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Abstract Book

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IRVINE PAGE PLENARY LECTURE

TRANSLATING BASIC RESEARCH ON 5-HT NEURON CONTROL AND SIGNALLING

Trevor Sharp
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5-hydroxytryptamine (5-HT, serotonin) mediates important brain functions and contributes to the pathophysiology of common and disabling psychiatric disorders. Many fundamental mechanisms of 5-HT neuron control and signalling have been identified, more are being discovered, and some have translated to give better insights into increased risk of psychiatric disorder or into the development of drug therapies. This presentation will give examples of our own such studies with a translational aim. One important 5-HT neuron control mechanism is the 5-HT transporter (SERT). Human gene association studies identify polymorphic variants of the SERT gene as anxiety and depression risk factors but these links are not always strong and there is no mechanistic explanation. Our studies using SERT overexpressing and knockout mice demonstrate that variation in SERT expression has a strong influence on anxiety behaviours, likely through impacting on the microcircuitry of the amygdala to modulate emotional learning. Another important mechanism of 5-HT neuron control is feedback inhibition by presynaptic 5 HT autoreceptors (5-HT1A, 5-HT1B) but evidence is emerging for postsynaptic 5 HT feedback mechanisms, some of which are inhibitory (5-HT2A, 5-HT2C, 5-HT3) and others excitatory (5-HT4, 5-HT6). Such findings have underpinned the development of multimodal pharmacological strategies to treat depression, starting with pindolol and recently exemplified by vortioxetine. Finally, knowledge of downstream signalling by 5-HT receptors is also generating therapeutic opportunities. Specifically, 5-HT2A receptor signalling via the phosphoinositide pathway utilises inositol monophosphatase (IMPase), which is implicated in the therapeutic actions of lithium. A screen of a library of compounds found to be safe in humans revealed the antioxidant ebselen as an IMPase inhibitor. This drug reduces 5-HT2A receptor function in molecular and behavioural assays and decreases impulsivity in different models. Ebselen is currently under clinical investigation for its potential to treat disorders characterised by loss of impulse control.

PAUL VANHOUTTE DISTINGUISHED LECTURE

PLATELET-DERIVED SEROTONIN: FROM BAD TO GOOD…

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When platelets release serotonin, the first cells that it encounters are those of the endothelial lining. The endothelium mediates relaxations of the underlying vascular smooth muscle by releasing endothelium-derived relaxing factors, which initiate endothelium-dependent vasodilatations. The best characterized is nitric oxide (NO) formed from l-arginine by endothelial NO synthase (eNOS). NO diffuses to the vascular smooth muscle where it stimulates soluble guanylate cyclase with, under normal conditions, the resulting production of cyclic GMP. The release of NO from the endothelium can be augmented by activation of endothelial cell membrane receptors linked to eNOS by either pertussis toxin-sensitive Gi- (e.g. α_2-adrenergic agonists, serotonin) and insensitive Gq- (adenosine diphosphate, bradykinin) proteins. Following injury or apoptotic death, the endothelium regenerates. However, in regenerated endothelial cells, there is an early selective loss of the pertussis-toxin sensitive NO release, in particular the response to serotonin. Functional studies suggest that the increased presence of oxidized LDL plays a key role in this selective dysfunction. Genomic studies demonstrate the emergence of fatty acid binding protein-A (A-FBP) and metalloproteinase-7 (MMP7) in regenerated endothelial cells. Inhibition of A-FABP curtails the occurrence of endothelial dysfunction in terms of the response to serotonin, and the resulting appearance of atherosclerotic lesions. Thus, the endothelial dysfunction in regenerated areas sets the stage for the occurrence of vasospasm to in response to serotonin released from aggregating platelets and thrombosis, as well as it permits the inflammatory response leading to atherosclerosis. In addition, in coronary arteries, hypoxia causes acute augmentations of vasoconstrictor responses to serotonin. This augmentation depends on the presence of NO and the biased activation of soluble guanylate cyclase which produces cyclic IMP rather than cyclic GMP. Since hypoxia is implicated in exaggerated vasoconstrictions observed in coronary artery disease, the emerging role of this non-canonical cyclic nucleotide may help identifying novel therapeutic targets.

MAURICE RAPPORT PLENARY LECTURE

5-HT RECEPTOR NOMENCLATURE: NAMING NAMES, DOES IT MATTER?

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The nomenclature of 5-HT receptors has a convoluted history. Maurice Rapport after synthesizing 5-HT (5-hydroxytryptamine) realized that enteramine and serotonin were identical. 5-HT D and 5-HTM receptors were distinguished by Gaddum & Picarelli (1954) in the guinea pig ileum, where most 5-HT receptors are expressed. S1/S2 or 5-HT 1/5-HT2 binding was described in the late 1970s, (*i.e. S. Peroutka, G. Fillion, D. Nelson, M. Hamon*). Distinct 5-HT 1-like, 5-HT 2 and 5-HT 3 receptors were acknowledged by Bradley and colleagues (1986); there was already clear evidence for subtypes of 5- HT 1 and 5-HT 2 receptors, based on rank orders of potency in binding and second messenger studies, but that was too early for such complexity. Then, Paul Vanhoutte, after creating the Serotonin Club, started modernizing IUPHAR and establishing receptor nomenclature committees. We met at the 1990 5-HT meeting in Basel with Paul Hartig, Pat Humphrey, Terri Branchek, Pramod Saxena, John Fozard amongst others, to constitute the 5-HT nomenclature committee (led by Pat Humphrey) and tackle the issue of 5-HT receptor complexity / diversity, with recommendations that may apply to other receptors. The resulting activities were frantic, enthusiastic, very collegial and facilitated by advances in molecular biology (5-HT 1A/G21 was the second cloned GPCR, followed by many others). Since much knowledge was covered by company IP, not everything could be revealed, but it was agreed not to lead the "competition" on the wrong track. By 1993, we proposed *structural, transductional and operational* principles for 5-HT receptor nomenclature (*Humphrey et al, 1993, TIPS, 14: 233-236*). By 1994, the official nomenclature paper was sanctioned by IUPHAR and published (*Hoyer et al, 1994, Pharmacol Revs, 46: 157-204*). By 1996, we recommended alignment with the human genome (*Hartig et al, 1996, TiPS, 17: 103-105; Hoyer & Martin, 1997, Neuropharmacology, 36: 419-428*) and further refinements made (*Hoyer et al, 2002, Pharmacology, Biochemistry and Behaviour, 71: 533-554*). The proposed nomenclature was widely adopted, the recommendations are still applicable, and the principles applied to other receptors. Out of this emerged multiple successful collaborations, between researchers working in competing Pharma and Biotech or Academia. Long lasting friendships were established and cultivated in numerous 5-HT meetings, where we had the privilege to meet Maurice Rapport. That is the beauty of Science, creating knowledge byhaving fun and making friends.

