serotonin production within the gut. Recent hypotheses suggest that neurodevelopment runs in parallel with establishment of an adult microbial profile and that disturbance or the lack thereof influences behaviour and brain function in adulthood by permanently altering CNS or GI function [2]. Here, we aimed to investigate whether exposure to antibiotic treatment or suboptimal diet, both of which significantly affect microbiota composition, resulted in long-lasting effects in the gut, gene expression, and behaviour independent of changes in GI serotonin turnover.
Male C57BL/6 mice were exposed to high-fat diet, cafeteria diet or antibiotic treatment for 21 days during adolescence and were tested for behavioural changes in adulthood. Whereas mice treated with antibiotics during adolescence showed increased anxiety-related behaviour in the elevated plus maze, dietary intervention did not change behaviour. No effects of treatment where seen in the open field, novel object recognition, social interaction or fear conditioning. In adulthood, drastic changes in gene expression in the amygdala of genes related to immune response, gut peptides, microglia, tight junction, neuroplasticity, synaptic transmission and short chain fatty acids, were observed in the brain of animals treated with either antibiotics or diets. Changes in gene expression were also seen in the gut. The GI serotonergic system, that was investigated as a indicator for proper GI functioning, was not permanently affected by adolescent treatments as colonic serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and 5-HIAA/5-HT ratio were not altered by any treatment.

Overall our results demonstrate that exposure to known microbial altering factors during adolescence can induce long-lasting changes in murine behaviour and the microbiota-gut-brain axis without perturbation to the colonic serotonergic system. Further research needs to be done to investigate the possible mechanisms involved

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POSTER # H-1

Establishing the CNS Serotonergic Contribution to the Molecular, Physiological and Behavioral Actions of Inflammatory Cytokine IL-1β

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Serotonergic circuits are involved in many complex behaviors where dysfunction is believed to support multiple neuropsychiatric disorders, including anxiety, depression, autism, and schizophrenia. Although often treated monolithically, serotonergic projections arise from subpopulations of neurons that ultimately confer specific behavioral outcomes following activation. One subpopulation of serotonergic neurons that has drawn recent attention, but for which little is understood, are those that support the transduction of peripheral immune system activation into changes in discrete behaviors. Prior RNA expression studies suggest non-uniform expression of the interleukin 1 receptor (IL-1R), indicating that specific pathways dictate the serotonergic contributions to the behavioral actions of the inflammatory cytokine IL-1β. Until recently, detection of expression of IL-1Rs at the protein level has proved difficult, and as such Il-1R modulated serotonin circuits have remained poorly defined. Using transgenic mouse lines in which we specifically eliminate IL-1Rs in serotoninergic neurons or restore serotonergic expression of IL-1Rs on an IL-1R KO background, coupled with immunohistochemistry, we have gathered evidence to support the existence of subsets of serotonergic neurons with the capacity to respond to IL-1β via IL-1R. Our current efforts are aimed at determining requirements for serotonergic IL-1R expression for the physiological and behavioral actions of IL-1β, elucidating the projections and inputs of IL-1R expressing serotonin neurons, and establishing the necessity of serotonergic IL-1R expression for Il-1β driven behavioral responses.

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POSTER # H-2

Sleep deprivation rapidly upregulates 5-HT$_{2A}$ receptor expression in the prefrontal cortex via the immediate early gene *Egr3*

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Dysfunction of serotonin 2A receptors (5-HT$_{2A}$Rs) has been implicated in the symptoms of schizophrenia, and levels of the receptor are reduced in schizophrenia patients' brains. Insufficient 5-HT$_{2A}$R receptor expression is one potential explanation for these findings. However, the molecular mechanisms that regulate 5-HT$_{2A}$R expression are unknown. We have previously reported that six hours of sleep deprivation upregulates expression of *Htr2a*, the 5-HT$_{2A}$R gene, in the mouse cortex. Furthermore, this induction requires the activity-dependent immediate early gene transcription factor, early growth response 3 (*Egr3*). In the current studies, we have used quantitative reverse transcription-PCR to show that sleep deprivation induces *Htr2a* in prefrontal cortex (PFC), but not in more anterior or posterior cortical regions, in wildtype mice, and that this requires *Egr3*. This is paralleled by an increase in 5-HT$_{2A}$R protein, detectable by autoradiography using the selective ligand [^{3}H]-M100907, after just eight hours of sleep deprivation. Chromatin immunoprecipitation demonstrated that neuronal activity significantly upregulates EGR3 binding to the *Htr2a* promoter in the mouse PFC. Furthermore, *in vitro,* EGR3 induces expression of luciferase reporter constructs driven by the *Htr2a* promoter containing either of two EGR3 binding sites. Finally, viral-mediated expression of *Htr2a* in the frontal cortex of *Egr3*-deficient (-/-) mice, which we have previously shown have reduced 5-HT$_{2A}$R levels, rescues anxiety-like behavioral abnormalities of these animals. These findings demonstrate that EGR3 can regulate expression of the 5-HT$_{2A}$R via direct binding to the *Htr2a* promoter, and strongly suggest that EGR3 may directly regulate 5-HT$_{2A}$R expression in the PFC in response to the physiologic stimulus of sleep deprivation. In addition, these results suggest that the reduced 5-HT$_{2A}$R levels in the brains of *Egr3-/-* mice are responsible for at least some of the behavioral abnormalities in these animals. Finally, these findings suggest the possibility that inadequate activation of EGR3 may contribute to the reduced 5-HT$_{2A}$R levels in schizophrenia patients' brains.

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POSTER # H-3

Contribution of raphe serotonin cells in encoding acute stress-related information to modulate behavioral states

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Stress is an etiologic factor in psychiatric disorders, especially those involving anxiety. The serotonin (5-HT) system is one of the first systems engaged following a stressful event and the response of this system to stress is particularly important in the context of stress-related neuropsychiatric disorders. However, while it has been demonstrated that 5-HT neurons are activated by many stressors, the underlying neural circuitry and the role of 5-HT neurons in generating stress-induced anxiety has not been elucidated. Further, whether this 5-HT system activation serves to promote, or inhibit, persistent anxiety is not clear

To examine the role of 5-HT cells in acute stress-induced anxiety, we implemented a restraint stress paradigm followed by anxiety testing. Following restraint stress, we observed a robust stress-induced anxiety phenotype and increased immediate early gene (c-fos) expression in dorsal raphe (DR), but not median raphe 5-HT neurons. Using *in vivo* recordings, our preliminary data indicates that restraint stress results in a trend towards increased activity during the stress acquisition. Perhaps more impressive is that this enhanced DR activity persists during anxiety testing resulting in increased anxiety. In addition, chemogenetic inhibition of DR-5-HT cells during restraint stress reduces restraint-induced anxiety, suggesting that DR-5-HT activity is needed during the stress experience to produce an anxiety state. Conversely, optogenetically activating DR-5-HT cells in stress naïve mice for the same duration as restraint stress is sufficient to mimic acute stress-induced anxiety-like behaviors and corticosterone increase. These results suggest that exogenously increasing DR 5-HT neural activity can create an anxiety state once the stimulation is removed. Taken together, our results suggest that restraint stress increases DR-5-HT cellular activity, influencing future behaviors by increasing anxiety- like behaviors.

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POSTER # H-4

Mono-allelic autoimmunity and serotonin

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Tolerance to self-antigens is a complex process that begins with the education of developing thymocytes by rare medullary thymic epithelial cells (mTEC) in the thymus. During this process mTECs express and display ~90% of the body's coding proteome in order to eliminate autoreactive thymocytes that could react with self-antigens and cause autoimmunity in the periphery. Crucial to this process is the Autoimmune Regulator (AIRE) protein, as it is responsible for the expression of hundreds of tissue-restricted antigens in mature mTECs. Indeed, dysfunction of the AIRE gene in humans results in rare multi-organ autoimmune syndrome, Autoimmune Polyendocrine Syndrome type 1 (APS-1). Historically, APS-1 has been shown to follow an autosomal recessive mode of inheritance, however, our recent data demonstrate that even mono-allelic mutations in *AIRE* can underlie organ-specific autoimmunity, characterized with later onsets and milder phenotypes than classical APS-1, yet with significantly higher prevalence. To better characterize the breakdown of self-tolerance by dominant-negative AIRE mutants, we have created a mouse model carrying a C311Y missense mutation in the *Aire* gene (C313Y in the mouse) on the NOD background. Interestingly, $Aire^{+/C313Y}$ heterozygous mice phenocopied traits of Aire knockout mice on this genetic background, such as wasting disease coupled with the development of organ-specific autoimmunity, increased frequencies of mature medullary thymic epithelial cells, decreased frequencies of thymic T regulatory cells and profound reduction in the expression of Aire-target genes in mTECs, including Tph1. This consequently resulted in a breakdown of tolerance to Tph1, which was characterized by autoimmune attack on Tph1-expressing enterochromaffin cells and concomitant and dramatic drop in serum serotonin levels in the $Aire^{+/C313Y}$ mice. These findings clearly demonstrate that mono-allelic mutations in Aire can give rise to devastating autoimmunity, and may have far-reaching consequences for many physiological processes including those dependent on peripheral serotonin.

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POSTER # I-1

Managing perinatal depression with fluoxetine: effect on maternal and neonatal microbiota

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Depression during pregnancy is associated with detrimental and long-lasting effects on the mother and her offspring. As a result, selective serotonin reuptake inhibitor antidepressants (SSRIs) are frequently used to treat maternal mood and anxiety disorders in pregnancy. However, perinatal exposure to SSRIs was shown to affect foetal brain development; *in utero* exposure to these medications has thus raised concerns about altered behavioural outcomes later in life. Recent findings suggest that SSRIs can also have a direct impact on the host microbiota. Transmission of altered microbiota from the mother to the infant can, in turn, affect early life programming of brain and behaviour. Using a rat model of perinatal depression, we sought to explore the effects of gestational stress (GS) and SSRI treatment on maternal and neonatal microbiota. Sprague-Dawley female rats were either left undisturbed or subjected to chronic unpredictable stress during the pregnancy. Half of dams were supplemented daily with fluoxetine (FLX) throughout the stress exposure. Microbiota composition was analysed in faecal samples and vaginal smears of dams just before and after giving birth, as well as in the colonic content of male pups. Administration of FLX to non-stressed dams altered maternal faecal microbiota: a few bacterial genera from *Lachnospiraceae, Ruminococcaceae* and *Erysipelotrichaceae* families of the *Firmicutes* phylum (*NK4A136* group, *Tyzzerella*, *Ruminiclostridium*, *Erysipelotrichaceae UCG-003*) were differentially altered in abundance in control rats. Administration of FLX to stressed dams had a distinct impact on maternal microbiota. FLX successfully counteracted some of the GS-induced changes: a reduction in *Bifidobacterium*, *Roseburia* and *Lachnospiraceae UCG-001*, and an increase in *Bacteroides* species. Furthermore, FLX exerted a GS-independent effect in stressed dams, differentially affecting the relative abundance of *Coriobacteriaceae*, *Clostridiales vadinBB60* group and *Erysipelotrichaceae* uncultured bacteria. These data suggest complex interaction effect of the GS and FLX on maternal faecal microbiota. On-going work is aimed at investigating whether GS/FLX-induced effects are seen in the vaginal microbiota of dams, and whether these changes can altogether affect vertical transmission of maternal bacteria and eventually alter microbial colonization of neonatal gut.

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POSTER # I-2

Delayed colonic transit in obese mice is normalized by dietary prebiotic supplementation: implication of gut microbiota

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Obesity is associated with significant alterations in colonic motility and intestinal microbiota. Gut bacteria can modulate intestinal transit through the production of short-chain fatty acids (SCFAs) and modulation of serotonin signalling in the host. Prebiotics are non-digestible dietary fibers which promote the growth of beneficial microbes in the gut. However, the effects of prebiotics on obesity-associated changes in colonic motility and serotonin metabolism have not been fully explored. Here we investigate whether dietary supplementation of a prebiotic fructooligosaccharides and galactooligosaccharides mixture (FOS/GOS) can change microbiota composition and improve impaired colonic transit in high fat diet-fed obese mice. C57/BL6 male mice were fed either a high fat (HFD) or a low fat diet (LFD) for 6 weeks; after which half of the animals were co-supplemented with FOS/GOS in drinking water for another 6 weeks. Colonic transit time was quantified *ex vivo* as the rate of spontaneous propagation of artificial pellet along the isolated colon. Caecal content was collected for the analysis of microbiota composition and SCFA levels. Colonic tissue was harvested to measure serotonin levels and for the gene expression analysis. Colonic transit was substantially impaired in HFD group. FOS/GOS co-supplementation improved the propulsive activity of the colon in HFD-fed mice, whilst having no effect in LFD-fed animals. The effect of prebiotic on the motility was not associated with an increase in SCFA levels in the gut lumen or serotonin levels in the colon. FOS/GOS altered caecal microbiota composition in HFD- and LFD-fed mice, decreasing bacterial species diversity and supporting the growth of *Bacteroidetes* at the expense of *Firmicutes* bacteria. Interestingly, a few bacterial taxa were affected by prebiotic in a diet-specific manner. To conclude, dietary enrichment with FOS/GOS can attenuate the deficit in colonic transit associated with the ingestion of high fat diet. These data suggest that prebiotics maybe a useful dietary adjunct for alleviating gastrointestinal symptoms in obesity.

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POSTER # I-3

Morphology and synaptogenesis of enteric neurons – the impact of serotonin receptor 5-HT7

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Intestinal inflammation is associated with structural and functional changes of the enteric nervous system (ENS), which can persist long after recovery, and may contribute to altered gut function in post-inflammatory irritable bowel syndrome occurring after infectious enteritis or active inflammatory bowel disease. We have previously shown that serotonin (5-HT) regulates neuronal morphology and plasticity via 5-HT receptor isoform 7 (5-HT7R)-mediated activation of RhoA and Cdc42 GTPases in CNS neurons. In the intestine, 5-HT7R is localized to enteric neurons. However, it is unresolved whether 5-HT7R is involved in intestinal neurons plasticity. The aim of the present study was to investigate the morphogenic role of 5-HT7R in enteric neurons.

