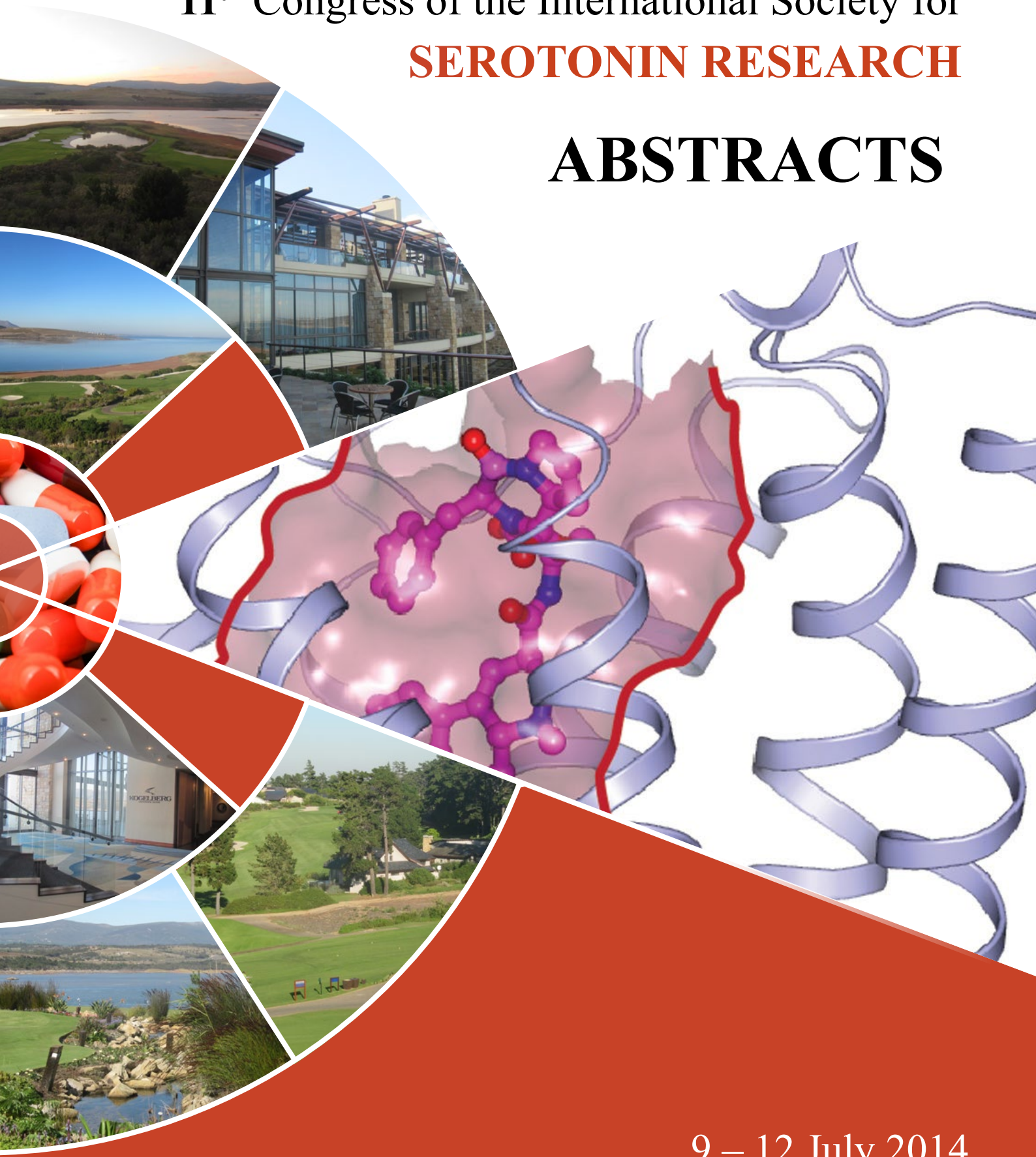
11th Congress of the International Society for SEROTONIN RESEARCH

ABSTRACTS



9 – 12 July 2014

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International Society for Serotonin Research

Formerly Serotonin Club

Serotonin

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

It gives me great pleasure to welcome you to the 11th meeting of the International Society for Serotonin Research. This year, our meeting will be held at the beautiful Arabella Estate near Capetown, South Africa, and is an official satelite conference of the 17th World Congress of Basic and Clinical Pharmacology to be held in that city.

The program of this year's meeting includes some of the leading serotonin researchers in the world, focusing on the role of serotonin in psychiatric illness, immune modulation, Alzheimer's disease, obesity and addiction, as well as serotonin neuroanatomy, neuropharmacology and receptor function. A particularly exciting aspect of the meeting is that a selection of highly talented graduate students and postdocs present their work as part of a symposium or in the special "Prodigees and Pioneers" session. As there are no parallel sessions, all registrants will have maximum opportunity to attend as many presentations as possible. The program also includes social events such as Cheese and Wine Tasting and a Gala Dinner.

The theme of the 2014 meeting is "A Serotonin Safari" and clearly this is a unique opportunity to extend your conference stay and explore the beautiful Western Cape or other parts of South Africa and its neighbouring countries. I am looking forward to seeing you in South Africa and hope you have a wonderful meeting.

Sincerely,

Maarten van den Buuse
President
International Society for Serotonin Research

A hearty welcome to a Serotonin Safari, Arabella, Western Cape, South Africa, 2014

It is a great pleasure to welcome you to the 11th Congress of the International Society for Serotonin Research (ISSR) at the Arabella Country Estate in the picturesque Western Cape Province of South Africa.

The theme of the conference is "A Serotonin Safari", and is an apt theme for the first ISSR meeting to be held on African soil. The ISSR and the Local Organizing Committee (LOC) have taken great effort in putting together what we believe to be a fantastic academic program that will appeal to all clinical and basic scientists that have a deep interest in the biology of serotonin. By bringing together the best speakers talking on the latest cutting edge research covering the physiology of serotonin in its broadest sense, ISSR 2014 will be the year's premier event for discussing the diverse roles of this important molecule, and for showcasing the latest research. We trust that it will impart new knowledge, initiate new areas of research, and mobilize new solutions to many ailments that challenge medical science today. The congress is purposefully placed as a pre-conference satellite meeting to the World Congress of Pharmacology 2014 (WCP2014) in order to allow delegates at ISSR 2014 to also attend the WCP2014 meeting in Cape Town. While the program caters for top academics, young aspiring talent is also afforded an opportunity to present their work at a special forum on pioneers and prodigies. But it's not just work and no play, and the LOC have also planned evening entertainment during the meeting that will complement the ambience of the venue and its surroundings, don't miss it.

The meeting presents as an ideal opportunity to network and to make contact with some of the leading minds in the field, so that ISSR 2014 will serve to encourage and empower young graduate students to reinforce their chosen careers, yet at the same time foster local and international relations and collaborations in the area of serotonin research. We not only wish you a very successful and valuable academic meeting, but that you will also be caught up in the raw beauty of South Africa, its people, its wild-life and its natural beauty. Please make the most of this opportunity, enjoy the environs, enjoy Cape Town, enjoy the "vibe" of the meeting, and may it be a boiling pot for long-term interaction and collaboration between Africa and the world.

Sincerely yours,

Brian Harvey
Chair
Local Organizing Committee
ISSR 2014

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SEROTONIN RESEARCH

BIOSKETCHES

9 – 12 July 2014

Emeritus Professor Charles A Marsden

Charles Marsden is Emeritus Professor of Neuropharmacology at the University of Nottingham UK and was previously Co-Director of The Institute of Neuroscience at Nottingham. He graduated in zoology from the University of London in 1966 and then studied for an MSc in Biochemical Pharmacology and then a PhD in invertebrate neurotransmission at the University of Southampton from 1966-1969. Charles Marsden worked at the University of Bergen, Norway (1969-72) and then at the Institute of Neurology, with Gerald Curzon, in London (1972-77) before moving to the University of Nottingham in 1978. In between London and Nottingham he spent an exciting and fruitful research period at the State University of Kansas in Lawrence working with Ralph Adams.

Charles Marsden was President of the British Association of Psychopharmacology (BAP) from 2000-2002 and of the International Serotonin Club from 2006-2008. In 2001 Charles Marsden was awarded the JR Vane medal by the British Pharmacological Society for an ”outstanding contribution to neuropharmacology” and in 2013 was given a Lifetime Achievement award by the BAP. Princess Maha Chakri Sirindhorn of Thailand presented him with a Global leadership award in 2008 for his work developing medical education in Thailand. Charles Marsden has supervised over 70 successful PhD students.

His research has centred on neurotransmitter control of behaviour with particular emphasis on in vivo assessment of neurotransmitter function during behaviour. This work over the past 30 plus years has made a significant contribution to the development of new technologies such as HPLC with electrochemical detection, in vivo voltammetry in vivo microdialysis and small animal functional magnetic resonance (fMRI). The work has contributed to our understanding of the neural mechanisms involved in stress, anxiety and depression with special attention to the role of serotonin.

Another major area of research has been the development of environmental animal models of psychiatric disorders including the isolation reared rat as a neurodevelopmental model, which has attracted wide international attention as a model of schizophrenia at both an academic and industrial level. Charles Marsden has also worked on models of depression and ADHD and the development of novel drug treatments for these disorders. Drugs of abuse have also been an important area of research with significant studies on ecstasy and cocaine particularly in regard to the mechanisms involved in potential neurotoxicity of such drugs.

Herbert Y. Meltzer, MD

Herb Meltzer, MD, is Professor of Psychiatry and Behavioral Sciences and Professor of Physiology at the Feinberg School of Medicine Chicago, Il. and visiting Professor at the Soochow University School of Medicine in Suzhou, China. He received his BA with Distinction from Cornell University, an MA in Chemistry from Harvard, and his MD from Yale University and has been President of the American College of Neuropsychopharmacology (ACNP) and the Collegium International Neuro-psychopharmacologicum (CINP). He served as editor of Psychopharmacology: The Third Generation of Progress and co-editor of the ACNP journal, Neuropsychopharmacology, and is a member of the editorial board of numerous scientific journals.

Dr. Meltzer is the recipient of the Efron and Paul Hoch Awards of the ACNP, the Noyes Prize of the Commonwealth of Pennsylvania, the Edward J. Sachar Award from Columbia University, the Lieber Prize for Schizophrenia Research from NARSAD, the Stanley Dean Award for Research in Schizophrenia of the American College of Psychiatry, the Gold Medal Award of the Society of Biological Psychiatry, the Earl Sutherland Prize for Achievement in Research of Vanderbilt University (2004), the Research Prize of the American Psychiatric Association (2005), the Grant Liddle Award for Clinical Research from Vanderbilt University (2008), and the Lifetime Achievement Award of the Winter Conference on Psychosis Research (2011). He has chaired the Young Investigator Grant Review Committee for the Brain and Behavior Research Foundation (formerly NARSAD) since its inception and the International Psychopharmacology Algorithm Project, a web-based algorithm to guide the treatment of schizophrenia (www.IPAP.org).

Dr. Meltzer's research interests include the discovery, development and optimal utilization of pharmacologic treatments for schizophrenia, the cause and treatment of cognitive impairment in schizophrenia (CIS), the elucidation of the mechanism of action of antipsychotic drugs and the discovery and development of novel treatments for schizophrenia and CIS, the development of genetic biomarkers for mental illness and personalized medicine, and the causes and prevention of suicide. His research has emphasized the importance of numerous serotonin receptors in the action of antipsychotic drugs and most recently, as targets for improving cognition. This research has been at basic, clinical and translational levels.

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SYMPOSIUM ABSTRACTS

9 – 12 July 2014

IRVINE PAGE PLENARY LECTURE

It's All In The (Serotonin Receptor) Family

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Paul Janssen is quoted in his interview in the 'Psychopharmacologists' that I was obsessed with serotonin, while stating his passion for dopamine as the key to understanding risperidone and other atypical antipsychotic drugs. I plead guilty, with pride, to this epithet , as I do to Sol Snyder's reference to me as 'Dr. Clozapine' after I challenged his view, at the time, that clozapine fit well into the dopamine D2 antagonist model that Arvid Carlsson, Phil Seeman, he and others once and some still do, as the only basis for antipsychotic activity. Unrepentant and stubborn, serotonin, clozapine, and the exceptional range of serotonergic drugs produced by those of us who recognize the central role of 5-HT, remain the sinews of the ongoing basic and translational research effort by my many colleagues and by me .

A premature belief that I knew something about 5-HT2A receptors began my academic career as a consultant , earning me a fancy dinner as my honorarium, as first year medical student, for discussing the role of 5-HT2A in the action of LSD with Dan Friedman . This lecture comes 46 years later, just as pimavanserin, a selective 5-HT2A inverse agonist, is about to be approved in the US for treating the dopamine-induced psychosis of Parkinson's disease. Its applications to treating schizophrenia, my overriding interest, and the behavioral disturbances of Alzheimer's disease, are well underway. The discovery of pimavanserin was inspired by, and its preclinical and clinical development were guided by, my hypothesis that clozapine, the first atypical antipsychotic drug, owed its atypical properties to more potent 5-HT2A than D2 receptor blockade, The major class of atypical antipsychotics grew from this and led to Bryan Roth and I finding that amisulpride, a different sort of atypical, owed some of its special properties to potent 5-HT7 antagonism. Appreciation of 5-HT2C agonism as a stand alone antipsychotic and the underutilization of 5-HT1A agonism for psychotropic drug development is imminent.

My ongoing obsession with serotonin has led to a variety of preclinical and translational studies which I will use to illustrate the way basic pharmacology and clinical experience has led to increased understanding of the role of 5-HT in relation to psychosis, learning and memory, mood and motor function, and important clinical applications. Thus, the development of severe extrapyramidal symptoms in one of my depressed patients participating in the first wave of fluoxetine trials was key to my appreciating the serotonergic regulation of dopaminergic function and how the two neurotransmitters might be involved in a complex disorder such as schizophrenia. My study of phencyclidine (PCP) as a model for motor neuron abnormalities in schizophrenia as part of my first research effort led to studies of PCP's effects on extracellular serotonin and dopamine, which have now culminated in a series of rodent and human studies revealing the contribution of 5-HT1A, 5-HT2A, 5-HT2C, and 5-HT7 receptors, among others, to understanding the glutamate-GABA hypothesis of cognitive impairment in schizophrenia and the opportunity to develop novel treatments for a wide range of behaviors that occur in many neuropsychiatric disorders. Prevention of the cognitive impairment of schizophrenia through serotonergic mechanisms and integrating this knowledge into personalized medicine are current interests. If achieved, those who gainsay serotonin and remain stuck in the dopamine only mode, will have to recognize the power of our (5-HT) Family. Irving Page would approve.

SEROTONIN, SCHIZOPHRENIA AND DEPRESSION

Role of 5-HT$_{1A}$ receptors in psychosis and sensory gating

Maarten van den Buuse

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Schizophrenia is a devastating mental illness with a complex set of symptoms including hallucinations, delusions, social withdrawal, negative affect and cognitive deficits. The neuropsychopharmacological literature on schizophrenia has been dominated by studies on dysfunction of dopaminergic and glutamatergic activity in different parts of the brain. However, it is clear that also serotonergic function, in particular the serotonin-1A (5-HT1A) receptor, plays an important part in some of the symptoms of schizophrenia and the action of antipsychotic drugs.

One fundamental deficit in schizophrenia is the inability to adequately filter sensory input so that the individual can focus on the relevant and is able to ignore irrelevant information. This filtering mechanism is often referred to as sensory gating, a multi-dimensional process that 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 5HT1A receptors in sensory gating.

In line with several previous studies, administration of 5-HT1A receptor agonists decreased PPI in rats, i.e. inducing a schizophrenia-'like' deficit. These drugs also reduce paired-pulse gating ratios (Thwaites, Van den Buuse & Gogos, Pharmacol Biochem Behav 2013). Surprisingly, depending on the strain, most of these drugs do not decrease or even increase PPI in mice. However, we have shown that altering baseline serotonergic activity can change the effect of 5-HT1A agonist drugs to induce decreases of PPI also in mice (Van den Buuse, ACS Chem Neurosci 2013).

The effect of the prototypical 5-HT1A receptor agonist, 8-OH-DPAT, on PPI in rats is mediated by downstream dopaminergic activation (Gogos et al., J Pharmacol Exp Ther 2010). Similarly, the decrease of paired-pulse gating induced by treatment with the serotonin releaser, MDMA, can be blocked by a dopamine D2 receptor antagonist, suggesting sequential activation of dopaminergic activity by serotonergic activation (Lee, Thwaites & Van den Buuse, unpublished). This 5-HT1A/dopaminergic interaction was further illustrated when we showed that chronic estrogen treatment could prevent 8-OH-DPAT-induced disruption of PPI most likely by an effect on downstream dopaminergic mechanisms (Gogos et al., J Pharmacol Exp Ther 2010; Chavez et al., Brain Res 2010). Of note, estrogen treatment also ameliorated the PPI reduction caused by the 5-HT1A receptor partial agonist, buspirone, in humans (Gogos et al., Neuropsychopharmacology 2006), although further experiments are needed to ascertain a dopaminergic involvement in this interaction.

The site of action of the 5-HT1A/dopaminergic interaction remains to be identified. In rats, serotonin depletion in the dorsal, but not ventral hippocampus, reduced baseline PPI (Kusljic, Copolov & Van den Buuse, 2003; Adams, Kusljic & Van den Buuse, 2008), suggesting a role for hippocampus serotonin. However, micro-injection of 8-OH-DPAT into the ventral tegmental area (VTA), the site of origin of the mesolimbic dopamine system, caused a small, but significant decrease of PPI, which could be prevented by pre-treatment with the dopamine receptor antagonist, haloperidol, suggesting a possible role of serotonin innervation of the VTA . Micro-injection of 8-OH-DPAT into the nucleus accumbens had no effect of PPI (Gogos, Kwek & Van den Buuse, unpublished).

These studies show that 5-HT1A receptors are involved in sensory gating, which is deficient in schizophrenia. This involvement is most likely mediated by downstream activation of mesolimbic dopaminergic activity, consistent with the prevailing hyperdopaminergia model of psychosis. Therefore, altered serotonin stimulation of 5-HT1A receptors may modulate dopaminergic hyperactivity in psychosis, which may also explain some of the beneficial effects of antipsychotic drugs with activity at 5-HT1A receptors.

Influence of social isolation of rats from weaning on serotonergic function, social withdrawal and memory as a potential model of schizophrenia

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Schizophrenia is a devastating mental illness with a prevalence of nearly 1%, whose symptoms include positive, negative and cognitive deficits. The aetiology of schizophrenia is unclear but evidence suggests both genetic predisposition and exposure to early-life adverse stress, such as maternal neglect, as risk factors. Exposing social mammals, such as rats, to social isolation from their littermates from the day of weaning, profoundly affects subsequent brain development and adult behaviour. The molecular mechanisms underlying these developmental adaptations are unclear but would improve our knowledge of the aetiology of the disorder and potentially enable identification of longitudinal biomarkers of dysfunction. Furthermore, social isolation rearing in rats may also be a valuable animal model with which to test reversal of symptoms with potential novel therapeutic compounds.

Rearing Lister hooded rat pups from weaning (post-natal day 21-23) in separate cages, so that they have auditory, visual and olfactory but no social interaction, causes profound, lasting alterations in behaviour and brain structure and function in the resultant adult rats compared with their group-housed littermates. These abnormalities include reduced habituation to a novel environment, impaired sensorimotor gating to an auditory stimulus and cognitive impairments in novel object recognition and contextual fear motivated learning paradigms. Furthermore when combined with neonatal exposure to the NMDA receptor antagonist, phencyclidine (10mg/kg s.c. post natal days 7, 9, 11), rats subsequently reared in social interaction show additional deficits in social interaction which may have translational relevance to the negative symptoms of schizophrenia. Isolation reared rats also show small but significant reductions in prefrontal cortical volume measured by MRI, and alterations in cortical serotonin and dopamine function compared with group housed littermates. Many of these behavioural, morphological and neurochemical abnormalities strongly resemble several core symptoms seen in schizophrenia.

The effect of atypical antipsychotics and potential novel therapeutic compounds, acting through modulation of serotonergic and glutamatergic and dopaminergic mechanisms, to reverse a battery of behavioural alterations produced in isolation reared rats will be shown. Of particular interest 5-HT_6 and D_3 receptor antagonists, nicotinic receptor modulators and drugs which modulate glutamate function reverse novel object recognition impairments while selective D_2 antagonists do not. The pro-cognitive effect of D_3 receptor antagonists may involve an action in the prefrontal cortex and be dependent on serotonergic function as it is prevented by serotonin depletion with 5,7-dihydroxytryptamine, while only drugs which modify glutamatergic function appear to reverse the alteration in social interaction.

The combined 'dual-hit' of early-life isolation rearing with neonatal administration of the NMDA receptor antagonist phencyclidine produced a wide-ranging impairment in learning and memory, and social interaction as well as hyperactivity in a novel arena thought to reflect mesolimbic dopamine hyperactivity and positive symptoms. Thus this dual-hit might provide a more comprehensive preclinical model to determine the neurobiological aetiology of schizophrenia than either treatment alone.

5-HT_2 receptor effects of a repurposed, lithium-mimetic with therapeutic potential for bipolar depression and related disorders

Ivi Antoniadou, Marianna Kouskou, Dominic Buchmeuller, Tasneem Arsiwala, Esha Johal, Nisha Singh, Sri Vasudevan, Philip Cowen[1], Grant Churchill and Trevor Sharp

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After serendipitous discovery 60 years ago, lithium remains the mainstay treatment for bipolar disorder and suicide prevention. Moreover lithium is licensed to treat aggression and self-harm, and reduces other impulsive behaviours in clinical trials. However, the full therapeutic potential of lithium is limited by poor tolerance and low treatment compliance due to adverse effects or symptoms of toxicity at concentrations marginally above the therapeutic range. A solution to this problem would be a drug that captures the unique therapeutic profile of lithium without its serious adverse effects. Lithium's mechanism is uncertain but a leading proposal is inhibition of inositol monophosphatase (IMPase), causing reduced neurotransmitter signalling via the phosphoinositide (PI) pathway. Recently, we "reprofiled" a library of drugs that have proved safe in humans but failed in efficacy trials for other indications. This work identified a potent IMPase inhibitor, ebselen, which was originally developed as an antioxidant and anti-inflammatory drug. Here we investigated the effect of ebselen in comparison to lithium in functional models of 5-HT_{2A} and 5-HT_{2C} receptors; Gq protein coupled PI signalling receptors associated with the actions of lithium and other psychotropic drugs, including antipsychotics.

Ebselen was compared with lithium in behavioural and molecular (immediate early gene) based on administration to mice of hallucinogenic 5-HT_{2A} receptor agonists (DOI and psilocin). Similar experiments were carried out using a 5-HT_{2C} receptor agonist (Ro 60-0175). Ebselen inhibited 5-HT_{2A} agonist-evoked behavioural and molecular responses, and these effects were maintained on repeated administration. Lithium also reduced 5-HT_{2A} agonist-evoked behavioural and molecular responses. Moreover, both ebselen and lithium attenuated 5-HT_{2C} agonist-evoked molecular responses. Finally, 5-HT_{2A} agonist-evoked behaviour was reduced by a putative selective IMPase inhibitor (L-690,330) but not an inhibitor of glycogen synthase kinase (AR-A014418), an action that has also been linked to the psychotropic effects of lithium.

The current data demonstrate that ebselen, like lithium, attenuates 5-HT_{2A} and 5-HT_{2C} receptor function, and suggest IMPase inhibition as the mechanism of action. This evidence that ebselen is a lithium-mimetic in neuropharmacological models supports its testing in relevant psychiatric patient populations. As ebselen has a known safety profile in man, such trials can be rapidly progressed.

Funding from the BBSRC and Rosetrees Trust, and scholarships from the Onassis Foundation and Greek Government supported this work.

SEROTONYLATION: REGULATION IN DIVERSE SYSTEMS; FROM TETRAHYMENA TO MAMMALIAN BRAIN

Common and Rare Alleles of the Serotonin Transporter Gene, SLC6A4, Associated with Tourette's Disorder

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To evaluate the hypothesis that functionally over-expressing alleles of the serotonin transporter (SERT) gene (solute carrier family 6, member 4, SLC6A4) are present in Tourette's disorder (TD), just as we previously observed in Obsessive- Compulsive disorder (OCD), we evaluated TD probands (N=151) and controls (N=858). We genotyped the refined SERT linked polymorphic region 5-HTTLPR/rs25531 and the associated rs25532 variant in the SLC6A4 promoter plus the rare coding variant SERT isoleucine-to-valine at position 425 (I425V). The higher expressing 5-HTTLPR/rs25531 LA allele was more prevalent in TD probands than in controls (X^2=5.75; P=0.017; odds ratio [OR] 1.35); and, in a secondary analysis, surprisingly, it was significantly more frequent in probands who had TD alone than in those who had TD plus OCD (Fisher's exact test; P=0.0006; OR, 2.29). Likewise, the higher expressing LAC haplotype (5-HTTLPR/rs25531/rs25532) was more frequent in TD probands than in controls (P=0.024; OR, 1.33) and also in the TD alone group versus the TD plus OCD group (P=0.0013; OR, 2.14). Furthermore, the rare gain-of-function SERT I425V variant was observed in 3 male siblings with TD and/or OCD and in their father. Thus, the cumulative count of SERT I425V becomes 1.57% in OCD/TD spectrum conditions versus 0.15% in controls, with a recalculated, family-adjusted significance of X^2= 15.03 (P < 0.0001; OR, 9.0; total worldwide genotyped =2914). This report provides a unique combination of common and rare variants in one gene in TD, all of which are associated with SERT gain of function. Thus, altered SERT activity represents a potential contributor to serotonergic abnormalities in TD. The present results call for replication in a similarly intensively evaluated sample

“Serotonin, dopamine and noradrenaline - more than neurotransmitters: transglutaminase-mediated monoaminylation of extracellular matrix proteins”

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Serotonin (5-hydroxytryptamine, 5-HT) was first discovered in the blood serum as a vasoconstrictor substance. Here, 5-HT is also covalently incorporated into distinct proteins involved in thrombus formation. This process is mediated by transglutaminases and has been termed “serotonylation”. Cross-linking of serotonylated procoagulant proteins to specific binding proteins is essential for blood clot formation.

In the central nervous system (CNS) 5-HT plays important roles in both embryonic development, as a mediator of neurogenesis, and in the mature brain, as a neurotransmitter. Disturbances in the 5-HT system have also been indicated in several psychiatric disorders, however, it is questionable whether this is only due to 5-HT acting as a classical neurotransmitter. Taking lessons from the fate of 5-HT during thrombin formation in the blood it is conceivable that also in the CNS 5-HT can serve as a substrate for transglutaminases to form cross-linked matrices, probably involved in synaptogenesis - a possibility which has not been investigated so far.