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LACK OF SEROTONIN IN THE RAT BRAIN ALTERS BDNF DURING ADUTHOOD.

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It is well established that alterations of the serotoninergic system may contribute to the pathophysiology of mood disorders. Using Zinc-finger nuclease technology we recently generated rats deficient in TPH2, the enzyme responsible for serotonin synthesis in the brain. We took advantage of this animal model, lacking serotonin in the brain, to investigate whether a vulnerable genotype can be associated with alterations of neuronal plasticity, which appear to be relevant for psychopathological risk. More specifically, we analyzed the expression of the neurotrophin brain-derived neurotrophic factor (BDNF) because of its important role in adult neuronal plasticity and its association with mood disorders.

The BDNF system is very complex both at transcriptional and translational levels. The *Bdnf* gene consists of nine 5' untranslated exons, each linked to individual promoter regions, and a 3' coding exon (IX), which codes for the BDNF protein. The transcription of each untranslated exon is driven by a separate promoter controlled by an array of signaling mechanisms.

We found that total *Bdnf* mRNA levels were significantly increased in the prefrontal cortex as well as in the ventral hippocampus of *Tph2*-deficient male rats, whereas in the ventral hippocampus we observed a significant increase of the long 3'UTR *Bdnf* transcript levels. Interestingly these changes were due to a significant upregulation of specific *Bdnf* isoforms.

In summary changes in *Bdnf* expression observed in *Tph2*-deficient rats can be relevant for behavioural abnormalities associated with the lack of serotonin in the brain.

THE SEROTONIN (5-HT) 5-HT$_{2C}$ RECEPTOR (5-HT$_{2C}$R) AGONIST LORCASERIN SUPPRESSES DRUG-SEEKING FOR COCAINE- OR OXYCODONE IN RODENTS

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Environmental contexts and stimuli become reliably associated with its use leading to durable conditioned responses ("cue reactivity") that can predict relapse as well as treatment success in humans with substance use disorders. Cue reactivity, as defined here, is the attentional orientation toward such drug-associated cues that is measurable as appetitive approach behaviors in humans. Investigational, selective 5-HT$_{2C}$R agonists suppress both cocaine intake and cue reactivity in self-administration assays. Lorcaserin is a first-in-class selective 5-HT$_{2C}$R agonist recently approved by the FDA for obesity. We tested the hypothesis that lorcaserin exhibits efficacy to suppress cue reactivity in rats trained to self-administer the psychostimulant cocaine or the opioid painkiller oxycodone. Male, Sprague-Dawley rats were trained to self-administer cocaine (0.25-0.75 mg/kg/inf) or oxycodone (0.1 mg/kg/inf). The reinforcing efficacy of cocaine or oxycodone was assessed on a fixed ratio schedule or reinforcement during the self-administration phase. Cue reactivity was assessed as lever presses for drug-associated cues in a within subject design (1-2 tests/wk; min 3 intervening self-administration sessions). Lorcaserin (0, 0.25, 0.5, 1 mg/kg; 15 min) was administered prior to each test session. Lorcaserin dose-dependently suppressed the reinforcing efficacy of cocaine and oxycodone vs vehicle; 1 mg/kg significantly suppressed active lever presses vs vehicle (p<0.05). Lorcaserin dose-dependently suppressed cue-reactivity in cocaine-trained and oxycodone-trained rats; 1 mg/kg significantly suppressed cue-reinforced lever presses vs. vehicle (p<0.05). In summary, lorcaserin suppressed both self-administration and cue reactivity in rats experienced in psychostimulant or opioid self-administration. Although the direct neural targets for psychostimulants and opioids to evoke their rewarding effects differ, the present data suggest that the incentive saliency of psychostimulant- and opioid-paired cues involve shared 5-HT$_{2C}$R-mediated mechanisms. Further investigation is warranted, but the clinical availability of lorcaserin now allows treatment efficacy analyses in psychostimulant or opioid use disorders.

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EXPRESSION OF *htr2A* GENE IDENTIFIES A NOVEL CLASS OF SEROTONIN-REGULATED EXCITATORY NEURONS IN THE CEREBRAL CORTEX

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Serotonin regulates the activity of the cerebral cortex by targeting both excitatory neurons, mostly pyramidal cells, as well as inhibitory interneurons. Excitatory cells and GABAergic interneurons are heterogeneous cell classes comprised of multiple neuronal subtypes that differentially contribute to the assembly of cortical networks. Thus an important challenge is to elucidate how serotonin regulates these different neuronal subtypes. Previous work has focuses predominantly in the serotonergic regulation of excitatory pyramidal cells of layer 5 and to a lesser extent the more superficial layers of cortex. In the current work we have taken advantage of a BAC transgenic mouse expressing EGFP (*htr2A*-EGFP) under the control of the 5-HT_{2A} receptor promoter to identify novel classes of serotonin-regulated neurons in the cerebral cortex. Examination of the cerebral cortex of the *htr2A*-EGFP mouse reveled expression of EGFP in layer 5 as well as in a narrow band corresponding to layer 6b. Whole cell recordings in *in vitro* cortical brain slices indicated that *htr2A*-expressing layer 6b cells are characterized by a distinctive physiology and pharmacology. Most notably, administration of serotonin to these cells results in a strong excitation that was blocked by ketanserin and reduced by MDL100907 indicating the involvement of 5-HT_{2A} receptors. To better understand these cells we injected an AAV virus expressing ChR-EYFP in a Cre dependent manner in layer 6b of the htr2A;Cre driver mouse. Infected neurons expressing the ChR-EYFP projected to the superficial layers of cortex where they made monosynaptic glutamate synapses with pyramidal cells and interneurons. Cells with similar characteristics could be found in rat as well as in several different regions of cortex. These results identify a ubiquitous but previously unidentified excitatory neuronal subtype regulated by serotonin in the cerebral cortex.

NETWORK ARCHITECTURE AND NEUROMODULATION OF THE LONG-RANGE DESCENDING INPUT FROM THE PREFRONTAL CORTEX TO THE DORSAL RAPHE NUCLEUS.

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Serotonin (5-HT) neurons located in the raphe nuclei modulate a wide range of behaviors by means of an expansive innervation pattern. In turn, the raphe receives a vast array of synaptic inputs and a remaining challenge lies in understanding how these individual inputs are organized, processed and modulated in this nucleus to ultimately contribute to the core coding features of 5-HT neurons. The details of the long-range, top-down control exerted by the medial prefrontal cortex (mPFC) in the dorsal raphe nucleus (DRN) are of particular interest, in part because of its purported role in stress processing and mood regulation. Here, we found that the mPFC provides a direct monosynaptic glutamatergic drive to both DRN 5-HT and GABA neurons and that this architecture was conducive of a robust feedforward inhibition. Remarkably, activation of cannabinoid receptors differentially modulated the mPFC inputs onto these cell types in the DRN, in effect regulating the synaptic excitatory-inhibitory balance governing the excitability of 5-HT neurons. Thus, the cannabinoid system dynamically reconfigures the processing features of the DRN, a mood-related circuit believed to provide a concerted and distributed regulation of the excitability of large ensembles of brain networks.