We used primary culture of isolated intestinal ganglia from adult C57BL/6J mice, which contains both myenteric as well submucosal neurons that survive up to 20 days. Using immunofluorescence, confocal microscopy and morphometric measurements, we analyzed neurite outgrowth at day in vitro 2 (DIV2), and the number of synapses at DIV12. Quantitative analysis revealed a significant increase in the length of neurites and the number of synaptophysin-positive puncta in cultures treated with 5-HT7R agonist 5-CT *vs.* control preparation. This morphogenic effect was fully inhibited by the specific 5-HT7R antagonist SB-269970. To assess the morphogenic role of endogenous 5-HT7R in enteric neurons *in vivo,* we performed morphological analysis of axon density in murine intestine after intra-peritoneal injection of SB-269970 for 35 day followed by immunofluorescence analysis of intestinal cryosections using confocal microscopy. Quantification of βIII-Tubulin-positive projections revealed a reduction of axonal density in mouse intestine.

Taken together, we have demonstrated that 5-HT7R-mediated signaling induces neuronal outgrowth and synaptogenesis of intestinal neurons, and might play an important role in the recovery and normalization of the ENS function after intestinal inflammation.

POSTER # I-4

Increased maternal extracellular serotonin levels beneficially influences offspring's anxiety- and anhedonia-like behaviour

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Serotonin is a critical player in brain development whereby serotonin neurotrophic actions can be regulated through maternal-foetal interactions. Hence, maternal rather than offspring's serotonergic genotype may determine variation in serotonin levels in the early foetal brain, which might result in downstream effects on the development of the brain and potentially influencing behaviour. Indeed, serotonin has been shown to be involved in psychiatric disorders such as autism, anxiety, and depression, but the nature of its etiology so far is unclear.

In our first study, we investigated whether changes in extracellular serotonin levels due to serotonin transporter (SERT) availability (SERT rat model) in the mother influenced the maternal care. Maternal care is a major constituent of early life environment and seems to be related to offspring's behaviour and serotonin levels. We observed that one of the most prominent forms, licking-grooming their offspring, is significantly less often performed by SERT knockout (KO) dams then SERT wildtype (WT) dams. Thus, variation in licking-grooming behaviour seems to be determined by maternal serotonergic genotype.

To delineate whether maternal serotonergic genotype influences offspring's development through changes in foetal serotonin levels and/or through changes in licking-grooming behaviour, we set up a breeding such that both these two questions could be answered. In this study, the offspring was subjected to several behavioural assessments. Our data showed that potential alterations in foetal serotonin levels (KO mother) and a decrease in licking-grooming behaviour (KO care) synergistically strengthen their impact on behaviour. More specifically, we observed diminished anxiety (elevated plus maze test) and diminished anhedonia (sucrose consumption test) in adult offspring from SERT KO mothers which received SERT KO care.

These findings indicate that genetically-induced increases in maternal extracellular serotonin levels has a beneficial effect on offspring's behaviour due to both potential alterations in foetal serotonin levels and decreased maternal licking-grooming behaviour. For this reason, maternal SERT genotype seems to be involved in the development of psychiatric disorders.
To understand in which direction the maternal SERT genotype alters foetal serotonin levels we are currently investigating serotonin metabolism in the placenta, and foetal forebrain and hindbrain, by high performance liquid chromatography.

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POSTER # J-1

Garcinia mangostana Linn displays antidepressant, antipsychotic and pro-cognitive effects in translational models of depression and schizophrenia: Role of serotonin and immune-inflammatory cascades

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The indonesian fruit, *garcinia mangostana Linn* (GML) or mangosteen, contains over 85 polyphenols, the best characterized of which are α-, β-, and γ-mangostin. Raw and purified extracts of GML have diverse anti-inflammatory, antioxidant and neuroprotective actions, while α- and γ-mangostin inhibit cyclic adenosine monophosphate phosphodiesterase and serotonin 5-HT_{2A} receptors, respectively. Disorganized redox balance and inflammation characterize major depression and schizophrenia, with antioxidant/anti-inflammatory agents representing novel treatments for these illnesses. We studied the therapeutic properties of chronic raw GML extract in two translational animal models, the Flinders Sensitive Line (FSL) rat model of depression and the maternal immune-activation (MIA) model of schizophrenia. Behaviour, as well as cortico-hippocampal monoamines, lipid peroxidation and plasma pro- and anti-inflammatory cytokines (interleukin-1, IL-1; tumour necrosis factor-α, TNF-α) were studied. We considered GML alone vs. imipramine or haloperidol, and as adjunctive treatment with haloperidol. Chromatographic fingerprinting of GML revealed the presence of α-mangostin (117 mg/g) and γ-mangostin (11 mg/g). FSL rats showed significant cognitive deficits and depressive-like behaviour, disordered cortico-hippocampal monoamines and elevated hippocampal malondialdehyde levels. GML (50 mg/kg/day x 14d) displayed antidepressant and pro-cognitive effects equal to imipramine (20 mg/kg x 14d) and reversed hippocampal lipid peroxidation. Behavioral and monoamine assessments suggest a serotonergic action for GML. Haloperidol (2 mg/kg x 14d) and GML (50 mg/kg x 14d) were equally effective in reversing MIA-induced deficits in sensorimotor gating and depressive behaviour, with haloperidol+GML more effective than either alone. MIA-induced elevated IL-6 and TNF-α levels and cortico-striatal lipid peroxidation were reversed by haloperidol, GML and haloperidol+GML. Prenatal MIA-induced sensorimotor gating deficits and depressive manifestations were reversed by haloperidol or GML, with depressive manifestations more responsive to GML. Again, haloperidol+GML were augmentative. GML presents with anti-inflammatory and antioxidant actions, having comparable antidepressant and antipsychotic efficacy vs. reference agents while bolstering the actions of the latter.

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POSTER # J-2

Adaptations of the Serotonin System in a Model of Epilepsy

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Dravet syndrome (DS) is a genetic form of severe-intractable epilepsy that begins in infancy. The most common cause is a mutation of a sodium channel gene, SCN1A. Although DS is refractory to several standard treatments, the serotonin-selective amphetamine fenfluramine has been effective in clinical trials, suggesting the relevance of serotonin neurotransmission to treatment strategies. In order to improve understanding of this issue, we sought to determine the state of the endogenous serotonin system in a mouse model of DS consisting of an *Scn1A* heterozygote loss of function allele bred on a mixed (129S6 X C57B/6J) background. First we analyzed monoamine content by HPLC of 6 brain areas including frontal cortex, striatum, amygdala, hippocampus, rostral hindbrain including the dorsal and median raphe nuclei and the ventral medial medulla in DS mice and their normal siblings. Assayed after seizures developed at postnatal day (P) 35, the most robust changes centered on the amygdala and included increased serotonin and it's metabolite 5-HIAA. There were no significant changes of any other monoamine in the frontal cortex, striatum, rostral hindbrain or ventral medial medulla, but a few modest changes were detected in the hippocampus. Histological analysis revealed that serotonin was increased in several subnuclei of the amgydala and extended into the piriform cortex. These changes were not detected before seizures developed at P14. However, at P14 there was reduced serotonin levels in the area including the dorsal and median raphe nuclei. In addition, DS mice at P35 exhibited altered receptor function in that they were hypersensitive to the effects to the 5-HT2A/2C agonist, DOI, exhibiting excessive head-shake/ear scratch responses. They were also hypersensitive to the 5-HT1A agonist 8-OH-DPAT, exhibiting excessive hypothermia in response to this drug. Taken together these findings reveal substantive alterations in endogenous serotonin signaling in DS and further they implicate the amgydala as particularly relevant to the expression of this disorder.

POSTER #J-3

Effects of perinatal fluoxetine exposure on circadian rhythmicity, 5HT-1A receptor sensitivity and affective behavior in female rats

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Depressive symptoms occur frequently during pregnancy and in some women lead to a major depression, making antidepressant treatment unavoidable. Serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants during pregnancy and are known to cross the placenta and reach the developing fetus. Serotonin plays an important role during early neural development, and SSRIs are known to affect both brain and behavior of the developing child. A likely contributor to neurodevelopmental changes may be the 5-HT_{1A} receptor. Furthermore, SSRIs are able to modulate aspects of circadian rhythmicity, with disturbances of circadian rhythms being a key symptom of mood and anxiety disorders.
In this study, our aim was to investigate whether perinatal treatment with the SSRI fluoxetine alters affective behavior, 5-HT_{1A} receptor sensitivity, circadian rhythmicity and phase shifts to non-photic or high-dose 5-HT_{1A} receptor stimuli in female rat offspring. Pregnant dams were treated daily with either 10 mg/kg fluoxetine or vehicle from gestational day 1 until postnatal day 21. When adult, female offspring was tested for home cage activity, and in the elevated plus maze, home cage emergence and forced swim test. In addition, circadian rhythmicity of activity and temperature was observed under normal and reversed day/night rhythm. Afterwards, phase shifts in response to total darkness followed by a high dose of the 5-HT_{1A} receptor agonist 8-OH-DPAT were tested. After stabilizing the circadian rhythm to a normal 12/12h rhythm, a dose-response of the 5-HT_{1A} receptor agonist F13714 was tested on hypothermia. Afterwards, a dose-response of the 5-HT_{1A} receptor antagonist WAY100635 was tested. Finally, a combination of F13714 and WAY100635 was given.
Rats exposed to fluoxetine early in development showed increased home cage activity. No differences were observed in affective behavior or in the sensitivity to the 5-HT_{1A} receptor agonist F13714, 5-HT_{1A} receptor antagonist WAY100635 or their combination in hypothermia. Interestingly, after 5 mg/kg 8-OH-DPAT treatment a decreased length of the circadian period (tau) was observed in fluoxetine exposed rats compared to vehicle treated rats, indicating that perinatal fluoxetine leads to alterations in the circadian system. The mechanisms underlying alterations in the circadian system are currently under investigation.

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POSTER # J-4

Serotonin$_{2A}$ receptor knockout mice exhibit stress response, fluoxetine-induced anxiety and corticosterone levels, and perturbed gene expression in a sexually dimorphic manner.

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Acute administration of antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) enhances serotonin levels and is often accompanied by enhanced anxiety. SSRI response seems to be facilitated by concomitantly blocking serotonin$_{2A}$ receptor (5-HT$_{2A}$R) with an antagonist. Further, 5-HT$_{2A}$R knockout mice (5-HT$_{2A}^{-/-}$) exhibit reduced anxiety, and increased firing rate of dorsal raphe serotonergic neurons. Thus, we hypothesized that the 5-HT$_{2A}^{-/-}$ mice might be resilient to stress and acute SSRI, fluoxetine-induced anxiety. Contrary to our predictions, our findings suggest, while 5-HT$_{2A}^{-/-}$ mice display normal chronic stress-induced depressive behaviour and anxiety response in open-field on acute exposure to fluoxetine, they are hypersensitive to environmental perturbation. We also find sexual dimorphism in 5-HT$_{2A}^{-/-}$ mice, with females exhibiting an altered stress axis response on acute fluoxetine administration. Sex-dependent effect was also seen in the cortical and hippocampal activity-dependent immediate early genes, such as *Bdnf, Egr2, Egr4, Fos, Fosb, Fosl2, Homer1-3* and *Jun*. While none of the genes analysed were regulated by acute fluoxetine in the males, *Bdnf, Egr1, Egr4* and *Homer3* showed a significant main effect of fluoxetine in the females, where all were down regulated. Our results suggest perturbed prefrontal network dynamics due to loss of 5-HT$_{2A}$ receptors along with compensatory changes might make 5-HT$_{2A}^{-/-}$ male mice less adaptable to environmental modification. Our findings encourage research to further understand estrogen-5-HT$_{2A}$R interactions that may synergistically modulate neuronal activity in females.

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Key words: Prefrontal cortex, Brain derived neurotrophic factor, gender-dependent, chronic mild stress, forced-swim test, tail suspension test.

POSTER # K-1

A large-scale computational modelling study to investigate serotonergic modulation of cortical columnar dynamics

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The prefrontal cortex (PFC) has been shown to play an important role in a range of high-level cognitive functions including attention, memory, decision-making, and mood regulation. The cortex, including the PFC, has been known to consist of local computational units in the form of cortical columns. Many anatomical, pharmacological, optogenetics, electrophysiological, and behavioural studies have indicated that serotonergic (5-HT) activity, particularly from the dorsal raphe nucleus (DRN), is important for the modulation of these functions, with differential effects on the prefrontal cortical columnar layers and neuronal types. However, it is still not completely known how 5-HT collectively modulates across the PFC layers, and the resultant emergent network dynamics.

Biologically realistic computational models become necessary to systematically understand such complex dynamics. To perform such large-scale computational simulations, we made use of the brain-inspired highly parallel hardware platform: SpiNNaker (Spiking Neural Network architecture) and investigate the relative contributions of 5-HT (5-HT_{1A}, 5-HT_{2A} and 5-HT_{3A}) receptor subtypes, neuronal types and cortical layers. We particularly focus on modelling tonic 5-HT effects on prelimbic (PL) PFC circuit given the relative abundance of experimental data available.

Our preliminary simulations show that constant 5-HT influence (baseline condition) found in separate experiments can co-exist in the PL network, and hence is consistent with experimental studies. We further explore how 5-HT receptor subtypes could differentially affect columnar dynamics. Future work would involve more explicit modelling of the DRN component of the network model and effects of 5-HT-based drugs. This work lays the foundation towards developing multiscale neuronal circuits to bridge from neuronal excitability to circuit dysfunctions.