Here we provide evidence for the serotonylation of neural proteins and proteins of the extracellular matrix such as fibronectin. In addition we show that the catecholamines dopamine (DA) and noradrenaline (NA) inhibit serotonylation of fibronectin and that DA and NA themselves can be selectively transamidated into fibronectin by TGase. These findings suggest a general mechanism of TGase-mediated ‘‘monoaminylation’’. In addition, possible physiological consequences of this newly discovered protein modification will be discussed.

Funded by the Deutsche Forschungsgemeinschaft

Serotonylation of Small G Proteins is Regulated by 5-HT_{2A} Receptors to Modify Dendritic Spines

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Serotonin receptor signaling, especially serotonin 2A receptors (5-HT_{2A}), plays an important role in psychiatric diseases and their treatments including schizophrenia and depression. Although several classes of drugs are available to treat these disorders, current medications are woefully inadequate due to long delays in therapeutic response, minimal impact on disease symptoms in many patients and adverse drug effects. Serotonylation is a novel signaling mechanism reported for 5-HT_{2A} receptors whereby activated transglutaminase catalyzes the transamidation of serotonin to a small G protein. We used A1A1v cells a rat cortical cell line and primary rat cortical neurons to explore serotonylation and transamidation in neuronal cells. In addition to activation of heterotrimeric G protein activation by 5-HT_{2A} receptors, we found that 5-HT_{2A} receptor stimulation activates the small G proteins Rac1 by serotonylation and Cdc42 by transamidation, possibly serotonylation in neurons. Stimulation of 5-HT_{2A} receptors with1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) transiently increases Rac1 transamidation and thereby activation in both A1A1v cells and primary rat cortical neurons and Cdc42 in A1A1v cells. Activation of Rac1 and Cdc42 by the 5-$HT_{2A/2C}$ receptor agonist DOI was prevented by inhibiting transglutaminase, the enzyme that catalyzes the transamidation reaction. The activation and transamidation of Cdc42 lasts longer than Rac1 following stimulation of 5-HT_{2A} receptors. Rac1 activity was elevated at 5 minute post-treatment, in contrast to Cdc42 in which activity remained elevated 2 hours after stimulation and returned to baseline by 3 hours post-treatment. Small G proteins of the Rho family, including Rac1, Cdc42 and RhoA, play a role in dendritic spine regulation in neurons. Activation of Rac1 and Cdc42 promotes spine formation, enlargement and maintenance, while RhoA induces spine retraction and loss. Dendritic spines are small protrusions on dendrites that contain the post-synaptic components of synapses. Spines are highly dynamic structures and this dynamic feature plays an important role in synaptic plasticity. Spine size and shape forms a continuum from filapodia to intermediate, stubby, thin and lastly mushroom shaped spines. This continuum reflects a progressive maturation in spines and this maturation results in an increase in synaptic strength and stability of the spines. To determine if the changes in small G protein activity following 5-HT_{2A} receptor stimulation alter dendritic spine morphology, we used primary cortical cell cultures (from day 18 embryonic rats day cultured for 21 days in vitro) which display mature dendritic spines. Primary neurons were double-labeled with Alexa Fluor® 568 Phalloidin to label actin and with antibodies against 5-HT_{2A} receptors. Double-labeled neurons were visualized by confocal microscopy to examine the morphology of dendritic spines. Dendrites and individual spines on dendrites were traced, and the area, maximum length and head width of each spine, and spine density were measured using Cell Profiler cell image analysis software. Dendritic spine area but not density is transiently increased 30 min after DOI treatment. Studies are underway to determine whether 5-HT_{2A} receptor-induced spine enlargement is dependent on 5-HT_{2A} receptor-mediated transamidation by using the transglutaminase inhibitor cystamine. Mutations at glutamine 61 and 74 of Rac1 prevent the DOI-induced Rac1 transamidation, suggesting either glutamine 61 or 74 or both are modified by transglutaminase. Constructs containing a single mutation at glutamine 61 and 74 have been made and will be used to determine which residue or if both are transamidated. Furthermore, those constructs will be used to determine the role of Rac1 transamidation in 5-HT_{2A} receptor-induced spine enlargement. In sum, these results suggest a potential role of Rac1 and Cdc42 transamidation in the dendritic spine regulation in 5-HT_{2A} receptor neurons. Numerous studies suggest that perturbations in dendritic spines contribute to the pathophysiology of schizophrenia and depression. Transamidation is a novel target for altering dendritic spines that can inform the development of new therapeutic approaches for treatment of perturbations in dendritic spines in these devastating disorders.

Funded by a grant from NIMH RO1-068612-011.

SEROTONIN: A MODULATOR OF INNATE IMMUNE CELLS

Serotonin modulates the functional polarization and the transcriptional signature of human macrophages.

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Given the importance of the macrophage functional plasticity in tissue homeostasis and repair, and in inflammatory responses, we evaluated whether Serotonin (5-HT) modulates human macrophage polarization. 5-HT inhibited the LPS-induced release of pro-inflammatory cytokines without affecting IL-10 production, upregulated the expression of M2(anti-inflammatory) polarization-associated genes and reduced the expression M1(pro-inflammatory)-associated genes. Both $5HT_{2B}$ and $5HT_{7}$ receptors mediated the pro-M2 skewing effect of 5-HT, whereas only $5HT_{7}$ mediated the inhibitory action of 5-HT on pro-inflammatory cytokine release. Moreover, blockade of both receptors prevented the acquisition of M2 polarization markers during *in vitro* monocyte-to-macrophage differentiation. These results demonstrate that 5-HT modulates macrophage polarization and contributes to the maintenance of an anti-inflammatory state via $5HT_{2B}$ and $5HT_{7}$, whose identification as functionally-relevant markers for anti-inflammatory/homeostatic human M2 macrophages suggests their potential therapeutic value in inflammatory pathologies. The determination of the 5HT- and $5\text{-}HT_{2B}$-dependent gene expression profile in human macrophages has now allowed the identification of the effectors (growth factors, growth factor receptors, cytokines) and signalling axis that might contribute to the growth promoting and immunomodulatory functions of 5HT, and revealed that $5\text{-}HT_{2B}$ ligation affects the expression of several genes that govern antiviral responses by human macrophages.

Serotonergic modulation of microglia function

Josef Priller

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Microglia are the resident innate immune cells of the central nervous system. They originate from the yolk sac and play crucial roles in neurological and psychiatric disorders. Here, we have tested the effects of various selective serotonin reuptake inhibitors (SSRIs) on microglia function in vitro. Moreover, we have assessed the impact of the SSRI fluoxetine on experimental autoimmune encephalomyelitis. Microglia also express dopamine receptors under pathological conditions, suggesting that microglia can sense and respond to monoamine neurotransmitters.

Role of serotonin-microglia interaction in sickness behavior

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The neurotransmitter serotonin (5-HT) is involved in numerous physiological functions (mood, sleep, body temperature, food intake regulations...) and pathological situations (pain, depression). Most of these physiological functions are transiently dysregulated in case of peripheral infection, resulting in so-called "sickness response" and "sickness behavior". This transient depressive-like state points to a role of the serotonergic system in shaping this behavior.

Microglia, brain resident macrophages, can be activated following a peripheral infection. Since sickness behavior following an infection is particularly striking in aged patients, whose microglial cells are thought to be in a "primed" state, our hypothesis is that these behavioral effects involve both serotonin-secreting neurons and microglia. Having observed that microglia express mainly the 5-HT_{2B} receptor, and that its expression is regulated with a specific kinetics by inflammatory stimuli, we compared sickness responses induced by a peripheral lipopolysaccharide injection in wild-type and 5-HT_{2B} KO mice. Our results show that 5-HT_{2B} KO mice react stronger than wild-type to this treatment. Thus, this serotonin receptor is necessary to prevent excessive behavioral response to inflammation. In further experiments, we will investigate whether this abnormal sickness response is directly related to a lack of control of microglia by serotonin.

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CELL-TYPE DISSECTION OF THE SEROTONERGIC SYSTEM AND OF ITS DEVELOPMENTAL TARGETS

Role of the 5-HT6R on pyramidal neuron migration.

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In humans, monkeys and rodents deregulation of the serotonin system during development is linked to increased vulnerability to psychiatric-related phenotypes. Serotonin acts as a developmental signal that regulates a variety of cellular processes involved in the formation of cortical circuits, including neuronal migration. The coordinated migration of different subtypes of excitatory neurons into specific layers is key in the assembly and subsequent function of cortical microcircuits. Work in the field has shown that the cyclin-dependent kinase 5 (Cdk5) is a master regulator of pyramidal neuron migration. Interestingly recent data indicates that the serotonin 6 G protein-coupled receptor (GPCR) binds to Cdk5. In this presentation I will provide evidence that the serotonin 6 receptor plays a critical role in the positioning and migration of pyramidal neurons during mouse corticogenesis. Collected data indicates that constitutive expression of the 5-HT6R controls pyramidal neuron migration through an agonist-independent mechanism that requires cyclin-dependent kinase 5 activity. Taken together these data support an *in vivo* role of constitutive activity at a GPCR for neocortical radial migration.

A serotonin receptor/Cdk5 complex controls neuronal differentiation

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The 5-HT_6 receptor is a promising target for treating cognitive deficits of schizophrenia that are often linked to neuro-developmental alterations and is implicated in cortical neuron migration. However, the signalling mechanisms underlying its control of neuro-developmental processes remain poorly characterized. Here, we addressed this issue by a proteomic approach aimed at identifying signaling proteins associated with the receptor. We show that the 5-HT_6 receptor interacts with Cyclin-dependent kinase (Cdk)5 and some of its substrates, which are known to control neuro-developmental processes such as migration and neurite growth. Expressing 5-HT_6 receptors in NG108-15 neuroblastoma cells induced neurite growth and expression of voltage-gated Ca^{2+} channels, two hallmarks of neuronal differentiation. These effects were not further enhanced by treating cells with an agonist and were prevented by SB258585, a specific 5-HT_6 antagonist, which also impaired association of 5-HT_6 receptor with Cdk5. Moreover, 5-HT_6 receptor-elicited neurite growth required receptor phosphorylation at ^{350}Ser and activation of Cdc42 by Cdk5. Supporting a role of native receptors in neuronal differentiation, neurite length of striatal and hippocampal neurons in primary culture was reduced by either SB258585 treatment or silencing 5-HT_6 receptor expression. Furthermore, expressing a Ser^{350}Ala 5-HT_6 receptor mutant or a Cdc42 dominant negative mutant inhibited neurite growth in primary neurons. Collectively, the present findings show that 5-HT_6 receptors promote neuronal differentiation *via* an agonist-independent mechanism and reveal a critical role of receptor-associated Cdk5. They provide novel insights into molecular substrates underlying their control of neuro-developmental processes relevant to the physiopathology and treatment of early-onset psychiatric disorders such as schizophrenia.

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Specificity in the projection pattern of the different raphe subnuclei. Looking for developmental cues.

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Serotoninergic innervation of the central nervous system is entirely provided by the raphe nuclei, which are notorious for having extensive projections with broad modulatory effects. The raphe neurons are distributed into different hindbrain subnuclei (B1-B9). However, the extent to which each of the nuclear subdivisions within the rostral (B4-B9) clusters correspond to distinct topographic organization of their projections is still unclear, because individual 5-HT neurons have highly collateralized axons. To determine the innervation territories of individual raphe subnuclei, we used selective anterograde tracing using adeno-associated viruses that conditionally express GFP under the control of the serotonin transporter gene. Small groups of 5-HT neurons were labelled in the dorsal (B7d) ventral (B7v), lateral (B7L), and caudal (B6) subcomponents of the dorsal raphe group, as well as in the rostral and caudal median raphe (B8 and B5), and in the supralemniscal (B9) cell groups. We performed a comprehensive description of the main targets of these raphe sub-groups. We confirm the existence of distinctive and largely non overlapping projections of the dorsal (B7) versus the median (B8) raphe. B7 projects essentially to basal part of the forebrain, such as the amygdala and piriform cortex, whereas the latter is the main 5-HT source to the hippocampus, septum, mammillary bodies and dorsal tegmental nuclei, emphasizing its potential importance in learning and memory. Moreover, distinct subsets of B7 had preferential brain targets: the ventral B7 is the main source of cortical innervation, while the dorsal B7 and lateral wings have distinctive projections to hypothalamus and lateral geniculate nucleus. Finally, we clarify the projections of the supralemniscal B9 cell group, that specifically target aminergic neurons of the brainstem and fore. This anatomical organization of the raphe nuclei is likely to underlie the different functional roles in which serotonin has been implicated in the brain. We will present preliminary evidence indicating how guidance molecules helps raphe neurons select these specific targets.

Serotonin 1B receptors affect neural circuits underlying aggression during development, but modulate impulsivity circuits during adulthood.

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The serotonin 1B receptor (5-HT1B R) has been implicated in the modulation of aggressive and impulsive behavior. Polymorphisms in 5-HT1B R are associated with aggression and impulse control disorders like pathological gambling alcohol and drug addiction. Also, constitutive 5-HT1B R knock-out mice are more aggressive, more impulsive and more vulnerable to drug abuse. However, the localization of the neural circuits mediating these behaviors, and whether 5-HT1B R have a developmental impact, are unknown. In order to answer these questions, we have generated a novel targeted transgenic mouse that permits temporal and spatial regulation of 5-HT1B R. Using a bigenic strategy with tetracycline operator (tetO) and tetracycline-dependent transcriptional silencer (tTS) transgenes, our mouse model allows investigation into the sensitive period and cell-type specificity of the effect of 5-HT1B R on behavior.

Sensitive period: First, our data show that whole brain, whole life knock-down (in β-Actin-tTS/tetO1B mice) of 5-HT1B R results in aggressive behavior as measured by increased male-male fighting. These mice are also highly impulsive, unable to inhibit responding in operant conditioning paradigms. Interestingly, rescue of receptor expression in adulthood with doxycycline reverses the impulsive, but not the aggressive, phenotype. The aggressive phenotype is reversed only by rescue of 5-HT1B R during postnatal development. This suggests that developmental expression of 5-HT1B R modulates the formation of circuits that contribute to adult aggression, while adult expression of 5-HT1B R contributes to modulation of impulse control circuits.

Localization: Next, using tissue-specific promoters, we were able to rule out the involvement of 5-HT1B autoreceptors in the raphe (using the Pet-1 promoter) in mediating both aggressive and impulsive behaviors. However, knockout of a subset of forebrain receptors (including cortical and striatal receptors using the CaMKII promoter) resulted in aggressive, but not impulsive behavior. We are currently using cell-type specific promoter- and viral-mediated knockdown to target GABAergic neurons in the cortex or striatum in order to localize sites of 5-HT1B R mediated inhibition.

Overall these data show that expression of 5-HT1B R heteroreceptors (in the cortex or striatum) during postnatal development contributes to the normal maturation of neural circuits underlying aggression. On the other hand, expression of a different population of 5-HT1B R heteroreceptors during adulthood contributes to the regulation of impulsive behavior. Taken together these data suggest that distinct circuits underlie the effects of the 5-HT1B R on aggressive and impulsive behaviors, and contribute to the identification of the neural substrates underlying serotonergic modulation of aggression and impulsivity.

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SEROTONIN, CORTICAL MICROCIRCUITRY AND COGNITION

Localization of 5-HT receptor subtypes within the cortical microcircuitry

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Beginning in the 60's the development of chemical neuroanatomical tools, a tremendous wealth of information has been generated on the anatomical components of the serotonergic system, at the microscopic level in the brain including the prefrontal cortex (PFC). The PFC presents a widespread distribution of 5-HT terminals from the median and dorsal raphe nuclei. 5-HT receptors were first visualized using radioligand autoradiography in the 80's and showed, in contrast to the 5-HT innervation, a quite differential heterogeneous distribution of the binding sites corresponding to the different 5-HT receptor subtypes. Thanks to the isolation and cloning of the different 5-HT receptor subtype genes in the late 80's it was possible, by using in situ hybridization, to localize the cells expressing the mRNA coding for these receptors. Double in situ hybridization and immunohistochemistry allowed for the chemical characterization of the phenotype of the cells expressing 5-HT receptors. Based on these data maps reflecting our current understanding of the different circuits where 5-HT receptors can modulate the electrophysiological, pharmacological and behavioural functions of the PFC have been constructed. Recent brain imaging techniques have made possible visualizing some of the 5-HT receptors in the brain of living animals and human in normality and disease.

We will review current knowledge regarding the cellular localization of the most abundant serotonin receptors in mammalian PFC and their possible functions in the neuronal circuits of the PFC. We will present data generated in our laboratory as well as in others, focussing on 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{3} and 5-HT_{4} localization in the pyramidal and GABAergic neuronal cell populations in different mammalian species using molecular neuroanatomy.

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Serotonin influences on prefrontal cortex function: neurons, networks, and circuits

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Higher-order executive tasks such as learning, working memory, and behavioral flexibility depend on the prefrontal cortex (PFC), the brain region most elaborated in primates. The PFC is an associational cortical area that receives remarkably dense inputs from serotonin neurons of the dorsal and median raphe nuclei, suggesting that serotonin exerts powerful influences on its function. However, despite decades of intense research, the role of serotonin in PFC function is still largely unresolved. In this seminar, I will present data accumulated over the last decade on the role of serotonin and serotonin receptors 5-HT1A, 5-HT2A, and 5-HT3 in the modulation of PFC neural activity of single neurons, neural networks, and the PFC-raphe circuit *in vivo* in the rat. More specifically, we have found that serotonin receptors show an exquisite pattern of expression in distinct populations of pyramidal neurons and inhibitory interneurons of the PFC, where they exert powerful actions on spiking activity. Stimulation of serotonin 5-HT1A and 5-HT2A receptors decreases and increases, respectively, the activity of pyramidal neurons and fast-spiking interneurons, with a clear predominance of 5-HT1A-mediated inhibitory actions, while serotonin 5-HT3 receptors increase the activity of slow-spiking interneurons. Moreover, serotonin modulates slow and fast neural oscillatory activities in the PFC. It regulates the frequency and amplitude of slow oscillations (< 2 Hz) induced by chloral hydrate anesthesia that resemble the slow rhythms of natural slow-wave sleep, likely via stimulation of 5-HT2A receptors. Serotonin also plays a role in regulating the amplitude of gamma oscillations (30-80 Hz) via both 5-HT1A and 5-HT2A receptors. Interestingly, gamma oscillations may be generated by the synchronous discharge of fast-spiking interneuron networks. Thus, we propose that 5-HT1A- and 5-HT2A-expressing fast-spiking interneurons may contribute to PFC gamma rhythms. Lastly, I will discuss the implications of this work for our understanding of the actions exerted by neuropsychiatric treatments that target serotonin receptors in the PFC.

Cognitive alterations in serotonin transporter knockout rats: for better and for worse.

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Serotonin affects many types of behavior, including cognition. This influence is dependent on serotonin levels in the brain, which can vary among individuals due to differences in dietary intake of tryptophan, exposure to serotonergic pharmacological agents, stress, or genetic factors. Within the serotonergic system the serotonin transporter plays a central role, it is solely responsible for serotonin reuptake and to an important extent determines serotonin levels in the synaptic cleft. Humans carry a polymorphism in the serotonin transporter gene, of which the short allelic version has been associated with reduced activity of the serotonin transporter. Although evidence in humans is controversial, studies employing serotonin transporter knockout rodents have demonstrated that reduced expression of the transporter leads to higher extracellular serotonin levels in various brain areas, including the prefrontal cortex. Serotonin is well known to interact with the environment, and as such, the behavioral profile of human s-allele carriers can be adaptive as well as maladaptive, depending on whether individuals have been exposed to a favorable or aversive environment. However, the definition of adaptive and maladaptive, and favorable and aversive, is ambiguous, hampering the understanding of the role of the serotonin in the regulation of cognition. It is my aim to clarify this role, using rats lacking the serotonin transporter. As such, when using rewarding reinforcers, the serotonin transporter knockout rats show cognitive improvements like increased behavioral (reversal learning) and cognitive flexibility (attentional set shifting), as well as improved decision making (iowa gambling task). However, goal-directed behavior (reward devaluation task) is impaired in these rats. Their memory for appetitive stimuli is very strong, albeit a failure to extinguish the memory can become maladaptive. When using negative reinforcers, the knockout rats either show an improvement in active avoidance, or a failure to extinguish conditioned fear. To reconcile these findings I propose the ‘for-better-and-for-worse’ concept by which the trait of being sensitive to environmental influences has its limitations when environmental stimuli are not available or cannot be used to steer behavior.

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Optogenetic modulation of the prefrontocortical-dorsal raphe microcircuit bidirectionally biases socioaffective decisions after social defeat

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It has been well established that modulating levels of serotonin (5-HT) in humans and animals affects affective perception and response to social threats. However, the circuit mechanisms that control social interaction are not well understood. Understanding these underlying mechanisms could provide the groundwork to develop therapeutic interventions to more precisely treat socioaffective disorders. We examined the organization and plasticity of the reciprocal microcircuit formed by 5-HT neurons in the dorsal raphe nucleus (DRN) and the ventromedial prefrontal cortex (vmPFC) and its role in social approach-avoidance decisions. We used a chronic social defeat stress (CSDS) model that results in a long lasting form of social aversion that is reversible by antidepressants. Using viral tracing in population specific Credriver mice we showed that excitatory vmPFC projections primarily localized to GABA-rich areas of the DRN. Next, using optogenetics with both cFos mapping and whole cell electrophysiology we established the functional effects of vmPFC-driven glutamatergic activity in the DRN. We provide the first direct evidence that vmPFC axons drive synaptic activity and immediate early gene expression in genetically identified DRN GABA neurons through an AMPA-dependent mechanism and that these GABA neurons locally inhibit 5-HT neurons. We also show that CSDS drove GABAergic sensitization that strengthened inhibition of 5-HT neurons in mice that were susceptible, but not resilient, to CSDS. Finally we demonstrate using optogenetics that increasing vmPFC input to the DRN during sensory exposure to an aggressor's cues respectively enhances avoidance bias. In contrast, optogenetically decreasing vmPFC drive of the DRN or GABAergic neuronal activity within the DRN prevented the acquisition of an avoidance phenotype after CSDS. These results clarify the functional organization of vmPFCDRN pathways and identify GABAergic neurons as a key cellular element filtering top-down vmPFC influences on affect-regulating 5-HT output.

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PIONEERS AND PRODIGIES - NIDA TRAVEL AWARDEES

Regulation of Neuronal Primary Cilia Morphology in Striatal Neurons by 5-HT_6 Receptors

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The primary cilium is a sensory organelle stemming from the cell body of most mammalian neurons. The cilium is an antenna-like, microtubule-supported structure that receives both chemical and mechanical signals from other cells and the surrounding environment. These signals are transduced by a discrete set of membrane-bound receptors localized to the primary cilium. Recently, neuronal primary cilia have become a major target of research as they play crucial roles in a variety of disorders known as "ciliopathies"; they have also been implicated in Huntington's and Alzheimer's diseases. However, the role of primary cilia in normal cognitive functions is not understood, but there is evidence that impairments of ciliary signaling produce cognitive deficits. The small set of receptors that localize to cilia have consensus residues that permit them to be selectively trafficked into cilia by unique ciliary trafficking mechanisms and intraflagellar transport. One such receptor is the 5-HT_6 serotonin receptor, which is an excitatory, G_S-coupled receptor that is heavily expressed in striatum; it is the only serotonin receptor that localizes to neuronal primary cilia. 5-HT_6 receptors modulate learning and memory, and are linked to a range of cognitive processes and neuropsychiatric syndromes including mood disorders, addiction, and dementia. Our goal is to confront the question of whether the unique functional properties of 5-HT_6 receptors are dependent upon its localization to cilia. Utilizing primary cultured striatal neurons we are able to evaluate the effects of wild-type and mutated 5-HT_6 receptors on the structure and function of primary cilia. We use striatal neurons dissected from P0-P1 mouse neonates that were maintained in culture for 10-12 days. Nearly all 5-HT_6 receptors co-localize with adenylyl cyclase III, a selective marker for primary cilia. Inhibition of 5-HT_6 receptors with the selective 5-HT_6 receptor antagonists such as SB258585 reduced primary cilia length in a time- and concentration-dependent manner by up to ~50% in neurons from wild-type but not 5-HT_6 KO mice. 5-HT_6 knockdown with shRNA produces similar changes in wild-type neurons. However, we did not observe a lengthening effect associated with agonist or overexpression, suggesting that either there is a ceiling effect and perhaps residual 5-HT in the growth medium; we continue to test this. Currently, we are studying whether manipulating 5-HT_6 receptor expression and localization also alters cilia morphology and signaling. Our preliminary results suggest that a small deletion mutation disrupts trafficking to cilia; we are currently exploring the functional consequences of subcellular localization of 5-HT_6 receptors using primary neuron culture and soon in vivo gene transfer. Furthermore, we are investigating how 5-HT_6 receptors modulate the activity of other signaling pathways in primary cilia of striatal neurons. Our results so far suggest that 5-HT_6 receptors are unique among serotonin receptors due to cilia localization and may represent a distinct, extrasynaptic mechanism of serotonin modulation of cell signaling, excitability, and behavior control.

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Antagonist-mediated down-regulation of 5-HT$_7$ serotonin receptors is regulated by C-terminal domains and interaction with GASP1

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The human 5-HT$_7$ serotonin receptor is a G-protein-coupled receptor (GPCR) that activates adenylyl cyclase constitutively and upon agonist-activation. Some inverse agonists towards the 5-HT$_7$ receptor can induce both homo- and heterologous desensitization, similar to agonist-stimulation, while others can induce receptor internalization. However, only a subset of these targeted 5-HT$_7$ receptors for lysosomal degradation. These results demonstrated that various ligands differentially activated regulatory processes governing receptor desensitization, internalization and degradation in addition to signal transduction, providing support for the concept of functional selectivity at the 5-HT$_7$ receptor, where different ligands stabilize different receptor conformations leading to differential effects.

Interestingly, the important atypical antipsychotics clozapine and olanzapine inhibited G-protein activation (as expected) and, surprisingly, induced both internalization and lysosomal degradation of 5-HT$_7$ receptors. We wanted to determine the mechanism of clozapine- and olanzapine-mediated internalization and lysosomal sorting of 5-HT$_7$ receptors.