MINING IMMUNE SEROTONIN CROSSTALK FOR INSIGHTS INTO NOVEL THERAPEUTICS IN AUTISM

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Autism spectrum disorder (ASD) has been associated with changes in both serotonin (5-HT) homeostasis and elevated immune system activation, though the connections between the two have not been mechanistically linked. Previously (Sutcliffe et al, 2005), we identified five rare coding variants in the presynaptic 5-HT transporter (SERT) and demonstrated that all five conferred elevated 5-HT uptake activity in subject lymphoblasts and transfected cells, either through enhanced transporter surface expression or elevated transport function. Independently, studies in our lab identified PKG and p38 MAPK linked pathways that could drive elevated 5-HT uptake of wildtype human and rodent SERT. Additionally, we have found that a p38 MAPK pathway can be triggered in cells and mouse brain preparations via immune system activation, specifically involving the inflammatory cytokine IL-1□. That these two lines of research were possibly related became apparent when we found that the most common of the ASD-associated SERT coding variants, Ala56, lacked sensitivity to both PKG and p38 MAPK activation, and displayed p38 MAPK-dependent hyperphosphorylation. These findings support the hypothesis that the mutant transporter is locked in a state normally reserved for the transiently activated wildtype protein upon PKG/p38 MAPK activation. Subsequent studies with the SERT Ala56 knock-in mouse model (Veenstra-VanderWeele et al, 2012) revealed hyperserotonemia, elevated *in vivo* SERT activity, hypersensitive 5-HT1A and 5-HT2A receptors, elevated p38 MAPK-dependent SERT phosphorylation and behavioral changes suggestive of ASD-like perturbations. Additionally, 5-HT neuron-specific conditional elimination of p38□ MAPK (Baganz et al, 2015) eliminates the ability of innate immune system activation with LPS to increase SERT activity. In my presentation, I will review these studies and discuss how the dialog between inflammatory signaling pathways and SERT, specifically linked to IL-1□/p38□ MAPK, may be modulated pharmacologically for therapeutic benefit in developmental and adult onset disorders linked to perturbed 5-HT signaling, including ASD, OCD and depression.

PLATELET-DERMED SEROTONIN IN AUTOANTIBODY MEDIATED INFLAMMATION

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Platelets survey blood vessels, searching for endothelial damage and preventing loss of vascular integrity. However, there are circumstances where vascular permeability increases, suggesting that platelets sometimes might not fulfill their expected function. Human inflammatory arthritis is associated with tissue edema attributed to enhanced permeability of the synovial microvasculature. Murine studies have suggested that such vascular leak facilitates entry of autoantibodies and may thereby promote joint inflammation. Whereas platelets typically help to promote microvascular integrity, we examined the role of platelets in synovial vascular permeability in murine experimental arthritis. Using an in vivo model of autoimmune arthritis, we confirmed the presence of endothelial gaps in inflamed synovium. Surprisingly, permeability in the inflamed joints was abrogated if the platelets were absent. This effect was mediated by platelet serotonin accumulated via the serotonin transporter (SERT) and could be antagonized using serotonin-specific reuptake inhibitor antidepressants. As opposed to the conventional role of platelets to microvascular leakage, this demonstration that platelets are capable of amplifying and maintaining permeability adds to the rapidly growing list of unexpected functions for platelets.

Title: Prenatal exposures and placental serotonin: a potential pathway for the developmental programming of mental diseases.

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Abstract:

In addition to its role in the pathophysiology of numerous psychiatric disorders, serotonin (5-HT) is a crucial modulator of neurodevelopment. Prenatal insults that alter 5-HT availability in the maternal, placental and fetal compartments, such as maternal depression, antidepressant exposure, as well as maternal inflammation and infections, can affect fetal brain development and have long-term consequences on adult offspring brain function. Using a mouse model and the widely-prescribed SSRI Citalopram (CIT), we assessed maternal-fetal drug disposition throughout pregnancy and examined the developmental effects of maternal depression with and without CIT exposure on the fetal brain. Results revealed that maternal depression and prenatal CIT exposures differentially impact fetal brain neurochemistry and circuit formation. In other studies, we investigated the mechanisms linking maternal inflammation and infections during pregnancy with increased risk of neurodevelopmental disorders in the offspring. We observed that maternal inflammation triggered by the viral-mimic poly(I:C) in mid-pregnancy results in an upregulation of tryptophan conversion to 5-HT within the placenta, leading to exposure of the fetal forebrain to increased concentrations of this biogenic amine. This resulted in altered serotonergic axon growth in the fetal forebrain with long-term consequences on offspring behavior. The data provide a new understanding of placental function playing a key role in fetal brain development, and how this process is altered by adverse prenatal events such as maternal stress, therapeutic drug exposure and inflammation. The results uncover important future directions for understanding the early developmental origins of mental disorders.

BRAIN SEROTONIN DEFICIENCY: STRESS VULNERABILITY AND TREATMENT RESISTANT DEPRESSION-LIKE SYMPTOMS

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The biological factors that determine whether an individual develops depression-related mental illness or responds adequately to pharmacotherapy still remain enigmatic. Converging evidence suggests that hypofunction of the brain serotonin (5-HT) system. To evaluate the importance of brain 5-HT levels in determining stress susceptibility and antidepressant responses, we have used a genetic model of 5-HT deficiency, the tryptophan hydroxylase 2 (R439H) knock-in (Tph2KI) mouse. These animals, which express a loss-of-function mutant form of the *Tph2* gene identified in humans display 60-80% reductions in the extracellular levels of brain 5-HT (Zhang et al., Neuron 2004; Jacobsen et al., *Mol. Psychiatry*, 2012). Our results show that brain 5-HT deficiency reduces the threshold at which mice display social avoidance behavior following repeated psychosocial stress in the paradigm of social defeat. In addition, we also demonstrate that 5-HT deficiency prevents the ability of chronic fluoxetine administration to reverse stress-induced behavioral avoidance. Associated with the phenotypes are changes gene expression in nucleus accumbens, frontal cortex and amygdala. Interestingly, inhibiting the activity of the lateral habenula with an inhibitory DREADD was sufficient to reverse stress-induced behavioral avoidance in both WT and fluoxetine-resistant Tph2KI animals, highlighting the potential of lateral habenula inhibition to overcome treatment resistance in depression.

We also used Tph2KI mice as a model to validate the concept of SSRI-augmentation therapy for treatment resistant depression (TRD), which in humans, is a significant unmet need. A limited number of older small clinical studies have suggested that the immediate precursor of 5-HT, 5-HTP, has SSRI augmenting properties but its poor pharmacodynamics profile prohibits its use as an effective therapy. Using a slow release mode of 5-HTP (5-HTP-SR), we show in mice that 5-HTP-SR synergistically augments the 5-HT_{Ext} elevating effects of chronic SSRI treatment with absolutely no adverse effects. Thus, 5HTP-SR becomes a high value therapeutic candidate for TRD.