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POSTER # K-2

The level of maternal serotonin during pre-implantation pregnancy is crucial for the formation of an anxious and depressive-like behavior in the offspring

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Preclinical and clinical investigations suggest that serotonin (5-HT) is related to the aetiology and treatment of different neuropsychiatric disorders including depression, generalized anxiety disorders, schizophrenia, PTSD, alcoholism, ADHD and Alzheimer's disease. There are some evidences that manifestation of such behavioral abnormalities/disorders is affected by the 5-HT level within forming brain. However, serotonin synthesis enzymes, transporters and receptors are present in the reproductive system of the mother as well as in the developing embryo long before the appearance of the first nerve elements. Our recent findings demonstrated that increased level of 5-HT as early as in cleaved blastomers and blastula stage modulates the expression of serotonin-dependent behavioral programs in the progeny of model freshwater mollusc *Lymnaea stagnalis*. High conservatism inherent for the components of the serotonergic system allows us to suggest presence of similar phenomenon in the mammalian development.
We utilized well known model to increase the 5-HT level within the mother organism via oral presentation of 5-HT precursor 5-hydroxytryptophan (5-HTP) during five days of the pre-implantation pregnancy in BALB/c and CBA inbred mouse strains. Immunohistochemical staining and HPLC measurements confirmed the 2-7 folds increase of the 5-HT content within ovary, oviduct and uterus tissues of treated mother mice. Behavior experiments were performed in offspring males and females, 1, 2 and 3 months old. We used «open field test», «white/black box» and «marble burying test» to assay the anxiety and fear, and forced swimming procedure to evaluate the depressive-like behavior. The progeny of 5-HTP-treated mice demonstrated statistically significant reduction of anxious responses starting from the age of 1 month which can be traced up to the age of 3 months, that is, up to young sexually mature animals. In addition, pre-implantation pregnancy administration of 5-HTP weakened the depressive-like reactions and strengthened defensive stereotyped behavior of the 1 month old progeny. The manifestation of the observed behavioral changes was strain-specific and sex-specific.
Similarities in the observed modulation of progeny behavior in invertebrate and vertebrate models suggest conservative mechanisms involved in long lasting effects of serotonin during early cleavage or pre-implantation pregnancy. We discovered that transglutaminase-mediated serotonylation of specific proteins underlay behavioral changes in molluscan offspring. This allows to expect that similar mechanisms are active during mammalian early development as well.

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POSTER # K-3

Tryptophan depletion potentiates moral emotions to scenarios of social harm

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Morally relevant emotions provide a link between moral standards and socially appropriate behaviour. Moral standards prohibit behaviours that are likely to have negative consequences for the well being of others. A wealth of empirical literature shows serotonin plays a role in behavioural inhibition in the face of aversion, and theoretical accounts suggest a role in inhibiting negative thought. In the social realm, serotonin depletion disinhibits aggressive impulses in the face of perceived injustice, and raising serotonin can have prosocial effects. Studying moral emotions is important for understanding psychopathology and people's adherence to moral standards. This area has focused mostly on the self-conscious emotions of guilt and shame. We employed a novel task, part of the EMOTICOM neuropsychological testing battery, to measure feelings of guilt, shame, annoyance, and feeling "bad". Healthy participants were presented with cartoons of social scenarios – Pavlovian cues – in which someone was harmed, either intentionally or unintentionally. We then interrogated emotional reactions to these scenarios by asking participants to self-reflect, depending whether they were the victim or agent of harm. We tested the effects of dietary manipulation of acute tryptophan depletion, which temporarily lowers brain serotonin levels by depleting its precursor, tryptophan, in a double-blind randomised placebo-controlled between groups study (n = 37, ATD; n = 36, placebo). We predicted that serotonin depletion would enhance emotional reactions. All four emotions – feeling guilty, ashamed, annoyed, and "bad" – were indeed potentiated following tryptophan depletion. This was the case irrespective of agency and intention to commit harm. Critically, mood ratings were not affected. Serotonin depletion, in other words, potentiated Pavlovian reactions to social cues. This extends previous literature showing serotonin depletion modulates Pavlovian responses to neutral cues predicting punishment, and instrumental reactions to social scenarios of unfairness. These results additionally inform the neurochemical basis of psychopathology associated with excessive emotions such as guilt and shame.

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POSTER # K-4

GABAergic, not serotonergic, alterations appear to underlie reduced efficacy of 5-HT_6 receptor antagonists in a dual-hit neurodevelopmental model for schizophrenia

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Schizophrenia has a complex aetiology involving early-life environmental factors. One approach to improved preclinical modelling (to understand disease and evaluate therapeutics) incorporates dual neurodevelopmental 'hits', like neonatal phencyclidine then post-weaning isolation rearing of rats (PCP-Iso). Hippocampal slices from PCP-Iso show attenuated glutamate responses to the 5-HT_6 receptor antagonist SB-399885 (but not the $mGluR_7$ antagonist MMPIP; King et al., 2015). The current study extends this by cognitive evaluation of SB-399885 in PCP-Iso versus single-hit isolates (Veh-Iso), then immunohistochemistry for hippocampal 5-HT and calbindin-positive GABAergic interneurons (that express 5-HT_6 receptors; Helboe et al., 2015) to investigate reduced activity of SB-399885 in PCP-Iso.

41 male Lister-hooded rats (Charles River UK) received vehicle or PCP (10mg/kg s.c.) on postnatal day (PND) 7, 9 and 11, before housing in groups (Gr) or isolation from weaning (PND21). They underwent novel object discrimination (NOD: King et al., 2018) three times, following vehicle, SB-399885 or MMPIP (10mg/kg i.p.) on separate days (1-2 week intervals; PND57-80). Brains were collected immediately after the final NOD for immunohistochemistry. Data (n=13-14) were analysed by ANOVA with Sidak's/Tukey's post-hoc.

Veh-Gr discriminated the novel object irrespective of acute treatment ($P<0.05$-0.01). Veh-Iso and PCP-Iso-induced impairments ($P>0.05$) were reversed by MMPIP ($P<0.01$-0.001), but SB-399885 was only effective in Veh-Iso ($P<0.001$), not PCP-Iso ($P>0.05$). PCP-Iso had unaltered hippocampal 5-HT-immunoreactivity, but fewer calbindin-positive cells throughout the dorsal hippocampus ($P<0.0001$), particularly in strata oriens ($P<0.0001$) and radiatum ($P<0.01$) of CA1. Calbindin (but not 5-HT) immunoreactivity correlated with NOD performance following SB-399885 ($P<0.01$).

Reduced cognitive and glutamatergic responses to SB-399885 in PCP-Iso do not appear due to reduced 5-HT tone, but instead loss or dysfunction of the predominant 5-HT_6 expressing hippocampal interneurons. This study highlights the importance of improved understanding for selection of appropriate preclinical models, especially when disease neuropathology impacts upon pharmacological effects of potential therapeutics.

Funded by the University of Nottingham

POSTER #K-5

Lack of brain serotonin affects feeding and differentiation of newborn cells in the adult hypothalamus

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Serotonin is a crucial signal in the neurogenic niche microenvironment. Deregulation of the serotonin system leads to neurogenic decline and mood disorders but also to changes in appetite and metabolic rate. Tryptophan hydroxylase (*Tph*)2-deficient (*Tph2*$^{-/-}$) mice depleted of brain serotonin display alterations in these parameters, e.g., increased food consumption, modest impairment of sleep and respiration accompanied by a less anxious and highly active phenotype. The newly discovered neural stem cell niche of the adult hypothalamus has potential implications for homeostatic functions. Positioned between the third ventricle and the median eminence, specialized radial glial tanycytes are thought to regulate the hypothalamic in- and output of circulating hormones and nutrients to maintain body homeostasis. Cell genesis in the adult hypothalamus may have an important role in feeding and reproduction control, in mediating stress responses, and in energy metabolism. Serotonin impacts a variety of these functions. In a direct approach, we use *Tph2*$^{-/-}$ mice to elucidate the role of serotonin in feeding and cell genesis in the adult hypothalamus. Specifically, we examine precursor cell proliferation and survival in *Tph2*$^{-/-}$ mice at baseline and following six weeks of high cholesterol diet (HCD). Our main finding is increased food intake of *Tph2*$^{-/-}$ mice independent of the diet without affecting body weight. Wild type mice under dietary challenge increase body weight accompanied by a decline in proliferation and survival of newly generated cells in the hypothalamic niche. In contrast, increased food consumption of *Tph2*$^{-/-}$ mice does not come along with decreased cell numbers. However, lack of brain serotonin results in a shift of precursor cell phenotypes that was abolished under HCD. We show that precursor cells in the hypothalamus retain fate plasticity and respond to changes in the environment. A novel link between serotonin signaling and cell genesis in the hypothalamus could be exploited as therapeutic target in metabolic disease.

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POSTER # K-6

Differential effects of the stimulation of median raphe nucleus on the power and frequency of hippocampal theta oscillations

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Ascending serotonergic projections from the median raphe (MR) nucleus to networks generating hippocampal theta rhythm have a suppressive effect manifested mainly in decreasing theta amplitude and power. Theta oscillation is critical for hippocampal function establishing transient coupling between hippocampus and a number of other structures involved in diverse functions. For example, coherent theta rhythm links hippocampus with prefrontal cortex in cognitive tasks and with amygdala during processing emotional information. Drugs affecting cognitive and emotional processing are known to differentially modulate theta; most procognitive compounds increase theta power whereas anxiolytics decrease theta frequency. The goal of this study therefore was to test and compare the effects MR stimulation on different attributes of theta rhythm, namely its power and frequency. The experiments were performed in rats (n=22) anesthetized with urethane and equipped for field potential recordings in the hippocampus (electrodes placed above the CA1 pyramidal layer and in the DG, below the hippocampal fissure, in both sides), and for electrical stimulation of the MR and the pontine reticular formation (RPO), the major theta-eliciting site in the brainstem. Electrical stimulation at different intensities was applied either separately to MR or RPO, or concurrently (combined in different proportions of intensities) to the two structures. A subset of rats (n=4) also received microinjections of the 5-HT2 receptor antagonist injected in the septum at different concentrations. We found that unlike RPO stimulations increasing theta power and frequency, in parallel, MR stimulation suppressed theta power while increasing its frequency. MR-induced theta acceleration was similar to that induced by RPO and the two effects were additive in combined stimulations. SB-242084 increased theta amplitude and decreased its frequency in a dose-dependent manner. The results indicate that MR stimulation has a complex effect on hippocampal oscillations that goes beyond the commonly known “desynchronization” effect, i.e. switching from theta to non-theta state.

POSTER # K-7

Serotonergic *Pet1-Tac1* neurons project to spinal cord motor areas and modulate respiration

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Serotonin (5-HT)-producing neurons located in caudal raphe nuclei demonstrate extensive axonal projections, targeting specific brainstem areas and descending to the most caudal levels of the spinal cord (SC). These descending projections are implicated in motor function as well as modulation of physiological processes, from cardiorespiratory control to thermoregulation. To map and functionally define specific brainstem-SC circuits responsible for such homeostatic modulation, we have used intersectional genetic lineage tracing and transcriptomics genome-wide, revealing a caudal raphe 5-HT (*Pet1*+) neuron subset identified by co-expression of *Tachykinin1* (*Tac1-Pet1* neurons). This provides genetic access for visualizing the precise innervation patterns of a major caudal raphe subtype along rostrocaudal and dorsoventral SC axes-previously unexplored. Using a mouse genetic intersectional strategy that results in expression of synaptophysin-GFP in *Tac1-Pet1* neurons, we found *Tac1-Pet1* innervation of ventral horn lamina throughout cervical, thoracic, lumbar and sacral SC levels, providing evidence for *Tac1-Pet1* involvement in motor function. Interestingly, *Tac1-Pet1* neurons exhibit focal innervation of the intermediolateral region of the SC at thoracic levels, implicating *Tac1-Pet1* neurons in modulation of the sympathetic nervous system. We previously demonstrated *Tac1-Pet1* neuron innervation of respiratory motor areas of the brainstem, therefore, we queried if *Tac1-Pet1* neurons innervate respiratory motor areas of the SC. Indeed, we found *Tac1-Pet1* innervation of phrenic motor neurons (PMNs), which regulate the breath via motor input to the diaphragm. Given anatomical targets and a role of *Tac1-Pet1* neurons in the respiratory chemoreflex, we next probed the role of *Tac1-Pet* neurons in eupneic respiration, utilizing a chemogenetic strategy (CNO-hM3Dq) to acutely activate *Tac1-Pet1* neurons in a whole-body plethysmography assay. Initial results indicate that activation of *Tac1-Pet1* neurons increases respiratory rate. Increased locomotor activity was not observed within the plethysmograph upon neuron activation, however, increases in both ambulation and locomotion were observed using a comprehensive lab animal monitoring system. Together, these findings implicate *Tac1-Pet1* neurons in modulation of baseline room-air respiration, potentially coordinating the breath with locomotor and sympathetic systems. Future directions include probing *Tac1-Pet1* circuitry to elucidate specific post-synaptic cellular partners and signaling mechanisms of *Tac1-Pet1* neurons at different respiratory and motor target areas to gain insight into how these neurons may differentially modulate distinct target areas.

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POSTER # K-8

Seasonal Effects on Serotonin Markers During Pregnancy

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Major psychiatric disorders such as schizophrenia, bipolar disorder, major depressive disorder and autism are, fundamentally, disorders of brain circuitry. To help prevent these disorders, understanding how fetal brain circuitry is first established is of utmost importance. During pregnancy, serotonin (5-HT) synthesized from maternal tryptophan (TRP) at the placenta plays a critical role in how brain circuitry develops (Bonnin & Levitt, 2011). A separate line of work has shown that serotonin/tryptophan metabolism is highly seasonal at temperate latitudes (Carlsson, Svennerholm, & Winblad, 1980; Maes et al., 1995). We investigated seasonal differences in plasma tryptophan, large neutral amino acids (LNAAs), and kynurenine at 15-18 weeks of pregnancy in 20 highly seasonal and 35 non-seasonal women participating in the Ontario Birth Study in Toronto, Canada. Placentae from 7 highly seasonal and 15 non-seasonal women collected in the fall-winter period were also analyzed to measure rate-limiting enzymes of the indoleamine pathway. Results indicated a significant seasonality-group by season interaction for plasma tryptophan and LNAAs, and a significant main effect of season for plasma kynurenine. In highly seasonal women only, tryptophan levels were significantly lower in fall compared with winter or spring, and significantly higher in winter compared with summer. No pairwise group differences in LNAAs were observed. Across all mothers, levels of kynurenine were significantly lower in the summer compared with winter. Fall-winter term placental MAOA protein was significantly greater in highly seasonal women compared with non-seasonal women. Other mRNA and protein levels of key indoleaminergic enzymes were not significantly different between seasonality groups. These findings suggest that both season of the year and the seasonality status of pregnant women (high or low) influence plasma tryptophan, LNAAs, and kynurenine levels and placental MAOA protein expression during pregnancy. Future work will establish the relevance of these findings for child neurodevelopment.