In the C-terminus of the 5-HT$_7$ receptor, we identified two important YXXΦ motifs, two conserved residues (LR) and the palmitoylated cysteine-anchor as potential sites involved in receptor internalization and recruitment of lysosomal sorting proteins, such as GPCR-associated sorting protein 1 (GASP1). Mutating one or both YXXΦ motifs, the LR residues or the cysteine-anchor inhibited clozapine- and olanzapine-mediated lysosomal sorting of 5-HT$_7$ receptors. In addition, we demonstrate that GASP1 binds to the 5-HT$_7$ receptor and that over-expression of the C-terminus of GASP1 inhibited clozapine-mediated degradation of 5-HT$_7$ receptors, indicating that GASP1 is recruited to these domains of the 5-HT$_7$ receptor and is involved in lysosomal sorting. The identified domains are located in helix VIII of the 5-HT$_7$ receptor and we are currently building a structural model to clarify the functional effects caused by the mutated residues and determine how GASP1 interacts with this structure.

Taken together, our data demonstrate that binding of clozapine or olanzapine to the 5-HT$_7$ receptor leads to antagonist-mediated internalization and lysosomal degradation by exposing key residues in the C-tail that interact with GASP1.

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Htr2a expression responds rapidly to environmental stimuli in an Egr3-dependent manner suggesting a functional link between two schizophrenia susceptibility genes.

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The serotonin system has been implicated in the etiology of schizophrenia, through numerous pharmacological and genetic findings. However the mechanisms by which serotonin influences schizophrenia susceptibility are poorly understood. The serotonin 2A receptor (5-HT2AR) has been of particular interest as agonists of the receptor cause psychosis in normal individuals and second generation antipsychotic medications have a high affinity for 5-HT2A Rs. In addition, Htr2a, the gene that encodes 5-HT2AR, is one of the most well-replicated schizophrenia candidate genes based on genetic association studies. Furthermore, numerous *in vivo* and post-mortem studies have found decreased levels of 5-HT2ARs in the brains of schizophrenia patients including in medication-naïve individuals.

We recently reported that mice lacking the immediate early gene (IEG) early growth response 3 (Egr3) show an approximately 70% decrease in 5-HT2AR binding in the prefrontal cortex, paralleling the 5-HT2A R deficits in schizophrenia patients. Egr3 is a transcription factor involved in synaptic plasticity that is rapidly activated in the brain after environmental events such as stress and it regulates downstream target genes to modulate the brain's response to these stimuli. Thus, dysfunction in Egr3 may account for both environmental and genetic influences on schizophrenia risk. We have previously shown that mice lacking Egr3 display schizophrenia-like behavioral abnormalities and responses to antipsychotics. In humans, variations in EGR3 has been associated with schizophrenia risk in numerous populations, and levels of EGR3 mRNA are reduced in the brains of patients with schizophrenia.

Our findings of decreased levels of 5-HT2AR binding in Egr3 -/- mice provide a mechanistic link between two schizophrenia candidate genes, and suggest how they may be affected by changes in the environment. The data presented here test the hypothesis that Egr3 rapidly modulates 5-HT2AR in response to environmental stimuli. If Egr3 directly regulates expression of Htr2a, EGR binding motifs should be present in the promoter region of the Htr2a gene. We therefore used the MEME suite FIMO tool analysis software to identify putative EGR response elements in the 2 Kb upstream of the Htr2a gene. This analysis revealed two highprobability EGR binding sites located at -2791-2778 bp and -75-62 bp. A second requirement would be that EGR3 protein must be expressed in the same cells as the 5-HT2AR. Since anti-5-HT2ARs antibodies do not show cell-specific labeling in the brain, to address this we employed a transgenic mouse that expresses EGFP under control of the Htr2a promoter. This showed overlapping expression of EGFP with anti-EGR3 antibody staining in the frontal cortex.

To determine if Htr2a is regulated by environmental stimuli, we used sleep deprivation, a stimulus known to induce expression of Egr3. This was necessary because Egr3 expression is stimulus-dependent and expressed at low levels under basal conditions. We compared levels of Htr2a mRNA in the frontal cortex of Egr3-/- and WT littermate mice at baseline and after sleep deprivation, by quantitative RT-PCR. This revealed no significant difference in Htr2a expression between WT or Egr3-/- mice under undisturbed conditions. However, following sleep deprivation, Egr3-/- mice showed decreased Htr2a expression in cortex as compared to WT. These data suggest Egr3 may rapidly modulate Htr2a in response to acute environmental stimuli. These findings are significant because they are one of the first demonstrations of rapid modulation of a serotonin receptor in response to environmental stimuli. Furthermore, they provide a functional link between two schizophrenia candidate genes and a possible explanation of how both genetic and environmental factors influence risk for schizophrenia.

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Permanent depletion of serotonin increases risky decision-making and impairs acquisition of the rat gambling task

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Human and animal research strongly implicates serotonin (5-hydroxytryptamine, 5-HT) in decision-making and altered functioning of the 5-HT system may disrupt the ability to learn from loss or punishment. We used the rat gambling task (rGT; Zeeb et al., 2009), a rodent analogue of the Iowa Gambling Task (IGT), to test the hypothesis that 5-HT contributes to decision-making by modulating the significance of punishment-related signals. In both the rGT and IGT, disadvantageous options are associated with larger immediate reward, but greater loss; whereas the optimal strategy is to choose preferentially from advantageous options that yield smaller immediate gains but less loss and therefore greater long-term reward.

Male Long Evans rats received either a sham surgery or a permanent 5-HT depletion accomplished by an intracerebroventricular infusion of 5,7-dihydroxytryptamine (5,7-DHT), which depleted 5-HT throughout the forebrain by 75-85%. Surgery was conducted either prior to or following rGT training. rGT testing took place in 5-hole operant chambers in daily 30 min sessions, during which animals chose between four different options. Each option was associated with a different magnitude of reward (sucrose pellets), different frequency of reward delivery, and a different duration of a timeout period. During the timeout, rats were not rewarded and initiation of the next trial was halted until the end of the punishment. Therefore—similar to losing on the IGT—timeouts result in less reward earned per unit time.

5-HT depleted rats did not differ in their choice preference compared to the sham-control group when the lesion occurred following rGT training. In contrast, rats that received pre-training 5,7-DHT lesions demonstrated a marked impairment in task acquisition. 5-HT depleted rats were slower to learn the optimal strategy and chose the disadvantageous, risky options more often than controls. Following 44 training sessions, compared to sham-control rats, animals with 5,7-DHT lesions continued to significantly choose the disadvantageous options—associated with greater loss—more than advantageous options. Additionally, the ability for amphetamine to increase choice of the options leading to less loss was absent in rats with 5,7-DHT lesions. Likewise, an acute injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT moderately decreased choice of the disadvantageous options in sham animals, an effect blocked by the 5-HT depletion. These results demonstrate that 8-OH-DPAT may alter decision-making by acting on pre-synaptic autoreceptors in the control group. Furthermore, amphetamine's ability to bias animals toward options associated with less punishment can be partially attributed to its interaction with the 5-HT system.

In conclusion, long-term depletion of 5-HT may impair the ability of animals to develop an optimal decision-making strategy possibly by dampening the significance of loss, biasing animals to choose disadvantageously despite the negative consequences associated with these options. As impairments in decision-making processes are observed in a wide range of psychiatric disorders (including depression, pathological gambling, and substance abuse) the results presented here may provide insight into the potential role of 5-HT in decision-making in these populations in which the 5-HT system may be compromised.

NOVEL INSIGHTS INTO SEROTONIN RECEPTOR STRUCTURE AND FUNCTION

5-HT2A receptors: biased phosphoproteomes following activation by hallucinogens and non-hallucinogenic agonists

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The 5-HT_{2A} receptor is a primary target of psychedelic hallucinogens such as lysergic acid diethylamine, mescaline and psilocybin, which reproduce some of the core symptoms of schizophrenia. An incompletely resolved paradox is that only some 5-HT_{2A} receptor agonists exhibit hallucinogenic activity, whereas structurally related agonists with comparable affinity and activity lack such a psychoactive activity. Using a quantitative phosphoproteomics approach, we compared the phosphoproteome in HEK-293 cells transiently expressing the 5-HT_{2A} receptor and exposed to either vehicle or the synthetic hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) or the non-hallucinogenic 5-HT_{2A} agonist lisuride. Among the 5,995 identified phosphopeptides, 16 sites were differentially phosphorylated upon exposure of cells to DOI *vs.* lisuride. These include a serine (Ser^{280}) located in the third intracellular loop of the receptor, a region important for desensitization. Specific phosphorylation of Ser^{280} by hallucinogens, compared with non-hallucinogenic agonists, was further validated by quantitative mass spectrometry on purified receptor and by using a phosphosite specific antibody. Moreover, administration of DOI, but not lisuride, to mice enhanced 5-HT_{2A} receptor phosphorylation at Ser^{280} in prefrontal cortex. Correspondingly, exposure to hallucinogens induced less pronounced desensitization of receptor-operated signaling and receptor internalization than exposure to non-hallucinogenic agonists. Moreover, mutation of the serine into aspartate (to mimic phosphorylation) reduced receptor desensitization by non-hallucinogenic agonists, while its mutation to alanine increased the ability of hallucinogens to desensitize the receptor. This study reveals biased phosphorylation of 5-HT_{2A} receptor by hallucinogenic *vs.* non-hallucinogenic agonists, which might underlie their distinct behavioural responses upon long-term treatment.

FRET- and FRAP-based evidence for ligand-independent preassociation of 5-HT_7 receptors and G_s

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Background

How G-protein-coupled receptors, G proteins and effectors interact is important for their biological function and their function as pharmacological targets. We have previously compared the signaling properties of the G_s-coupled 5-HT_4 and 5-HT_7 serotonin receptors and found that 5-HT_4 functions by classical collision coupling, whereas the pharmacological properties of 5-HT_7 indicate preassociation with G_s in the absence of ligand. In this study, we directly examine the interaction between 5-HT_7 receptors and G_s by FRET (Fluorescence Resonance Energy Transfer) and FRAP (Fluorescence Recovery After Photobleaching) techniques, and try to determine the intracellular domains of the receptor responsible for the preassociation.

Methods

FRET and FRAP experiments were performed in HEK293 cells transfected with β_1-adrenergic, 5-HT_4 or 5-HT_7 receptors and G protein subunits labeled with YFP or CFP. To identify the intracellular structural determinants of the receptor responsible for the preassociation, we performed similar experiments on a series of chimeric receptors, in which the intracellular segments of the 5-HT_7 receptor were systematically exchanged with corresponding segments from the 5-HT_4 receptor.

Results

Agonist activation of β_1-adrenergic or 5-HT_4 receptors increased the FRET signal, consistent with the expected interaction by collision coupling. In contrast, for the 5-HT_7 receptor, FRET experiments with either labeled G_α or G_γ G protein subunits indicated an initial conformational change within and a subsequent dissociation of a preassociated complex of 5-HT_7 receptor and G_s. Consistent with this, FRAP experiments with antibody-immobilized receptors demonstrated that 5-HT_7, in contrast to 5-HT_4 receptors, prevented or delayed G_s diffusion in the cell membrane, confirming preassociation of 5-HT_7 receptors with G_s. Both FRET and FRAP experiments with the chimeric constructs identified the C-tail and, to some extent the intracellular loop 3 as responsible for the preassociation, as these segments of the 5-HT_4 receptor converted 5-HT_7 into a collision-coupled receptor that associated with G protein upon agonist activation.

Conclusion

Taken together these data indicate that the 5-HT_7 receptor preassociates with G_s, and that this preassociation is dependent on the C-tail and intracellular loop 3 of the receptor.

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Structural Features for Functional Selectivity at Serotonin Receptors

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The psychedelic drug lysergic acid diethylamide (LSD) is known to act through G protein-coupled 5-Hydroxytryptamine (5-HT, serotonin) receptors. The molecular details of drug action at these receptors, however, remains poorly understood. Drugs active at G protein-coupled receptors (GPCRs) can differentially modulate either canonical or non-canonical signaling pathways via a phenomenon known as functional selectivity or biased signaling. Initiated from structural studies, we find that compared to the endogenous agonist 5-HT, LSD elicits markedly different signaling at 5-HT receptors, and acts as a full agonist, β-arrestin biased agonist or even antagonist at different 5-HT receptors. Crystal structures of the 5-HT_{1B} and 5-HT_{2B} receptor bound to the LSD derivative Ergotamine further reveal the binding mode, important receptor-ligand interactions, and the structural basis for the different signaling properties of LSD at different 5-HT receptors. Molecular insight into different GPCR signaling pathways is important to better understand both adverse and favorable therapeutic activities.

THE SEROTONIN RECEPTORS: FROM FUNCTION TO STRUCTURE AND BACK

5HT Receptors from structure to function: A long journey.

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The recent reports on 3D atomic crystal structures of 5-$HT_{1B/2B}$ receptors (and other GPCRs) link 5-HT receptor structure, pharmacology and function. Although, orthosteric binding pockets of 5-$HT_{1B/2B}$ receptors show clear similarities, there are interesting differences: ergotamine/ DHE bound receptors reveal an extended binding pocket compared to 5-HT. In contrast to GPCRs, knowledge of the atomic structure of 5-HT_3 receptors, pentameric ligand-gated ion channels (members of Cys loop family) is limited. Crystal structures of a binding protein engineered to recognize 5-HT and granisetron offer first structural perspectives. Do such studies provide a comprehensive basis for understanding 5-HT receptor-ligand interactions, allowing the design of subtype- or even pathway selective ligands? Are structural and functional data consistent? Can a binding protein accurately represent a neurotransmitter-binding site? Will information from 5-HT GPCRs help understanding structure–function relationships in 5-HT_3 receptors? Such questions will be addressed in the present symposium.

Since the 1994 proposal for a nomenclature for 5-HT receptors and its three pillars, structure, transduction and pharmacological signature (Hoyer et al, 1994, Pharmacol Revs, 46, 157), much knowledge has accumulated and it is timely for the initial proposal to be adapted. Numerous GPCRs activate more than one G-protein heterotrimer and/or may signal in a G-protein-independent manner. The consequences of such diversity are multiple: the pharmacological profile, both for agonists and antagonists can vary significantly depending on signal transduction mechanism studied. Thus, rank orders of potency and efficacy can vary in a ligand-, transduction pathway- and cell-dependent manner: compounds may act as agonists at one and inverse agonists at another pathway. Biased signaling may not be the exception, but rather the rule: true neutral antagonists are rare species, as illustrated with ligands interacting with beta-adrenergic and other receptors. GPCRs may also form homo- or heterodimers, which may present different pharmacological profiles; some homomeric receptors may not signal well at all. The recent wealth and diversity of structural data collected with GPCRs of the 3 main families, A, B and C, do reveal similarities but also profound differences in how receptors and ligands interact, both at orthosteric and allosteric sites. Moreover, although there are only a few examples of GPCRs co-crystallised with G protein heterotrimers and/or additional accessory proteins, it is to be expected that conformations will vary significantly depending on the interacting proteins as is clear for RAMP-interacting receptors, where the pharmacological signature is highly variable although the primary 7TM receptor protein is the same (calcitonin or CRLR). Even when the binding pocket is very similar (e.g. 5-HT_{1B} / 5-HT_{2B} receptors), compounds may behave rather differently at one or the other receptor. Thus, biased signaling may be prominent for e.g. norfenfluramine at 5-HT_{2B}, but not at 5-HT_{1B} receptors. Further, highresolution NMR spectroscopy applied to GPCRs does provide convincing evidence for the existence of multiple states driven by a given compound at one receptor: e.g. carvedilol and other beta-blockers or agonists at beta adrenoceptors. The field is moving at great pace, but solution and crystal-derived structures may reveal marked differences; further, membrane lipids, thus cell nature, play a significant role in receptor-ligand complex conformation (recombinant versus native expression systems?).

Finally, structure based drug design will gain from 3D atomic structure knowledge, but the conformational diversity of ligand-receptor complexes still represents a major challenge, since the actual conformation of the complex depends on ligand, receptor (homo or heterodimer), G-protein and other interacting partners (including membrane lipids).

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Molecular Recognition of Serotonin Receptors

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Serotonin or 5-hydroxytryptamine (5-HT) manifests a myriad of physiological effects in humans mediated through 14 distinct receptor subtypes, of which 13 are G-protein coupled receptors (GPCRs). Recent GPCR crystal structures of the 5-HT_{1B} and 5-HT_{2B} receptors reveal similar binding modes for ergotamine, an ergoline with antimigraine properties and cardiomyocyte proliferative side-effects. In both receptors, ergotamine occupies the orthosteric site, which encompasses the indole nucleus and amine portion of the ergoline scaffold, and an extended binding region, which encompasses the amide and peptide portion. Mutagenesis studies in the 5-$HT_{2A/2B/1B}$ receptors reveal that two conserved residues in the orthosteric site, $A/S^{5.46}$ and $T^{3.37}$, recognize the indole *N*(1)-H of both ergolines and tryptamines. Further studies with the 5-HT_{2B} receptor methylergonovine, an agonist, and methysergide, an antagonist, show that orthosteric residues 5.46 and 3.37 may also serve to function as a putative activation switch to confer agonist/antagonist activity. The extended binding region encompasses the extracellular portion of transmembranes (TM) 5, 6, 7, and extracellular loop 2 (EL2), where a conserved hydrophobic residue lines the extended binding pocket. Mutagenesis studies with leucine209 and leucine229 in the EL2 of the 5-HT_{2B} and 5-HT_{2A} receptors, respectively, interact with the diethylamide of lysergic acid diethylamide (LSD), an ergoline with hallucinogenic properties, in a stereospecific fashion. Finally, current studies with the 5-HT_{2B} receptor have revealed that EL2 mutants severely dampen the β-arrestin recruitment activity for ergotamine, a β-arrestin biased agonist. These studies combining mutagenesis with receptor crystallography provide a comprehensive structural basis for elucidating receptor-ligand interactions and designing subtype-selective and functionally selective serotonergic drugs.

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5-HT_3 receptors

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The 5-HT_3 receptor is a Cys-loop ligand-gated ion channel and is structurally and functionally distinct from the other six classes of 5-HT receptors whose actions are mediated via G-proteins. 5-HT_3 receptors are pentamers, and five classes of subunit (A to E) have been identified. These are widely distributed, both in the nervous system and in other tissues. 5-HT_3 receptor activation opens a cation-selective ion channel, and receptor function can be modulated by a range of compounds including anesthetics, opioids and alcohols. 5-HT_3 receptors play a major role in the vomiting reflex, regulate gut motility, secretion, and peristalsis in the enteric nervous system, and are involved in information transfer in the gastrointestinal tract. Studies implicate the malfunction of 5-HT_3 receptors in a range of neurological and gastrointestinal disorders. Here I review the latest developments in our understanding of the structure and function of these receptors.

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NEW CONTRIBUTIONS OF SEROTONIN RECEPTORS AND THEIR MOLECULAR NETWORKS IN SYNAPTOGENESIS, DEGENERATION AND ADDICTION

Serotonin$_{2C}$ receptors modulate dopamine transmission in the nucleus accumbens independently of dopamine release: studies with cocaine

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The serotonin$_{2C}$ receptor (5-HT$_{2C}$R), in keeping with its ability to control the mesoaccumbens dopamine (DA) pathway, plays a key role in mediating the behavioral and neurochemical effects of drugs of abuse. Studies assessing the influence of 5-HT$_{2C}$R agonists on cocaine-induced responses have suggested that 5-HT$_{2C}$Rs can modulate mesoaccumbens DA pathway activity independently of accumbal DA release, thereby controlling DA transmission in the nucleus accumbens (NAc). In the present study, we assessed this hypothesis by studying the influence of the 5-HT$_{2C}$R agonist Ro 60-0175 on cocaine-induced behavioral, neurochemical and molecular responses. The intraperitoneal (i.p.) administration of 1 mg/kg Ro 60-0175 inhibited hyperlocomotion induced by cocaine (15 mg/kg, i.p.), had no effect on cocaine-induced DA outflow in the shell and increased it in the core subregion of the NAc. Also, Ro 60-0175 inhibited the late-onset locomotion induced by the subcutaneous administration of the DA-D$_2$R agonist quinpirole (0.5 mg/kg), as well as cocaine-induced increase in c-Fos immunoreactivity in NAc subregions. Finally, Ro 60-0175 inhibited cocaine-induced phosphorylation of the DA and c-AMP regulated phosphoprotein of Mr 32kDa (DARPP-32) at threonine residues in the NAc core, this effect being reversed by the selective 5-HT$_{2C}$R antagonist SB 242084 (0.5 mg/kg, i.p.).

Altogether, these findings demonstrate that 5-HT$_{2C}$Rs are capable of modulating mesoaccumbens DA pathway activity at post-synaptic level, by specifically controlling DA signaling in the NAc core subregion. This interaction, in keeping with the tight relationship between locomotor activity and NAc DA function, could participate in the inhibitory control of cocaine-induced locomotor activity. On their whole, the obtained results afford additional knowledge into the prominent role of the 5-HT$_{2C}$R into the regulatory neurochemistry of mesoaccumbens DA functions, and provide new information allowing a better understanding of the mechanisms underlying the 5-HT$_{2C}$R-dependent control of cocaine-induced responses.

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Does modulation of the serotonergic system by ecstasy impact the expression of symptoms in the monkey model of Parkinson's disease?

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Convergent studies performed in humans have underlined an involvement of the serotonergic (5-HT) system in Parkinson's disease (PD). Correlations were evidenced between alterations of the 5-HT system and the expression of motor (tremor, dyskinesia) symptoms. Moreover, animal studies performed by our team evidenced an increase of striatal dopamine (DA) and serotonin (5-HT) in the striatum of parkinsonian monkeys recovering from their motor symptoms, suggesting that 5-HT could participate to compensatory mechanisms. To investigate the involvement of 5-HT on the expression of parkinsonian symptoms and behavioral effects induced by L-DOPA, the main symptomatic treatment for PD, we developed a new monkey model of PD exhibiting a double DA/5-HT lesion due to sequential use of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) followed by 3,4-methylenedioxy-N-methamphetamine (MDMA or Ecstasy). The impact of MPTP and MDMA was characterized with an *in vivo* brain imaging approach in combination with post-mortem immunohistochemistry. Depending on the mode of MPTP administration (acute or progressive), animals exhibited stable or transient motor symptoms and were therefore divided into stable or recovered groups. MDMA lesion did not evoke reappearance or worsening of parkinsonian symptoms, suggesting that the 5-HT system does not play a compensatory role. But the 5-HT lesion counteracted the expression of rigidity when present in stable animals and favored it in recovered ones. Before MDMA, chronic L-DOPA evoked severe dyskinesia in stable animals and behavioural hyperactivity in recovered ones. Interestingly, both responses were drastically reduced after MDMA. The impact of L-DOPA treatment was evaluated here again with an *in vivo* brain imaging approach in combination with post-mortem immunohistochemistry. In conclusion, our results highlight a causal role of 5-HT system in L-DOPA-induced dyskinesia and behavioural hyperactivity but refute the hypothesis that the 5-HT is involved in motor compensatory mechanisms.

Selecting one's addiction to cocaine, anorexia or food depends n the activity state of serotonin 4 receptors

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How does the brain make inappropriate eating decision to the point of death by starvation, overeating or abuse of cocaine even though it has evolved to survive by favoring adapted eating behavior? This mystery poses a timely and vital challenge for treating adolescents with motivation disorders. The present studies mainly focus on the nucleus accumbens (NAc), which may influence decisions by an expectation of rewards.

In this context, we previously found that stimulation of 5-HTR_4, in the NAc, favors anorexia and motor hyperactivity by activating the anorectic peptide called cocaine- and amphetamine-regulated transcript (CART) through a c-AMP- protein kinase A (PKA) signaling pathway. Considering next that anorexia shares common signals with addiction, we questioned the analogy between overeating and addiction, and tested whether a single pathway triggers both anorexia and overeating. Here, we found a common molecular mechanism that underlies the transition from under- to over-eating. This mechanism depends on a peculiar property of few G-protein coupled receptors (GPCR). The 5-HTR_4 displays this particular property. In some instances, these receptors exhibit an autonomous capacity to regulate their own intracellular signaling pathways, without agonist stimulation. This autonomous capacity (or agonist-independent activity) defines their constitutive activity. Inhibiting this constitutive activity, *i.e.* inhibiting the autonomous capacity of 5-HTR_4 to regulate their owns intracellular signaling pathways, stabilizes their inactive form (G-protein uncoupled: Symbolized by R) in the plasma membrane. In other words, agonists enrich active form (G-protein coupled, symbolized by R*) whereas inverse agonists stabilize R, and antagonists equilibrate R/R*. We found that inactivating totally ("silencing") the NAc-5-HTR_4, *i.e.* injecting a specific inverse agonist of 5-HTR_4 in the NAc of behaving mice, provoked overeating. In order to analyze the effects on food intake induced by a high constitutive activity of 5-HTR_4, we engineered a specific mutation in the *mHtr*4 gene encoding the 5-HTR_4. This mutation "locked" the 5-HTR_4 to 5-HT by mutating the gene *mHtr*4 [D100A: codon encoding aspartate (D) was mutated in the codon encoding alanine (A), at position 100)]. This defines the 5-HTR_4 Activated Solely by Synthetic Ligand: R_4ASSL. This mutation enhances the constitutive activity of 5-HTR_4. A non-viral procedure (Polyplus technology) has been used to deliver the R_4ASSL gene in the NAc of behaving mice. Injecting R_4ASSL in the NAc increased the levels of cAMP and CART in the NAc, and decreased food intake in freely moving animals. Results suggest that at two extremes of the R*/R of 5-HTR_4, in the NAc, correspond to two extremes of feeding patterns: restrictive diet and overeating. Analyses of downstream molecular signals show that silencing NAc-5-HTR_4 decreases the levels of cAMP, decreases CART and increases the mRNA levels of the orexigenic neuropeptide Y (NPY). siRNA-mediated NPY knock-down in the NAc suppresses overeating induced by silencing 5-HTR_4. Finally, in an operant intravenous self-administration model under a progressive ratio schedule of reinforcement, we found that the 5-HTR_4 knock-out mice display decreased motivation for cocaine while increased motivation for food reward. Detailed molecular analyses show a down-activity of the pCREB/ΔFosB/FosB signals and a decreased number of dendritic spines in the NAc of 5-HTR_4 knock-out mice treated with cocaine. In conclusion, finding provides indication on the physiological consequences of the R*/R transition ('toggling') of the 5-HTR_4, which may favor the transition from anorexia to overeating, while the absence of the 5-HTR4 could "hijack" cocaine abuse in food abuse.

SEROTONIN NEURONS COME OF AGE WITH 5HT1A RECEPTOR FUNCTION

An early postnatal critical period for transcriptional maintenance of *Htr1a* autoreceptor expression.