DORSAL RAPHE SEROTONERGIC NEURONS SIGNAL REWARDS AND PUNISHMENTS

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Serotonergic neurons are thought to be involved in many behaviors. One of the challenges in understanding their functions has been observing their action potentials during well-controlled behavioral tasks. To address this, we have used optogenetic "tagging" to identify dorsal raphe serotonergic neurons in mice engaged in tasks in which availability of reward changes slowly over time. We found that serotonergic, as well as unidentified, dorsal raphe neurons signaled reward value using tonic firing rates, and reward-predicting cues using phasic firing rates. These results suggest that serotonergic neurons can regulate reward behavior across multiple timescales.

SEROTONIN, KYNURENINE AND AGGRESSIVE BEHAVIOR: FROM NEUROBIOLOGY TO BIOMARKERS

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Aggressive behavior is one of the most challenging symptoms in psychiatry. Serotonin (5-HT) is the most studied neurotransmitter related to the pathophysiology of aggression. The old dogmatic view of a low 5-HT activity linked to aggression appears true only for certain types of aggressive behavior such as impulsive behavior. The complex neurobiology of the 5-HT pathway in aggression thus needs clarifications and especially lacks of large cohort human studies. Growing evidence is suggesting that impairments in the 5-HT pathway observed in several neuropsychiatric disorders may derive by an altered metabolism of its precursor tryptophan (Trp) along the kynurenine (Kyn) pathway. We thus explored the serum levels of Trp and its metabolites via 5-HT and Kyn in a large cohort of aggressive inmates and several psychosocial factors linked to aggressive behavior including mental and personality disorders, global assessment of functioning (GAF), intelligent quotient (IQ), impulsivity, and adult attention-deficit/hyperactivity disorder (ADHD). We also sought to validate which biological and psychopathological factors can be considered biomarkers of aggression. Our findings suggest that in aggressive individuals there is a reduction of peripheral Trp which leads to a reduced availability of the amino acid in the brain for the synthesis of 5-HT and that there is an activation of the Kyn pathway in more aggressive individuals (the ratio Kyn/Trp was positively correlated to the number of severe aggressive acts: $r=0.617$, $P<0.001$). Importantly, they also indicate that only a combination of biological (5-HT/Trp ratio) and psychopathological (antisocial behavior and GAF) markers discriminates between aggressive and non-aggressive behavior (area under the ROC curve of 0.851 (95% CI 0.806-0.895)), thus confirming the heterogeneous construct of aggression. According to these data, the conversion of Trp into 5-HT and Kyn in the periphery may be a novel potential target for therapeutic intervention in individuals with aggressive behavior.

CONNECTIVITY OF THE ASCENDING RAPHE: THE DEVIL IS IN THE DETAILS

Dr. Kathryn Commons

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Breaking large complex systems down into their constituent components can give important insight into how those systems work. Yet sometimes it can also be helpful to examine how constituent components relate to each other to get a better understanding of how they might work together. The dorsal and median raphe nuclei (DR and MR) encompass large groups of neurons that can be broken down into many different subgroups. Here we asked how different neuroanatomical subregions might relate to each other with respect to afferent input. By analyzing the relative distribution of afferents within subregions of the DR and MR, a prominent pattern emerged. That was, axons that innervated the rostral pole of the DR often dissipated when reaching the caudal third of the nucleus, which is also known as B6 and named 'dorsal raphe caudal' and 'dorsal raphe interfasicular' on rodent atlases. Likewise axons that tend to innervate the MR appear to continue on a dorsal trajectory and ramify within B6. Thus subregions within the rostral two thirds of the DR appear similar to each other in afferent input, whereas the MR and B6 contrast in receiving innervation from different areas. As a consequence of these different streams of neural information, these areas could be called into action under different behavioral circumstances. Indeed, this could explain our prior results showing reciprocal patterns of activity in the rostral and caudal poles of the DR produced by nicotine exposure vs. withdrawal. These observations and others lead us to conclude that it may be worthwhile to distinguish B6 from the remainder of the DR, since it actually seems more similar to the MR. While there may be many important subtypes of neurons within these two areas, rostral DR and B6/MR may be useful umbrella groups that could help us understand the context of these subtypes.

SEROTONIN PRODUCTION BY TROPHOBLAST CELLS: ROLE IN PLACENTAL AND EMBRYO DEVELOPMENT AND HEMATOPOISESIS

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In humans, anaemia is the most common disorder of the blood. Anaemia includes excessive break down of red blood cells (hemolysis) or deficient red blood cell production (ineffective erythropoiesis) in the bone marrow. Little is known regarding the mechanism of action of monoamines including serotonin (5-HT) in bone marrow during stem cell differentiation and maturation although 5-HT was hypothesized long ago to have a strong erythropoietic effect in mice. Published data together with recent results demonstrated that lack of peripheral 5-HT consecutive to the disruption of the Tph1 gene causes macrocytic anaemia in adult mice and the death of 20% of $Tph1^{-/-}$ embryos at E13.5. As both placenta and fœtal liver are critical sources of red blood cell progenitors, we hypothesized that a serotonergic network was present within the two tissues and contribute to regulate red blood cells differentiation during embryonic development. In $Tph1^{-/-}$ embryos, erythroid progenitors colonize fœtal livers and placenta but are unable to generate sufficient numbers of late erythroid cells, resulting in anaemia. More precisely, fœtal liver and placental index were significantly reduced in $Tph1^{-/-}$ embryos and using flow cytometry analysis, we observed an increase in erythroid progenitors that is not accompanied by an increase in red blood cells production in $Tph1^{-/-}$ fœtal liver and placenta. We relate our findings to human health issues, as using purified human $CD34^{+}$ cord blood cells, we revealed the existence of specific serotonergic components that influence erythroid differentiation. Our findings should provide new perspectives for clinical studies of blood disorders specifically in anaemia and myeloproliferative/myelodysplastic disorders in which defects in red blood cell production have been reported.