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POSTER # K-9

Characterization of a specialized serotonergic neuron subtype responsive to dopamine and central to social behavior

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Serotonergic (5-HT) neurons modulate diverse behavioral and physiological functions. Increasingly, 5-HT neurons are described as distinct subpopulations that are specialized to regulate distinct biological processes and functions. One such subpopulation that modulates aggression and hyperactivity in mice is distinguished by expression of the type-II dopamine receptor (Drd2) and the pan serotonergic transcription factor Pet1. We refer to these as Drd2-Pet1 5-HT neurons. *In vivo* silencing of Drd2-Pet1 5-HT neurons drives heightened aggression and increased activity. While brain slice electrophysiology demonstrates that their excitability is inhibited cell-autonomously via Drd2 signaling, the requirement for Drd2 receptor activity in these serotonergic neurons for behavior modulation is unknown. To query the functional requirement for Drd2 receptor in Drd2-Pet1 5-HT neurons, we generated mice with serotonin specific deletion of *Drd2* (Drd2-CKO) and administered a panel of behavioral assays. We find that Drd2-CKO males exhibit altered aggressive and social dominance behavior. Further, Drd2-CKO females display altered acoustic startle responses compared to control littermates. These findings suggest an additional role for Drd2-Pet1 5-HT neurons in the modulation of auditory processing and/or sensorimotor gating. Interestingly, Drd2-Pet1 5-HT neurons have axonal projections to many brain regions involved in auditory processing. To further probe the circuitry involving Drd2-Pet1 5-HT neurons, we have generated novel viral vectors for intersectional transsynaptic tracing. The identification of their pre- and post-synaptic partners is ongoing. These experiments will inform upon the molecular, cellular, and circuit pathways that underlie these behavioral phenotypes while generating and testing novel viral-genetic tools for studying neuronal connectivity.

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POSTER # K-10

The microbiota defines the gastrointestinal serotonergic response to acute stress in a sex- and region-dependent manner

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Background/Purpose: Gut-derived serotonin (5-HT) produced following stress can exert physiologically and clinically-important local and systemic effects. We have previously demonstrated a murine strain-dependent temporal response of the gastrointestinal serotonergic system following acute stress, but the role of the gut microbiome in modulating this profile is unknown. We therefore sought to define the role of the gut microbiome in the gastrointestinal serotonergic system response to acute stress.

Methods: Adult male and female C57/BL6 conventional and germ-free mice were randomly allocated to the unstressed control or stress group. Stressed animals were subjected to 15min of restraint stress and sacrificed immediately or 45min post-stressor. Plasma corticosterone was assayed using ELISA. Gastrointestinal serotonin (5-hydroxytryptamine; 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined using HPLC. Results were analyzed by student's t-test or ANOVA, where applicable, and statistical significance was set at $p<0.05$.

Results: In the control group, corticosterone levels were significantly greater than in GF animals than conventional mice. Corticosterone levels were significantly elevated in all mice immediately after restraint stress. The acute stress-induced increase in colonic 5-HT in male animals, was abolished in germ-free animals. Male but not female germ-free colonic 5-HIAAwas significantly lower at baseline and post-stress compared to same-sex conventional mice. Moreover, colonization of male germ-free mice restored the colonic 5-HT response to acute stress. Interestingly, baseline ileal 5-HT and 5-HIAA was significantly greater in male and female germ-free mice compared to conventional animals, a feature maintained for 5-HIAA both immediately and 45min post-stress.

Conclusions:

The gut microbiome defines the set point of the gastrointestinal serotonergic system and is required for the immediate response to acute stress in a region- and sex-dependent manner. Further studies are required to understand the mechanisms underpinning the impact of acute stress and colonization and the implications of these findings for the control of stress-induced 5-HT-mediated gastrointestinal symptoms.

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POSTER # L-1

The psychostimulant (±)-*cis*-4,4'-dimethylaminorex (4,4'-DMAR) interacts with human SLC6A4 and other plasmalemmal and vesicular monoamine transporters in a manner similar to MDMA

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(±)-*cis*-4,4'-dimethylaminorex (4,4'-DMAR) is a new psychoactive substance (NPS) that has been associated with 31 fatalities and other adverse events in Europe between June 2013 and February 2014. However, the pharmacology of 4,4'-DMAR remains largely unexplored.
We used *in vitro* uptake inhibition and transporter release assays to determine the effects of 4,4'-DMAR on human high-affinity transporters for serotonin (SERT, SLC6A4), norepinephrine (NET, SLC6A2) and dopamine (DAT, SLC6A3). In addition, we assessed its binding affinities to monoamine receptors and transporters. Furthermore, we investigated the interaction of 4,4'-DMAR with the vesicular monoamine transporter 2 (VMAT2, SLC18A2) in rat phaeochromocytoma (PC12) cells and synaptic vesicles prepared from human striatum.
4,4'-DMAR inhibited uptake mediated by human SERT, NET and DAT in the low micromolar range (IC_{50} values < 2 µM). Transporter release assays identified 4,4'-DMAR as a substrate type releaser, capable of inducing transporter-mediated reverse transport via SERT, NET and DAT. This study confirmed 4,4'-DMAR as a potent serotonin-norepinephrine-dopamine releasing agent (SNDRA). In contrast to the known effects of its parent substances aminorex and 4-methylaminorex, 4,4'-DMAR exerts profound effects on human SERT. Furthermore, 4,4'-DMAR inhibited both the rat and human isoforms of VMAT2 at a potency similar to 3,4-methylenedioxymethylamphetamine (MDMA). The activity at VMAT2 suggests that chronic abuse of 4,4'-DMAR may result in long-term neurotoxicity.
Compared to other amphetamine-type stimulants and its predecessors, 4,4'-DMAR has a very pronounced serotonergic profile of action similar to MDMA. Strikingly, 4,4'-DMAR was often mislabeled and sold as ecstasy, even though it is a more potent releasing agent. The deaths can therefore be apprehended as overdoses, acutely causing serotonin and norepinephrine toxicity.

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POSTER # L-2

Sustained activation of postsynaptic 5-HT_{2A} receptors gates long-term depression in prefrontal cortex

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Abstract

The prefrontal cortex (PFC) plays a key role in many high-level cognitive processes. It is densely innervated by serotonergic neurons originating from the dorsal and median raphe nuclei, which profoundly influence PFC activity. Among the 5-HT receptors abundantly expressed in PFC, 5-HT_{2A} receptors located in dendrites of layer V pyramidal neurons control neuronal excitability and mediate the psychotropic effects of psychedelic hallucinogens, but their impact on glutamatergic transmission and synaptic plasticity remains poorly characterized. We previously demonstrated that the activation of presynaptic 5-HT_{2A} receptors located at thalamocortical synapses enhances NMDA transmission and gates the induction of spike timing-dependent long-term depression (t-LTD) mediated by presynaptic NMDA receptors, which might underlie their influence on specific cognitive tasks such as associative memory retrieval (Barre et al. Proc Natl Acad Sci U S A. 2016 Mar 8;113(10):E1382-91). Here, we show that a prolonged (20 min) exposure of mouse PFC slices to serotonin or the 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) produces a long-lasting depression of evoked AMPA excitatory postsynaptic currents (EPSCs) in layer V pyramidal neurons. DOI-elicited long-term depression of synaptic transmission is absent in slices from 5-HT_{2A} receptor-deficient mice, is rescued by viral expression of 5-HT_{2A} receptor in pyramidal neurons and occludes classical electrically-induced LTD. Furthermore, 5-HT_{2A} receptor activation promotes phosphorylation of GluA2 AMPA receptor subunit at Ser^{880} and AMPA receptor internalization, indicating common mechanisms with electrically-induced LTD. Mimicking the effect of prolonged exposure of PFC slices to 5-HT_{2A} receptor agonists, sub-chronic administration of fluoxetine to mice, which results in a sustained increase in extracellular concentration of serotonin and, consequently, a prolonged activation of 5-HT_{2A} receptors, also occludes electrically-induced LTD. Collectively, these findings provide one of the first examples of LTD gating under the control of a G protein-coupled receptor that might lead to imbalanced synaptic plasticity and memory impairment following a non-physiological elevation of extracellular serotonin that occurs after the onset of SSRI treatment in depressed patients.

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POSTER #L-3

Sphingolipid control of serotonin balance in depression

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Depression is a major psychiatric disorder that frequently emerges comorbid with alcohol abuse. Pathogenesis as well as pharmacological treatment approaches classically focused on protein dysfunctions. Thereby, the molecular components of the serotonergic system have proven to be essential. Together with cholesterol and glycerophospholipids, sphingolipids are the most common lipids in brain membranes. Sphingolipids form lipid rafts and signaling platforms, which are membrane compartments enriched in G-protein-coupled receptors. Acid sphingomyelinase (ASM) hydrolyses sphingomyelin to ceramide and phosphorylcholine and, thus, represents a major regulator of sphingolipid metabolism. We found that overexpression of ASM in mice (tgASM) reduces serotonin (5-HT) tissue levels in many brain areas, reduces hippocampal neurogenesis and induces depression-like behaviour. tgASM mice were found to drink significantly more alcohol and escalate consumption after withdrawal than WT mice. Free-choice alcohol drinking, but not forced alcohol exposure, normalized 5-HT levels and reduced depression-like behaviour selectively in depressed animals by normalization of ASM activity. However, extracellular 5-HT levels were preserved in naïve tgASM mice as measured by in-vivo microdialysis, but the acute 5-HT response to an alcohol challenge or a preferred food was attenuated in the hippocampus. This evidence suggests a strong control of 5-HT activity by the sphingolipid rheostat in the brain that may, when dysfunctional, lead to emotional disorders and enhanced preference for drugs that interfere with them.

POSTER # L-4

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Serotonin 2A receptors as a target for alcohol-use disorder

17 million adults in the United States ages 18 and older had an alcohol use disorder (AUD) in 2012. Current FDA-approved treatments for AUD are limited to disulfiram, naltrexone, and acamprosate, and many patients are refractory to these treatments. Hallucinogenic drugs were used in the 1960s to treat AUD, and a number of studies supporting their use were published. Moreover, recent studies have documented impressive effects of psilocybin for tobacco cessation and ayahuasca for the prevention of ethanol-induced behavioral sensitization. Therefore, herein, we investigated the effects of the serotonin 2A receptor agonist and hallucinogen 2,5-dimethoxy-4-iodoamphetamine (DOI) in widely accepted animal models of ethanol abuse. We found that DOI was effective in reducing ethanol-induced place conditioning and decreased ethanol consumption without affecting total fluid intake. As there are clear clinical limitations to the use of hallucinogenic drugs, we went on to explore whether computational chemistry approaches could be used to develop biased agonists of serotonin 2A receptors with reduced hallucinogenic activity, and whether such compounds suppressed ethanol consumption. Ligand-based computational approaches, including molecular modeling tools for pharmacophore modeling (Phase), docking (Glide), property prediction (QikProp), and QSAR z95-8, revealed the atypical beta blocker carvedilol as a potential biased agonist candidate. *In vivo* and *in vitro* studies demonstrated that carvedilol is an agonist of 5-HT2A receptors with minimal hallucinogenic activity. Moreover, carvedilol was also effective in reducing ethanol consumption without affecting total fluid intake. These studies document a new model system in which we can now investigate the neurobiological and pharmacological mechanisms through which hallucinogens reduce ethanol consumption and appetitive conditioning. They also show that carvedilol may be an excellent prototype for a medicinal chemistry program to develop therapeutic non-hallucinogenic serotonin 2A receptor agonists.

POSTER # M-1

Aptamer field-effect transistors to monitor serotonin *in vivo*

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Monitoring serotonin flux associated with complex neuropathological processes *in vivo* and in real-time necessitates chemically specific neurotransmitter sensors that approach the spatiotemporal resolution of neuronal signaling while differentiating structurally similar neurochemicals. To address this challenge, we employ rationally designed oligonucleotide sequences, termed aptamers as artificial receptors for molecular recognition. Aptamers can be designed for a range of target detection, signal transduction, response speeds, and *in vivo* stability. Beyond the challenge of discovering recognition elements with high specificity and selectivity for neurotransmitters, translation of electronic biosensing platforms such as field-effect transistors for *in vivo* environments has been hindered by the Debye length, the sensing distance beyond semiconducting channel surfaces wherein changes in local electric fields affect the distributions of channel free-charge carriers to the greatest extent. Structure-switching aptamers undergo large conformational changes upon target capture that involve rearrangement of their highly negatively charged backbones in close proximity to semiconducting channels resulting in measurable changes in channel conductances. Coupling serotonin-specific aptamers to field-effect transistors, we detected unprecedented femtomolar concentrations of serotonin in undiluted buffers (phosphate-buffered saline and artificial cerebrospinal fluid). Serotonin-functionalized sensors show excellent selectivity with the capacity to differentiate precursors (*L*-tryptophan and *L*-5-HTP), metabolites (5-HIAA), and other neurotransmitters at physiological concentrations. We have conducted measurements *ex vivo* in brain tissue homogenates of Tph2 knockout mice that lack brain serotonin to test device responses upon controlled addition of serotonin, while assessing biofouling of devices for acute measurements. With the goal of *in vivo* sensing, we have designed and fabricated miniaturized field-effect transistors on silicon microprobes (120 μm in width). Optimization of these aptamer-field-effect transistor neuroprobes will lead to *in vivo* detection of serotonin in the brain to link serotonin signaling with complex behaviors.

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POSTER # M-2

ONLY POSTER PRESENTATION

Evidence for 5-HT$_4$ signal transduction via phosphodiesterases (PDEs) in transgenic mice

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We have generated transgenic mice with overexpression of 5-HT4 receptors targeted to the heart (TG). In these mice, in contrast to wild type mice (WT), exogenous serotonin (5-HT) exerted a positive inotropic effect (PIE) and increased the beating rate. Others (Afzal et al. Brit J Pharmacol 2008) noted in rats with myocardial infarction or in ventricular cardiac preparations from patients with end-stage heart failure: the PIE of 5-HT was potentiated by the non-selective PDE inhibitor IBMX or the PDE3 inhibitor cilostamide but not by the PDE2 inhibitor EHNA or the PDE4 inhibitor rolipram. The effect was augmented in the combined presence of rolipram and cilostamide. Hence, signal transduction may involve also inhibition of PDEs or PDEs might be an innocent bystander. Hence, we performed concentration response curves (CRC) for 5-HT in electrically driven (1 Hz) left atrial preparations from TG and WT in the absence and presence of PDE inhibitors. In contrast to Azfal et al. 2008, we noted that IBMX (10 µM), cilostamide (1 µM), or EHNA (1 µM) alone or combinations of rolipram (1 µM) and cilostamide and EHNA did not reveal a previously hidden PIE in WT to 5-HT (0.1 nM to 1 µM). Cilostamide potentiated the PIE of rolipram in WT and TG. The shift was most pronounced when clostamide and rolipram were combined. These data suggest that 5-HT4 signals may be act at least in part via inhibition of PDE activity. In TG, rolipram shifted the CRC of 5-HT to the right whereas rolipram combined with EHNA shifted the PIE to the left. 1 µM cilostamide increased the PIE of rolipram (0.1 µM) with the result that additionally applied 5-HT was ineffective. In summary, no latent 5-HT-mediated PIE in WT could be uncovered by inhibition of PDEs. The PIE of 5-HT in TG is suggested to be antagonized by the combined action of endogenous PDE3 and PDE4 but might also involve activation of PDE4 whereas PDE3 alone seems irrelevant.