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Altered maintenance of serotonergic gene expression has been implicated in mood and anxiety disorders. For example, suppression of 5-HT1a autoreceptor (Htr1a) expression from P14-30 (but not in adulthood) increased anxiety-like behaviors later in life (Donaldson et al., 2014. Neuropsychopharmacology), suggesting an early critical period for Htr1a control of anxiety. Gene regulatory programs that initiate 5-HT synthesis and 5-HT neuron Htr1a expression in fetal life have been identified. However, far less is known about the regulatory mechanisms that function later in life to maintain these key functions and thereby preserve serotonergic neurotransmission. We have developed genetic approaches to investigate the gene regulatory mechanisms controlling the initiation and maintenance of serotonergic gene expression. The Pet-1 ETS transcription factor is required for upregulation of the Htr1a gene expression in maturing fetal 5-HT neurons. We further investigated the dependence of Htr1a gene expression on early Pet-1 function. Paraformaldehyde-fixed 25µm adult tissue sections were collected from the entire rostrocaudal extent of +/+ and Pet-$1^{-/-}$ dorsal raphe nucleus (DRN) for in situ hybridization analysis (ISH). Htr1a gene expression was virtually eliminated in the Pet-$1^{-/-}$ DRN with only weak hybridization signals visible in some sections after prolonged signal development. These findings demonstrate that seemingly all DRN 5HT neurons fail to express Htr1a in Pet-1 deficient mice and, therefore, few if any DRN 5-HT neurons are resistant to loss of Pet-1 for acquisition of Htr1a differentiation feature. In contrast, we showed previously with conditional targeting of Pet-1, that maintenance of Htr1a gene expression is not dependent on Pet-1 in adult 5-HT neurons. These findings led us to investigate further the critical period for Pet-1-dependent regulation of Htr1a. We developed an approach to target Pet-1 expression at various postnatal stages with AAV-Cre injection into the DRN of Pet-$1^{loxP/loxP}$ mice. Injection of AAV-Cre at P0 produced a severe loss of Pet-1 expression when analyzed by ISH at P21. Interestingly, a comparable severe loss of Htr1a gene expression resulted from the neonatal targeting of Pet-1. These findings suggest that an early postnatal critical period exists for transcriptional maintenance of Htr1a expression in the DRN and that this critical period may overlap with the early critical period for Htr1a autoreceptor-dependent control of later life anxiety behaviors. Thus, genetic or environmentally driven variation in the transcriptional maintenance of early postnatal Htr1a expression might be a previously unrecognized path for development of anxiety or mood disorders. Further studies are currently being done to precisely define the critical period for transcriptional maintenance of Htr1a expression in DRN 5-HT neurons.

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Using optogenetics to understand Dorsal Raphe serotonergic synaptic transmission

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The 5-HT synthesizing neurons of the Dorsal Raphe nucleus (DRN) are the main source of serotonergic inputs to the forebrain including the cerebral cortex. Work during the last few decades has defined the anatomy of forebrain DRN afferents, elucidated many of the determinants of serotonin release from these projections, and provided important clues of their functional role at the whole organism level. Yet, remarkably, we still know very little about the cellular physiology of serotonergic synaptic transmission and essential link between serotonin cell firing, serotonin release and behavior.

The reason for this gap in our knowledge is technical. Serotonin neurons constitute a very small fraction of all the neurons in the CNS. Therefore electrical stimulation, the technical cornerstone of synaptic physiology, cannot be readily used to study serotonergic synaptic transmission. This limitation however has become less important with the advent of optogenetics, which allows for the use of light to activate (or inhibit) genetically targeted neurons. These advances now make it possible to address longstanding questions regarding synaptic transmission from serotonergic neurons of the DRN.

There are multiple ways to implement expression of channelrhodopsin (ChR) in serotonergic neurons including virally-mediated gene expression as well as strictly generic strategies. We have tried several of these approaches and found that, while effective, each offers distinct advantages and disadvantages including expression levels, cell specificity and regional selectivity of expression. We have taken advantage of the different strengths of these approaches to address three fundamental question concerning serotonergic neuron physiology: the synaptic basis of autoinhibition in the DRN, the role of reuptake in shaping serotonergic synaptic transmission onto 5-HT_{1A} autoreceptors, and the extent of serotonin-glutamate co-transmission onto different postsynaptic targets. In this talk I will present recent work from our lab addressing each of these questions.

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Genetic mechanisms underlying variation in 5-HT1A receptors in the human developing and adult brain

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The complex effects of serotonin 1a receptors (5-HT1A) on mood and behavior are mediated by both development-specific and brain region-specific effects of receptor activation. Work in mouse models has demonstrated that the behavioral roles of different neural populations of 5-HT1A change across the course of development. For instance, post-natal variation in autoreceptors, but not forebrain heteroreceptors, influences anxiety levels, while adult autoreceptors modulate stress coping, but not anxiety.

Furthermore, multiple manipulations have demonstrated that even small, ethologically relevant differences in receptor levels in a single brain region and/or during a particular developmental period can impact anxiety and depression-related phenotypes. Thus, understanding the factors that contribute to normal variation in receptor levels across the life course will help elucidate the mechanisms underlying 5-HT1A as a risk factor for mood and anxiety disorders. In humans, a G/C single nucleotide polymorphism (rs6295) located 1 kb upstream of the 5-HT1A gene, which has been linked with depression and antidepressant responsiveness, is hypothesized to mediate differences in neural 5-HT1A levels through the altered binding of a number of transcription factors, including Deaf1, c-Jun, and Hes5. *In vitro* work suggests that this SNP has region-specific and development-specific effects on the expression of 5-HT1A. Thus, in order to determine the transcriptional relationship between rs6295 and 5-HT1A within the human brain, we measured the relative allelic expression from the G and C alleles in postmortem human brain tissue. Within the prefrontal cortex of control subjects, more mRNA is derived from the C- compared to the G-allele. However, this effect is region-specific, as the G- and Callele produce equivalent amounts of mRNA in the hippocampus and in the raphe.

Because the Hes transcription factors are thought to interact with rs6295 selectively during neuronal differentiation, we also examined the G:C expression ratio in human fetal cortex (gestational week 17). Similar to the adult prefrontal cortex, we observed more mRNA produced from the C-allele, indicating that rs6295 is associated with differences in 5-HT1A expression during gestation, potentially impacting important developmental events. Interestingly, in individuals with major depressive disorder, we found that the normal pattern of transcription was disrupted in the prefrontal cortex.

Together, these findings support the hypothesis that rs6295 has region-specific effects on transcription across multiple developmental stages and that the normal transcriptional profile is disrupted in disease states. This work, along with a humanized mouse model of rs6295 that we recently developed, will provide important insight into mechanisms by which common genetic variation in the human 5-HT1A gene might potentiate risk for mental illness.

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SEROTONIN: A NEW HOPE IN ALZHEIMER'S DISEASE

Serotonin signaling lowers amyloid-beta levels and plaques in transgenic mice and humans

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Accumulation of amyloid-beta (Abeta) within the extracellular space as plaques is a pathological hallmark of Alzheimer's disease (AD). Abeta generation is regulated by synaptic activity via several mechanisms, including activation of neurotransmitter receptors which lead to signaling cascades that down-regulate processing of APP into Abeta. Increasing serotonergic signaling, either by infusing serotonin into the brain or by administering selective serotonin re-uptake inhibitors (SSRI) antidepressants, acutely reduces brain interstitial fluid (ISF) Abeta levels in a mouse model of AD. Chronic administration of SSRIs also significantly reduce plaque load in mice. Recently, we have utilized in vivo Abeta microdialysis to assess the effect of agonists that are selective for particular serotonin receptor (5HT-R) subtypes. Reverse microdialysis treatment with agonists has shown 5HT-R4, 6, and 7 activation reduces ISF Abeta levels while 5HT-R1, 2, and 5 agonists have no effect. Importantly, 5HT-R4, 6, and 7 all activate a similar Gs-protein coupled receptor which leads to activation of protein kinase A (PKA) signaling pathways. Reverse microdialysis infusion of selective PKA inhibitors increases Abeta levels and negates the effects of the SSRI, citalopram. We have also administered a single dose of citalopram or placebo to young, cognitively-normal individuals while performing staple isotope labeling kinetic (SILK) studies to assess Abeta metabolism within the CSF. The citalopram-treated group had significantly lower Abeta levels and their production rate of Abeta was slower than the placebo-treated group. This data is consistent with serotonin signaling suppressing Abeta generation in humans and suggests long-term treatment with serotonin-enhancing agents could be a promising therapeutic for AD.

5-HT_4 receptor agonists: novel promising agents for AD prevention

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5-HT_4 agonists have been proved to exert procognitive effects in rodents and to induce the non-amyloidogenic processing of the amyloid precursor protein (APP), leading to an increase of soluble APPα (sAPPα). Therefore, 5-HT_4 receptors (5-HT_4R) could be of interest to delay AD progression. Following these observations, we decided to study the action mechanism of 5-HT_4R ligands and analysed their effects on Aβ production and amyloid plaque formation. COS-7 cells were stimulated with 5-HT_4R agonists and sAPPα release quantified through ELISA. Chronic administration of 5-HT_4 agonists was performed in an aggressive mouse model of AD, the 5XFAD, during the prodromal phase preceding the appearance of behavioural deficits. Following treatments, amyloid plaque load and Aβ burden were measured through ELISA and thioflavin T staining. Cerebrospinal fluid (CSF) was also collected and sAPPα and $Aβ_{42}$ analysed through ELISA. Besides, astroglial inflammation and microglia activation associated to plaques were revealed through GFAP and Iba-1 staining. Our results clearly show that 5-HT_4R agonists induced an increase of sAPPα release both in cell cultures and in the CSF of 5XFAD mice. Indeed, the chronic and prodromal administration of 5-HT_4R agonists to 5XFAD mice reduced the production of Aβ peptides and slowed down the formation of plaques. These effects were prevented by a co-treatment with a specific 5-HT_4R antagonist that was ineffective by itself, demonstrating that the effects observed are specific to 5-HT_4 receptors. Astroglial inflammation was also markedly reduced after 5-HT_4R agonist administration. Finally, these treatments reversed cognitive deficits in novel object recognition test. In summary, chronic treatments promoting sAPPα release via the stimulation of 5-HT_4 receptors clearly hinder plaque formation and Aβ load while jointly attenuating inflammation processes and improving cognitive outcome. We conclude that 5-HT_4 agonists administration could represent an interesting and promising strategy for AD prevention.

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Mechanisms contributing to lack of antidepressant efficacy in juveniles and adolescents

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Depression is a major health problem for which most patients are not effectively treated. This problem is further compounded in children and adolescents where only two antidepressant drugs are currently approved for clinical use. Both are selective serotonin (5-HT) reuptake inhibitors (SSRIs), which are often less therapeutically efficacious in this young population compared to adults. Consistent with clinical literature, we found that antidepressant-like effects of SSRIs in mice aged 21 days post-partum (P21, juvenile) was reduced relative to adult mice; however, there was no difference in expression of hippocampal 5-HT transporter (SERT), the target protein of SSRIs, to account for the reduced SSRI efficacy. The increase in extracellular 5-HT following SSRI administration is thought to trigger downstream events required for therapeutic effects. Thus, our data raise the possibility that transporters capable of 5-HT uptake other than SERT may be present in disproportionately higher levels during juvenile and adolescent periods thereby preventing extracellular 5-HT from climbing to therapeutically relevant levels following SSRI treatment. Decynium-22 (D22) is a blocker of organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT), low affinity, but high capacity transporters for 5-HT. We found that in juvenile and adolescent mice, the density of [^{3}H]D22 binding sites in hippocampus are greater than in adults. Western blot analysis using specific antibodies revealed that increased [^{3}H] D22 binding was most likely driven by increased PMAT expression in young mice relative to adults. These data suggest that D22 may have antidepressant activity in juvenile and adolescent mice. In our preliminary studies we found that D22 (0.01mg/kg) produced antidepressant-like effects in juvenile but not adult mice. Using *in vivo* chronoamperometry, an electrochemical technique which allows for sub-second measurements of region specific 5-HT clearance in brain, studies are underway to determine whether the antidepressant-like effects of D22 are related to its ability to inhibit 5-HT clearance. Our results suggest that significant uptake of 5-HT by PMAT and/or OCTs may limit the therapeutic efficacy of SSRIs, providing a mechanistic basis for poor treatment response to SSRIs particularly in juveniles and adolescents. This work was supported by National Institutes of Health Grants R01-MH064489-S1 (LCD), R01-MH093320 (LCD, WK), NARSAD Independent Investigator Award (LCD), and a NIDA travel award 1R13DA033783-01.

Mice with compromised 5-HTT function lack phosphotyrosine-mediated inhibitory control over prefrontal 5-HT responses

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The activity of the prefrontal cortex is essential for normal cognition and emotional processing, which are strongly modulated by serotonin (5-HT). Yet, little is known about the regulatory mechanisms that control the effects of prefrontal 5-HT receptors. It is also not known whether disruptions in such regulatory mechanisms would perturb prefrontal cortical functions. Here, we characterized alterations in the regulation of prefrontal 5-HT receptor electrophysiological signaling in mouse models of disrupted serotonin transporter function, a risk factor for emotional and cognitive disturbances in both humans and rodents.

We identified a novel tyrosine-kinase dependent mechanism that regulates 5-HT-mediated inhibition of prefrontal pyramidal neurons. We report that mice with compromised serotonin transporters (even if this disruption occurs only transiently during development due to treatment with selective serotonin reuptake inhibitors) have amplified, inhibitory, 5-HT_{1A} receptor-mediated currents in adulthood with enhanced downstream coupling to Kir3 channels.

Such amplified 5-HT_{1A} responses can be mimicked through inhibition of Src family tyrosine-kinases in normal mice and rapidly normalized through inhibition of tyrosine-phosphatases in the mouse models examined. Our findings implicate tyrosine phosphorylation in regulating the electrophysiological effects of prefrontal 5-HT_{1A} receptors with implications for neuropsychiatric diseases associated with emotional and cognitive dysfunction, such as anxiety and depressive disorders.

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DRUG ADDICTION AND IMPULSIVITY: IS SEROTONIN INVOLVED?

Cortical 5-HT_{2A}R:5-HT_{2C}R involvement in convergent impulsivity and cocaine cue reactivity

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Cocaine abuse and addiction continue to extract considerable personal, health and societal tolls in the world. The cycling progressive nature of this disorder stymies efforts to stay abstinent of relapse, precipitated by impulsive action (predisposition toward rapid, unplanned reactions to stimuli without regard to negative consequences) and craving due to exposure to cocaine-associated cues (cocaine cue reactivity). We have data to suggest that convergence of the impulsivity trait with the dynamic state of cue reactivity is a synergy that contributes to greater vulnerability to relapse in animal models. Our data suggest that impulsive action and cue reactivity are mechanistically-linked to disrupted serotonin (5-HT) signaling through the 5-HT_{2A} receptor (5-HT_{2A}R) and 5-HT_{2C}R localized to prefrontal-striatal-thalamic circuitry. Impulsive action and cocaine cue reactivity were evaluated in the 1-choice serial reaction time task and cocaine self-administration (SA)/forced abstinence (FA) paradigm, respectively. We employed immunoblots to detect the synaptosomal protein expression of 5-HT_{2A}R and 5-HT_{2C}R in the medial-prefrontal-cortex (mPFC) of phenotypically differentiated rats. Experimental disruption of the 5-HT_{2A}R:5-HT_{2C}R balance was achieved following genetic deletion of the 5-HT_{2C}R in the mPFC; rats were then subjected to behavioral tests. High impulsive rats exhibited a higher ratio of 5-HT_{2A}R to 5-HT_{2C}R protein expression in synaptosomes from mPFC *vs.* low impulsive rats ($p<0.05$). Rats with a knockdown of the 5-HT_{2C}R in the mPFC expressed significantly elevated impulsive action relative to control rats ($p<0.05$) and enhanced potency of the 5-HT_{2A}R antagonist M100907 to suppress impulsive action ($p<0.05$). In rats trained to SA cocaine (0.75 mg/kg/inf; FR 5; 14 days) and then assessed for cue reactivity on FA Day 1 or FA Day 30, we observed elevated cue reactivity and a late-forming increase in the ratio of 5-HT_{2A}R to 5-HT_{2C}R mPFC synaptosomal protein expression at FA Day 30 ($p<0.05$). Thus, we have uncovered potential neurobiological mechanisms of importance in driving relapse vulnerability. Because the ultimate output of 5-HT neurotransmission in the mPFC is driven by a myriad of regulatory epigenetic, transcriptional, translational and topological processes, we are currently evaluating these aspect of 5-HT_{2A}R and 5-HT_{2C}R function in these behavioral phenotypes. We propose that restoration of the 5-HT_{2A}R:5-HT_{2C}R balance will repair corticostriatal deficits and ameliorate relapse during abstinence from cocaine addiction.

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5-HT_{1B} and DREADD regulation of relapse to cocaine seeking.

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Serotonin has complex effects on drug reward, reinforcement, and drug seeking after abstinence from cocaine. Several receptors are likely to mediate these effects, including 5-HT_{1B} receptors. Medium spiny neurons in the dorsal and ventral striatum express 5-HT_{1B} receptors, which are located on axon terminals in the projection targets of these neurons in areas such as ventral tegmental area. We found that cocaine exposure regulates 5-HT_{1B} mRNA expression in striatum in a complex manner. We then manipulated 5-HT_{1B} expression in rat nucleus accumbens (NAc) neurons and measured the resulting effects on cocaine self-administration and cocaine seeking behavior after extinction or forced abstinence. We found that increased expression of 5-HT_{1B} receptors in these neurons increased the motivation for rats to self-administer cocaine. However, if the animals had extended abstinence from cocaine or extinction training, then the same intervention consistently reduced cocaine seeking. This effect was observed for drug cue and drug prime, but not stress, induced reinstatement of cocaine seeking.

In order to examine the effects of G -coupled receptor effects on cocaine self-administration and seeking, we have recently been using DREADD receptor expressed in corticostriatal neurons. These engineered receptors were expressed using an intersectional viral vector strategy with AAV-DIO-hM_4Di into cortex and CAV2-Cre injected into NAc; this combination leads to selective expression of the inhibitory DREADD receptors only in corticostriatal neurons. Similar to the case of 5-HT_{1B} receptors in NAc neurons, activation of DREADDs in PFC corticostriatal neurons also enhanced the motivation to take cocaine but reduced reinstatement of cocaine seeking after extinction. We interpret these data to suggest that there is a fundamental reorganization of NAc-mediated excitability in regard to cocaine seeking after an interval of forced abstinence or extinction training. The locus of the plasticity involved in this reversal of receptor regulation of drug seeking is not known but seems to be a generalized phenomenon rather than one particular to a single receptor subtype.

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Serotonergic manipulation in specific subtypes of impulsivity and compulsivity in humans

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Serotonin regulates diverse motor, cognitive, decisional and affective brain functions. Convergent results from animal and human studies suggest that reducing serotonin (5-hydroxytryptamine, 5-HT) neurotransmission promotes impulsive behavior and can influence compulsivity. However, the precise function of serotonin in regulation of different types of impulsivity and compulsivity is still unclear. To formally address this question, we employed the dietary acute tryptophan depletion procedure (TD) to reduce 5-HT neurotransmission in healthy volunteers. This procedure has been shown to produce a transient reduction of central serotonin transmission in the brain, as tryptophan is the amino acid precursor of serotonin.

First, we examined the role of serotonin in impulsive action and impulsive choice. We used a novel translational analogue of a rodent 5-choice serial reaction time task (5-CSRTT) and a reward delay discounting questionnaire to measure effects on these different forms of 'waiting impulsivity'. There was no effect of TD on impulsive choice as indexed by the reward delay-discounting questionnaire. In contrast, TD significantly increased premature responses (or impulsive action), which is remarkably similar to the previous findings of effect of serotonin depletion on rodent 5-CSRTT performance. TD also improved the accuracy of performance and speeded responding, possibly indicating enhanced attention and reward processing.

Second, we also assessed the role of diminished 5-HT neurotransmission on the decisional impulsivity using a risk-taking task in which subjects choose between a sure choice and a gamble, focusing on the anticipation of risky outcomes across a range of probabilities. The task also included two independent counterbalanced sessions with Reward and Loss conditions. There was no effect of TD on decision making under the risk in the Reward condition. However, the TD group exhibited significantly lower certainty equivalents in high Loss condition, which corresponds to a lower risk taking tendencies.

Third, we assessed the role of serotonin in the balance between goal-directed model-based behaviours and habitual model-free behaviours using a two-step choice discrimination task. We show that serotonin depletion shifts the relative balance towards habitual model-free learning for reward outcomes and towards goal-directed model-based learning for loss outcomes.

Overall, the results of these studies indicate that manipulation of serotonin produces dissociable effects on different measures of impulsivity. We further show that serotonin influences risk taking and the balance between goal-directed and habitual behaviours as a function of valence. These results suggest considerable specificity in its neuromodulatory role and have implications for understanding the neurochemical basis of impulsive compulsive disorders.

THE 5HT$_{2C}$ RECEPTOR AT THE INTERFACE OF OBESITY AND ADDICTION

Characterisation of Appetite-Regulating 5-HT2C Receptors

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The 5-HT$_{2C}$ receptor (5-HT$_{2C}$R) is an important regulator of appetite; the clinical significance of which has recently been realised with the launch of the 5-HT$_{2C}$R agonist lorcaserin for obesity treatment in the USA. Efforts to delineate the underpinnings of 5-HT$_{2C}$R appetite suppression have largely focused upon melanocortin pro-opiomelanocortin (POMC) within the hypothalamus. However, there is a second neuroanatomical population of POMC neurons that is located within the nucleus of the solitary tract (NTS). To investigate the specific contribution of NTS 5-HT$_{2C}$Rs to appetite, we generated a 5-HT$_{2C}$R-Cre:yellow fluorescent protein (YFP) mouse line. We then crossed this with a POMC-dsRed reporter mouse line and determined that 5-HT$_{2C}$Rs are also anatomically positioned to influence NTS POMC activity. Next, we observed that 5-HT$_{2C}$R agonists such as lorcaserin at concentrations that reduce food intake increase c-fos immunohistochemistry in POMCds-Red neurons. Using patch-clamp electrophysiology, we then determined that 5-HT and 5-HT$_{2C}$R agonists directly depolarise and increase the firing rate of approximately 40% of NTS POMCds-Red neurons. To examine the physiological significance of these findings, we utilised a chemogenetic technique, designer receptors exclusively activated by designer drugs (DREADD) to probe the discrete appetite suppressive function of NTS 5-HT$_{2C}$R neurons. Designer G$_q$ receptor (AAV8-hSyn-DIO-hM3Dq-mCherry) was bilaterally injected into the NTS of 5-HT$_{2C}$R-Cre mice producing 5-HT$_{2C}$R-Cre:hM3D$_q$-expressing neurons exclusively within the NTS. The selective activation of these neurons by designer drug clozapine-N-oxide (CNO) significantly suppressed feeding. To evaluate the contribution of POMC to the observed appetite suppression, 5-HT$_{2C}$R-Cre:hM3D$_q$ mice were pretreated with the melanocortin 4 receptor antagonist SHU9119, which prevented CNO-induced hypophagia. These findings provide new insight into the circuits engaged by 5-HT$_{2C}$Rs to impact food intake and suggest that the little studied population of NTS POMC neurons play a role in 5-HT$_{2C}$R appetite suppression.

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Novel therapeutic opportunities for anti-obesity 5-HT_{2C} receptor agonists in psychostimulant abuse and nicotine dependence

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Central serotonin (5-HT) systems have long been associated both with the control of investigative behaviour, and regulating the behavioural effects of psychostimulants, opioids, alcohol and nicotine. During the 1990's, the 5-HT releaser/reuptake inhibitor (dex)fenfluramine was widely approved for treatment of obesity, later followed by the 5-HT/NA reuptake inhibitor sibutramine. However both drugs were subsequently withdrawn due to safety concerns related to cardiac valvulopathy and/or pulmonary hypertension creating regulatory challenges to the development of subsequent anti-obesity drugs. For some years now the 5-HT_{2C} receptor has emerged as the principal target for the anorectic properties of (dex)fenfluramine.

Selective 5-HT_{2C} receptor agonistic drugs have been identified and developed to more directly examine this potential. The most advanced drug of this class is lorcaserin which will be the primary focus of this presentation. The selective 5-HT_{2C} agonist, lorcaserin, was approved by the FDA in June 2012 for the treatment of obesity – the first approval since sibutramine for such use. In addition to the regulation of food intake, preclinical evidence also supports the potential for the 5-HT_{2C} agonist drug class to regulate behaviours and neurochemical effects of psychostimulants, alcohol and nicotine – consistent with earlier literature. Indeed there is complete overlap in lorcaserin doses and plasma exposure necessary to influence ingestive/feeding behaviour and regulating the behavioural effects of nicotine. This presentation will highlight this evidence and further suggest that overlapping neurobiological systems may contribute to both an anti-addictive potential and anti-obesity property.

Developments in areas of target selectivity, positive allosteric modulation and functional selectivity may offer even greater therapeutic potential for this drug class, as would the identification of biomarkers to define target engagement. We have recently identified potential differences between lorcaserin and another 5-HT_{2C} agonist drug, CP809101, in terms of tolerability which might conceivably lead to improvements in 5-HT_{2C} agonist based therapies. The availability of selective 5-HT_{2C} agonists in the clinic now provides an important opportunity to evaluate their potential as treatments for nicotine dependence or psychostimulant abuse, in addition to obesity.

Taking Two to Tango:

A Role for 5-HT$_{2C}$ Receptor Heterodimerization in Food Intake and Reward

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Heterodimerization of GPCRs involved in appetite signalling may explain functional crosstalk observed between neuropeptide systems mediating food intake and food reward. We have recently shown compelling evidence for the existence of a novel heterodimer between the 5-HT$_{2C}$ receptor and the GHS-R1a receptor, with both receptors implicated in the homeostatic control of appetite and satiety and in neurocircuits involved in the rewarding aspects of food. In this study, it was shown that GHS-R1a-mediated calcium mobilization following ghrelin treatment was attenuated following interaction with the 5-HT$_{2C}$ receptor. The attenuated GHS-R1a receptor signalling was completely restored following pharmacological blockade of the 5-HT$_{2C}$ receptor. Interestingly, it has also been demonstrated that direct PVN administration of 5-HT in rats attenuates ghrelin's orexigenic effect, which supports a potential significant role for the GHS-R1a/5-HT$_{2C}$ receptor dimer pair in appetite regulation *in vivo*. In line with these findings, we have shown that blockade of 5-HT$_{2C}$ receptor signalling potentiates ghrelin mediates orexigenic effect (unpublished), which substantiates a physiological relevant role of a GHS-R1a/5-HT$_{2C}$ interaction. These findings may uncover novel mechanisms important for the future pharmacological targeting of the GPCRs in the homeostatic regulation of body weight as well as hedonic appetite signalling, which both play a significant role in the development of obesity.