SEROTONIN AND THE MICROBIOTA-GUT-BRAIN AXIS

John F. Cryan

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The brain-gut axis is a bidirectional communication system between the central nervous system and the gastrointestinal tract. Serotonin functions as a key neurotransmitter at both terminals of this network. Accumulating evidence points to a critical role for the gut microbiome in regulating normal functioning of this axis. In particular, it is becoming clear that the microbial influence on tryptophan metabolism and the serotonergic system may be an important node in such regulation. There is also substantial overlap between behaviours influenced by the gut microbiota and those which rely on intact serotonergic neurotransmission. The developing serotonergic system may be vulnerable to differential microbial colonisation patterns prior to the emergence of a stable adult-like gut microbiota. At the other extreme of life, the decreased diversity and stability of the gut microbiota may dictate serotonin-related health problems in the elderly. The mechanisms underpinning this crosstalk require further elaboration but may be related to the ability of the gut microbiota to control host tryptophan metabolism along the kynurenine pathway, thereby simultaneously reducing the fraction available for serotonin synthesis and increasing the production of neuroactive metabolites. The enzymes of this pathway are immune and stress-responsive, both systems which buttress the brain-gut axis. In addition, there are neural processes in the gastrointestinal tract which can be influenced by local alterations in serotonin concentrations with subsequent relay of signals along the scaffolding of the brain-gut axis to influence CNS neurotransmission.
Recently we have investigated the role of the microbiota-gut-brain axis in a mouse model of autism the BTBR mouse. BTBR mice demonstrate delayed intestinal transit time (as estimated by Carmin Red assay in vivo) and significantly decreased colon length, as well as water content in faecal matter in comparison with C57BL/6 strain. These changes, indicative of constipation, were accompanied by alterations to the expression levels of genes tightly involved in serotonin turnover, which is a key regulator of peristaltic and propulsive activity of gastrointestinal tract. In particular, BTBR mice exhibit a 20-60% decrease to Tph1 and Ido1 and a 2.5-fold increase to Slc6a4 (Sert) expression in distal ileum and proximal colon samples (assessed by relative real-time RT-PCR). These findings suggest a deficiency in production and synaptic content of serotonin in the BTBR gut. The role of the microbiota in these effects is being investigated. Overall, therapeutic targeting of the gut microbiota might be a viable treatment strategy for serotonin-related brain-gut axis disorders.

POSITIVE ALLOSTERIC MODULATORS OF THE SEROTONIN (5-HT) 5-HT$_{2C}$ RECEPTOR (5-HT$_{2C}$R) AS NOVEL NEUROTHERAPEUTICS

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Allosteric modulation of G protein-coupled receptors (**GPCR**s) has gained traction as a pharmacological strategy to impact GPCR function. At present, allosteric modulators are defined operationally as positive (**PAM**) or negative allosteric modulators (**NAM**) or neutral allosteric ligands (**NAL**) with the possibility of the additional property of allosteric agonism (agonist effects consequent to binding to allosteric sites). We propose that PAMs of the serotonin GPCR 5-HT$_{2C}$ receptor (**5-HT$_{2C}$R**) may provide therapeutically useful in disorders associated with dysfunctional 5-HT$_{2C}$R systems, including addiction, eating and mental health disorders. An accumulation of evidence has shown that decreased 5-HT$_{2C}$R signaling capacity is a regulatory factor in neurobehavioral processes that may underlie these chronic health issues and we hypothesize that selective 5-HT$_{2C}$R PAMs will be useful to restore signaling capacity and thereby improve underlying aberrant neurobiology.

We have designed and synthesized a series of novel 5-HT$_{2C}$R allosteric modulators based on the structure of synthetic 5-HT$_{2C}$R PAM PNU-69176E and have identified pharmacological activity in *in vitro* functional and radioligand binding assays. Several compounds have been shown to enhance intracellular calcium (**Ca$_i^{++}$**) release and activation of extracellular signal related kinase$_{1/2}$ (**ERK$_{1/2}$**) induced by the endogenous ligand 5-HT or the selective 5-HT$_{2C}$R agonist WAY 163909. Additional cellular screening indicates that these PAMs do not exhibit intrinsic 5-HT$_{2A}$R or 5-HT$_{2C}$R agonist activity nor potentiate 5-HT$_{2A}$R-mediated signaling. Preliminary rodent studies indicate a promising behavioral profile for a lead compound assessed in the modified open field (locomotor activity), 1-choice serial reaction task (motor impulsivity) as well as drug discrimination and cocaine self-administration/cue reactivity assays. Optimization of our newly identified 5-HT$_{2C}$R PAMs and further evaluation of these molecules in preclinical models are ongoing. Taken together, our target-based drug design and development efforts of 5-HT$_{2C}$R PAMs open new avenues to allow analyses of 5-HT$_{2C}$R function and discovery of novel neurotherapeutics.

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GENETIC AND ENVIRONMENTAL MANIPULATIONS OF MONOAMINES AND ALTERATIONS OF SOCIAL AND COMMUNICATIVE BEHAVIORS

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We generated mouse lines to allow the imaging and translational profiling of serotoninergic neurons. Because of the hypothesized link between serotonin and autism, we conducted a comparative analysis to identify hundreds of transcripts enriched in these neurons, and screened these genes for association to ASD in humans. We found suggestive evidence implicating the RNA binding protein, Celf6, and mutation of Celf6 in mice lowered monoamine levels, including serotonin, and robustly disrupted both early communicative behavior(pup vocalization) and adult conditioning in mice. To understand whether the serotoninergic alterations mediated the communicative phenotype, we conducted both conditional deletion of Celf6 only in serotonergic neurons, and a Gene by Environment study of the interaction between Celf6 genotype and a suspected environmental risk factor for ASD, exposure to SSRIs during neurodevelopment. While we found that loss of Celf6 in serotonergic neurons was not sufficient to disrupt vocalization, transient exposure to SSRIs, even in wildtype mice, is sufficient to completely phenocopy the most severe aspects of the Celf6 communicative deficit, and alter social and repetitive behaviors in adult animals long after exposure. Both models now provide the opportunity to understand the molecular and cellular circuits mediating social and communicative behaviors.

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MORE THAN MEETS THE EYE: MAPPING MOLECULAR AND FUNCTIONAL SUBTYPES OF SEROTONIN NEURONS

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Cellular heterogeneity within the serotonergic system is proving substantial and likely parallels the wide diversity of effects modulated by serotonin - from sensory processing to cognitive control and motivated behaviors to autonomic responses. Anatomical topography has been used to subdivide groups of serotonin neurons, first into the dorsal (DR), median (MR), and medullary raphe, then into subnuclei (e.g. DR comprised of subnuclei B7, B6, B4; MR-B8, B5), then further into DR subgroups – rostral, ventral, dorsal, lateral, caudal, and interfascicular. In this presentation, genetic tools and findings will be presented that refine further the serotonergic structure-function map, superimposing onto topography new serotonin cell-subtype information, from molecular expression to efferent bouton locations to modulated organismal behavior. Specifically, our new mapping resolution plots (1) serotonergic developmental lineage (which includes coordinate position and signature gene expression of the ancestral serotonergic progenitor cell in the developing hindbrain), (2) cell groups based on unbiased clustering analyses of global gene expression profiles, (3) cellular electrophysiological properties measured in slice preparations and registered within the context of lineage and transcriptomic information, (4) efferent projections of the serotonergic neuron molecular subtypes including terminal and en passant bouton locations, as a first step in connectivity mapping, and (5) specific organismal behaviors and physiological processes altered upon in vivo, neuron-subtype silencing. Highlighted will be multiple distinct molecular and functional subtypes of serotonergic neurons mapped within the DR, as well as four molecular subtypes of serotonergic neurons mapped within the MR.