POSTER # M-3

Altered 5-HT_{2A} receptor-mediated $G\alpha_q$ signalling in postmortem prefrontal cortical membranes in opiate addicts

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Bacground: Serotonergic dysfunction is thought to contribute to the pathophysiology of psychiatric disorders and addiction vulnerability. The present study examined whether 5-HT_{2A} receptor-mediated $G\alpha_q$ signalling is altered in opiate addicts.
Methods: 5-HT_{2A} receptor-mediated $G\alpha_q$ activation was determined by means of [^{35}S]GTPγS binding/immunoprecipitation assay (Odagaki et al., J. Neural Transm. 124: 1123-1133, 2017) in postmortem prefrontal cortical membranes obtained from 20 opiate addicts and age- and sex-matched 20 controls.
Results: Addition of increasing concentrations (1 nM to 100 μM) of 5-HT stimulated the specific [^{35}S]GTPγS binding to $G\alpha_q$ in prefrontal cortical membranes in a concentration-dependent manner through 5-HT_{2A} receptor subtype. From each concentration-response curve, maximal percent increase (%E_{max}), concentration eliciting half-maximal response (EC_{50}), and slope factor were determined. The %E_{max} values of opiate addicts were 58.7 ± 7.9 % (mean ± SEM), significantly lower than those of the control subjects (89.6 ± 10.6 %) ($P < 0.05$). Additionally, the negative logarithm of EC_{50} (pEC_{50}) values of the opiate addicts were 6.75 ± 0.10 (EC_{50} = 177 nM), significantly different from those of the controls (pEC_{50} = 7.17 ± 0.11, EC_{50} = 67.0 nM) ($P < 0.01$). The slope factor was not significantly different between the two groups.
Conclusions: The $G\alpha_q$-mediated signalling via 5-HT_{2A} receptor is disturbed in the prefrontal cortex of opiate addicts. Although it is unclear whether these disturbances are consequences of prolonged exposure to opiate or inherent pathological alterations underlying opiate addict vulnerability, 5-HT_{2A} receptor-mediated $G\alpha_q$ signalling may be of relevance in the cellular and molecular process of opiate addiction.

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POSTER # M-4

Transcriptome profiling of postmortem human infant serotonin neurons and brainstem raphe nuclei – towards a molecular understanding of sudden infant death syndrome.

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Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant in the first year of life, generally occurring during sleep, which is unexplained by death scene investigation and autopsy. It is the leading cause of death for infants between one to twelve months of age, and has a peak incidence between two to four months. Numerous abnormalities in serotonin related signaling and metabolic pathways have been reported in SIDS cases in the medullary brainstem, an important autonomic control center in the brain. These findings, together with known environmental risk factors and observations of cardiorespiratory distress in SIDS infants, have lead to the hypothesis that impaired central homeostatic control mechanisms may contribute to fatality in SIDS. Recent studies have begun to reveal aberrant expression in other brainstem neurochemical pathways, for example in Substance P mediated neurotransmission, as well as other nodes of central homeostatic regulatory networks, such as the hippocampus, suggesting that there are likely wider, yet perhaps related, brain circuit abnormalities in SIDS yet to be identified. In pursuit of further elucidating serotonergic and broader neural circuit abnormalities in SIDS we have begun to apply a single nuclei droplet-based microfluidic RNA-sequencing approach to screen postmortem human infant brainstem tissue for molecular and cellular differences between SIDS and controls. Here we report the initial findings of a pilot study of two SIDS and two non-SIDS control cases, profiling both the medullary raphe and surrounding regions, as well as the midline rostral pons and surround. While the number of cases presently examined prohibits claims of statistically significant differences between SIDS and non-SIDS controls, we demonstrate feasability of the methods, showing that we are able to reliably identify serotonin neurons as well as other cell types, including oligodendrocytes, astrocytes, microglia, and endothelial cells. We also show that our approach is sensitive enough to identify molecular signatures likely related to cause of death, as suggested by detected cell type-specific elevation of heat shock protein encoding transcripts in the brainstem of an infant that died of hyperthermia. Moreover, we show that comparison of human and mouse serotonin neuron transcriptome profiles indicates conserved differential expression of a number of molecular markers in human serotonin neurons corresponding to serotonin neuron subtypes with distinct projection targets, functions, and developmental lineages, in mice. Linking human serotonin neurons to mouse serotonin neurons, for which we have greater depth of knowledge, potentially allows for inferences to be made about functional deficits that may result from dysfunction of any given 5HT neuron subtype, and therefore help to explain cause of death as well as suggest means of preventing future SIDS deaths.

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POSTER # N-1

The anxiolytic and antidepressant effects of fluoxetine are mediated by specific regions along the longitudinal axis of the hippocampus

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Depression is currently the leading cause of disability worldwide and yet antidepressant treatments remain suboptimal. This is due in part to our incomplete understanding of the neurobiology underlying the pathophysiology and successful treatment of depression. However, human neuroimaging studies suggest that the hippocampus area of the brain likely plays a key role. Accumulating studies in rodents suggest that the hippocampus is functionally segregated along its longitudinal axis into a dorsal region (dHi) which is predominantly involved in spatial learning and memory, and a ventral region (vHi) which regulates anxiety, a symptom often co-morbid with depression. Gene expression studies suggest that the area between these areas, the intermediate hippocampus (iHi) might also be functionally independent but few studies have interrogated its function. Similarly, little is known about the roles of these specific hippocampal subregions in the antidepressant response. Thus, the present study investigated the impact of ibotenic acid-induced lesions of the dHi, iHi or vHi on the regulation of anxiety and depressive-like behaviour in the absence or presence of the antidepressant and selective serotonin reuptake inhibitor, fluoxetine. In the absence of fluoxetine, vHi lesions reduced anxiety, while none of the lesions affected depressive-like behaviour under these conditions. On the other hand, only vHi lesions prevented the acute antidepressant effects of fluoxetine in the tail suspension test. Similarly, only vHi lesions prevented its anxiolytic effects in the novelty-induced hypophagia test. Interestingly, only iHi lesions prevented the antidepressant effect of chronic fluoxetine treatment in the forced swim test. dHi lesions did not impact antidepressant- or anxiety-related behaviour either in the absence or presence of fluoxetine. Taken together, the present data demonstrate that the vHi plays a key role in anxiety and its modulation by chronic fluoxetine treatment, and that both the iHi and vHi play distinct roles in fluoxetine-induced antidepressant-like behaviour.

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POSTER # N-2

Alcohol intake in maternally stressed serotonin transporter knockout rats

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Alcohol use disorder (AUD) affects approximately 32 million adults in the USA. Serotonin has been linked to AUD and especially the serotonin transporter (5-HTT). A polymorphism in the promoter region of the 5-HTT gene is characterized by a long (L) and a short (S) allele, resulting in different 5-HTT transcriptional activity. The S- allele has been associated to AUD, implicating that the S-allele is a risk factor for the development of AUD. The effect of environmental factors have also been studied, however results concerning 5-HTT are inconsistent. The exact biological mechanisms by which 5-HTT and the environment interact to increase vulnerability to AUD remains elusive. Therefore, we studied the interactive effects of 5-HTT deficiency and early life stress on alcohol intake in rats.
Methods: Male and female 5-HTT wildtype (5-HTT$^{+/+}$), heterozygous (5-HTT$^{+/-}$), and knockout (5-HTT$^{-/-}$) rats underwent maternal separation (MS) or control handling (CH) from postnatal day 2 till 15. When adult, rats were housed individually and exposed to a 20% alcohol solution, according to an intermittent-every-other day schedule for 7 hours/day for three days/week, for four weeks. After this period rats had access to the 20% alcohol solution for 24 hours/day for three days/week, for another four weeks.
Results: Our preliminary findings indicate that MS is associated with increased alcohol consumption in 5-HTT$^{-/-}$ males, but not females. However, females 5-HTT$^{-/-}$ rats consumed significant more alcohol then 5-HTT$^{+/+}$ rats, independent of maternal handling.
Conclusion: Early-life stress and 5-HTT deficiencies seem to affect alcohol consumption in males and females differently. While males seem more sensitive to increase their alcohol intake after MS, females seem more resistant to this stressor. Underlying mechanisms contributing to these sex differences are now under investigation.

POSTER # N-3

Development of the prefrontal to raphe circuit. Role of Serotonin

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The medial prefrontal cortex (mPFC) plays a major role in emotional control, a large part of which appears to be mediated by feedback connections to brainstem monoaminergic neurons, in particular to serotoninergic neurons in the dorsal raphe nucleus (DRN). Alterations in the developmental trajectory of these neurons could play an important role in the psychopathology of anxiety-depressive disorders.
We recently determined that PFC-to raphe neural circuits transiently express *SLC6A4*, the gene encoding the monoamine serotonin transporter (SERT) that ensures high affinity reuptake of 5-HT, and is the major target of antidepressants. We demonstrated that subsets of layer 5-6 pyramidal in the mPFC express SERT from E18- to P10. These neurons establish synaptic connections with subcortical targets including serotoninergic and GABAergic neurons in the DRN.
Complete or cortex specific ablation of *SLC6A4*, SERT induces an increase excitatory synaptic input from the PFC onto both 5-HT and GABA neurons in the raphe. This indicated that SERT is cell-autonomously controlling the development of PFC-raphe connectivity. To decipher the underlying mechanisms we are analyzing the postnatal development of the PFC-raphe circuit. This shows that PFC axons reach the raphe by P3 but terminal innervation continues to increase over the first 3 postnatal weeks. Current experiments using in utero electroporation of a synaptic-Tdtomato fusion protein into the SERT+PFC neurons allows determination of the onset of synaptogenesis and of the hyper-innervation phenotype in the SERT-KO mice.
In parallel we are investigating the role of the 5-HT7 receptor in this hyper-innervation phenotype. This G_s and G_{12} coupled receptor has been reported to control synapse formation. It shows a peak of expression during the period of PFC SERT expression and is down-regulated in the PFC of SERT-KO mice.
Overall our findings indicate a critical role of SERT in the postnatal period for the maturation of PFC-raphe circuits and suggest that a tight control of 5-HT receptor activation could be key in controlling synapse numbers in PFC targets.

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POSTER # N-4

High-density recording of DRN neurons in-vivo

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The midbrain dorsal raphe nucleus (DRN) contains the majority of the forebrain-projecting 5-hydroxy-tryptamine (5-HT) neurons in the brain. These neurons are highly heterogenous in terms of their molecular characteristics, and they interact with multiple types of neighbouring non-5-HT neurons in ways that are not yet fully documented. One way to understand the functional implications of this heterogeneity is to collect large-scale data-sets of DRN neural activity and to use computational methods to help analyse the complex neural circuitry. Here, we commence to collect such a data-set through high-density multi-site silicon electrode recordings in the DRN.

Recordings (Open Ephys) were made in urethane-anaesthetised mice using a silicon probe (Cambridge NeuroTech, 32 channels) stereotaxically implanted into the DRN. EEG electrodes (3 channels) were placed bilaterally over the frontal cortex and right occipital cortex to record brain state. After 1 h of baseline recording, neurons were screened for evidence of 5-HT_{1A} receptor-mediated autoinhibition by administration of the selective serotonin reuptake inhibitor citalopram (10mg/kg i.p.). Recordings were continued for a further 1 h. Raw data from 32 channels were filtered and single units were identified automatically using Kilosort and verified by manual clustering using Phy. Spike trains were further analysed using a suite of custom-written Python scripts to reveal spike waveform characteristics, firing rate and firing regularity. Spike-sorted neurons then underwent clustering analysis to reveal groups of neurons with similar firing properties.

An initial analysis (~170 neurons) revealed multiple simultaneously recorded neurons (~35 neurons/mouse). Although much diversity in baseline firing properties was evident, clustering analysis revealed 3 prominent groups of neurons; regular slow firing neurons previously identified as putative 5-HT containing, irregular slow firing neurons, and fast firing neurons previously identified as putative GABA containing. Citalopram inhibited all regular slow firing neurons and some irregular slow firing neurons, while some of the latter were also excited.

Overall, the current high-density in vivo recordings show evidence of heterogeneity in the baseline properties of DRN neurons as well as heterogeneity in their response to citalopram administration. Ongoing experiments are expanding the size of the data-set to increase the power of the clustering analysis and to commence computational analysis of DRN neuron interactions. Future experiments will incorporate optotagging to aid chemical identification of the principal neuron clusters.

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POSTER # O-1

Early life growth retardation and increased fat metabolism in *Tph2*-deficient rats

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Serotonin has long been known as a major player in the pathophysiology of a broad spectrum of psychiatric disorders. Only in recent years, central serotonin has also been linked to fat metabolism, suggested by a possible interplay between the serotonergic and noradrenergic systems.
Genetic deletion of tryptophan hydroxylase 2 (TPH2), the rate limiting enzyme for serotonin synthesis in the central nervous system, results in an almost complete lack of brain serotonin and a prominent postnatal growth retardation.
The aim of our study is to elucidate the link between central serotonin depletion and the growth retardation in *Tph2*-deficient rats.
First differences in body weight and size of *Tph2*-deficient and wildtype rat pups are evident starting from 3-4 days after birth. This phenotype persists throughout the first postnatal weeks, however both body weight and size are normalized later in life.
The appearance of lacteals is normal demonstrating unaltered milk fat absorption in *Tph2*-deficient pups compared to wildtype littermates. Nevertheless, body composition analysis revealed that *Tph2*-deficient pups are not only smaller, but also have a decrease in body fat content in comparison to wildtype animals of the same age.
5-Hydroxytryptophan (5-HTP) treatment was able to restore brain serotonin levels in *Tph2*-deficient pups and rescue the growth retardation phenotype. Body weight of 5-HTP treated pups at 17-18 days of age was significantly higher than the one of saline injected littermates. Clorgyline treatment on the other hand resulted in only partial body weight recovery.
Stress-sensitive systems have been reported to contribute to poor postnatal development in clinical studies. 5-HTP treatment showed a tendency of decreasing the blood corticosterone levels in *Tph2*-deficient pups compared to saline injected littermates.
We conclude that metabolic alterations, including increased energy consumption and possibly alterations in the stress response regulation, contribute to the growth retardation phenotype of *Tph2*-deficient rats.