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THE ROLE OF SEROTONIN SYSTEMS IN THE ADVERSE NEUROPSYCHIATRIC SIDEEFFECTS AND RECREATIONAL USE OF HIV-1 ANTIRETROVIRAL DRUGS

Antiretrovirals as emerging drugs of abuse: serotonergic neuropharmacology of efavirenz and other antiretroviral drugs

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Almost 1 in 200 people worldwide live with HIV/AIDS and new cases are reported every twelve seconds. Due in large part to highly efficacious antiretroviral (ARV) medicines, the number of people living with HIV continues to increase as does the median age of those infected with the virus. Efavirenz is widely prescribed because it is highly effective at suppressing HIV-1 (Sierra-Madero et al., 2010), even though over half of HIV patients taking this medication under prescribed dosing conditions experience neuropsychiatric side effects which can include depression, anxiety, aggressive behavior, night terrors, hallucinations, psychosis and delusions (Sustiva, 1998). Though these side effects are thought to subside after several weeks they apparently can persist (Lochet et al., 2003; Fumaz et al., 2005) and the role that age and genotype plays is entirely unknown; this may be relevant now that HIV is being treated as a chronic disease rather than a terminal illness. There have also been reports of efavirenz being diverted for recreational use in South Africa, where pills are crushed and smoked for a high (Gallagher, 2007; Marwaha, 2008; Sciutto, 2009). Molecular profiling of the receptor psychopharmacology of efavirenz has pinpointed interactions with multiple established sites of action for other known drugs of abuse including catecholamine and indolamine transporters (DAT, SERT, VMAT2) as well as $GABA_A$ and $5\text{-}HT_{2A}$ receptors (Gatch et al., 2013). Despite having only weak partial agonist activity at $5\text{-}HT_{2A}$ receptors measured as IP_3 turnover, a lysergic acid diethylamide (LSD)-like interaction with the $5\text{-}HT_{2A}$ receptor appears to dominate efavirenz's behavioral profile in rodents. Both LSD and efavirenz depress open field activity in a novel environment in the same mouse strain. LSD occasions level pressing in rats trained to discriminate efavirenz from saline, and conversely, efavirenz occasions level pressing in rats trained to discriminate LSD from saline (Gatch et al., 2013). The later effect is abolished by selective blockade of the $5\text{-}HT_{2A}$ receptor. Though receptor profiling indicated interactions with a number of potential molecular targets within a similar concentration range, behavioral profiling in rodents indicated a prevailing psychopharmacological profile for efavirenz most consistent with LSD-like psychoactivity mediated via the $5\text{-}HT_{2A}$ receptor. This finding correlates with subjective reports from HIV patients experiencing efavirenz-induced neuropsychiatric side effects, such as hallucinations, night terrors and abnormal dreaming, and provides a rationale for its recreational use. Studies focused on other serotoninergic targets have revealed additional interactions. The most pronounced being with $5\text{-}HT_3$ and $5\text{-}HT_6$ receptors and monoamine oxidase A, adding to the already complex neuropsychopharmacology of efavirenz. No interactions at $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$ or $5\text{-}HT_6$ receptors were detected for two other HIV-1 antiretroviral drugs, zidovudine and emtricitabine, though some modest $GABA_A$ receptor inhibitory effects were noted.

Serotonin 5-HT_{2A} receptor involvement in the hallucinogen-like actions of efavirenz in mice

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In clinical studies, over half of HIV patients taking the antiretroviral drug efavirenz reported serious psychiatric effects such as hallucinations, paranoia and aggressive behavior. The Gq/11 protein-coupled serotonin 5-HT_{2A} receptor has been implicated in the molecular mechanism of action of hallucinogenic drugs of abuse, such as lysergic acid diethylamide (LSD), mescaline and psilocybin. We previously showed that only 5-HT_{2A} receptor agonists with hallucinogenic potential in humans activate a significant head-twitch behavior in wild-type mice, but not in 5-HT_{2A} knockout (KO) mice. These data suggest that head-twitch behavior induced by LSD-like drugs is useful as a mouse behavioral proxy of human hallucinogenic action. We tested the effects of efavirenz on head-twitch behavior in wild-type and 5-HT_{2A}-KO mice. Mice were intraperitoneally injected with efavirenz (15 mg/kg), or vehicle, and immediately after, they were videotaped for 15 min. We found that administration of efavirenz produced a significant head-twitch response in wild-type mice, but not in 5-HT_{2A}-KO mice. We next examined whether efavirenz acts as a 5-HT_{2A} receptor agonist in HEK293 cells transfected to transiently express the human 5-HT_{2A} receptor (pcDNA3.1-c-Myc-5HT2A). Using inositol phosphate (IP) accumulation as a measure of Gq/11 activation, we found that efavirenz has partial 5-HT_{2A} receptor agonist properties when compared to stimulations induced by LSD or serotonin. The effect of efavirenz on IP accumulation was prevented by the 5-HT_{2A} receptor antagonist methysergide. Although further investigation is needed to understand the mechanisms and consequences of these findings in vitro and in vivo, it is tempting to speculate that efavirenz has psychoactive properties similar to those induced by the hallucinogenic 5-HT_{2A} receptor agonist LSD.

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Preclinical assessment of the abuse potential of antiretroviral drugs: the role of serotonin mechanisms

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Efavirenz has antiretroviral activity and is used extensively in the treatment of HIV. Efavirenz can have adverse psychiatric effects (e.g. nightmares) and in South Africa it is reportedly diverted for recreational use, typically being crushed and smoked with other psychoactive substances. Based on its affinity for molecular targets (e.g. serotonin [5-HT] receptors) that can mediate abuse related effects of drugs, efavirenz was compared with reference drugs in well-established preclinical models that are predictive of abuse liability. There was partial overlap in the discriminative stimulus effects (Gatch et al, 2013) of efavirenz and the 5-HT_{2A} receptor agonist, and known hallucinogen, LSD. Rats receiving 0.1 mg/kg LSD responded partially on the drug lever after receiving efavirenz and rats receiving 18 mg/kg efavirenz responded partially on the drug lever after receiving LSD. Cocaine (0.32 mg/kg/infusion i.v.) was self-administered at a high rate and significantly more than saline; up to a dose of 10.0 mg/kg/infusion, efavirenz did not maintain a responds above what was obtained with saline. Efavirenz and drugs that are known to act directly at 5-HT receptors (DOM, quipazine) were studied alone and in combination with drugs that are reportedly co-abused with efavirenz (heroin, nicotine, THC) under procedures that are sensitive to different aspects of impulsivity (5-choice serial reaction time test and delay discounting). Taken together, with negative results in self administration and conditioned place preference studies, the results of these preclinical studies fail to provide clear evidence for any adverse behavioral effects of efavirenz and provide only limited data that would predict significant abuse liability. Factors other than or in addition to its pharmacodynamic activity might play a major role in the recreational use of efavirenz.

FROM OPTOGENETICS TO THE MICROBIOME: EMERGING STRATEGIES TO UNDERSTAND THE ROLE OF SEROTONIN IN ANXIETY AND ADDICTION

5-HT moderates coritcostriatal control of cognition

Andrew Holmes

NIAAA. Supported by NIAAA intramural program

The serotonin system has long been established to play a key role in modulating brain systems subserving behavior. However, emerging behavioral, genetic and imaging technologies are providing important new insights into how serotonin actively shapes the development and function of neural circuits critical for the control of cognition and emotion. This presentation will present two parallel lines of research linking the serotonin system to prefrontal-striatal mediated forms of learning. First, convergent data will be shown from behavioral, in vivo electrophysiological and optogenetic experiments demonstrating the importance of medial prefrontal cortex and amygdala in mediating inhibitory fear learning, in the form of fear extinction. In the second part, studies will be shown demonstrating how pharmacological blockade of the serotonin transporter produces facilitation of inhibitory fear learning, in the form of fear extinction, via the promotion of endocannabinoid signaling and synaptic plasticity in prefrontal-amygdala circuits. These data will be discussed in terms of possible implications for understanding the neural mechanisms underlying the anti-anxiety effects of serotonin transporter blockers.

Anxiety-related regulation of non-coding RNAs: a role for 5-HT receptors

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Altered regulation of serotonin receptors including 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} has been implicated in stress-related disorders such as anxiety and affective disorders. The 5- HT_{2C} receptor is the only receptor of the 5-HT family that is subject to extensive processing mediated in part by interactions with the small nucleolar RNA (snoRNA) MBII-52 and RNA editing (A to I) by adenosine deaminases acting on RNA (ADAR). Since previous studies including our own have revealed that increased 5-HT_{2C} receptor activation induces anxiogenic effects in animals and humans, we initially focused on snoRNA interaction and hypothesized that predisposition to and/or maintenance of enhanced anxiety may be associated with altered MBII-52-mediated processing of the 5-HT_{2C} receptor ultimately leading to increased receptor function. We first revealed evidence of a spatial and temporal dynamic regulation of the brain specific snoRNAs MBII-52 and MBII-85 in the prefrontal cortex, hippocampus and amygdala of C57BL/6N mice after exposure to stress, an important trigger and pathophysiological factor in anxiety and affective disorders, pointing towards functional significance of these snoRNAs in stress-processing. Using a psychopathological mouse model of enhanced anxiety with comorbid depression (HAB), we observed lower levels of MBII-52 (which is a negative 5-HT_{2C} receptor regulator) in the amygdala and hippocampus of HAB mice when compared to their normal anxiety behavior (NAB) counterparts. Furthermore, 5-HT_{2C} receptor editing was reduced in the amygdala of HABs as compared with NAB controls. These two findings suggest an upregulated 5-HT_{2C} receptor function in HAB mice that may contribute to their behavioral hyperanxiety. This compliments previous results where overexpression of the 5-HT_{2C} receptor in the brain leads to elevated anxiety and administration of the 5-HT_{2C} receptor agonist MCPP increases neuronal activation in the amygdala, an important brain region for the expression of fear and anxiety. Taken together, these findings have highlighted mechanisms leading to dysregulated 5-HT_{2C} receptor function in high anxiety individuals.

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The Gut Microbiome: A Key Regulator of Brain Serotonin and Behavior

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Bacterial colonisation of the intestine has a major role in the post-natal development and maturation of the immune and endocrine systems. These processes are key factors underpinning central nervous system (CNS) signalling. Regulation of the microbiome-gut-brain axis is essential for maintaining homeostasis, including that of the CNS. However, until recently there was a paucity of data pertaining to the influence of microbiome on the serotonergic system. Germ-free (GF) animals represent an effective preclinical tool to investigate such phenomena. We have recently shown that male GF animals have a significant elevation in the hippocampal concentration of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid, its main metabolite, compared with conventionally colonised control animals. Moreover, this alteration is sex specific in contrast with the immunological and neuroendocrine effects which are evident in both sexes. Concentrations of tryptophan, the precursor of serotonin, are increased in the plasma of male GF animals, suggesting a humoral route through which the microbiota can influence CNS serotonergic neurotransmission. Interestingly, colonisation of the GF animals post weaning is insufficient to reverse the CNS neurochemical consequences in adulthood of an absent microbiota in early life despite the peripheral availability of tryptophan being restored to baseline values. In addition, reduced anxiety in GF animals is also normalised following restoration of the intestinal microbiota. Finally, GF mice have autism-like deficits in sociability and social cognition and increases in repetitive behaviour. Taken together these data suggest that modulation of the gut microbiota may be a novel strategy for stress-related and neurodevelopmental disorders.

In line with this we define a psychobiotic as a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness. As a class of probiotic, these bacteria are capable of producing and delivering neuroactive substances such as gamma-aminobutyric acid and serotonin, which act on the brain-gut axis. Preclinical evaluation in rodents suggests that certain psychobiotics possess antidepressant or anxiolytic activity. Effects may be mediated via the vagus nerve, spinal cord, or neuroendocrine systems. Results from large scale placebo-controlled studies are awaited.

Serotonin inputs to the bed nucleus of stria terminalis shape network function and behaviors relating to fear memory and anxiety

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Serotonin neurons originating from the dorsal raphe nucleus (DRN) innervate a variety of limbic structures involved in feeding, mood regulation, reward-related and avoidance behavior. The bed nucleus of stria terminalis (BNST) is one critical output of the dorsal raphe with a well-defined role in stress-induced relapse and anxiety associated with drug dependence and acute withdrawal states. Using slice electrophysiology in SERT-cre mice stereotaxically injected with a DIO-Ch2-eYFP viral construct into the DRN, we found that optogenetic stimulation of serotonin terminals in the ventrolateral BNST (vBNST) depolarizes cells via activation of 5HT2c receptors (5HT2cRs). Furthermore, colocalization of serotonin-positive fibers with CRF neurons in the vBNST was observed in a Cre-dependent CRF reporter mouse line, suggesting a role for CRF neurons in the excitatory effects of serotonin in the vBNST. Slice electrophysiology in this CRF reporter line confirms that bath applied 5HT2c-R agonists can depolarize cells within the vBNST, in agreement with our findings in SERT-cre mice.

Next we investigated the role of this serotonin-CRF interaction in retention of fear memory in a cued fear conditioning paradigm. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), can potentiate freezing behavior during presentation of the cued stimulus alone. Previous studies have shown that this fear enhancing effect of serotonin is localized to the BNST and is 5HT2c-R dependent, implicating CRF neurons as a potential neural substrate. Using a Designer Receptor Exclusively Activated by Designer Drug (DREADD) approach, we stereotaxically injected a viral DIO-hM4D-mCherry construct into the BNST of CRF-cre mice, which restricted expression of this Gi-coupled receptor to CRF neurons. Peripheral injection of clozapine N-oxide (CNO, 3 mg/kg) will then selectively silence CRF neurons within the BNST that express the DREADD construct. Our results show CNO injection in DREADD-expressing mice blocks the effect of fluoxetine on fear memory.

Additional data from our laboratory indicates that withdrawal from chronic intermittent ethanol (CIE) induces anxiety-like behavior on the social interaction test in a 5HT2c-R dependent manner. Furthermore, bath application of 5HT2c-R antagonists in slice blocks CIE-induced enhancement of excitability in the vBNST. Taken together, these converging lines of evidence suggest that serotonin release in the BNST, which can be triggered by stress or withdrawal from drugs of abuse, can activate neural circuits governing learned fear and anxiety behavior.

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FAST MEASUREMENTS OF SEROTONIN IN BRAIN: IMPLICATIONS FOR PSYCHIATRIC DISEASE AND DRUG ABUSE

Serotonin Transporter Function in Lymphoblast Cell Lines

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Introduction: Due to challenges associated with obtaining and studying human brain tissue, we are exploring blood cells as peripheral surrogates of central nervous system serotonin transporter (SERT) function. Platelets are known to express high levels of SERT. Lymphoblast cell lines (LCLs) are prepared from B-lymphocytes that have been transformed with Epstein- Barr virus. Immortalized LCLs can be viably frozen and grown in culture. Furthermore, LCLs provide a nearly infinite supply of genetic material and preserve rare genotypes. Like primary lymphocytes, LCLs have been reported to express functional SERT.

Methods: We used chronoamperometry to quantify serotonin (native substrate) uptake with high temporal resolution. Uptake of the non-native fluorescent transporter substrate, APP+ (IDT307; Beikmann et al., *ACS Chem Neurosci*, 2013), by flow cytometry was used to resolve uptake on a cell-by-cell basis. We compared SERT function in LCLs to non-native SERTtransfected HEK293 cells (HEK-SERT) and native SERT expressing platelets and lymphocytes.

Results: Addition of serotonin (500 nM) to LCL solutions produced no changes in current with respect to time measured by chronoamperometry. Increasing the numbers of lymphoblasts from 10 million to 25 million cells/ sample did not result in measurable serotonin uptake. To investigate SERT function by flow cytometry, we incubated LCLs with APP+ in the absence or presence of 1 μM paroxetine (PRX), clomipramine, or citalopram. We observed a small (10%) decrease in APP+ fluorescence in the presence of SERT inhibitors. By contrast, in platelets, >90% inhibition of APP+ fluorescence occurred in the presence of 100 nM PRX. Lymphoblasts had measurable but low SERT mRNA levels compared to SERT-transfected HEK cells determined by RT-qPCR. Since LCLs appeared to express small amounts of SERT, we investigated genotype-related SERT function. No 5-HTTLPR- or I425V-associated differences were detected in SERT-specific uptake of APP+.

Given that only 10% of LCL uptake of APP+ uptake was blocked by SERT inhibitors, we investigated contributions from other transporters. No significant reductions in APP+-associated fluorescence were observed in the presence of DAT or NET inhibitors. Moreover, a high concentration of dopamine (DA) failed to inhibit APP+ uptake by LCLs. Results of further experiments suggested that APP+ is not a substrate for organic cation or cation/carnitine transporters (OCTs or OCTNs), which are expressed by LCLs. Finally, we used the biotinylated SERT ligand IDT318 to measure surface SERT expression. Like APP+, IDT318 binds to surface SERT but is not transported. Streptavidin-conjugated quantum-dots were coupled to bound biotinylated IDT318 to detect surface SERT with single-molecule resolution. HEK-SERT cells showed significantly greater surface fluorescence compared to non-transfected HEK293 cells, primary lymphocytes, and LCLs by flow cytometry.

Conclusions: We conclude that LCLs express small amounts of SERT and possess measurable but low SERT function, particularly compared to platelets and transfected SERTHEK cells. Similar to primary lymphocytes, non-SERT-mediated transport of APP+ occurs via an unidentified mechanism that is not associated with DAT, NET, OCTN1, OCTN2, or the OCTs. Genotype-related differences in SERT function were not differentiated using APP+ and flow cytometry, possibly because SERT-specific uptake in LCLs is minimal. Thus, while LCLs are useful genetic repositories, they appear less-than-ideal for studies aimed at investigating human SERT function.

Serotonin, fear and the amygdala: the challenge of studying serotonin in a region receiving mixed monoamine innervation.

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Fast-scan cyclic voltammetry (FCV) enables measurement of monoamine release with high spatial and temporal resolution. FCV measurement of dopamine (DA) has greatly advanced our understanding of DA release dynamics, regulation and DAergic behavioural control. By contrast, FCV study of serotonin has proved more challenging, despite a well-recognised need and much work toward this goal (Jennings, 2013 ACS Chem Neurosci 4:704-14).

One significant challenge facing FCV measurement of serotonin is that of isolating a serotonin signal in regions that also receive catecholaminergic innervation, such as the amygdala. Serotonin function in the amygdala is of much interest because reduced serotonin transporter (SERT) availability, as well as genetic variants that drive reduced SERT expression (Heils *et al*, 1996 J Neurochem 66:2621-4), have been linked to enhanced amygdala activity (Rhodes *et al*, 2007 J Neurosci 27:9233-7; Hariri *et al*, 2002 Science 297:400-3 but see Murphy *et al*, 2013 Mol Psychiatry 18:512-20). Altered amygdala activity might therefore underpin reported genotype-phenotype associations between SERT polymorphisms and psychiatric disorders and predisposing personality traits. At present, such associations are correlative and remain controversial; understanding the neurobiological underpinnings would be a significant advance. Supporting a causal relationship between SERT availability, amygdala activity and behaviour, we recently demonstrated that mice with increased SERT expression (SERT OE) show impaired fear learning along with decreased amygdala hemodynamic responses and oscillatory activity (Barkus et al 2013 Biol Psychiatry *epub*). Here, we set out to explore the neurobiological mechanisms driving this effect. Given that SERT OE mice show reduced tissue and extracellular levels of serotonin in several regions (Jennings *et al*, 2006 J Neurosci 26:8955-64, Jennings *et al*, 2010 J Neurochem 115:965-73), we hypothesized that reduced amygdala serotonin may lead to altered network activity in this region. To test this hypothesis, a strategy to isolate the FCV serotonin signal in amygdala was needed, as the region receives a substantial DA innervation (Asan *et al*, 2013 Histochem Cell Biol 139:785-813).

As a starting point, optogenetic strategies were chosen to selectively evoke serotonin in acute slices containing amygdala and negate the problem of a mixed monoamine signal. However, we experienced difficulty in establishing virally-mediated expression of *ChR2* and in evoking detectable serotonin release in mice with constitutive, *TpH2*-driven *ChR2* expression. We next explored whether distinct anatomical loci in the amygdala demonstrated a serotonin-specific signal and could be reliably identified. However, the majority of sites examined showed evidence of a mixed monoaminergic signal. Finally, we used a pharmacological approach to isolate the serotonin component of the mixed signal. Application of the D2 agonist, quinpirole, significantly reduced the magnitude of electrically evoked monoamine signals, suggesting a DAergic component to the signal that could be supressed by autoreceptor activation (Bull *et al* 1991 Neurosci Lett 134:41-4). The remaining signal was significantly elevated by the serotonin uptake blocker citalopram (300 nM) to a varying degree across sites, confirming the presence of serotonin in the remaining signal. Although the DA uptake blocker nomifensine (250 nM) caused a small elevation in signal in some sites, this effect was not significant, suggesting a minimal residual DA component of the signal. Importantly, the monoamine signal detected under drug-free conditions was not smaller in SERT OE mice versus wildtype littermates (WT). Nor was the signal after quinpirole application any smaller in SERT OE than WT mice. Collectively, our data currently suggest that SERT overexpression causes minimal dysregulation of amygdala monoamine transmission in adulthood. Interestingly, and in contrast to our published findings in other brain regions, our data give no indication that serotonin levels are reduced in the amygdala of SERT OE mice. In light of this unexpected result, current experiments are underway to validate that this method can reliably detect reductions in amygdala 5-HT.

How much extracellular serotonin matters: Deconvoluting serotonin clearance kinetics in vivo.

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Serotonin is a key neurotransmitter regulating mood. Dysfunction of serotonergic neurotransmission is linked to numerous psychiatric disorders. The strength and duration of serotonin neurotransmission is thought to be largely controlled by high-affinity uptake of serotonin via the serotonin transporter (SERT). Not surprisingly then, disorders associated with serotonin hypofunction are most commonly treated with drugs that block SERT (e.g. selective serotonin reuptake inhibitors (SSRI)). However, a major challenge in treating psychiatric disorders, in particular depression, is that most patients are not effectively treated with SSRIs. Helping to resolve this puzzle, emerging evidence points to a previously unsuspected role for "non-SERT" membrane transporters in serotonin uptake. These transporters may serve to buffer the ability of SSRIs to bolster extracellular serotonin to therapeutically relevant levels. It is now clear that norepinephrine (NET), dopamine (DAT), organic cation (OCT) and plasma membrane monoamine (PMAT) transporters are among the transporters that can take up serotonin. Here we apply high-speed chronoamperometry, in vivo, to better understand conditions where these transporters become engaged in serotonin clearance from extracellular fluid in brain. High-speed chronoamperometry is an electrochemical technique that has high temporal and spatial resolution, affording sub-second measurement of serotonin clearance in discrete regions of brain. Coupled to pressure-ejection of exogenous serotonin, these qualities allow us to quantify the kinetics of serotonin clearance, without an associate "release" component.

Using a combination of pharmacologic, genetic and neurotoxic lesion approaches, we found the ability of blockers selective for SERT, NET, DAT as well as OCTs/PMAT to inhibit serotonin clearance in the brain was highly dependent on extracellular concentration of serotonin as well as expression of SERT relative to other transporters. Using 5,7-dihydroxytryptamine (5,7-DHT) or 6-hydroxytrypamine (6-OHDA) to destroy serotonergic and noradrenergic neurons, respectively, we showed substantial uptake of serotonin by NET in hippocampus. Similarly, studies in SERT heterozygous and knockout (KO) mice revealed significant clearance of serotonin from hippocampus via decynium-22-sensitive transporters (OCTs/PMAT). In addition, pharmacologic blockade of DAT in striatum inhibited uptake of serotonin, suggesting that a significant fraction of serotonin clearance in this brain region is regulated by DAT.

Of note, SSRIs failed to inhibit serotonin clearance once extracellular concentrations rose to levels of around 2 micromolar, a concentration that is below the Km value for serotonin clearance as determined in vivo. In contrast, blockers of NET, DAT and OCTs/PMAT robustly inhibited serotonin clearance when extracellular concentrations were in this range. These data add mechanistic support to clinical data suggesting that triple SERT, NET and DAT blockers have greater therapeutic efficacy in the treatment of depression than either SSRIs, or dual SERT-NET blockers. In addition, they reveal OCTs/PMAT as new targets for the development of novel antidepressant drugs with improved therapeutic efficacy.

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Identifying subsets of serotonergic neurons that selectively modulate aggressive and social behaviors in the mouse

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Central serotonin-producing neurons are heterogeneous – differing in embryonic origin, location in the adult brain, morphology, electrophysiological properties, and association with clinical disorders – but the underpinnings and functional implications of this heterogeneity are just beginning to be explored. To resolve the relevance of this cellular diversity, we have generated and employed intersectional genetic tools that allow multiple neuronal features to be measured and linked with physiology and/or behavior.

Here, we are interested in delineating those serotonergic neurons contributory to aggressive behaviors. We reveal that aggressive and social behaviors in male mice are modulated by two small subsets of serotonergic neurons. Suppressing vesicular neurotransmission from, and thus silencing chemically, one or the other of these subsets escalates aggression. Axonal projection mapping, via a novel synaptophysin-GFP allele, revealed that these serotonergic neuron subtypes innervate distinct and partially overlapping brain regions, including limbic and auditory centers. This work identifies subtypes of serotonergic neurons that modulate social behaviors, defines these neurons molecularly and hodologically, and provides genetic access for further mechanistic study. This work shows more broadly that patterned gene expression within the serotonergic system can reflect a functional and cellular modularity with potential for therapeutic selectivity

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MAURICE RAPPORT PLENARY LECTURE

Serotonin and Behaviour – What is the link?