CLINICAL STUDIES USING SEROTONERGIC AGENTS IN PARKINSON'S DISEASE (PD)

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The serotonergic system plays an integral role in both the motor and non-motor symptoms of PD. In terms of movement control, reducing involuntary movements secondary to chronic levodopa, called levodopa-induced dyskinesia (LID), has been the main therapeutic target. Several studies targeting presynaptic $5HT_{1A}$ receptors, to reduce aberrant dopamine release from surviving serotonergic terminals have been performed. These include mixed $5HT_{1A}$ agonists, sarizotan, buspirone and eltoprazine. Issues relate to 5HT receptor selectivity and other side-effects however have limited efficacy, to date. Post synaptic $5HT_{2A}$ receptors have also been implicated in LID and may underlie the efficacy of atypical antipsychotic, clozapine in reducing LID without worsening motor function. Clozapine can also reduce PD tremor, which may reflect $5HT_{2A}$ properties.

Non-motor symptoms that may respond to serotonergic targets include PD psychosis. There is preclinical and clinical evidence for a role for 5HT2A receptors in PD psychosis. Drugs that target these receptor and maybe effective include clozapine, and the new $5HT_{2A}$ inverse agonist, pimavanserin. Mood disorders, including anxiety and depression respond to serotonergic agents in PD e.g. SSRIs. Dementia is a major issue in PD, and the recent 5HT6/5HT2A dual antagonist (SYN120) is also being evaluated in PD dementia.

CONSTITUTIVE AND ACQUIRED HYPOSEROTONERGIA ALTERS MEMORY AND HIPPOCAMPAL SYNAPTIC PLASTICITY

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Besides its well-established role in the regulation of emotional and affective responses, serotonin (5-HT) neurotransmission has been implicated in cognitive functions such as learning and memory that are altered in several neurological and psychiatric disorders. However, the mechanisms involved and causal relationships between reduced 5-HT transmission and memory have not been established. Here, we used mice with a constitutive depletion of 5-HT brain levels, (Pet1-KO mice) to analyze the contribution of 5-HT depletion to different learning tasks. We find that Pet1-KO mice have a profound and selective impairment of both short and long-term object memory, that corresponds to a form of declarative memory in humans. On the other hand, contextual object memory; motor learning and operant conditioning were not altered in the Pet1-KO mice. A similar object memory deficit was mimicked by adult depletion of 5-HT neurotransmission and by the acute pharmacogenetic silencing (DREADDs) of the medial raphe (MR) but not when DREADDs targeted the dorsal raphe (DR) neurons. This indicated that neural activity of MR neurons, the main source of 5-HT afferents to the hippocampus, is selectively required for object learning. To determine the neurobiological correlates of this effect we performed *in vivo* electrophysiological recordings in the hippocampus of freely moving mice, during the acquisition of the object memory task. Pet1-KO mice showed enhanced synaptic potentiation responses of the CA3-CA1 synaptic circuit relative to controls, suggestive of a saturation of LTP mechanisms. These changes could be related to a lack of inhibitory drive via 5-HT1A receptors in CA1, since acute administration of 5-HT1A agonist 8-OHDPAT rescued the memory deficits of the Pet1-KO mice. Overall these results established a direct causal link between recognition memory deficits and loss of 5-HT innervation in the hippocampus , moreover they open the way to specific therapeutic intervention for specific memory disorders linked to reduced serotonin transmission.

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ENTERIC SEROTONERGIC SIGNALING, SERT, AND AUTISTIC SPECTRUM DISORDER

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Throughout evolution, from cyclostomes to humans, serotonin has been involved in enteric signaling. In mammalian gut, serotonin is stored in epithelial enterochromaffin (EC) cells and neurons. Although survival is possible when both serotonin depots are lacking, each is needed for normal gut function. Tryptophan hydroxylase 1 (TPH1) is required for serotonin biosynthesis in EC cells and TPH2 in neurons. Epithelial serotonin is a paracrine messenger that regulates intestinal motility and inflammation and is an endocrine hormone that regulates bone deposition; therefore, deletion of TPH1 (epithelial serotonin) results in abnormal motility, diminished inflammation, deficient resistance to infection, and increased bone density. Enteric neuronal serotonin is a neurotransmitter but also a growth factor; therefore, deletion of neuronal serotonin leads to deficiencies in enteric neurogenesis/neuroprotection, increased severity of inflammation, and diminished propulsive motility. Because serotonin is so fundamental to intestinal physiology, disorders that affect serotonergic signaling disturb gastrointestinal function. Autism spectrum disorder (ASD), for example, is often accompanied by gastrointestinal dysfunction. Hyperfunctional coding variants in the serotonin transporter (SERT, *SLC6A4*) have been identified in ASD. When the most common of these, SERT Ala56, is expressed in mice, the resulting increase in serotonin clearance leads to a hypoplasia of the enteric nervous system (ENS), which is similar to that seen in mice lacking TPH2. Accompanying functional deficits include slow intestinal transit, deficient peristaltic reflexes, and reduced proliferation of crypt epithelial cells. The phenotype of SERT Ala56 mice is the opposite of that of mice lacking SERT. These reciprocal phenotypes suggest that serotonergic signaling regulates enteric neuronal development. Administration of a 5-HT_4 agonist throughout development rescues the SERT Ala56 phenotype, implying that the hyperfunctional SERT Ala56 leads to a deficit of ligand at the 5-HT_4 receptors that drive enteric neurogenesis/neuroprotection. Defective serotonergic signaling during development may thus contribute to gastrointestinal manifestations of ASD. Supported by NIH grant NS15547.

Epigenetic mechanisms of 5-HT_{2A} receptor-dependent antipsychotic action.

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In some patients with schizophrenia, antipsychotic drug treatment produces complete remission of psychotic symptoms, including hallucinations and delusions. Cognitive deficits, however, are present in the majority of schizophrenia patients, with absence of improvement or even deterioration upon antipsychotic drug treatment across several domains of executive function. NF-kB is a nuclear factor that has been recognized to play fundamental roles in the regulation of neuropsychological and structural aspects related to normal brain processes and cognitive capabilities. Our data suggest functional dysregulation of the NF-kB pathway as a principal mediator responsible for deleterious effects of chronic antipsychotic drug treatment along numerous behavioral and physiological traits. Chronic treatment with atypical antipsychotic drugs selectively enhanced NF-kB (p65) translocation into the nucleus of pyramidal neurons in both mouse and human frontal cortex (trafficking event that was triggered via serotonin 5-HT_{2A} receptor-dependent down-regulation of expression of the NF-kB repressor *IkB*a). Such up-regulation of NF-kB activity occurred in association with its increased binding at the promoter region of the *Histone deacetylase 2 (Hdac2)* gene, an epigenetic recruitment that led to a decrease in synaptic structural complexity. Selective deletion of HDAC2 function in forebrain pyramidal neurons prevented the unfavorable effects of chronic antipsychotic drug treatment on cortical synaptic remodeling and cognitive processes. Conversely, virally mediated activation of cortical pyramidal NF-kB-dependent transcriptional activity minimized the formation of mature spine structures, decreased synaptic plasticity, and exacerbated psychosis-related behaviors and cognitive deficits through a signaling mechanism that required up-regulation of HDAC2. Together, our results suggest that activation of the NF-kB pathway by chronic atypical antipsychotic drug treatment increases HDAC2-dependent negative effects on synaptic plasticity and behavior. These observations may aid in efforts to develop therapeutic strategies that improve the currently poor outcome in schizophrenia patients.