POSTER # O-2

Novel insights into antidepressant action reveal the interplay of serotonin, stress axis, neurogenesis and BDNF signaling

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Serotonin is well known as the "molecule of happiness". Serotonin-based anti-depressants are used with the immediate action to alter the synaptic availability of the monoamine. Yet, clinical improvement only becomes apparent after several weeks that is accompanied by increased hippocampal neurogenesis. For a long time, research on major depressive disorder has been focused almost exclusively on neurotransmitters, e.g. serotonin. In contrast, current biological theories, which are partly intertwined, center on dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis, impaired neurogenesis/neuroplasticity, and altered neurotrophin signaling (e.g. BDNF). The precise role of serotonin in mediating these effects has remained undefined. Here, we used mice deficient in tryptophan hydroxylase 2 (*Tph2*$^{-/-}$) to compare the linked signaling systems whose effects can be mediated by SSRI, but also SSRE (-enhancer) treatment. Specifically, we examined adult neurogenesis and BDNF signaling in absence of serotonin 21 days after treatment with either citalopram (SSRI), tianeptine (SSRE) or saline. Our data reveal that the known antidepressants differentially affect cell survival in wild type animals, while, surprisingly, all treated *Tph2*$^{-/-}$ groups, including saline, revealed an increase in adult neurogenesis. BDNF levels were not altered by SSRI/SSRE treatment in the hippocampus of either genotype. We further examined parameters of the HPA-Axis in *Tph2*$^{-/-}$ mice at baseline, and in response to long term treatment. Preliminary data reveal different ACTH and CORT plasma levels in *Tph2*$^{-/-}$ mice as opposed to levels in SERT$^{-/-}$ mice, that lack the serotonin transporter, accompanied by alterations in glucocorticoid- and mineralocorticoid receptor levels in the examined brain regions. Our study gives new insights into mechanisms of antidepressant action and supports theories involving alternative pathways that can be used as novel targets in antidepressant therapy.

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POSTER # O-3

Chemogenetic activation of serotonin neurons in the dorsal raphe

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Serotonin (5-hydroxytryptamine; 5-HT) is critical for mood regulation in health and disease. However, the neuronal circuitry involved and how 5-HT activity translates into behaviour are yet to be fully elucidated. To explore the effect of specifically manipulating 5-HT circuitry on anxiety behaviour, we have utilised the chemogenetic (DREADD) approach to target 5-HT neurons. Viral constructs containing either the excitatory DREADD hM3Dq or control mCherry were delivered to the dorsal raphe nucleus (DRN) of Slc6a4-Cre mice. Using immunohistochemistry, we confirmed that both constructs were selectively expressed in 5-HT neurons (>95% specificity), identified by colocalization with tryptophan hydroxylase (TPH2). Once selective targeting of 5-HT neurons was established, we tested functionality by measuring the effect of the DREADD activator, clozapine-N-oxide (CNO), on expression of the immediate early gene c-Fos. We found that CNO increased c-Fos expression in the DRN, and this was localised in TPH2-positive neurons. In addition, CNO increased c-Fos expression in the basolateral amygdala, suggesting 5-HT activation of downstream anxiety-related circuits. Once 5-HT-selective and functional DREADD expression was confirmed, we tested the effect of CNO on DREADD-transfected animals in various anxiety models. On the elevated plus maze, CNO administration caused an increase in time spent in the open arms in DREADD-transfected versus control mice, and this effect was not confounded by changes in locomotor activity as shown in measurements in a home-cage-like environment. In two other models (light and dark box and novelty supressed feeding) however, CNO had no significant effect. These findings suggest chemogenetic activation of 5-HT neurons reduces anxiety, at least under certain conditions. Together, these data indicate the successful chemogenetic targeting of DRN 5-HT neurons to explore the role of 5-HT circuitry involved in anxiety behaviour.

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POSTER # O-4

Further studies investigating the pharmacology of CSTI-300; a novel 5-HT_3 receptor partial agonist with potential to treat patients with irritable bowel syndrome and carcinoid syndrome

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Diarrhoea-predominant irritable bowel syndrome (IBS-d) is associated with increased levels of 5-hydroxytryptamine (5-HT) that may contribute to the symptoms. Current treatment for IBS-d is limited. Whilst the 5-HT_3 receptor antagonist alosetron delivers symptomatic relief, patients often experience constipation and more rarely potentially life-threatening ischemic colitis. It is reasoned that a 5-HT_3 receptor partial agonist would reduce some of the consequences of increased 5-HT levels, but by still retaining some 5-HT_3 receptor activity, the side effect profile is predicted to be better than simple antagonists. The efficacy of CSTI-300 is at least comparable to a current therapeutic, the 5-HT_3 receptor antagonist alosetron, in an *in vivo* rat model replicating the viscera hypersensitivity patients with IBS-d experience (Roberts et al, 2018, this meeting). Our studies have evaluated some of the 5-HT_3 receptor partial agonist properties of CSTI-300, and the potential of the drug to treat IBS-d (Roberts et al, 2018, this meeting). Here we further investigate the *in vitro* pharmacology of CSTI-300.Radioligand binding studies, functional intracellular calcium assays and whole-cell patch clamp electrophysiology were utilised to investigate the pharmacology of CSTI-300 at the human (h) 5-HT_3A and 5-HT_3AB receptors expressed in HEK293 cells. In radioligand binding assays, CSTI-300 displayed high affinity for the h5-HT_3A and h5-HT_3AB receptors, with a K_i of approximately 2.0 nM compared to around 400 nM for 5-HT. 5-HT and CSTI-300 allowed the cryptic orthosteric modulator 5-chloroindole to compete for h5-HT_3A receptor binding sites (an action not evident with antagonists). In comparison to 5-HT, CSTI-300 displayed potent partial agonist activity (measured by an increase in $[Ca^{2+}]_i$) at the h5-HT_3A and h5-HT_3AB receptors (approximately 30-40% intrinsic efficacy). Preincubation with the selective 5-HT_3 receptor antagonist granisetron (500 nM) blocked either 5-HT or CSTI-300 induced responses at the h5-HT_3A or h5-HT_3AB receptors, demonstrating the selectivity of these ligands for the 5-HT_3 receptors in this assay. Electrophysiology experiments demonstrated that the CSTI-300 induced current had a slower rise time in comparison to 5-HT at the h5-HT_3A receptor; with peak effect of approximately 20% relative to 5-HT.

In summary, CSTI-300 displayed actions of a partial agonist at h5-HT_3 receptor isoforms that support its therapeutic potential to reduce the symptoms experienced by patients with IBS-d, with the added benefit of likely reduced adverse effects mediated by high level inhibition of the 5-HT_3 receptor.

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POSTER # P-1

Orthosteric and Allosteric Activation Mechanisms of the Serotonin 5-HT_{2B} Receptor

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The serotonin 5-HT_{2B} receptor is an important off-target receptor where chronic activation by former anti-migraine medications (e.g. methysergide) has been linked to cardiac valvulopathy. Here we present four structures of the 5-HT_{2B} receptor in complex with methylergonovine, methysergide, lisuride, and LY266097 and biochemical data that identify and characterize key ligand-receptor interactions in the orthosteric and an allosteric binding site responsible for modulating 5-HT_{2B} serotonin receptor activation. These structures reveal that activation mechanisms can occur via the orthosteric binding pocket by ligand engagement with T3.37 or A5.46 at the TM3/TM5 junction, and also via the extended binding pocket with L7.35 in TM7. Importantly, mutations in these regions reveal divergent effects on β-arrestin recruitment, and provide a potential mechanism of ligand bias occurring via ligand-contact with TM7 in the extended binding pocket. These results illuminate structural determinants essential for the serotonin receptor activation and biased signaling, and shed light on atomic activation mechanisms that ultimately lead to cardiac valvulopathy.

POSTER # P-2

Serotonin Modulates a Novel Microcircuit in the *Drosophila* Visual System

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Serotonergic projections densely innervate the visual system of the fruit fly *Drosophila melanogaster*, however the role of serotonin signaling in the optic lobe is unknown. Here, we characterize cells expressing serotonin receptors and identify a novel microcircuit modulated by serotonin. *Drosophila* have five serotonin receptors that are homologous to mammalian 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B and 5-HT7. We found that 5-HT1A, 5-HT1B and 5-HT7 are expressed in serotonin-immunoreactive projections, presumably functioning as autoreceptors. We also identified several neurons that house serotonin heteroreceptors, including lamina monopolar cell 2 ('L2'), expressing excitatory 5-HT2B and 5-HT7 receptors, and 'T1', expressing inhibitory 5-HT1A and 5-HT1B receptors. L2 and T1 neurons respond to applied or optogenetically evoked serotonin with calcium and/or voltage changes that are ablated by receptor knockdown. Serotonin neurons were not found to synapse onto L2 or T1 neurons, indicating signaling via volume transmission. However, L2 and T1 neurons both synapse onto serotonergic projections, in contrast to several other serotonin receptor-expressing cells we examined. Functionally, L2 depolarization elicits an excitatory response in serotonin neurons, whereas T1 induces an inhibitory effect. Thus, serotonergic projections interact with L2 and T1 to create a feed forward circuit. Intriguingly, there are reciprocal synaptic connections between L2 and T1 neurons themselves. Thus, serotonin receptors independently modulate each visual neuron in an overall feed-forward manner, while the L2/T1 synaptic connection acts as a potential braking mechanism. L2 and T1 both form reciprocal synapses with lamina monopolar cell one ('L1'), together encompassing the light-OFF and -ON visual pathways. This is the first description of serotonin modulating visual processing neurons that function as inputs to fly visuomotor behaviors. The *Drosophila* visual system's well-characterized circuitry provides an ideal model system to inform our understanding of long range serotonergic signaling and reveal basic principles of modulatory network function.

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POSTER # P-3

Human Serotonin type 3 receptor subunit D (5-HT3D): A novel negative regulator of Wnt signaling.

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The serotonin type 3 receptor (5-HT_3R) belongs to the class of ligand-gated ion-channels, consisting of five subunits. The human genome harbors five genes (*HTR3A-E*) encoding five 5-HT3 subunits (5-HT3A-E). Subunits are characterized by a ligand-binding-domain (LBD) followed by four transmembrane regions. However, 5-HT3D differs and lacks a signal-peptide and LBD. Human 5-HT3 subunits are broadly expressed, particularly within the gastrointestinal tract. In addition, 5-HT_3 receptors have been implicated in various human diseases including anxiety, depression and irritable-bowel-syndrome. Recently, we have unraveled a role of 5-HT_3 in canonical Wnt-signaling during left-right development of *Xenopus* embryos. Here we show evidence for a 5-HT_3-Wnt interaction in humans.
In subsequent studies in *Xenopus*, we found that heterologous mis-expression of human *HTR3D* was capable to block *Wnt3a* induced signaling, in contrast to other 5-HT3 subunit genes. Wnt activation was not repressed by 5-HT3D when downstream factors like dishevelled or ß-catenin were applied, suggesting that 5-HT3D acts at the level of receptor or ligand. 5-HT3D and 5-HT3A deletion-mutants as well as chimeric constructs of the two, demonstrated that Wnt-inhibition is specific to and depends on its subcellular localization. Surprisingly, a functional channel was not required for *Wnt*-inhibition. Finally, we will present data indicating that Wnt-inhibition by 5-HT3D might be relevant in the human system as well.

POSTER # P-4

Large scale monitoring of neural activity in dorsal raphe nucleus of freely moving mice

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Serotonin (5-hydroxytryptamine; 5-HT) neurons in microcircuits of the midbrain dorsal raphe nucleus (DRN) are critical to the normal regulation of emotion. Despite the strong links between 5-HT and emotional control, much is unknown about the emotional information that 5-HT neurons signal. Progress has been hampered by the lack of methods to record the activity of identified 5-HT neurons in behaving animals. Here we report preliminary data from experiments which combine multiunit tetrode recordings with optotagging to monitor the activity of identified 5-HT neurons in awake mice.

Channelrhodopsin (ChR2) was delivered via a viral vector (AAV-EF1a-DIO-hChR2(E123T/T159C)-EYFP) into the DRN of adult male SERT-cre mice (B6.Cg-TG(Slc6a4-cre) prior to an implantation of a microdrive mounted with a multi-tetrode array and an optic fiber for light delivery by blue laser (473 nm). Post surgery tetrodes were lowered into place and neural activity was then recorded over several weeks whilst mice were in the home cage or placed in a novel environment. On each recording day, following measurement of baseline activity light pulses (1-10Hz, 5ms duration) were delivered to test for optically activated neurons. In some experiments the selective serotonin reuptake inhibitor citalopram was administered (10 mg/kg i.p.) to test for 5-HT_{1A} autoreceptor-mediated inhibition of neuron activity followed by administration of the selective $5HT_{1A}$ receptor antagonist WAY-100635 (0.1 mg/kg i.p.). Electrophysiological data is analysed to identify spike clusters, baseline firing characteristics of individual neurons, response to optical stimulation, changes in firing during behaviour and introduction of novel environment, and drug administration.

Preliminary recordings revealed the firing of individual DRN neurons, some of which could be recorded over a period of several days. DRN neurons exhibited a diversity of baseline firing properties comprising at least 3 categories. These were i) highly regular ‘clock-like’ neurons (coefficient of variation of interspike intervals <0.5) with a frequency of ~4-8Hz, ii) less regular slow-firing neurons (1-10 Hz) and iii) fast-firing neurons (>10Hz). Experiments detected examples of clock-like neurons that were optically activated, thereby supporting their 5-HT neuron identity. Also, as expected of 5-HT neurons, the firing rate of clock-like neurons was inhibited in response to administration of citalopram, an effect reversed by WAY-100635. Interestingly, over time the regularity and firing rate of the clock-like neurons decreased when mice engaged in feeding or exploratory behaviour, and when moved to the novel environment.