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The English poet Philip Larkin (1922-1985) started his poem Annus Mirabilis with the famous line *''Sexual intercourse began in nineteen sixty three (which was rather late for me)"* which is the year I went to University and discovered the existence of serotonin (5-HT); could there be a link between sex and 5-HT? By 1966 I was a postgraduate student looking for dopamine and 5-HT neurones in the cerebral ganglia (brains) of leeches and snails using fluorescence histochemistry and helped by a compound called p-chlorophenylalanine (p-CPA) to deplete 5-HT. A little later a study at Babraham, just outside Cambridge, showed p-CPA increased mounting in male rats. What was the link between this behaviour and 5-HT and indeed between all the other emerging possible links between 5-HT and behaviour – most forcibly demonstrated by antidepressant drugs that blocked 5-HT re-uptake and improved mood? How could such links be demonstrated in animal models? It had been drummed into me that demonstrating Ca^{++} release of a putative neurotransmitter was one of the key criteria for establishing a substance as a transmitter. What was wanted now was to show the dependence of a particular behavioural or drug response on release of a specific neurotransmitter. This objective remained my major research concern from then onwards – an objective that took me almost around the world in search of technologies, ideas and above all fantastic collaborators. A key step was to find a highly sensitive and very specific analytical method to measure 5-HT. I first meet Ralph Adams, a brilliant and imaginative electrochemist with a fascination for the brain, in 1977 in a wine bar close to The Institute of Neurology in London**.** Within months myself, Lucilla and our two young sons found ourselves in Lawrence, Kansas – Ok it's not NY, Washington or San Francisco but the science was great as were the people. I returned to England, not to London but to start a new post at The University of Nottingham, the proud owner of an electrochemical detector and a "black box" for *in vivo* chronoamperometry. The next few years were a research challenge as ascorbic and uric acid got in the way of amine voltammetry while Prime Minister Margaret Thatcher believed the only good science led to immediate financial gain leading to the rise in molecular biology. Luckily we were protected by long-tem Wellcome Trust funding, the immediate research impact of HPLC with electrochemical detection to measure amines and some outstanding PhD students and postdocs. One, Trevor Sharp, went to a meeting in Holland, met Tyra Zettersrom from the Karolinska Institute and the link between our HPLC method and Urban Ungerstedt's microdialysis probe was born (Zetterstrom et al 1983, J..Neurochem. **41**, 1769-73) and soon we were using both voltammetry and dialysis alongside each other (Sharp et al 1984, Neuroscience **12**, 1213-21). The initial work was all in anaesthetised animals and it took longer to convert the microdialysis method to measure extracellular 5-HT in freely moving rats. There was problem as all the initial studies were with dopamine, there was so much of it in the striatum making it so easy to measure, but 5-HT was a different matter as the levels were lower and the assay less sensitive because it was retained by the column for longer. It was not until 1991 that PhD student Ian Wright finally cracked it and measured an increase in ventral hippocampal extracellular 5-HT when rats were placed on the elevated plus maze (Wright et al 1992, Psychopharmacology **109**, 336-46). At last we had moved from drug induced changes in 5-HT to behaviour induced changes and we all could really begin to answer important questions including the one posed at the start of this abstract. These questions will be the subject of my talk .

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Brain serotonin deficiency: physiological and behavioral analysis of a *Tph2*-knockout rat

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Tryptophan hydroxylase 2 (TPH2) is the rate limiting enzyme of serotonin-synthesis in the brain. We have previously shown that in mice genetic deletion of *Tph2* is correlated with growth retardation, increased mortality, and multiple alterations in autonomic functions. Moreover, these mice exhibit exaggerated response to serotonin receptor agonists and show signs of sensitization of GABAergic system. These mice also show increased aggression, loss of aversive and anxiety-like behavior, whereas the evaluation of depression-like phenotype revealed controversial results in different tests, such as tail-suspension and forced swim test.

However, further analysis of this model was hampered by poor ability of the mouse for complex social interactions and learning capacity. The rat is the preferred animal model for the analysis of cardiovascular and behavioral phenotypes and results in this species can be more reliably transferred to the human situation than mouse data. Therefore, we created 2 different rat models: a doxycycline-inducible *Tph2*-shRNA knockdown (TetO-shTPH2) and a complete *Tph2*-knockout (*Tph2*$^{-/-}$) generated via a zinc-finger-nuclease (ZFN) based gene-deletion.

Two weeks-long Dox-treatment of TetO-shTPH2 rats resulted in downregulation of TPH2 expression by half at both, mRNA and protein level. However, serotonin content in the brain were decreased only by 20% in different brain areas of TetO-shTPH2 rat.

In contrast, analysis of serotonin content in *Tph2*$^{-/-}$ rats revealed no detectable levels of the monoamine in the brain, but unchanged levels in the blood, confirming strict duality of serotonin system. *Tph2*$^{-/-}$ rats were born in a Mendelian ratio, but exhibited growth retardation during first postnatal weeks, similar to *Tph2*$^{-/-}$ mouse. We tested *Tph2*$^{-/-}$ females for their hedonic behavior and analyzed cardiovascular and behavioral parameters in different stress-situations, i.e. handling, mating, open field test and positive conditioning, and, thereby, provide an initial cardiovascular and behavioral phenotyping of the first rat model lacking serotonin in the brain.

P 02

SEROTONIN AND DOPAMINE RELEASE AND REUPTAKE BY FAST MICRODIALYSIS

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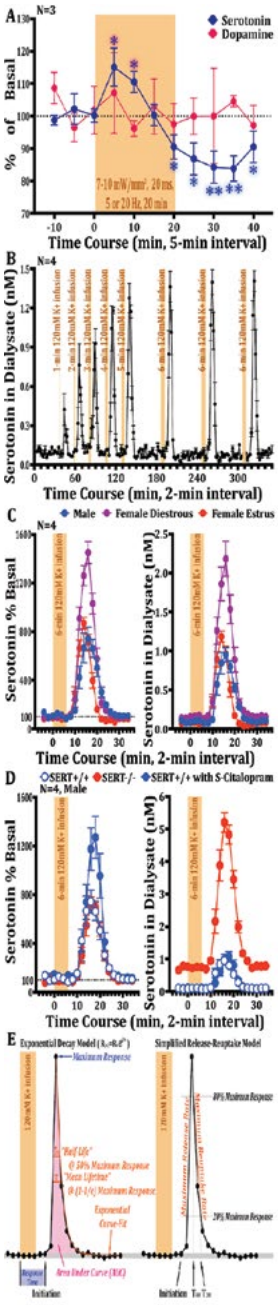

Fast microdialysis is unique in its ability to detect near real-time basal and stimulated chemical neurotransmission involving multiple neurotransmitters in the intact brains of freely behaving subjects. By optimizing chromatographic separation and electrochemical detection conditions, we recently established online "fast" microdialysis methods that enable concurrent detection of rapid changes in serotonin and dopamine in dialysate samples from awake mice with a sampling rate of 1-3 min using commercial HPLC instrumentation. This dramatic increase in temporal resolution facilitated the observation of transient and subtle changes in serotonin neurotransmission associated with stress and circadian rhythm that were previously unobserved [1].

Here, we show how fast microdialysis can be used to differentiate release and reuptake kinetics during transient release events. Changes in serotonin and dopamine levels were measured in the ventral striatum using optical stimulation in the dorsal raphe of Tph2-EYFP-ChR2 mice. Channel rhodopsin-2 was selectively expressed in sertononergic neurons and stimulated via exposure to blue light. In separate studies, we used reverse dialysis to deliver short pulses of aCSF containing 120 mM K^+ into the ventral striatum. We investigated various stimulation durations and effects with respect to sex, estrous phase, and serotonin transporter (SERT) expression levels.

Optical stimulation of dorsal raphe serotonergic neurons in awake mice caused a significant increase in serotonin but not dopamine in the ventral striatum during the early part of the 20-min stimulation period (Panel A). This was followed by a significant decrease in serotonin levels. This pattern was not altered by pretreatment with 5-HT1A or 5-HT1B antagonists. For chemical stimulation, we varied the length and frequency of high K^+ pulses. In doing so, the amplitude, duration, and initiation thresholds of release events were manipulated (Panel B). Using this paradigm, we elucidated significantly different release and reuptake patterns between male and female wildtype mice, and between female mice in diestrous vs. estrous phases (Panel C). Furthermore, we observed differences between male SERT+/+ and SERT-/- mice that were not reproduced by acute perfusion of S-citalopram in wildtype mice (Panel D). We are currently developing algorithms to quantify response times, half times, mean lifetimes, maximal release, and reuptake rates (Panel E). Moreover, we are using fast microdialysis to investigate encoding of emotionally salient stimuli by serotonin and dopamine.

1. Yang H, Thompson AB, McIntosh BJ, Altieri SC, Andrews AM. (2013) Physiologically relevant changes in serotonin resolved by fast microdialysis. ACS Chem. Neurosci. 4(5):790-798. PMCID: PMC3656759.

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P 03

Negative Allosteric Modulation of the Human 5-HT_3A Receptor; Strategy for the Treatment of Irritable Bowel Syndrome

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Allosteric modulation of receptors is seen increasingly as an attractive therapeutic approach as it provides a mechanism to modulate receptor activity whilst relying on receptor activation by the endogenous ligand, thereby retaining physiological control of receptor function.

We have recently reported the identification of a relatively potent positive allosteric modulator (PAM), 5-Cl-indole, that displays selectivity for the human 5-HT_3A receptor over other Cys-loop LGICs such as the human α-7 nAChR (Newman et al., 2013). The allosteric binding site of this receptor is a potential molecular target for the treatment of irritable bowel syndrome although negative allosteric modulators (NAMs) rather than PAMs are more likely to offer therapeutic benefit with a reduced risk of inducing nausea. We have identified a series of halogenated indole derivatives as potential allosteric modulators of the 5-HT_3A receptor with the objective to identify novel drugs that act as NAMs of the 5-HT_3 receptor. Systematic modification of the core molecule has led to the identification of 24 additional molecules with PAM activity and importantly, one that acts as a NAM in functional assays using human recombinant 5-HT_3 receptor.

In addition, we have screened a series of unrelated novel diketopiperazine-based compounds and have identified several molecules that inhibit h5-HT_3 receptor function but lack affinity for the orthosteric binding site on the h5-HT_3A receptor (i.e. potential NAMs). Our systematic approach has identified key features of the structure-activity relationship of these compounds, and we have synthesized three molecules – NMB7-37B, NMB7-83 and NMB7-89 – which display NAM-like activity in functional assays using human recombinant 5-HT_3A receptor.

These complementary approaches may lead to the identification of novel potential therapeutics, which would be predicted to dampen excessive 5-HT_3 receptor signaling without complete blockade even at supra-maximal concentrations and hence retain some physiological control of receptor function. It is anticipated that this pharmacological strategy will retain clinical efficacy in patients with diarrhoea-predominant irritable bowel syndrome with a reduced risk of constipation and ischemic colitis.

P 04

Development of the normal physiology of serotonin neurons and the 5-HT_{1A} receptor-effector complex.

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Trauma during early life is a major risk factor for the development of anxiety disorders and suggests that the developing brain may be particularly sensitive to perturbation. Increased vulnerability most likely involves altering neural circuits involved in emotional regulation. The role of serotonin in emotional regulation is well established, but little is known about the postnatal development of the raphe where serotonin is made. Using whole cell patch clamp recording and immunohistochemistry, we tested whether serotonin circuitry in the dorsal and median raphe was functionally mature during the first three postnatal weeks in mice. Serotonin neurons at postnatal day four were hyper-excitable. The increased excitability was due to depolarized resting membrane potential, increased resistance, increased firing rate, lack of 5-HT_{1A} autoreceptor response, and lack of GABA synaptic activity. Over the next two weeks membrane resistance decreased and resting membrane potential hyperpolarized due in part to potassium current activation. The 5-HT_{1A} autoreceptor-mediated inhibition did not develop until P21, even though the effector complex was fully functional. The frequency of spontaneous inhibitory and excitatory events increased as neurons extended and refined their dendritic arbor. Serotonin co-localized with vGlut3 at P4 as in adulthood suggesting enhanced release of glutamate alongside enhanced serotonin release. Because serotonin affects circuit development in other brain regions, altering the developmental trajectory of serotonin neuron excitability and release could have many downstream consequences. We conclude that serotonin neuron structure and function change substantially during the first three weeks of life during which external stressors could potentially alter circuit formation.

P 05

The Genetic and Epigenetic Aetiology of Anxiety Proneness

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Anxiety disorders are among the most prevalent psychiatric disorders among both adults and adolescents. Comorbidity with other psychiatric disorders and other anxiety disorders is common and impose a high burden of distress and daily impairment. The aetiology of anxiety disorders is multifactorial, comprising proximal and distal aetiological factors that interact to culminate in illness. Individual trait characteristics, such as anxiety sensitivity (AS) and trait anxiety (TA), have been found to possess predictive potential for a number of anxiety disorders, and individuals with high levels of both TA and AS are classified as anxiety prone (AP). Genetic factors have been found to play a role in the development of both AS and TA; and certain genetic variants have been found to interact with the degree of childhood trauma to mediate increased anxiety proneness, and subsequently increase the risk for the development of anxiety disorders. Several genes have been implicated in the genetic aetiology of anxiety proneness, including the serotonin transporter gene (*5HTT*) which has a 43bp deletion/insertion polymorphism (*5HTTLPR*) ,Brain Derived Neurotrophic Factor (*BDNF)*, Neuropeptide Y Receptor (*NPYR)*,Neuropeptide S receptor (*NPSR)* and FK506 binding protein 5 (*FKBP5)*. This study aims to investigate the interaction between childhood trauma and *5HTTLPR* in mediating increased levels of anxiety proneness. Adolescents (13y-18y) have been screened by a research psychologist to determine levels of self-reported childhood maltreatment/trauma and anxiety-proneness and anxiety sensitivity. Saliva was obtained from eligible participants, and the DNA from 978 samples was extracted using the PrepIT-L2P (DNAgenotek) protocol. *5HTTLPR* and rs25531 were genotyped using a two-step, phase-certain genotyping protocol. This research will contribute to existing data utilizing a much larger sample size thereby strengthening statistical power. Furthermore, genotype data will help to elucidate possible new genetic links to anxiety disorders.

P 06

Impact of 5-HT_{2A} and 5-HT_{1A} receptors on the murine serotonin syndrome

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The serotonin (5-HT) syndrome in man is a side-effect of drugs in over-dose that increase the synaptic 5-HT concentration or directly activate specific 5-HT receptors. It is characterized by a triad of signs including mental state alterations, neuromuscular excitation, and autonomic dysregulation. In mice, a set of behavioural and autonomic responses can be induced by the same serotonergic drugs as used in man.

The role of the 5-HT_{1A} receptor for the 5-HT syndrome in mice has been extensively studied and responses like backward walking, flat body posture, forepaw treading, head weaving, hind limb abduction, Straub tail, tremor and hypothermia have been attributed to 5-HT_{1A} receptor activation. The role of the 5-HT_{2A} receptor is less clear, and it has been mainly restricted to the induction of head twitches and hypothermia.

Hence, the aim of the present study was to define and differentiate the impact of the 5-HT_{2A} and the 5-HT_{1A} receptor for the different 5-HT responses. To that end, the effects of the full 5-HT_{1A} receptor agonist 8-OH-DPAT and the partial 5-HT_{1A} agonist buspirone as well as the 5-HT_{2A} receptor agonist TCB-2 were investigated in male NMRI mice. The effects of the three compounds were compared with the effects induced by 5-HTP which increases the extracellular 5-HT content and therefore activates all 5-HT receptor subtypes.

In our study flat body posture, hind limb abduction, Straub tail, tremor, piloerection and decreased rearing were observed following the administration of 8-OH-DPAT. A similar set of responses were seen after treatment with buspirone. However, the Straub tail response did not occur, probably due to the lower efficacy of buspirone at postsynaptic 5-HT_{1A} receptors. As expected TCB-2 induced head twitches, but we also observed flat body posture, hindlimb abduction, and piloerection, as well decreased numbers of rearings and defecation boli. To our knowledge such a broad spectrum of responses has not been previously demonstrated in mice after the activation of 5-HT_{2A} receptors. Apart from the Straub tail response all previously described responses were induced by 5-HTP plus fore paw treading and hunched back.

In summary, the Straub tail response seems to be a specific sign for 5-HT_{1A} receptor activation in NMRI mice. In addition, we demonstrated that the 5-HT_{2A} receptor has more impact on the 5-HT syndrome than suggested. By inducing the broadest spectrum of signs, 5-HTP seems to be the most suitable compound as a positive control when investigating the 5-HT syndrome in mice.

Robert Haberzettl received financial support from the Elsa-Neumann-Doctoral-Fellowship from the Land Berlin. We also want to thank M. Fox for her technical advice.

P 07

Desensitization of ventricular 5·HT_4 receptors

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Background: In the human heart, serotonin (5-HT) exerts inotropic and chronotropic effects via 5-HT_4 receptors. Signaling includes at least in part stimulation of adenylate cyclases. The murine receptors are not functional in wild type (WT) mice. Hence, we generated transgenic mice (TG) with cardiac specific expression of the human 5-HT_{4a} receptor coupled to a His-tag for transgene detection by immunohistochemistry. We have previously shown that this receptor responds to exogenous 5-HT with inotropic and chronotropic effects in isolated atrial cardiac preparations. Furthermore, we could show that in TG, the inotropic effects in left atrial preparations and the chronotropic effects in right atrial preparations to 5-HT could be desensitized. Next we wanted to know whether desensitization occurs in the ventricle.

Methods and results: Using a Vew2100 echocardiographic system, anaesthetized (2% isoflurane) WT and TG mice were studied. Ejection fraction (EF) amounted to 57.5% before and 68% ($p<0.05$, N=8) after intraperitoneal injection of 100µ1 1mM 5-HT. TG were desensitized using 100µl 100mM 5-HT which increased EF to 82%. About 40 min thereafter, 100µ1 100mM 5-HT failed to increase EF. In isolated perfused TG hearts (Langendorff system), 10µM 5-HT increased force of contraction (F) to 164±18% compared to WT. A high concentration of 5-HT (100µM) for 30min was used to desensitize the receptors and increased F to 153±34%. After washout, 10µM 5-HT increased F only to 106±13%. lsoprenaline (1µM) increased F to 135%. In WT, 5-HT was ineffective whereas isoprenaline increased F to 224±24% which is more than in TG. Similar data were obtained for effects on maximum rate of tension development.

Conclusions: These data indicate that in accordance with previous data, 5-HT cannot only desensitize atrial but also ventricular receptors in vitro and in vi'IO. In atrial preparations partial heterologous desensitization to B-adrenoceptors was noted, evidence for the inwlvement of G-protein coupled receptor kinases were obtained. It remains to be elucidated whether similar pathways are active in ventricular desensitization. Clinically, our model might simulate conditions of persistent high 5-HT levels like some carcinoid tumours which continuously secrete 5-HT and are expected to desensitize human 5-HT_4 receptors in the heart.

P 08

Comparing the expressional patterns of placental magnesium/phosphorus transporting channels between healthy and preeclampsia pregnancies

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Background: Preeclampsia is a pregnancy-specific disorder characterized by de novo development of concurrent hypertension, proteinuria, and placental oxidative stress. During pregnancy, mineral (such as magnesium, inorganic phosphate) homeostasis are essential for fetal development and pregnant women's health. At the last trimester of gestation, maternal-to-fetal transport of minerals is dramatically increased and tightly mediated by ion channels. The ion channels of magnesium, TRPM6, TRPM7, are highly permeable to various divalent cations such as Ca^{2+}, Mg^{2+}, and Zn^{2+}. PIT-1 and PIT-2, and plays a fundamental housekeeping role in phosphate transport such as absorbing phosphate ions from interstitial fluid for cellular functions. However, the regulations of magnesium/ inorganic phosphorus transport channels in the placenta are incompletely understood.

Methods: We examined the expression and regulation of magnesium/inorganic phosphorus channels (MPCs) in the placenta of pregnant women suffering from preeclampsia and in human placental primary cells subjected to oxidative stress. Duodenal- and renal MPCs mRNA and protein expression were examined using real-time PCR and Western blot analysis. The localization of MPCs was investigated by Immunohistochemistry.

Results: The levels of MPCs (TRPM6, TRPM7, PIT-1, and PIT-2) were down-regulated in the preeclampsia placenta tissues at pre-term labor. In parallel with this down-regulation, the expression of MPCs at term labor was also down-regulated but TRPM7 expression in the central placenta and PIT-2 expression in whole placenta was unchanged or up-regulated. In addition, MPCs expression in the placental primary cells was reduced in hypoxia conditions. The spatial expression of TRPM6, TRPM7, and PIT-1 was predominantly detected in the syncytiotrophoblast layers of the placenta. In contrast, PIT-2 was abundantly expressed in the connective tissues of placental intra-villous.

Conclusion: Taken together, our findings indicated that placental MPCs are down-regulated in preeclampsia and hypoxia conditions, which may contribute to a better understanding of the interrelationship between magnesium / inorganic phosphorus imbalances and preeclampsia during pregnancy.

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P 09

Impact of 5-HT_{2A} and 5-HT_{1A} receptors on the murine serotonin syndrome

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In summary, the Straub tail response seems to be a specific sign for 5-HT_{1A} receptor activation in NMRI mice. In addition, we demonstrated that the 5-HT_{2A} receptor has more impact on the 5-HT syndrome than suggested. By inducing the broadest spectrum of signs, 5-HTP seems to be the most suitable compound as a positive control when investigating the 5-HT syndrome in mice.

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P 10

Characterization of decynium-22 analogs in the inhibition of plasma membrane monoamine and organic cation transporter functions: Novel targets for the development of new antidepressant drugs

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Dopamine (DA), serotonin (5-HT), and norepinephrine (NE) mediate an array of brain behaviors and physiological functions. Extracellular levels of these neurotransmitters are tightly regulated by reuptake mechanisms via transporters of two varieties – a high-affinity, low-capacity (uptake-1) and low-affinity, high-capacity (uptake-2). Increased extracellular neurotransmitter levels are achieved from the inhibition of uptake-1 transporters, either by abused psychostimulants (e.g. cocaine) or therapeutic agents (e.g. antidepressant reuptake blockers). The uptake-2 system in the brain is less studied; however, it is rapidly gaining recognition for its contribution to neurotransmitter clearance that can negatively impact drug abuse liability and therapeutic benefits. Investigations of uptake-2 transporters, which include the plasma membrane monoamine transporter (PMAT) and organic cation transporters (OCTs), are limited by lack of selective ligands. To date, the only commercially available uptake-2 inhibitor is decynium-22 (D-22), a non-selective antagonist. In efforts to overcome this limitation, we have partnered with chemists to evaluate the pharmacological properties of novel D-22 analogs with the goal of finding ligands selective for each of these uptake-2 transporters. The compounds were initially screened in mice for their effect on locomotor activity and potential antidepressant like activity in the tail suspension test. These analogs display a wide range of potencies to inhibit MPP^+ uptake into hippocampal synaptosomal preparations, with some having greater potency in comparison to the parent D-22 compound. Here, we continue characterization of the analogs using a combination of synaptosome preparations and transiently transfected heterologous cells. We assess the PMAT/OCT binding affinity of the analogs (IC_{50} and K_i values) via displacement of [^{3}H]D-22 and evaluate D-22 ligands in the competition of [^{3}H]5-HT uptake in comparison with classic serotonin reuptake inhibitors. With these studies we will learn more about the pharmacological profile of these novel compounds for their therapeutic potential in the treatment of depression and drug abuse.

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P 11

Serotonin transporter gene variants are associated with increased risk of suicide in an HIV-positive Ugandan population

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The HIV epidemic has particularly devastated sub-Saharan Africa, which, despite having just over 10% of the world's population, is home to 67% of all people living with HIV. In Uganda, one of the countries hardest hit by the epidemic, an estimated 6.4% of adults nationwide are living with the HIV infection. The increasing access to highly effective antiretroviral therapy (ART) for HIV-positive individuals has delayed HIV disease progression and prolonged survival. Recent focus has therefore been on the issues of quality of life of these individuals, including mental and emotional wellbeing.

HIV/AIDS is associated with a considerable burden of major depressive disorder (MDD). Notably, it has been found that depression is one of the foremost mental health disorders representing a barrier to ART adherence, which is subsequently associated with poorer outcome. Suicide is a particular concern in the context of MDD, and MDD has been found to be highly predictive of suicidal ideation in HIV-positive individuals. In addition, lifetime suicidal ideation has been found to be increased amongst individuals with HIV. There is growing evidence suggesting that genes play an important role in the predisposition to suicidal behaviour, as indicated by genetic and epidemiological studies. Genes in the serotonergic system have garnered special interest in this area, because disturbances in serotonin transmission are the most frequently reported neurobiological substrates associated with suicidal behaviour.

The aim of the present study was to investigate whether gene variants within the gene encoding serotonin transporter (5-HTT) were associated with suicidality in a Ugandan population with HIV/AIDS.

Five hundred and sixty-nine HIV-infected Ugandan participants were included in this study. Suicidality and comorbid psychiatric disorders were assessed using the Mini International Neuropsychiatric Interview (M.I.N.I). Risk of suicide was defined as being none-to-low, moderate or high, according to the suicidality module of the M.I.N.I.

Three functional variants were investigated. An insertion-deletion polymorphism and single nucleotide polymorphism, rs25531, which are both located within the 5-HTT-linked polymorphic region (*5-HTTLPR*) were analysed as a triallelic polymorphism, based on results from previous publications. Here, the L_A variant has been found to be associated with an increased expression of 5-HTT, whilst the L_G and *S* alleles have been found to be associated with a reduced expression of *5-HTT*. A variable number of tandem repeats polymorphism (VNTR) in intron 2 of the gene (*STin2*) was also investigated.

We used mixed-effects logistic regression to analyse for suicidality susceptibility. These models enabled us control for confounders such as age and gender as well as other socio-demographics, psychiatric co-morbidity and clinical variables as fixed effects; and to correct for the variability caused by the different recruiting centres as random effects.

The higher-expressing L_A-allele was found to be significantly associated with increased risk of committing suicide ($p \leq 0.001$). The *STin2* VNTR 12-repeat allele was also found to be associated with increased risk of committing

suicide (p = 0.046). Haplotype analysis revealed that the L_A-12 allele combination was found to be significantly associated with increased risk of suicide (p = 0.002), compared to the S_A-12 allele combination.

The present study represents a preliminary genetic study, investigating the role that variants in the *5-HTT* gene may play in increasing risk for suicide. The high-*5-HTT* expressing alleles, i.e. L_A and the *STin2.12* were found to represent risk factors for increased risk of suicide in an HIV-positive Ugandan population. The results are interesting in light of previous publications implicating *5-HTT* in suicidal behaviour and suicide-related phenotypes. Understanding the risk factors, including those pertaining to biological risk, for suicidality in HIV/AIDS in the African socio-cultural context is an important step towards understanding locally appropriate interventions for suicidality. Future studies should thus include gene-environment interaction analyses in order to reveal a better understanding of the role of 5-HTT in suicidality.