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INHIBITING PERIPHERAL SEROTONIN SYNTHESIS REDUCES OBESITY, NON-ALCOHOLIC FATTY LIVER DISEASE AND INSULIN RESISTANCE BY PROMOTING BROWN ADIPOSE TISSUE THERMOGENESIS

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Brown adipose tissue (BAT) is an organ which has long been known to exist in rodents and infants but only recently was discovered in adult humans. BAT has the unique capability to burn large amounts of sugar and fat and effectively dissipate this energy as heat. Mitochondrial uncoupling protein 1 (UCP1) is enriched within interscapular BAT and beige/brite adipose tissue but its thermogenic potential is reduced with obesity and type 2 diabetes, for reasons that are not understood. Thus identifying ways to increase the metabolic activity of BAT is considered a potential therapeutic strategy for treating obesity and type 2 diabetes. Serotonin is a highly conserved biogenic amine that resides in distinct peripheral and central tissue pools that are specifically regulated via tryptophan hydroxylase (Tph) 1 and 2, respectively. Recent findings suggest that peripheral serotonin and polymorphisms in *Tph1* may be associated with obesity but whether this is directly related to reduced BAT theromogenesis and obesity is not known. We find that Tph1 deficient mice fed a high fat diet (HFD) are protected from obesity, insulin resistance and non-alcoholic fatty liver disease while exhibiting increased energy expenditure by BAT. Small molecule chemical inhibition of Tph1 in HFD-fed mice mimics the benefits ascribed to *Tph1* genetic deletion; effects that are dependent on UCP1-mediated thermogenesis. The inhibitory effects of serotonin on energy expenditure are cell autonomous as serotonin blunts □-adrenergic induction of the thermogenic program in brown and beige adipocytes *in vitro*. As obesity increases BAT serotonin content the inhibition of Tph1 or serotonin in adipose tissue may be an effective treatment for obesity and related diseases.

TONIC ENDOCANNABINOID SIGNALING GATES HEBBIAN PLASTICITY IN THE DORSAL RAPHE NUCLEUS THROUGH PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

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The Endocannabinoid (eCB) system composed of cannabinoid receptors and their endogenous agonists, 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (Anandamide or AEA) is an important modulator of stress homeostasis, anxiety and mood. In the dorsal raphe nucleus (DRn), activation of serotonin (5-HT) neurons leads to the synthesis and release of the eCB, 2-AG, which subsequently activates presynaptic CB1 receptors to dampen neurotransmitter release. This phasic mode of eCB signaling enables DRn 5-HT neurons to exert a feed-back control of their excitatory and inhibitory synaptic inputs.

In addition to the activity-driven eCB synthesis and release, recent studies have revealed that 2-AG and AEA are constitutive synthesized and release from numerous cell type, including neurons, which may control synaptic transmission. However, the presence of such mode of eCB signaling and its role in controlling synaptic plasticity in the DRn remain unknown. In the present study, we used an electrophysiological approach to address these issues. The results of this study revealed that blockade of CB1 receptors with AM 251 or NESS profoundly increased the strength of glutamate synapses onto DRn 5-HT neurons by increasing glutamate release. In contrast, inhibition of monoglyceride lipase (MGL) or fatty acid amid hydrolase (FAAH), enzymes that degrade 2-AG and AEA, respectively, reduced glutamatergic transmission to DRn 5-HT neurons, indicating the presence of tonic eCB signaling at glutamate synapses onto DRn 5-HT neurons.

We next examine the role of tonic eCB signaling in controlling the plasticity of glutamate synapses onto DRn 5-HT neurons. We found that in control condition, repetitive pairing of presynaptic stimulation with back propagation action potentials in DRn 5-HT neurons readily induced a spike-timing dependent long-term potentiation (t-LTP) of EPSCs. Remarkably, inhibition of 2-AG degradation leading to transient increase in tonic eCB signaling blocked t-LTP. Similarly, acute exposure to the stress hormone corticosterone or swim stress, manipulations known to increase tonic eCB release in the DRn, prevented tLTP. The blockade of tLTP induced by tonic eCB signaling was not signaled by CB1 receptors, but rather by peroxisome proliferator-activated receptors (PPARs). As such, the present results unravel a novel role of eCB signaling in controlling synaptic function in the DRn and indicate that tonic eCB signaling at glutamate synapses gates spike timing dependent plasticity. Pharmacological or behavioral manipulations that alter this mode of signaling will affect the plasticity properties of synapses in the DRn.

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REGULATION OF HALLUCINOGEN-INDUCED BEHAVIORAL RESPONSES BY MGLU2/3 AND MGLU5 RECEPTORS

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Metabotropic glutamate (mGlu) receptors have been suggested to play a role in schizophrenia and depression. Because serotonergic hallucinogens increase glutamate release and mGlu receptors modulate the response to serotonin (5-HT)$_{2A}$ activation, the interactions between serotonin 5-HT$_{2A}$ receptors and mGlu receptors may prove to be important for our understanding of these disease states, and may help to unravel the mechanisms underlying the potential therapeutic effects of hallucinogens.

One series of experiments assessed whether the head twitch response (HTR) induced by the highly selective 5-HT$_{2A}$ agonist 25CN-NBOH in C57BL/6J mice is modulated by acute or chronic treatment with the mGlu2/3 agonist LY379268. In the acute experiment, mice were treated with LY379268 (0.1-10 mg/kg SC) 30 min prior to administration of 25CN-NBOH (1 mg/kg SC). In the chronic experiment, mice were treated with vehicle or LY379268 (10 mg/kg/day SC) for 21 days, and then challenged with 25CN-NBOH after a 48-h washout period. We also tested whether deletion of the mGlu5 gene in mice alters the locomotor hyperactivity induced by the 5-HT$_{2A}$ agonists DOM and TCB-2.

The HTR response induced by 25CN-NBOH was significantly attenuated by acute treatment with 10 mg/kg LY379268. When mice were treated with LY379268 for 21 days and then challenged 48-h later with 25CN-NBOH, the HTR was attenuated 26.7% relative to mice treated chronically with vehicle. The locomotor hyperactivity induced by DOM and TCB-2 was potentiated in mGlu5 knockout mice relative to their wild-type littermates.