In conclusion, the current study utilised multiunit recording combined with optotagging to monitor neuronal activity of 5-HT neuons and other neurons in the DRN of awake-freely moving mice. A further detailed physiological and pharmacological analysis of a larger population of DRN neurons is ongoing.

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POSTER # P-5

Role of 5-HT in maternal behaviour.

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In mammals, maternal care has an essential role to ensure offspring survival. Triggering the repertoire of behavioural responses for adequate maternal care implies functional changes in brain circuits linked to numerous physiological, cellular and molecular modifications. Serotonin (5-HT) neurotransmission plays an important role in this plasticity. In genetic models with constitutive lack of brain 5-HT, maternal behaviour is perturbed and litter survival is compromised *(1) (2) (3)*. However, the mechanisms involved are not known, and in particular whether developmental changes are implicated. To explore this question, we reduced 5-HT transmission in adult female mice using Cre-recombinase AAV delivery in the dorsal (B7) or the median (B8) raphe of *Vmat2*$^{fl/fl}$ mice (B7$^{Vmat2-/-}$; B8$^{Vmat2-/-}$). In parallel, we reinvestigated the maternal behaviour of Pet1$^{-/-}$ mice. Surprisingly, in all genotypes, pup retrieval and nesting behaviours were not different from controls, indicating that motivated maternal response is maintained in 5-HT depleted dams. However, pup survival was strongly reduced in litters of Pet1$^{-/-}$ (as in ref *2*) and of B7$^{Vmat2-/-}$ mice. Pet1$^{-/-}$ and B7$^{Vmat2-/-}$ mothers showed a significant reduction in the time dedicated to nursing although the time spent in the nest was comparable to control dams. Similar results were obtained in primiparous Pet1$^{-/-}$. Altogether, these data suggest that 5-HT transmission from B7 is required at the time of parturition for the onset of mother/pup interactions during lactation, even in experienced mothers. Ongoing experiments aim to identify altered circuits involved in this phenotype.

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POSTER # P-6

Genetically encoded photocross-linkers locate the heteromeric interface in a serotonin GPCR heteromer.

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Protein-protein interactions represent a fundamental process involved in many aspects of cell physiology. G protein-coupled receptors (GPCRs) were initially assumed to exist and function as monomeric plasma membrane proteins. Nevertheless, more recent findings suggest that GPCR homodimeric/homomeric assemblies may occur in living cells. Of particular interest is also the observation that GPCRs form heteromeric complexes, which may ultimately affect GPCR trafficking, pharmacology and function.

We previously reported that the family A serotonin 2A (5-HT_{2A}) receptor and the family C metabotropic glutamate 2 (mGlu2) receptor are assembled as a GPCR heteromeric complex in living mammalian cells. Our previous mutagenesis studies reported that three residues (A4.40, A4.44 and A4.48) located at the intracellular end of the transmembrane domain 4 (TM4) of the mGlu2 receptor were necessary to form the 5-HT_{2A}-mGlu2 receptor heterocomplex. Although interesting, these data do not indicate directly whether these three residues interact physically with the 5-HT_{2A} protomer of the 5-HT_{2A}-mGlu2 heteromeric complex. This is particularly relevant when considering that previous crystal structures of mGlu receptor dimers revealed a TM1-TM1 homodimeric interface, whereas findings based on a cysteine crosslinking protocol suggested that the main mGlu2 receptor homodimeric interface is formed by TM4 and TM5.

With the final goal of mapping precisely the mGlu2 receptor residues that interact physically with the 5-HT_{2A} receptor, we applied a relatively novel receptor-based targeted photocross-linking approach. Activation by ultraviolet light induces covalent cross-linking of the interacting proteins, which can be detected by immunoblotting. As compared to the classical cysteine crosslinking approach, the major advantages of photocross-linking are greater specificity of cross-linking owing to the short lifetimes of the excited intermediates, and absence of the need for introduction of mutations at highly-conserved cysteine residues potentially involved in GPCR structure and function. We used amber codon suppression to introduce the photoreactive unnatural amino acid (UAA) *p*-azido-L-phenylalanine (azF) at selected positions in mGlu2 receptors. The mGlu2 variants were expressed in mammalian HEK293 cells and retained their pharmacological ligand binding and functional properties. Notably, we found that mGlu2 variants with azF in certain positions located at the intracellular end of TM4 cross-linked efficiently to the 5-HT_{2A} protomer. These results show for the first time the residues that interact physically at a GPCR heteromeric complex interface, using a targeted photocross-linking strategy with genetically encoded UAAs. This method will be important in future studies that will provide additional functional and structural insights into serotonin GPCR complexes.

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POSTER # P-7

Advantages and disadvantages of DREADD technology in serotonin related investigations

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Spinal cord transection results in damage to the descending pathways from the brain, which are responsible for the control of movement. In the case of locomotion, serotonergic (5-HT) cells of the medulla facilitate the initiation and control of locomotion. Our research involves an attempt to replace the lost control of locomotion from the brainstem by grafting the neurons that are the source of the descending locomotor command pathway into the spinal cord, where they make connections and can facilitate locomotion in paraplegic rats. We previously demonstrated that grafts of embryonic brainstem neurons into the sublesional spinal cord improve locomotor activity, and this improvement involves 5-HT_{2A} and 5-HT_7 receptors. Activation of the grafted cells requires exteroceptive stimulation such as pinching the tail, and spontaneous expression of locomotion in the open field rarely occurs. We have attempted to activate the grafted neurons using DREADD technology (designer receptors exclusively activated by designer drugs). We have successfully transduced the cells for grafting with the DREADDs using adeno-associated viral (AAV) vectors for delivery of an excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs. We have shown that the DREADD-containing cells survived and sent projections to appropriate targets in the host spinal cord. Delivery of the designer drug (Clozapine-N-oxide; CNO) had a positive effect on locomotion in cases where we used an excitatory DREADD, and suppression of locomotor activity was observed when we transfected the graft with an inhibitory DREADD. We found that CNO, which is now known to act via conversion to clozapine rather than penetrating the CNS itself, also has direct inhibitory effects due to clozapine antagonism of 5-HT receptors involved in the action of serotonin in the spinal locomotor system. We also examined the effects of the grafts on the expression of certain key receptors (5-HT_{2A} and 5-HT_7) in paraplegic rats, and we showed that the presence of grafts normalized receptor expression.
Conclusions: New insights into the actions of the DREADD ligands revealed that DREADD studies that use locomotion as an outcome measure must consider inhibitory effects of clozapine antagonism in planning and interpreting the results of such experiments.

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POSTER # P-8

5-HT modulation of cortical parvalbumin-positive interneurons

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The cellular basis of cognition lies within cortical microcircuits which comprise complex ensembles of excitatory and inhibitory neurons. These microcircuits receive serotonergic (5-hydroxytryptamine; 5-HT) input from the midbrain. Of particular interest is the interaction between 5-HT and parvalbumin-positive (PV^+) interneurons, which are powerful regulators of pyramidal neurons and other neurons in the microcircuit, and generate synchronised neural network oscillations (especially gamma oscillations; 30 - 80 Hz) that are considered crucial for normal cognition. The mechanisms by which 5-HT interacts with cortical PV^+ interneurons are not yet fully clear. The aim of the current study was to investigate the effect of 5-HT on cortical PV^+ interneurons that were genetically identified using PV-Cre x td-Tomato transgenic mice. Whole-cell patch clamp recordings were made from visualised PV^+ neurons in slices of the prefrontal cortex, and the effects of bath application of 5-HT were studied. Bath application of 5-HT (50µM) induced a significant depolarization of resting membrane potential of the PV^+ interneurons, and this effect was accompanied by an enhancement of current-evoked firing rate, input resistance and rheobase (n=8). These effects were greatly reduced, but not completely abolished, by bath application of the selective 5-HT_{2A} receptor antagonist M100907 (150 nM) (n=6). Overall, these results indicate a 5-HT_{2A} receptor-mediated excitation of PV+ interneurons in the prefrontal cortex. Optogenetic experiments are underway to compare the effects of bath applied 5-HT on cortical PV^+ interneurons with physiologically activated 5-HT neuron inputs.

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POSTER # P-9

SSRIs target prefrontal-raphe circuits during development to modulate synaptic connectivity and emotional behavior

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Selective serotonin reuptake inhibitors (SSRIs) antidepressants that block the serotonin transporter (Slc6a4/SERT) improve mood in adults but have paradoxical long-term effects when administered during perinatal periods, increasing the risk to develop anxiety and depression. The basis for this developmental effect is not known. Here, we show that during an early postnatal period in mice (P0-P10), Slc6a4/SERT is transiently expressed in a subset of layer 5-6 pyramidal neurons of the prefrontal cortex (PFC). PFC-SERT+ neurons establish glutamatergic synapses with subcortical targets, including the serotonin (5-HT) and GABA neurons of the dorsal raphe nucleus (DRN). PFC-to-DRN circuits develop postnatally, coinciding with the period of PFC Slc6a4/SERT expression. Complete or cortex-specific ablation of SERT increases the number of functional PFC glutamate synapses on both 5-HT and GABA neurons in the DRN. This PFC-to-DRN hyper-innervation is replicated by early life exposure to the SSRI, fluoxetine (P2 to P14) that also causes anxiety/depressive-like symptoms. We show that silencing the PFC-SERT+ neurons enhances the emotional alterations caused by early life exposure to SSRIs, increasing passive coping responses. Overall, our data identify specific PFC descending circuits that are targets of antidepressant drugs during development. We demonstrate that developmental expression of SERT in this subset of PFC neurons cell-autonomously controls synaptic maturation of PFC-to-DRN circuits, and that remodeling of these circuits in early life modulates behavioral responses to stress in adulthood.

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POSTER # P-10

Serotonin$_{2B}$ receptors exert a GABA-mediated inhibitory control on dorsal raphe nucleus serotonin neuron activity: a microdialysis study in the rat

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The central serotonin$_{2B}$ receptor (5-HT$_{2B}$R) is a well-established modulator of dopamine (DA) neuron activity in the rodent brain, and is currently considered as a potential pharmacological target for treating DA-dependent neuropsychiatric disorders such as schizophrenia or drug addiction. Recent electrophysiological and microdialysis studies in rats have shown that the ability of 5-HT$_{2B}$R antagonists to modulate the mesocorticolimbic DA system results from a primary action at the level of the dorsal raphe nucleus (DRN), where they activate 5-HT neurons innervating the medial prefrontal cortex (mPFC).
The present study aimed at investigating the mechanisms underlying this interaction in the DRN, using intracerebral microdialysis in isoflurane anaesthetized rats, an experimental procedure allowing the simultaneous monitoring of 5-HT outflow in the DRN and the mPFC. When locally administered, drugs were delivered into the DRN by reverse dialysis.
We found that intraperitoneal (0.16 mg/kg) or intra-DRN (1 µM) administration of the 5-HT$_{2B}$R antagonist RS 127445 increases 5-HT outflow in both brain regions, these effects being prevented by the intra-DRN perfusion of the GABA-A R antagonist bicuculline (100 µM), as well as after the subcutaneous (0.16 mg/kg) or the intra-DRN (0.1 µM) administration of the selective 5-HT$_{1A}$R antagonist WAY 100635. Also, the increase in DRN 5-HT outflow induced by the intra-DRN administration of the selective 5-HT reuptake inhibitor citalopram (0.1 µM) was potentiated by the intra-DRN administration (0.5 µM) of RS 127445 only in the absence of bicuculline perfusion.
These results support the view that 5-HT$_{2B}$Rs exert an indirect GABA-mediated inhibitory control on DRN 5-HT neuron activity and suggest a possible location of 5-HT$_{2B}$Rs on DRN GABA interneurons.

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POSTER # P-11

The open chromatin landscape of 5-HT neurons

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Serotonergic function requires precise spatiotemporal control of gene expression in 5-HT neurons and altered expression patterns in these neurons are thought to contribute to psychiatric disorders. Serotonergic gene expression depends on proper patterns of open and closed chromatin states to permit appropriate transcription factor interactions with the promoters and enhancers of 5-HT expressed genes. As is the case with virtually all neuron-types, nothing is known about how 5-HT neurons acquire patterns of open chromatin to enable expression of genes needed for 5-HT neuron function. 5-HT neurons mature upon their initial postmitotic differentiation at about embryonic day 11 through three weeks of postnatal life. During this prolonged stage, expression of hundreds of genes is upregulated to support acquisition of mature characteristics while other genes needed at earlier stages of development are turned off. We hypothesize that dynamic changes in patterns of 5-HT neuron open chromatin underlies the dynamic patterns of gene expression that support differentiation and maturation of 5-HT neurons. To investigate this idea, we developed a novel pipeline for assay of Tn5 transposase-mediated insertion of sequencing adaptors (ATAC-seq) into native chromatin using flow sorted fetal and early postnatal 5-HT neurons. Our pipeline, 5HT-ATAC-seq, has produced highly reproducible maps of the open chromatin landscape in maturing 5-HT neurons. Comparing 5-HT neurons and non-5-HT cells, over 20,000 5-HT neuron-specific open chromatin regions were identified across the genome. 5-HT-specific open regions mark putative transcriptional regulatory elements controlling 5-HT expressed genes, including *Pet1, Tph2, Ddc, Gch1, Gchfr, Slc6a4, Slc22a3, Maoa,* and *Maob*. Our working 5HT-ATAC-seq pipeline enables investigation of how maturation of 5-HT open chromatin relates to maturation of 5-HT gene expression patterns, what is the impact of environmental and genetic factors on maturation of 5-HT open chromatin patterns, and what are the regulatory mechanisms that establish 5-HT neuron-type open chromatin landscapes.