1 Uganda AIDS Commission, 2011

P 12

RNA Editing-Mediated Regulation of Serotonin 2C Receptor Expression

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Pre-mRNA transcripts encoding the 2C subtype of the serotonin receptor ($5HT_{2C}$) can be differentially edited by RNA-specific adenosine deaminases at five closely spaced positions within exon 5. These A-to-I editing events can generate as many as 24 protein isoforms that differ by up to three amino acids within the predicted second intracellular loop of the receptor, a region essential for G protein coupling. Functionally, the highly edited $5HT_{2C}$ isoforms (e.g. $5HT_{2C\text{-}VSV}$ and $5HT_{2C\text{-}VGV}$) exhibit reduced constitutive activity and altered subcellular localization in comparison to the genomically-encoded isoform ($5HT_{2C\text{-}INI}$). Furthermore, alterations in $5HT_{2C}$ editing have been observed in patients diagnosed with schizophrenia, in suicide victims with a history of major depression, and in response to antidepressant and antipsychotic treatments, suggesting that improper editing of $5HT_{2C}$ transcripts may be a contributing factor in neuropsychiatric illness. Recent work in our lab has shown that genetically modified mice solely expressing the fully edited $5HT_{2C}$ receptor isoform ($5HT_{2C\text{-}VGV}$) produce an anomalous 40- to 70-fold increase in receptor density without a concurrent change in steady-state $5HT_{2C}$ mRNA, indicating that the relative expression of $5HT_{2C}$ protein isoforms is not represented by the steady-state level of edited $5HT_{2C}$ mRNAs. The molecular mechanism(s) underlying this novel disparity between mRNA and protein isoform expression have yet to be elucidated. To confirm whether such a disparity exists between edited $5HT_{2C}$ transcripts and their encoded protein products in wild-type mice, I will use affinity purification methods to isolate $5HT_{2C}$ receptors, followed by a mass spectrometry-based proteomic analysis to quantify the relative expression levels of $5HT_{2C}$ protein isoforms. Due to the low expression of this G-protein coupled receptor in most regions of the mouse brain, I am using a recently developed CRISPR/Cas9n-based approach to generate transgenic mice in which hexahistidine- and *Strep-II*® affinity tags have been "knocked-in" to the endogenous $5HT_{2C}$ locus. Purification efficacy has already been validated in a heterologous system (HEK 293) expressing this affinity-tagged $5\text{-}HT_{2C}$ receptor. Identification of disparities between $5HT_{2C}$ RNA and protein isoform expression will have immediate implications for human studies of disease-related alterations in $5HT_{2C}$ RNA editing, in which inferences about receptor isoform expression and function have been based solely upon edited mRNA distribution profiles. These findings will also imply the existence of a novel post-transcriptional mechanism for regulating $5HT_{2C}$ receptor expression which may be mediated by differences in translation efficiency and/or protein stability among distinct edited isoforms. Subsequent work will focus on identifying the mechanistic basis underlying this disparity between RNA and protein expression.

P 13

Contribution of serotonergic and dopaminergic mechanisms to the physiological and behavioural effects of mephedrone following binge dosing in the rat

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The recreationally used synthetic cathinone derivative 4-methylmethcathinone (mephedrone) has similar psychoactive effects to 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). Both compounds are non-selective substrates for monoamine transporters, stimulating neurotransmitter release and blocking reuptake (Simmler et al. 2012). Mephedrone has a short duration of action and users often ingest repeated doses within a single session, but in the case of MDMA users report tolerance to the desired effects and binge use appears to increase the risk of adverse effects (Parrott, 2005) which include severe hyperthermia. Although the predominant thermoregulatory effect of mephedrone following acute administration in the rat is hypothermia (Shortall et al. 2013; Wright et al. 2012) two studies have reported hyperthermia following binge dosing (Baumann et al. 2012; Hadlock et al. 2011). However, there is little information on the temporal profile of this hyperthermia or its alteration with repeated injections, and behavioural and neurochemical responses to repeat administration of the same dose within a short space of time have yet to be examined.

In the current study male Lister hooded rats (190-300g; Charles River UK) received three i.p. injections of saline vehicle (1ml/kg) or mephedrone HCl (10mg/kg) at 2h intervals, with continual measurement of locomotor activity and core body temperature using radiotelemetry, or extracellular 5-HT and dopamine levels in the striatum using *in vivo* microdialysis. To further examine the contribution of serotonergic and dopaminergic mechanisms to the physiological and behavioural effects of mephedrone, separate rats received bilateral intracerebroventricular injections of 5,7-dihydroxytryptamine (75µg/5µl) or 6-hydroxydopamine (150µg/5µl), to deplete 5-HT or dopamine respectively, 21 days prior to radiotelemetry studies. A final experiment investigated the specific 5-HT receptors which mediated the thermoregulatory effect of mephedrone, by assessing the impact of the 5-HT_{1A} receptor antagonist WAY-100635 (0.5mg/kg i.p.) or 5-HT_7 receptor antagonist SB-258719 (10mg/kg i.p.) on mephedrone-induced alterations in rectal temperature.

Binge-type administration of mephedrone to individually housed rats at normal ambient temperature (20-21ºC) caused a rapid onset of hyperactivity, hypothermia, and elevated striatal dopamine efflux, the magnitude of which was similar on each occasion and returned to vehicle control levels between injections. Mephedrone-induced hyperactivity was markedly attenuated and hypothermia completely abolished by prior 5-HT depletion (-35% from control in striatum and -58% in hypothalamus), and mephedrone-induced decreases in rectal temperature were also blocked by 5-HT_{1A} (but not 5-HT_7) receptor antagonism. In contrast mephedrone-induced hyperactivity was slightly enhanced but hypothermia unaffected by dopamine depletion (-48% in striatum, but -15% dopamine and -50% noradrenaline in hypothalamus).

This study found no evidence for rapid sensitisation or tolerance to the physiological, behavioural or neurochemical effects of mephedrone during a single binge dosing session in the rat. The limited depletion of hypothalamic dopamine makes it impossible to exclude a role of this monoamine in mediating mephedrone-induced hypothermia based only on the current findings. However, mephedrone-induced hypothermia is completely unaffected by dopamine D_2 receptor blockade and actually prolonged by dopamine D_1 receptor antagonism (Shortall et al. 2013). Taken together the available evidence therefore suggests mephedrone-induced increase in dopamine efflux is unlikely to contribute to the drug-induced decrease in body temperature. In contrast, modulation of central serotonergic neurotransmission plays a key role in mediating both the hyperlocomotor and hypothermic effects of mephedrone. Although 5-HT_{1A} receptors are implicated in the hypothermic response their involvement is almost certainly a consequence of 5-HT release and/or inhibition of reuptake, since the low affinity of mephedrone for the 5-HT_{1A} receptor (Ki>20µM; Simmler et al. 2012) makes any direct effect unlikely.

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P 14

The role of serotonin in DCS-induced fear extinction in an animal model of PTSD

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Background

Posttraumatic stress disorder (PTSD) is a severe, chronic and debilitating psychiatric disorder that can occur after exposure to a traumatic event. D-cycloserine (DCS), a partial N-methyl-D-aspartate (NMDA) receptor agonist, has been found to be effective in facilitating fear extinction; however, the precise molecular mechanisms are unknown. This study aimed to elucidate the molecular mechanism of DCS in facilitating fear extinction in a rat model of PTSD by investigating gene expression profiles in the left dorsal hippocampus (LDH).

Serotonin (5-HT) is one of the neurotransmitters involved in the pathophysiology of PTSD and is responsible for various functions in the central nervous system (Southwick et al., 1999). Studies have shown that 5-HT influences various systems, including anxiety, fear, learning, aggression, arousal, sleep and appetite (Dubovsky 1994). Furthermore, 5-HT is the principle regulator of mood, with lower platelet levels corresponding to depressed mood and vice versa (Williams et al., 2006). Research has also found lower 5-HT-uptake sites in the platelets of PTSD patients vs. controls (Arora et al., 1993). In a recent predator exposure/psychosocial stress rodent model of PTSD lower 5-HT levels in the hippocampus of the PTSD group vs. control group were observed (Wilson et al., 2014).

Methods

The modified PTSD animal model described by Siegmund and Wotjak (2007) was used. Male Sprague-Dawley rats were categorised into four groups: *Fear* + *saline*, *Fear* + *DCS*, *Control* + *Saline* and *Control* + *DCS*. Animal behavioural tests were conducted to determine which rats displayed anxiety-like behaviour. Next-generation RNA-sequencing and subsequent bioinformatics analyses were performed on the LDH to identify genes that were differentially expressed between the animal focus groups (Fear + DCS well-adapted [FDW] vs. Fear + saline maladapted [FSM]).

Results

Comparing gene expression results between the FDW and FSM group, 424 genes were found to be significantly downregulated and 27 significantly upregulated ($\geq$1.5 fold change, $p \leq 0.05$). Of these differentially expressed genes, 120 of the downregulated genes and nine of the upregulated genes were predicted to be biologically relevant to PTSD. Within this set of differentially expressed genes, four genes had direct associations with 5-HT secretion and binding (*Crhr2*, *Htr2c*, *Cd300a* and *Lilrb3l*); with the co-administration of DCS and behavioural fear extinction resulting in increased 5-HT secretion and binding in the LDH.

Conclusion

One of the possible molecular mechanisms whereby DCS facilitates fear extinction is through the upregulation of 5-HT secretion and binding in the LDH. Repeated HPA axis activation and excessive glucocorticoid production may result in reduced hippocampal 5-HT levels, resulting in dysregulated serotonergic transmission and in turn contributing to heightened fear, depressed mood, and reduced resilience (Wilson et al., 2014). It is therefore hypothesised that DCS administration together with behavioural fear extinction facilitates the upregulation of 5-HT in the hippocampus and thereby contributes to fear extinction, improved mood and resilience.

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P 15

Chronic constant light alters dopamine and serotonin activity in the rat brain

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Background: Depression is a debilitating mood disorder, negatively affecting an individual's health and well-being. Despite this, the aetiology of depression remains poorly understood. Consistently, depression treatments continue to induce unwanted side effects in addition to being inefficient. As a result, light therapy has been increasingly used as an alternative treatment to study and treat depression; however the biological mechanisms of light therapy are unclear.

Method: Control (C CCL) and maternally separated (MS CCL) rats were exposed to chronic constant light (CCL) for 3 weeks during adolescence while control (C) rats and rats subjected to maternal separation (MS) did not receive light therapy. At postnatal day 80 (adulthood), rats were decapitated and brain tissue collected for analysis of glutamate- and potassium-stimulated [^{3}H] dopamine release in the nucleus accumbens using in vitro superfusion. Serotonin (5-HT) levels in the hypothalamus and prefrontal cortex (PFC) were determined using Enzyme-Linked ImmunoSorbent Assay (ELISA).

Results: MS and MS CCL rats had significantly reduced glutamate-stimulated [^{3}H] dopamine release in the nucleus accumbens in comparison to C and C CCL rats. In the hypothalamus, 5-HT levels were significantly lower in MS CCL rats than in MS rats. Conversely, MS CCL rats had significantly higher 5-HT levels than MS rats in the PFC. Similarly, C CCL rats had elevated PFC 5-HT levels compared to C rats. Moreover, 5-HT was significantly increased in MS compared to C rats.

Conclusion: Present data indicate that MS as a rat model of depression alters the activity of the dopamine and 5-HT systems in the rat brain. However, a combination of MS and CCL further exacerbates the effect of MS on the 5-HT system and results in impaired function of the monoamine system.

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P 16

5-HT_{1B} receptors activate Erk1/2 in a neuronal cell line.

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5-HT_{1B} receptors are known to inhibit adenylate cyclase via their interactions with $G_{i/o}$. However, these receptors have also been implicated in activating other signaling pathways including kinases such as Erk1/2. Further, we and others have shown that 5-HT_{1B} autoreceptors enhance serotonin transporter activity, but the intervening signaling mechanisms have not been elucidated. 5-HT_{1B} receptors are also expressed in a variety of other neuron types but their signaling pathways have not been explored in detail. Therefore, this study explores whether 5-HT_{1B} receptors can activate kinase signaling pathways using a well-characterized neuronal cell line that lacks endogenous 5-HT_{1B} expression.

We chose to use Neuro2A cells for these studies because they endogenously express a variety of genes found in serotonergic cells, including Pet1, AADC, VMAT2, and SERT. After confirming that no endogenous 5-HT_{1B} receptors were expressed using qRT-PCR analysis, rat 5-HT_{1B} receptors were stably transfected into Neuro2A cells and these cells were routinely compared with wild-type Neuro2A cells. Kinase pathway analysis was performed using phospho-specific antibodies and quantitative western blots. Activation of 5-HT_{1B} receptors with selective agonists CP93129 and CP94523 induced a time and dose-dependent increase in phosphorylated Erk1/2; this was inhibited completely by the selective antagonist, SB224289. However, 5-HT_{1B} activation had no effect on total Erk1/2 levels. Ongoing experiments are focused on identifying proteins that may interact directly with 5-HT_{1B} receptors and whether other kinase pathways are similarly activated by these receptors.

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P 17

Optogenetic dissection of the serotonergic circuits controlling emotional behaviors

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The serotonergic system regulates an extended array of behaviors, ranging from basic physiological responses like breathing to more complex behaviors like anxiety. In the first part of this work we used mice that express channelrodopsin2 exclusively in serotonergic cells (Pet1-Cre, Rosa:floxedChR2-YFP) to dissect serotonergic circuitry involved in the regulation of anxiety and mood-related behaviors. We found that optogenetic stimulation of dorsal raphe (DR) serotonergic neurons increases exploratory behavior, decreases fear responses and produces place-preference. In ongoing work, we proceeded to test which target regions are involved, being sufficient and/or necessary, in regulating these specific behaviors. For these studies we perform terminal stimulation and local infusion of serotonin-receptor antagonists in conjunction with DR stimulation. We are currently studying the involvement of the serotonergic signaling in the ventral tegmental area, medial prefrontal cortex, hippocampus and amygdala.

In the second part of this work, we investigate the functional integrity of the serotonin system in a developmental mouse model of anxiety and depression using the tools and parameters established in the first part. Specifically, we studied mice that had received fluoxetine during postnatal development (PNflx), which leads to anxiety- and depression-like behavior, as well as cognitive deficits. We find that optogenetic stimulation of the DR serotonergic neurons in PNflx treated mice produced a blunted behavioral response, when compared to the stimulation of the PNsaline mice. These results suggest that PNflx treatment permanently dampens the functional connectivity of the serotonergic system to its postsynaptic targets. Ongoing work investigates the functional connectivity to the ventral tegmental area, medial prefrontal cortex, hippocampus and amygdala.

Our work reveals functional serotonin circuit properties under normal conditions and in a developmental mouse model of depression/anxiety. These data should help to gain mechanistic insight into the etiology of psychiatric disorders and to improve serotonin-based treatment strategies.

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P 18

FRET- and FRAP-based characterization of 5-HT$_7$ receptor-G protein preassociation and low versus high potency G$_s$ activation

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How G-protein-coupled receptors (GPCRs), G proteins and effectors are structurally organized and whether their interaction is static or dynamic is important for their function and biological effects and conceivably for their function as targets for pharmaceutical intervention in diseases. We have previously compared the G$_s$-coupled 5-HT$_4$ and 5-HT$_7$ serotonin receptors and revealed fundamental differences in their interaction with G protein: whereas the function of 5-HT$_4$ receptors is consistent with collision coupling, the pharmacological properties of 5-HT$_7$ receptors best fit a model with receptor and G$_s$ preassociated in the absence of ligand. In this study, we wanted to directly examine if the 5-HT$_7$ receptor and G protein are truly preassociated and if so, determine the intracellular domain of the receptor responsible for this interaction. In addition, we describe a novel, bimodal, agonist-induced activation of G$_s$ by 5-HT$_7$ receptor with a large low-potency and a smaller high-potency component that could result from a preassociated receptor-G protein complex.

To test 5-HT$_7$ receptor interaction with G$_s$, we used FRET (Fluorescence Resonance Energy Transfer) and FRAP (Fluorescence Recovery After Photobleaching) with fluorescently labeled β_1-adrenergic, 5-HT$_4$ or 5-HT$_7$ receptors and different G protein subunits. Agonist-activation of β_1-adrenergic or 5-HT$_4$ receptors, as expected, increased FRET indicating an interaction by collision coupling. In contrast, FRET experiments with labeled γ and α G protein subunits indicated a conformational change within and subsequent dissociation of a preassociated complex of 5-HT$_7$ receptor and G$_s$. Consistent with this, FRAP experiments with antibody-immobilized receptors demonstrated that 5-HT$_7$, in contrast to 5-HT$_4$ receptors, also prevented G$_s$ diffusion in the cell membrane, confirming preassociation of 5-HT$_7$ receptors with G$_s$.

To identify the intracellular structural determinants of the receptors responsible for these differences in receptor-G protein interaction, we constructed chimeric receptors, in which intracellular segments of the 5-HT$_7$ receptor were exchanged with corresponding segments from the 5-HT$_4$ receptor. The chimeric exchanges only had minor effects on ligand binding affinity. However, exchange of either the C-tail or intracellular loop 3 selectively eliminated the low-potency component of the bimodal G$_s$ activation by the wild type 5-HT$_7$ receptor. In these constructs, agonists stimulated adenylyl cyclase with a high potency similar to the high-potency component of the wild type 5-HT$_7$ receptor. Both FRET and FRAP experiments identified the C-tail and ICL3 to be responsible for the preassociation of the 5-HT$_7$ receptor with G$_s$. Thus, the C-tail or ICL3 from the 5-HT$_4$ receptor converted the 5-HT$_7$ receptor from being preassociated with G$_s$ into a collision-coupled receptor that associated with G protein upon agonist activation.

In conclusion, the C-tail and the intracellular loop 3 have been identified to be responsible for the characteristic preassociation and bimodal G$_s$ coupling of the 5-HT$_7$ receptor.

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P 19

BDNF Val66Met polymorphism and plasma levels in acutely traumatised road traffic accident survivors

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Objective: Brain-derived neurotrophic factor (BDNF) modulates synaptic plasticity involved in memory and learning and has been associated with posttraumatic stress disorder (PTSD), however findings of BDNF levels in PTSD have been inconsistent and the relationship between BDNF and stress-related disorders remains unclear. This study prospectively evaluated a cohort of individuals who were road traffic accident (RTA) survivors to determine whether BDNF plasma levels and BDNF *Val66Met* carrier status were correlated in acutely traumatised individuals. We also wanted to determine if the BDNF Val66Met polymorphism and BDNF plasma levels were associated with an early post RTA acute stress disorder (ASD) diagnosis and working memory impairments.

Methods: 123 adult participants (age 33.18 ± 10.60; gender; 56.9% male) who had survived an RTA were recruited from four hospitals in Cape Town. Clinical and laboratory assessments were performed within 10 ± 4.86 days of RTA exposure. ASD was diagnosed in 50 (42%) of participants based on a cut-off of 56 on the Acute Stress Disorder Scale (ASDS). Digit span backwards (5.25 ± 2.29) was used to assess working memory performance. ELISA was used to measure early morning BDNF plasma levels and Val66Met genotyping was performed.

Results: 84 participants (68.3%) were *BDNF Val66Val* homozygous; 28 (22.8%) were heterozygous and 3 (2.4%) were *BDNF Met66Met* homozygous. *Met* carrier groups were combined for analysis. BDNF plasma levels (51.27 ± 41.63) were not significantly associated ($p \leq 0.05$) with BDNF Val66Met genotype. Neither BDNF Val66Met genotype nor plasma BDNF was significantly ($p \leq 0.05$) associated with the presence or severity of ASD or working memory impairments.

Conclusions: In acutely traumatised RTA survivors we found no association between BDNF *Val66Met* genotype, BDNF plasma level and ASD or working memory impairments. In acutely traumatised RTA survivors many factors can influence BDNF plasma levels, such as tissue injury, diet, medication and substance use. ASD can be diagnosed up to one month post-trauma and as individuals were assessed within two weeks, it is possible that additional ASD cases may have been missed. The relationship between BDNF Val66Met, BDNF plasma level, PTSD and working memory function may be more reliably detected sometime after trauma.

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P 20

A highly efficient approach for specific targeting of postnatal brain 5-HT synthesis

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Deficient serotonin (5-HT) signaling in the brain has been implicated in the pathogenesis of many devastating and highly prevalent psychiatric disorders. However, the importance of postnatal 5-HT for the maintenance of 5-HT neuron function and normal behavior is still poorly understood. This is partly due to the lack of specificity and efficiency of traditional techniques used to alter the 5-HT system in animal models. For example, many studies have employed genetic approaches to target transcription factors required for fetal 5-HT neuron development and function, which result in substantial decreases in the expression of *tryptophan hydroxylase 2* (*Tph2*), the rate-limiting enzyme in brain 5-HT synthesis, and deficiencies in 5-HT levels. However, those approaches also affect many other serotonergic genes not directly related to 5-HT synthesis, bringing into question the specific cause of the behavioral deficits seen in those models. Importantly, mouse models with germ line targeting of *Tph2,* in which brain 5-HT synthesis is never initiated, have confirmed that 5-HT deficiencies beginning in early fetal life are sufficient to cause behavioral abnormalities in adulthood. Still, it remains to be determined if maintenance of postnatal 5-HT levels is critical for the functional integrity of 5-HT neurons and normal behavior. To address these fundamental questions, we have developed an efficient approach to decrease brain 5-HT synthesis, specifically, at different postnatal time points. *Tph2* expression is targeted by stereotaxic injection of a viral vector expressing Cre recombinase, AAV-Cre, into the dorsal raphe nucleus (DRN) of adult *Tph2fl/Δ* mice. This technique results in highly reproducible and severe losses of postnatal Tph2 and 5-HT. Representative targeting in the adult DRN resulted in a 91.8% (±0.9) decrease in *Tph2* mRNA, a 99.5% (±0.3) reduction in Tph2+ neurons, and a 93.9% (±1.9) decrease in forebrain 5-HT levels. Expression of aromatic L-amino acid decarboxylase, the other enzyme involved in 5-HT synthesis, was unchanged in AAV-Cre-targeted 5-HT neurons marked with Rosa-YFP, demonstrating that 5-HT neurons remain in normal numbers and loss of Tph2 and 5-HT are not due to 5-HT neuronal death. Preliminary data suggests the development of gene expression changes following chronic loss of 5-HT, and we are investigating these alterations further. We are also examining the impact of postnatal 5-HT deficiency on anxiety-like behaviors, fear conditioning, behavioral inhibition, and the response to chronic stress.

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P 21

Social isolation rearing induces elevated tryptophan, anthranilic acid and 3-OH anthranilic acid and altered cortico-striatal serotonin metabolism –relevance for schizophrenia

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Schizophrenia is a complex illness causally linked to environmental and neurodevelopmental factors. The neuropathology of schizophrenia is closely associated with a pro-inflammatory state, implicating mitochondrial alterations, oxidative stress and altered tryptophan metabolism. Changes in dopamine (DA) and serotonin (5-HT) are a central construct in our understanding and treatment of the disorder. Previously, we have demonstrated that social isolation rearing (SIR) in rats, a neurodevelopmental animal model of schizophrenia, is associated with cognitive and other behavioural changes [1, 2] akin to schizophrenia, as well as altered cortico-striatal DA metabolism [2], decreased neuro-protection via the kynurenine pathway associated with altered cytokine release [2], and increased oxidative stress [1]. Some of these bio-behavioural changes were reversed by clozapine [1, 2] and/or N-acetyl cysteine (NAC) treatment [2]. Here we present new data describing the effect of clozapine and clozapine plus NAC on SIR induced effects on peripheral tryptophan and cortico-striatal 5-HT metabolism.

Male Sprague-Dawley (SD) rats were used (Ethical approval number NWU-0035-08-S5) for all the studies [1, 2] and exposed to either SIR or social rearing for 8 weeks. Rats received clozapine (5 mg/kg/day), NAC (150 mg/kg/day) or clozapine + NAC (150 mg/kg/day) for 14 days. Cortico-striatal levels of 5-HT and associated metabolites (using HPLC [2]), as well as peripheral levels of tryptophan-kynurenine metabolism (using LCMS [2]), were determined. Data were analysed using 2-way ANOVA with Bonferroni post hoc test.

This study demonstrated that SIR in rats significantly ($p<0.05$) decreased frontal cortical but increased striatal 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), as well as significantly ($p<0.01$) increased peripheral tryptophan, anthranilic acid and 3OH-anthranilic acid. Moreover, these changes we reversed by chronic treatment with clozapine, NAC as well as clozapine plus NAC, in some instances demonstrating an improved response following the latter. Together with our earlier behavioural and biological studies [1, 2, 3] these new findings emphasise that early-life adversity can produce a range of significant long-term changes in kynurenine metabolism with implications for increased oxidative stress and decreased neuro-protection via increased 3OH-anthranilic acid and anthranilic acid, respectively ($p<0.01$). An associated increase in tryptophan metabolism and altered cortico-striatal 5-HT release ($p<0.01$) may have relevance for the frontal-cortical deficits and affective symptoms noted in schizophrenia. This, plus that clozapine, NAC as well as clozapine + NAC treatment significantly ($p<0.01$) reversed most of these bio-behavioural alterations, emphasises the construct and predictive validity of the findings. Clozapine + NAC was in some instances more ($p<0.01$) effective than clozapine, suggesting the utility of NAC as an adjunctive treatment in schizophrenia.

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P 22

Fear memory is retrieved through the activation of serotonin 5-HT$_7$ receptor in the ventral hippocampus

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Patients with posttraumatic stress disorder or panic disorder are often troubled by inappropriate retrieval of fear memory. Moreover these disorders are often comorbid with irritable bowel syndrome. The main aim of the present study is to elucidate the involvement of hippocampal serotonergic systems in fear memory retrieval and stress-induced defecation. Microinjection of 5-HT$_7$ receptor antagonist, but not other 5-HT receptor antagonists (5-HT$_{1A, 2A, 2C, 3, 4, \text{and } 6}$ receptor antagonists), into the rat ventral hippocampus significantly suppressed the expression rate of freezing behavior, an index of fear memory retrieval, and decreased the number of feces, an index of stress-induced defecation, in the contextual fear conditioning test. Neither memory-independent fear-related behavior nor locomotor activity in the elevated plus maze test was affected by the microinjection of 5-HT$_7$ receptor antagonist while the number of feces was decreased. Electrophysiological data using whole-cell patch clamp recording techniques in brain slices indicated that the stimulation of 5-HT$_7$ receptor in the CA3 of ventral hippocampus would enhance the sensitivity to inputs by increasing the hyperpolarization-activated nonselective cation current. Moreover, *in situ* hybridization demonstrated that Htr7 mRNA is expressed abundantly in CA3 compared to other sub-regions of the hippocampus, and that these Htr7 mRNA-positive cells co-expressed hyperpolarization-activated cyclic nucleotide-gated (HCN) channel 2 and 4 mRNAs. These results indicated that the released 5-HT activates 5-HT$_7$ receptor in the CA3 region of ventral hippocampus and enhances the sensitivity to inputs, and thereby facilitates fear memory retrieval. Furthermore, 5-HT$_7$ receptor antagonist could be a target of drug development for the treatment of mental disorders involving fear memory and gastrointestinal problems, such as posttraumatic stress disorder and panic disorder comorbid with irritable bowel syndrome.