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POSTER # P-12

Serotonin – a regulator of neuronal mitochondrial energetics and biogenesis

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Serotonin (5-HT) modulates neuronal differentiation, growth and synaptic plasticity, however it's influence on mitochondrial physiology in neurons is largely unknown. In cortical cultures, 5-HT treatment evoked an increase in mitochondrial biogenesis, with enhanced mtDNA levels, increased expression (mRNA and protein) of specific mitochondrial markers, and increased mitotracker staining. We also observed that 5-HT treatment of cortical neurons increases cellular ATP levels and enhances basal as well as maximal respiration. The effects of 5-HT were mimicked by the 5-HT_{2A} receptor agonist, DOI, which enhanced mtDNA and ATP production. Pretreatment with a 5-HT_{2A} receptor selective antagonist MDL100,907 or cortical neurons cultured from 5-HT_{2A} receptor knockout mice showed a complete blockade of the effects of 5-HT on mitochondrial biogenesis/ function. We examined the role for SIRT1, a key regulator of mitochondrial biogenesis, in mediating the effects of 5-HT. In cortical cultures derived from SIRT1 knockout mice or treated with the SIRT1 inhibitor EX-527, 5-HT failed to elicit an increase in mitochondrial biogenesis/function. We also observed a robust induction of PGC-1α following treatment with 5-HT or the 5-HT_{2A} receptor agonist, DOI. PGC-1α is known to influence the transcription of the mitochondrial transcription factor (TFAM), which was upregulated by both 5-HT and DOI. Notably, the increase in PGC-1α and TFAM levels preceded the increase in mtDNA and ATP levels, demonstrating that 5-HT may influence mitochondrial physiology through modulation of mitochondrial biogenesis in neurons. In cortical neurons 5-HT decreased cellular ROS levels and enhanced anti-oxidant enzymes SOD2 and catalase, indicating a potential role in buffering cellular stress. We show that pretreatment with 5-HT has a neuroprotective effect in primary cortical neurons buffering excitotoxic and oxidative stress evoked by kainate and H_2O_2 respectively. Our results highlight the important influence of 5-HT in regulating neuronal mitochondrial physiology.

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POSTER # P-13

Modulation of host serotonin and the microbiota-gut-brain axis by the fermented milk drink, kefir

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Mounting evidence suggests a role for the gut microbiota in modulating brain physiology and behaviour through bi-directional communication along the gut-brain axis. As such, the gut microbiota represents a potential therapeutic target for influencing centrally-mediated events and host behaviour. The fermented milk drink kefir has recently been shown to modulate the composition of the gut microbiota in mice. In this study, we sought to investigate the potential role of kefir in positively modulating host reward-seeking behaviour through the microbiota-gut-brain axis in animal models. Two distinct kefirs (UK4 and Fr1) or milk control were administered to male adult mice for 15 weeks and reward-seeking behaviour was assessed using the saccharin preference test and female urine sniffing test. In addition, caecal microbiota composition and function were assessed by shotgun metagenomics, gut serotonergic signaling by HPLC and systemic immunity by flow cytometry. Both kefirs increased reward-seeking behaviour in the female urine sniffing test, while kefir Fr1 also increased reward-seeking behaviour in the saccharin preference test. Furthermore, kefir Fr1 increased the 5HIAA/5-HT ratio in the colon, indicating increase serotonergic activity, and additionally reduced circulating neutrophil levels, a marker of inflammation which has been reported to be affected by serotonergic signaling. Interestingly, only kefir UK4 significantly increased caecal microbiota alpha diversity; whereas both kefirs were able to affect caecal microbiota function. Altogether, these data show that kefir can signal through the microbiota-gut-brain axis and modulate reward-seeking behaviour, potentially through local serotonergic signaling and systemic immunity. In addition, our results show that different kefirs may potentially direct the microbiota toward distinct behavioural modulatory effects, highlighting the need for thorough kefir characterization. Overall, these results indicate that kefir could play a beneficial role on the microbiota-gut-brain axis in health and support the recent broadening of the definition of psychobiotic to include fermented foods such as the fermented milk drink, kefir.

POSTER # P-14

Non-canonical function of early serotonergic neurons in molluscan and annelid larvae: from neurite growth navigation to link between generations

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The most of molluscs and annelids (Lophotrochozoa: Bilateria) have complex life cycle including free swimming or encapsulated larvae prerequisite to adult form. Larval stage is particularly important for survival and dispersion strategies and possesses specialized nervous structures adapted for these purposes. It was generally accepted that early serotonergic neurons appear within central ganglia and mostly regulate larval behavior. Our recent findings demonstrate that the first neuronal elements differentiate much earlier than previously suggested. Early neurons are located at the periphery (before the onset of central ganglia formation) and serve much wider functions which are crucial for the neurogenesis, intra- and interspecific chemical communication and rapid adaptation to the changing environment.

Our studies focused on the early neurons functional morphology in the course of gastropods (*Lymnaea stagnalis* and *Helisoma trivolvis*), bivalves (*Mytilus trossulus* and *Crassostrea gigas*) and polychaete worms (*Phyllodoce maculata* and *Platynereis dumerilii*) development. In all species investigated the population of early neurons always includes serotonin (5-HT) producing cells. Two distinct sets of early 5-HT neurons can be distinguished: (1) peripheral neurons of episphere and hyposphere and (2) apical neurons. Despite variety in cell body location of peripheral cells, their extended axons spread through the larval body and mark the layout of future main central ganglia, connectives and commissures in a species-specific manner. Thus, we consider early peripheral cells as primary guiding cells - pioneer neurons. Apical cells form the sensory-humoral organ (ASO). ASO compact varicose neuropil constantly release 5-HT thus establishing the apical-caudal gradient of 5-HT in larval body. Disruption of 5-HT gradient knocking down the navigation of pioneer cell axons and affects the definitive nervous system morphology. Both peripheral and apical early neurons are sensory thus linking external signals with developmental and behavioral tuning. For example, 5-HT production increases in response to the chemical signals emitted by adults in conditions of starvation and overcrowding. As a result, larvae retard developmental tempo and intensify locomotion. Such adult-to-embryo communication gives larvae a chance to leave unfavorable environment before they metamorphose and became immobile or slowly moving adults.

Altogether our results demonstrate that early larval 5-HT cells are a part of complex and precise system that coordinates larval neurogenesis, developmental tempo and behavior in a constantly changing environment.

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POSTER # P-15

Novel Crosstalk and Heterodimerization of the Oxytocin Receptor and Serotonin 2A Receptor

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The oxytocin receptor (OTR) is known to play a major role in the development of sociability and mood and has been implicated in many disorders such as; anxiety, depression, autism and more recently schizophrenia. Similarly, the serotonin 2A (5-HT_{2A}) receptor has also been implicated in such disorders. *In vivo* studies have shown a possible link between these receptors and their neurotransmitters. Notably, OTR expression in serotonergic neurons has been shown to exert an anxiolytic effect, while administration of oxytocin results in altered serotonin innervation. Although both neurotransmitters and their receptors have been shown to affect one another, the exact mechanism of this is unknown.

Lentiviral plasmids encoding the OTR tagged with a red fluorescent protein were packaged into lentiviral vector (Lv-OTR-RFP) and transfected into HEK293A cells stably expressing the 5-HT_{2A} receptor tagged with a green fluorescent protein (HEK293A-5-HTR_{2A}-GFP). Co-localisation and ligand-mediated co-internalisation of the receptors were analysed using confocal and fluorescent microscopy. Flow cytometry based FRET (fcFRET) was used to further evaluate the physical interaction of these receptors. Functional assays, including calcium mobilisation and IP-1 accumulation were used to evaluate any alterations in receptor-dependent signalling.

Results demonstrate compelling evidence for 5-HT_{2A}/OTR heterodimer formation and crosstalk with remarkable downstream signalling consequences. A significant decrease in OTR Gq-mediated signalling in cells co-expressing the 5-HT_{2A} receptor is observed in both calcium mobilisation and IP-1 assays. Heterodimer formation is supported by co-localisation of the receptors on the cell membrane, co-internalisation of both receptors upon agonist treatment, and 23% fcFRET signal. The observed signalling changes following 5-HT_{2A}/OTR dimer formation are poised to have significant functional consequences *in vivo* and are highly likely to impact current and future pharmacotherapies targeting these receptors.

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POSTER # P-16

PZKKN-94 – a novel and potent dually acting 5-HT$_6$R antagonist/5-HT$_{1B}$R agonist with pro-cognitive, antidepressant and antiparkinsonian properties in animal models

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A number of preclinical and clinical studies indicate the therapeutic potential of 5-HT$_6$ receptor modulators in the treatment of cognitive disorders associated with Alzheimer's disease and behavioral and psychological symptoms of dementia. Several studies revealed that 5-HT$_{1B}$R agonist may play a role in modulating the mood, extrapyramidal motor functions and motor symptoms related to Parkinson's disease (PD) dyskinesia. In the present study we assessed drug-like properties and pharmacological properties of a novel dually acting 5-HT$_6$R antagonist/5-HT$_{1B}$R agonist – PZKKN-94.

In vitro experiments revealed that PZKKN-94 behaved as very potent 5-HT$_6$R antagonist (K_i = 2.2 nM, K_b = 1 nM) and 5-HT$_{1B}$R agonist (EC_{50} = 30 nM). The compound is characterized by satisfactory *in vitro* metabolic stability (Cl_{in} = 25 μl/min/mg) and good oral bioavailability in rats with C(max) of 116 ng/ml and ca. 3 folds preference for CNS at C(max). Behavioral studies showed ability of PZKKN-94 (0.3 or 0.1 mg/kg), also in combination with donepezil, to reverse the cognitive impairment in Novel Object Recognition (NOR) test caused by scopolamine or phencyclidine. Furthermore, PZKKN-94 (0.3 mg/kg) displayed pro-cognitive effect in aged animals (20 months) and no tolerance was developed after 14-day BID administration. PZKKN-94 (1 mg/kg) was able to improve attention in Attentional Set Shifting test (ASST), and showed antidepressant activity at a dose of 0.1 mg/kg in Force Swim Test (FST). Finally, PZKKN-94 decreased the haloperidol-induced catalepsy (at a dose of 3 mg/kg) in rat model of PD.

Conclusion: A new approach, based on dually acting 5-HT$_6$R antagonist**/**5-HT$_{1B}$R agonist – PZKKN-94, for the treatment of CNS disorders was proposed. Evaluation of PZKKN-94 properties revealed its high activity potential in *in vitro* and *in vivo* studies and pro-cognitive, antidepressant, and antiparkinsonian properties. Preferential drug-likeness profile and promising behavioral results justify further mechanistic studies of complementary modes of action of PZKKN-94 and its development as a potential therapy in CNS disorders.

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POSTER # P-17

A novel potent, selective and metabolically stable 5-HT$_7$ receptor inverse agonist PZ-1373 with antidepressant and anxiolytic properties

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The type 7 serotonin receptor (5-HT$_7$R) is the most recently identified member of serotonin family positively coupled with adenylyl cyclase through the stimulatory $G_{\alpha s}$ and $G_{\alpha 12}$ proteins.[1] Recent preclinical and clinical data support the hypothesis that 5-HT$_7$R antagonists may represent a valid alternative strategy for the treatment of affective disorders and neurodegenerative processes.[2]

Continuing our effort on the development of selective 5-HT$_7$R ligands, we have successfully demonstrated the possibility to replace the known long-chain arylpiperazine (LCAP) scaffold with the (aryloxy)ethyl alicyclic amine moiety which might be considered as a flexible biomimetic of LCAP.[3,4] The study identified compound PZ-1373, 4-fluoro-*N*-(1-{2-[2-(pyridine-3-yl)phenoxy]ethy}
piperidin-4-yl)benzenesulfonamide, as a lead structure.

Radioligand binding experiments determined in HEK293 cells overexpressing h5-HT$_7$R, revealed that PZ-1373 displayed high affinity for 5-HT$_7$R (K_i = 13 nM) and was selective over structurally related GPCRs and transporters (i.e., 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_6$, D$_2$, SERT, NET), and did not bind to off-targets receptors which are involved in potential cardiovascular and/or CNS side effects (i.e. M$_1$, α_1- β_1-adrenergic, H$_1$ and *h*ERG; % inhibition < 50% at 1 μM). Moreover, PZ-1373 behaved as moderate inverse agonist at 5-HT$_7$R in cAMP-based functional assays and displayed favorable physicochemical properties (water solubility, mice Cl_{in} = 22 μl/mg/min, rat Cl_{in} = 26 μl/mg/min, good BBB penetration).

Finally, PZ-1373 exerted antidepressant activity after acute or chronic administration in the forced swim test in rodents (MED = 0.625–2.5 mg/kg, *i.p.*) and anxiolytic properties in the Vogel test (MED = 1 mg/kg, *i.p.*) in rats. These findings warrant further studies to explore the therapeutic potential of PZ-1373 for the treatment of CNS disorders.

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POSTER # P-18

The ability of serotonin $(5\text{-HT})_{2C}$ receptor agonist to alleviate affective signs of nicotine withdrawal

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Preclinical data demonstrate that serotonin $(5\text{-HT})_{2C}$ receptor agonists inhibit nicotine self-administration, reinstatement of nicotine-seeking behavior and the locomotor effects of nicotine (e.g., Higgins et al., 2012; Levin et al., 2011; Zaniewska et al., 2009; Zeeb et al., 2015). However, there is limited data on the effect of 5-HT_{2C} receptor agonism on affective signs of nicotine withdrawal (Zaniewska et al., 2010). In the present study, we tested the hypothesis that nicotine withdrawal induced affective symptoms and that 5-HT_{2C} receptor agonism alleviated these aspects of nicotine withdrawal and drug-seeking behavior. Rats were subjected to nicotine (0.03 mg/kg/inf) self-administration under an increasing schedule of reinforcement (fixed ratio (FR)=1-5). Immediately after the last self-administration sessions, the anxiolytic and hyperlocomotive effects of nicotine were evaluated. After 21 self-administration sessions, animals were subjected to a withdrawal phase. At different time points (day 1, 3, 14 and 30 of nicotine withdrawal) the depression-like behaviour was assessed in the forced swim test (FST). At similar time points as in the FST, the level of anxiety was verified in the light/dark box test (LDB). Nicotine produced anxiolytic properties and locomotor hyperactivity. In the FST, we reported increased immobility time on day 1, 3 and 14 of nicotine withdrawal, and the strongest effect was observed during the first 3 days of withdrawal. This depression-like effect was not accompanied by the anxiety, as we did not observe changes in animals' behavior in the LDB during nicotine cessation. Administration of 5-HT_{2C} receptor agonist Ro 60-0175 (3 mg/kg) on day 3 of nicotine withdrawal shortened the enhanced immobility time in drug-weaned rats, but did not alter the reinstatement of nicotine-seeking behavior examined on day 15. To summarize, here we show that acute administration of 5-HT_{2C} receptor agonist alleviated the depression-like behavior during withdrawal from nicotine self-administration, which suggests the efficacy of 5-HT_{2C} receptor agonists in attenuating some affective aspects of nicotine cessation.

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