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P 23

Common and Rare Alleles of the Serotonin Transporter Gene, SLC6A4, Associated with Tourette's Disorder

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To evaluate the hypothesis that functionally over-expressing alleles of the serotonin transporter (SERT) gene (solute carrier family 6, member 4, SLC6A4) are present in Tourette's disorder (TD), just as we previously observed in Obsessive- Compulsive disorder (OCD), we evaluated TD probands (N=151) and controls (N=858). We genotyped the refined SERT linked polymorphic region 5-HTTLPR/rs25531 and the associated rs25532 variant in the SLC6A4 promoter plus the rare coding variant SERT isoleucine-to-valine at position 425 (I425V). The higher expressing 5-HTTLPR/rs25531 LA allele was more prevalent in TD probands than in controls (X^2=5.75; P=0.017; odds ratio [OR] 1.35); and, in a secondary analysis, surprisingly, it was significantly more frequent in probands who had TD alone than in those who had TD plus OCD (Fisher's exact test; P=0.0006; OR, 2.29). Likewise, the higher expressing LAC haplotype (5-HTTLPR/rs25531/rs25532) was more frequent in TD probands than in controls (P=0.024; OR, 1.33) and also in the TD alone group versus the TD plus OCD group (P=0.0013; OR, 2.14). Furthermore, the rare gain-of-function SERT I425V variant was observed in 3 male siblings with TD and/or OCD and in their father. Thus, the cumulative count of SERT I425V becomes 1.57% in OCD/TD spectrum conditions versus 0.15% in controls, with a recalculated, family-adjusted significance of X^2= 15.03 (P < 0.0001; OR, 9.0; total worldwide genotyped =2914). This report provides a unique combination of common and rare variants in one gene in TD, all of which are associated with SERT gain of function. Thus, altered SERT activity represents a potential contributor to serotonergic abnormalities in TD. The present results call for replication in a similarly intensively evaluated sample

P 24

Serotonin 1B receptors affect neural circuits underlying aggression during development, but modulate impulsivity circuits during adulthood.

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The serotonin 1B receptor (5-HT1B R) has been implicated in the modulation of aggressive and impulsive behavior. Polymorphisms in 5-HT1B R are associated with aggression and impulse control disorders like pathological gambling alcohol and drug addiction. Also, constitutive 5-HT1B R knock-out mice are more aggressive, more impulsive and more vulnerable to drug abuse. However, the localization of the neural circuits mediating these behaviors, and whether 5-HT1B R have a developmental impact, are unknown. In order to answer these questions, we have generated a novel targeted transgenic mouse that permits temporal and spatial regulation of 5-HT1B R. Using a bigenic strategy with tetracycline operator (tetO) and tetracycline-dependent transcriptional silencer (tTS) transgenes, our mouse model allows investigation into the sensitive period and cell-type specificity of the effect of 5-HT1B R on behavior.

Sensitive period: First, our data show that whole brain, whole life knock-down (in β-Actin-tTS/tetO1B mice) of 5-HT1B R results in aggressive behavior as measured by increased male-male fighting. These mice are also highly impulsive, unable to inhibit responding in operant conditioning paradigms. Interestingly, rescue of receptor expression in adulthood with doxycycline reverses the impulsive, but not the aggressive, phenotype. The aggressive phenotype is reversed only by rescue of 5-HT1B R during postnatal development. This suggests that developmental expression of 5-HT1B R modulates the formation of circuits that contribute to adult aggression, while adult expression of 5-HT1B R contributes to modulation of impulse control circuits.

Localization: Next, using tissue-specific promoters, we were able to rule out the involvement of 5-HT1B autoreceptors in the raphe (using the Pet-1 promoter) in mediating both aggressive and impulsive behaviors. However, knockout of a subset of forebrain receptors (including cortical and striatal receptors using the CaMKII promoter) resulted in aggressive, but not impulsive behavior. We are currently using cell-type specific promoter- and viral-mediated knockdown to target GABAergic neurons in the cortex or striatum in order to localize sites of 5-HT1B R mediated inhibition.

Overall these data show that expression of 5-HT1B R heteroreceptors (in the cortex or striatum) during postnatal development contributes to the normal maturation of neural circuits underlying aggression. On the other hand, expression of a different population of 5-HT1B R heteroreceptors during adulthood contributes to the regulation of impulsive behavior. Taken together these data suggest that distinct circuits underlie the effects of the 5-HT1B R on aggressive and impulsive behaviors, and contribute to the identification of the neural substrates underlying serotonergic modulation of aggression and impulsivity.

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P 25

Optogenetic modulation of the prefrontocortical-dorsal raphe microcircuit bidirectionally biases socioaffective decisions after social defeat

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It has been well established that modulating levels of serotonin (5-HT) in humans and animals affects affective perception and response to social threats. However, the circuit mechanisms that control social interaction are not well understood. Understanding these underlying mechanisms could provide the groundwork to develop therapeutic interventions to more precisely treat socioaffective disorders. We examined the organization and plasticity of the reciprocal microcircuit formed by 5-HT neurons in the dorsal raphe nucleus (DRN) and the ventromedial prefrontal cortex (vmPFC) and its role in social approach-avoidance decisions. We used a chronic social defeat stress (CSDS) model that results in a long lasting form of social aversion that is reversible by antidepressants. Using viral tracing in population specific Credriver mice we showed that excitatory vmPFC projections primarily localized to GABA-rich areas of the DRN. Next, using optogenetics with both cFos mapping and whole cell electrophysiology we established the functional effects of vmPFC-driven glutamatergic activity in the DRN. We provide the first direct evidence that vmPFC axons drive synaptic activity and immediate early gene expression in genetically identified DRN GABA neurons through an AMPA-dependent mechanism and that these GABA neurons locally inhibit 5-HT neurons. We also show that CSDS drove GABAergic sensitization that strengthened inhibition of 5-HT neurons in mice that were susceptible, but not resilient, to CSDS. Finally we demonstrate using optogenetics that increasing vmPFC input to the DRN during sensory exposure to an aggressor's cues respectively enhances avoidance bias. In contrast, optogenetically decreasing vmPFC drive of the DRN or GABAergic neuronal activity within the DRN prevented the acquisition of an avoidance phenotype after CSDS. These results clarify the functional organization of vmPFCDRN pathways and identify GABAergic neurons as a key cellular element filtering top-down vmPFC influences on affect-regulating 5-HT output.

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Regulation of Neuronal Primary Cilia Morphology in Striatal Neurons by 5-HT$_6$ Receptors

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The primary cilium is a sensory organelle stemming from the cell body of most mammalian neurons. The cilium is an antenna-like, microtubule-supported structure that receives both chemical and mechanical signals from other cells and the surrounding environment. These signals are transduced by a discrete set of membrane-bound receptors localized to the primary cilium. Recently, neuronal primary cilia have become a major target of research as they play crucial roles in a variety of disorders known as "ciliopathies"; they have also been implicated in Huntington's and Alzheimer's diseases. However, the role of primary cilia in normal cognitive functions is not understood, but there is evidence that impairments of ciliary signaling produce cognitive deficits. The small set of receptors that localize to cilia have consensus residues that permit them to be selectively trafficked into cilia by unique ciliary trafficking mechanisms and intraflagellar transport. One such receptor is the 5-HT$_6$ serotonin receptor, which is an excitatory, G_S-coupled receptor that is heavily expressed in striatum; it is the only serotonin receptor that localizes to neuronal primary cilia. 5-HT$_6$ receptors modulate learning and memory, and are linked to a range of cognitive processes and neuropsychiatric syndromes including mood disorders, addiction, and dementia. Our goal is to confront the question of whether the unique functional properties of 5-HT$_6$ receptors are dependent upon its localization to cilia. Utilizing primary cultured striatal neurons we are able to evaluate the effects of wild-type and mutated 5-HT$_6$ receptors on the structure and function of primary cilia. We use striatal neurons dissected from P0-P1 mouse neonates that were maintained in culture for 10-12 days. Nearly all 5-HT$_6$ receptors co-localize with adenylyl cyclase III, a selective marker for primary cilia. Inhibition of 5-HT$_6$ receptors with the selective 5-HT$_6$ receptor antagonists such as SB258585 reduced primary cilia length in a time- and concentration-dependent manner by up to ~50% in neurons from wild-type but not 5-HT$_6$ KO mice. 5-HT$_6$ knockdown with shRNA produces similar changes in wild-type neurons. However, we did not observe a lengthening effect associated with agonist or overexpression, suggesting that either there is a ceiling effect and perhaps residual 5-HT in the growth medium; we continue to test this. Currently, we are studying whether manipulating 5-HT$_6$ receptor expression and localization also alters cilia morphology and signaling. Our preliminary results suggest that a small deletion mutation disrupts trafficking to cilia; we are currently exploring the functional consequences of subcellular localization of 5-HT$_6$ receptors using primary neuron culture and soon in vivo gene transfer. Furthermore, we are investigating how 5-HT$_6$ receptors modulate the activity of other signaling pathways in primary cilia of striatal neurons. Our results so far suggest that 5-HT$_6$ receptors are unique among serotonin receptors due to cilia localization and may represent a distinct, extrasynaptic mechanism of serotonin modulation of cell signaling, excitability, and behavior control.

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Mice with compromised 5-HTT function lack phosphotyrosine-mediated inhibitory control over prefrontal 5-HT responses

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The activity of the prefrontal cortex is essential for normal cognition and emotional processing, which are strongly modulated by serotonin (5-HT). Yet, little is known about the regulatory mechanisms that control the effects of prefrontal 5-HT receptors. It is also not known whether disruptions in such regulatory mechanisms would perturb prefrontal cortical functions. Here, we characterized alterations in the regulation of prefrontal 5-HT receptor electrophysiological signaling in mouse models of disrupted serotonin transporter function, a risk factor for emotional and cognitive disturbances in both humans and rodents.

We identified a novel tyrosine-kinase dependent mechanism that regulates 5-HT-mediated inhibition of prefrontal pyramidal neurons. We report that mice with compromised serotonin transporters (even if this disruption occurs only transiently during development due to treatment with selective serotonin reuptake inhibitors) have amplified, inhibitory, 5-HT_{1A} receptor-mediated currents in adulthood with enhanced downstream coupling to Kir3 channels.

Such amplified 5-HT_{1A} responses can be mimicked through inhibition of Src family tyrosine-kinases in normal mice and rapidly normalized through inhibition of tyrosine-phosphatases in the mouse models examined. Our findings implicate tyrosine phosphorylation in regulating the electrophysiological effects of prefrontal 5-HT_{1A} receptors with implications for neuropsychiatric diseases associated with emotional and cognitive dysfunction, such as anxiety and depressive disorders.

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Antagonist-mediated down-regulation of 5-HT$_7$ serotonin receptors is regulated by C-terminal domains and interaction with GASP1

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The human 5-HT$_7$ serotonin receptor is a G-protein-coupled receptor (GPCR) that activates adenylyl cyclase constitutively and upon agonist-activation. Some inverse agonists towards the 5-HT$_7$ receptor can induce both homo- and heterologous desensitization, similar to agonist-stimulation, while others can induce receptor internalization. However, only a subset of these targeted 5-HT$_7$ receptors for lysosomal degradation. These results demonstrated that various ligands differentially activated regulatory processes governing receptor desensitization, internalization and degradation in addition to signal transduction, providing support for the concept of functional selectivity at the 5-HT$_7$ receptor, where different ligands stabilize different receptor conformations leading to differential effects.

Interestingly, the important atypical antipsychotics clozapine and olanzapine inhibited G-protein activation (as expected) and, surprisingly, induced both internalization and lysosomal degradation of 5-HT$_7$ receptors. We wanted to determine the mechanism of clozapine- and olanzapine-mediated internalization and lysosomal sorting of 5-HT$_7$ receptors.

In the C-terminus of the 5-HT$_7$ receptor, we identified two important YXXΦ motifs, two conserved residues (LR) and the palmitoylated cysteine-anchor as potential sites involved in receptor internalization and recruitment of lysosomal sorting proteins, such as GPCR-associated sorting protein 1 (GASP1). Mutating one or both YXXΦ motifs, the LR residues or the cysteine-anchor inhibited clozapine- and olanzapine-mediated lysosomal sorting of 5-HT$_7$ receptors. In addition, we demonstrate that GASP1 binds to the 5-HT$_7$ receptor and that over-expression of the C-terminus of GASP1 inhibited clozapine-mediated degradation of 5-HT$_7$ receptors, indicating that GASP1 is recruited to these domains of the 5-HT$_7$ receptor and is involved in lysosomal sorting. The identified domains are located in helix VIII of the 5-HT$_7$ receptor and we are currently building a structural model to clarify the functional effects caused by the mutated residues and determine how GASP1 interacts with this structure.

Taken together, our data demonstrate that binding of clozapine or olanzapine to the 5-HT$_7$ receptor leads to antagonist-mediated internalization and lysosomal degradation by exposing key residues in the C-tail that interact with GASP1.

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Htr2a expression responds rapidly to environmental stimuli in an Egr3-dependent manner suggesting a functional link between two schizophrenia susceptibility genes.

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The serotonin system has been implicated in the etiology of schizophrenia, through numerous pharmacological and genetic findings. However the mechanisms by which serotonin influences schizophrenia susceptibility are poorly understood. The serotonin 2A receptor (5-HT2AR) has been of particular interest as agonists of the receptor cause psychosis in normal individuals and second generation antipsychotic medications have a high affinity for 5-HT2A Rs. In addition, Htr2a, the gene that encodes 5-HT2AR, is one of the most well-replicated schizophrenia candidate genes based on genetic association studies. Furthermore, numerous *in vivo* and post-mortem studies have found decreased levels of 5-HT2ARs in the brains of schizophrenia patients including in medication-naïve individuals.

We recently reported that mice lacking the immediate early gene (IEG) early growth response 3 (Egr3) show an approximately 70% decrease in 5-HT2AR binding in the prefrontal cortex, paralleling the 5-HT2A R deficits in schizophrenia patients. Egr3 is a transcription factor involved in synaptic plasticity that is rapidly activated in the brain after environmental events such as stress and it regulates downstream target genes to modulate the brain's response to these stimuli. Thus, dysfunction in Egr3 may account for both environmental and genetic influences on schizophrenia risk. We have previously shown that mice lacking Egr3 display schizophrenia-like behavioral abnormalities and responses to antipsychotics. In humans, variations in EGR3 has been associated with schizophrenia risk in numerous populations, and levels of EGR3 mRNA are reduced in the brains of patients with schizophrenia.

Our findings of decreased levels of 5-HT2AR binding in Egr3 -/- mice provide a mechanistic link between two schizophrenia candidate genes, and suggest how they may be affected by changes in the environment. The data presented here test the hypothesis that Egr3 rapidly modulates 5-HT2AR in response to environmental stimuli. If Egr3 directly regulates expression of Htr2a, EGR binding motifs should be present in the promoter region of the Htr2a gene. We therefore used the MEME suite FIMO tool analysis software to identify putative EGR response elements in the 2 Kb upstream of the Htr2a gene. This analysis revealed two highprobability EGR binding sites located at -2791-2778 bp and -75-62 bp. A second requirement would be that EGR3 protein must be expressed in the same cells as the 5-HT2AR. Since anti-5-HT2ARs antibodies do not show cell-specific labeling in the brain, to address this we employed a transgenic mouse that expresses EGFP under control of the Htr2a promoter. This showed overlapping expression of EGFP with anti-EGR3 antibody staining in the frontal cortex.

To determine if Htr2a is regulated by environmental stimuli, we used sleep deprivation, a stimulus known to induce expression of Egr3. This was necessary because Egr3 expression is stimulus-dependent and expressed at low levels under basal conditions. We compared levels of Htr2a mRNA in the frontal cortex of Egr3-/- and WT littermate mice at baseline and after sleep deprivation, by quantitative RT-PCR. This revealed no significant difference in Htr2a expression between WT or Egr3-/- mice under undisturbed conditions. However, following sleep deprivation, Egr3-/- mice showed decreased Htr2a expression in cortex as compared to WT. These data suggest Egr3 may rapidly modulate Htr2a in response to acute environmental stimuli. These findings are significant because they are one of the first demonstrations of rapid modulation of a serotonin receptor in response to environmental stimuli. Furthermore, they provide a functional link between two schizophrenia candidate genes and a possible explanation of how both genetic and environmental factors influence risk for schizophrenia.

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Permanent depletion of serotonin increases risky decision-making and impairs acquisition of the rat gambling task

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Human and animal research strongly implicates serotonin (5-hydroxytryptamine, 5-HT) in decision-making and altered functioning of the 5-HT system may disrupt the ability to learn from loss or punishment. We used the rat gambling task (rGT; Zeeb et al., 2009), a rodent analogue of the Iowa Gambling Task (IGT), to test the hypothesis that 5-HT contributes to decision-making by modulating the significance of punishment-related signals. In both the rGT and IGT, disadvantageous options are associated with larger immediate reward, but greater loss; whereas the optimal strategy is to choose preferentially from advantageous options that yield smaller immediate gains but less loss and therefore greater long-term reward.

Male Long Evans rats received either a sham surgery or a permanent 5-HT depletion accomplished by an intracerebroventricular infusion of 5,7-dihydroxytryptamine (5,7-DHT), which depleted 5-HT throughout the forebrain by 75-85%. Surgery was conducted either prior to or following rGT training. rGT testing took place in 5-hole operant chambers in daily 30 min sessions, during which animals chose between four different options. Each option was associated with a different magnitude of reward (sucrose pellets), different frequency of reward delivery, and a different duration of a timeout period. During the timeout, rats were not rewarded and initiation of the next trial was halted until the end of the punishment. Therefore—similar to losing on the IGT—timeouts result in less reward earned per unit time.

5-HT depleted rats did not differ in their choice preference compared to the sham-control group when the lesion occurred following rGT training. In contrast, rats that received pre-training 5,7-DHT lesions demonstrated a marked impairment in task acquisition. 5-HT depleted rats were slower to learn the optimal strategy and chose the disadvantageous, risky options more often than controls. Following 44 training sessions, compared to sham-control rats, animals with 5,7-DHT lesions continued to significantly choose the disadvantageous options—associated with greater loss—more than advantageous options. Additionally, the ability for amphetamine to increase choice of the options leading to less loss was absent in rats with 5,7-DHT lesions. Likewise, an acute injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT moderately decreased choice of the disadvantageous options in sham animals, an effect blocked by the 5-HT depletion. These results demonstrate that 8-OH-DPAT may alter decision-making by acting on pre-synaptic autoreceptors in the control group. Furthermore, amphetamine's ability to bias animals toward options associated with less punishment can be partially attributed to its interaction with the 5-HT system.

In conclusion, long-term depletion of 5-HT may impair the ability of animals to develop an optimal decision-making strategy possibly by dampening the significance of loss, biasing animals to choose disadvantageously despite the negative consequences associated with these options. As impairments in decision-making processes are observed in a wide range of psychiatric disorders (including depression, pathological gambling, and substance abuse) the results presented here may provide insight into the potential role of 5-HT in decision-making in these populations in which the 5-HT system may be compromised.

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Genetic mechanisms underlying variation in 5-HT1A receptors in the human developing and adult brain

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The complex effects of serotonin 1a receptors (5-HT1A) on mood and behavior are mediated by both development-specific and brain region-specific effects of receptor activation. Work in mouse models has demonstrated that the behavioral roles of different neural populations of 5-HT1A change across the course of development. For instance, post-natal variation in autoreceptors, but not forebrain heteroreceptors, influences anxiety levels, while adult autoreceptors modulate stress coping, but not anxiety.

Furthermore, multiple manipulations have demonstrated that even small, ethologically relevant differences in receptor levels in a single brain region and/or during a particular developmental period can impact anxiety and depression-related phenotypes. Thus, understanding the factors that contribute to normal variation in receptor levels across the life course will help elucidate the mechanisms underlying 5-HT1A as a risk factor for mood and anxiety disorders. In humans, a G/C single nucleotide polymorphism (rs6295) located 1 kb upstream of the 5-HT1A gene, which has been linked with depression and antidepressant responsiveness, is hypothesized to mediate differences in neural 5-HT1A levels through the altered binding of a number of transcription factors, including Deaf1, c-Jun, and Hes5. *In vitro* work suggests that this SNP has region-specific and development-specific effects on the expression of 5-HT1A. Thus, in order to determine the transcriptional relationship between rs6295 and 5-HT1A within the human brain, we measured the relative allelic expression from the G and C alleles in postmortem human brain tissue. Within the prefrontal cortex of control subjects, more mRNA is derived from the C- compared to the G-allele. However, this effect is region-specific, as the G- and Callele produce equivalent amounts of mRNA in the hippocampus and in the raphe.

Because the Hes transcription factors are thought to interact with rs6295 selectively during neuronal differentiation, we also examined the G:C expression ratio in human fetal cortex (gestational week 17). Similar to the adult prefrontal cortex, we observed more mRNA produced from the C-allele, indicating that rs6295 is associated with differences in 5-HT1A expression during gestation, potentially impacting important developmental events. Interestingly, in individuals with major depressive disorder, we found that the normal pattern of transcription was disrupted in the prefrontal cortex.

Together, these findings support the hypothesis that rs6295 has region-specific effects on transcription across multiple developmental stages and that the normal transcriptional profile is disrupted in disease states. This work, along with a humanized mouse model of rs6295 that we recently developed, will provide important insight into mechanisms by which common genetic variation in the human 5-HT1A gene might potentiate risk for mental illness.

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Mechanisms contributing to lack of antidepressant efficacy in juveniles and adolescents

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Depression is a major health problem for which most patients are not effectively treated. This problem is further compounded in children and adolescents where only two antidepressant drugs are currently approved for clinical use. Both are selective serotonin (5-HT) reuptake inhibitors (SSRIs), which are often less therapeutically efficacious in this young population compared to adults. Consistent with clinical literature, we found that antidepressant-like effects of SSRIs in mice aged 21 days post-partum (P21, juvenile) was reduced relative to adult mice; however, there was no difference in expression of hippocampal 5-HT transporter (SERT), the target protein of SSRIs, to account for the reduced SSRI efficacy. The increase in extracellular 5-HT following SSRI administration is thought to trigger downstream events required for therapeutic effects. Thus, our data raise the possibility that transporters capable of 5-HT uptake other than SERT may be present in disproportionately higher levels during juvenile and adolescent periods thereby preventing extracellular 5-HT from climbing to therapeutically relevant levels following SSRI treatment. Decynium-22 (D22) is a blocker of organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT), low affinity, but high capacity transporters for 5-HT. We found that in juvenile and adolescent mice, the density of [^{3}H]D22 binding sites in hippocampus are greater than in adults. Western blot analysis using specific antibodies revealed that increased [^{3}H] D22 binding was most likely driven by increased PMAT expression in young mice relative to adults. These data suggest that D22 may have antidepressant activity in juvenile and adolescent mice. In our preliminary studies we found that D22 (0.01mg/kg) produced antidepressant-like effects in juvenile but not adult mice. Using *in vivo* chronoamperometry, an electrochemical technique which allows for sub-second measurements of region specific 5-HT clearance in brain, studies are underway to determine whether the antidepressant-like effects of D22 are related to its ability to inhibit 5-HT clearance. Our results suggest that significant uptake of 5-HT by PMAT and/or OCTs may limit the therapeutic efficacy of SSRIs, providing a mechanistic basis for poor treatment response to SSRIs particularly in juveniles and adolescents. This work was supported by National Institutes of Health Grants R01-MH064489-S1 (LCD), R01-MH093320 (LCD, WK), NARSAD Independent Investigator Award (LCD), and a NIDA travel award 1R13DA033783-01.

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Serotonin inputs to the bed nucleus of stria terminalis shape network function and behaviors relating to fear memory and anxiety

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Serotonin neurons originating from the dorsal raphe nucleus (DRN) innervate a variety of limbic structures involved in feeding, mood regulation, reward-related and avoidance behavior. The bed nucleus of stria terminalis (BNST) is one critical output of the dorsal raphe with a well-defined role in stress-induced relapse and anxiety associated with drug dependence and acute withdrawal states. Using slice electrophysiology in SERT-cre mice stereotaxically injected with a DIO-Ch2-eYFP viral construct into the DRN, we found that optogenetic stimulation of serotonin terminals in the ventrolateral BNST (vBNST) depolarizes cells via activation of 5HT2c receptors (5HT2cRs). Furthermore, colocalization of serotonin-positive fibers with CRF neurons in the vBNST was observed in a Cre-dependent CRF reporter mouse line, suggesting a role for CRF neurons in the excitatory effects of serotonin in the vBNST. Slice electrophysiology in this CRF reporter line confirms that bath applied 5HT2c-R agonists can depolarize cells within the vBNST, in agreement with our findings in SERT-cre mice.

Next we investigated the role of this serotonin-CRF interaction in retention of fear memory in a cued fear conditioning paradigm. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), can potentiate freezing behavior during presentation of the cued stimulus alone. Previous studies have shown that this fear enhancing effect of serotonin is localized to the BNST and is 5HT2c-R dependent, implicating CRF neurons as a potential neural substrate. Using a Designer Receptor Exclusively Activated by Designer Drug (DREADD) approach, we stereotaxically injected a viral DIO-hM4D-mCherry construct into the BNST of CRF-cre mice, which restricted expression of this Gi-coupled receptor to CRF neurons. Peripheral injection of clozapine N-oxide (CNO, 3 mg/kg) will then selectively silence CRF neurons within the BNST that express the DREADD construct. Our results show CNO injection in DREADD-expressing mice blocks the effect of fluoxetine on fear memory.

Additional data from our laboratory indicates that withdrawal from chronic intermittent ethanol (CIE) induces anxiety-like behavior on the social interaction test in a 5HT2c-R dependent manner. Furthermore, bath application of 5HT2c-R antagonists in slice blocks CIE-induced enhancement of excitability in the vBNST. Taken together, these converging lines of evidence suggest that serotonin release in the BNST, which can be triggered by stress or withdrawal from drugs of abuse, can activate neural circuits governing learned fear and anxiety behavior.

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Identifying subsets of serotonergic neurons that selectively modulate aggressive and social behaviors in the mouse

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Central serotonin-producing neurons are heterogeneous – differing in embryonic origin, location in the adult brain, morphology, electrophysiological properties, and association with clinical disorders – but the underpinnings and functional implications of this heterogeneity are just beginning to be explored. To resolve the relevance of this cellular diversity, we have generated and employed intersectional genetic tools that allow multiple neuronal features to be measured and linked with physiology and/or behavior.

Here, we are interested in delineating those serotonergic neurons contributory to aggressive behaviors. We reveal that aggressive and social behaviors in male mice are modulated by two small subsets of serotonergic neurons. Suppressing vesicular neurotransmission from, and thus silencing chemically, one or the other of these subsets escalates aggression. Axonal projection mapping, via a novel synaptophysin-GFP allele, revealed that these serotonergic neuron subtypes innervate distinct and partially overlapping brain regions, including limbic and auditory centers. This work identifies subtypes of serotonergic neurons that modulate social behaviors, defines these neurons molecularly and hodologically, and provides genetic access for further mechanistic study. This work shows more broadly that patterned gene expression within the serotonergic system can reflect a functional and cellular modularity with potential for therapeutic selectivity

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