These studies demonstrate that mGlu2/3 and mGlu5 receptors modulate the behavioral responses induced by 5-HT$_{2A}$ activation. Additionally, we found that repeated activation of mGlu2/3 receptors can reduce the response to a hallucinogen. These studies provide additional support for the link between the serotonergic and glutamatergic systems. Additional studies are necessary to understand why 5-HT$_{2A}$ responses are altered by chronic mGlu2/3 activation and by the loss of mGlu5 signaling.

PRECLINICAL EVIDENCE TO SUPPORT LORCASERIN AS A TREATMENT FOR NICOTINE DEPENDENCE

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Lorcaserin was approved by the FDA for the treatment of obesity in 2012. Its origin can be traced to an understanding that the anorectic properties of (dex)fenfluramine are most likely mediated through increased 5-HT_{2C} receptor signalling. As a functionally selective orthosteric 5-HT_{2C} receptor agonist, lorcaserin was expected to share this same property without the cardiac valvulopathy and pulmonary hypertension that resulted in the withdrawal of (dex)fenfluramine. This expectation has since been confirmed in the clinic.

Given the complexity and redundancy of neuronal systems that regulate feeding behavior, energy expenditure and weight control, it is likely that drugs with multiple modes of action are most likely to produce a therapeutic benefit in obese individuals. Based on accumulating preclinical evidence, we propose that lorcaserin (and 5-HT_{2C} receptor agonists in general), may treat obesity through multiple mechanisms, namely affecting (1) feeding behaviour and metabolism, (2) reward processing and (3) impulse control. In support of (2) and (3), at doses and plasma exposures at least equivalent to those affecting food intake (i.e. 0.3-1 mg/kg SC; plasma C_{max} 28-88 ng/ml), lorcaserin similarly reduces nicotine motivated behaviours (self-administration, reinstatement, motor stimulant properties), responding for electrical self-stimulation and measures of impulsive action in the rat.

This presentation will review these preclinical findings, which suggest that at doses equivalent to those used to treat obese individuals, lorcaserin may also have potential to treat nicotine dependence and support smoking cessation, as well as other substance abuse disorders. Lorcaserin thus represents a valuable tool with which to probe this potential in suitable clinical populations.

AGGRESSIVE BEHAVIOUR AND COCAINE ADDICTION IN TPH2 KNOCKOUT RATS

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Serotonin plays a key role in a variety of psychiatric disorders, but its precise role is not fully understood. Serotonergic knockout rodents can help to uncover serotonin's role in endophenotypes of these disorders. We employ rats, given their elaborate behavioural repertoire and cognitive and social skills. In the past we extensively characterized serotonin transporter knockout (5-HTT$^{-/-}$) rats, which exhibit high extracellular serotonin levels. We found that these rats display increased anxiety, depressive-like or anti-depressive behaviour (depending on environmental stimuli), increased flexibility, reduced aggression, reduced social (play, sexual) behaviour, and increased cocaine self-administration behaviour. These endophenotypes correspond to those associated with anxiety-related disorders, drug addiction, and potentially autism, although the increased flexibility does not seem to fit. We believe that these 5-HTT$^{-/-}$ endophenotypes all result from environmental sensitivity. As counterparts we are now phenotyping rats lacking tryptophan hydroxylase 2 (TpH2$^{-/-}$). These rats exhibit very low central serotonin levels. Preliminary experiments show that TpH2$^{-/-}$ rats are highly aggressive, even without prior training or environmental triggers. Furthermore, male TpH2$^{-/-}$ rats mount each other, suggesting abnormal sexual behaviour. When combined with 5-HTT$^{-/-}$ data, these findings imply that central serotonin levels are directly linked to social behaviour: high serotonin levels result in low levels of aggression and social behaviour, and low levels of serotonin result in high levels of aggression and abnormal social behaviour. The TpH2$^{-/-}$ rats are currently tested for intravenous cocaine self-administration. Because cocaine-induced serotonin release is strongly reduced in 5-HTT$^{-/-}$ rats self-administering high amounts of cocaine, and cocaine-induced serotonin release likely also will be low in TpH2$^{-/-}$ rats, we hypothesize that TpH2$^{-/-}$ rats will self-administer high amounts of cocaine as well. Data will be presented at the meeting. In conclusion, comparing genetic rodent models exhibiting opposing central serotonin levels helps us to uncover the association between serotonin levels and psychiatric endophenotypes.

SEROTONIN AND DYSKINESIA, ROADMAP TO CURRENT AND NEW THERAPIES

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In this presentation, we will review the pre-clinical studies that underlie the "false neurotransmitter" hypothesis and will discuss the role of the serotonin (5-HT) system and 5-HT_{1A} agonists in L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia in Parkinson's disease (PD).

L-DOPA-induced dyskinesia is a disabling complication of chronic dopamine replacement therapy in PD. The 5-HT system is considered a vector whereby dopamine is released within the striatum following each L-DOPA dose. However, unlike the dopaminergic system, the 5-HT system lacks the autoregulatory mechanisms that are required for physiological dopamine release, leading to pulsatile dopamine release and alternating peaks and troughs of striatal dopamine levels. This constitutes the false neurotransmitter hypothesis. This pulsatile dopamine release following each L-DOPA intake is regarded as an important pathophysiological factor in dyskinesia.

Stimulation of 5-HT_{1A} receptors with 5-HT_{1A} agonists is a way to modulate dopamine release to reduce peaks following administration of L-DOPA. Thus, administration of the 5-HT_{1A} agonist 8-OH-DPAT in combination with L-DOPA to 6-hydroxydopamine (6-OHDA)-lesioned rats leads to a reduction of striatal dopamine levels. At the behavioural level, in the 6-OHDA-lesioned rat and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned non-human primate, administration of 5-HT_{1A} agonists consistently alleviates L-DOPA-induced dyskinesia, although a deleterious effect on L-DOPA anti-parkinsonian action is often encountered. Collectively, these studies suggest that striatal dopamine release by 5-HT terminals is involved in L-DOPA-induced dyskinesia, but might also be involved in mediating the anti-parkinsonian action of L-DOPA.

CLINICAL PSILOCYBIN STUDIES: CANCER ANXIETY/DEPRESSION AND TOBACCO ADDICTION

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This presentation will describe results from recent studies examining therapeutic effects of psilocybin. One study is a recently completed clinical trial examining psilocybin in the treatment of cancer related anxiety and depression. Results show that high dose psilocybin, compared to a low dose comparison condition, significantly decreased measures of anxiety and depression. Another trial to be described is an open-label pilot study using psilocybin with cognitive behavioral therapy to treat tobacco addiction. Results showed 80% of participants were abstinent at 6-month follow up, a success rate substantially higher than conventional treatments. Collectively, these results suggest psilocybin holds considerable promise as a therapeutic behavior change agent.

THE JUNCTIONAL ZONE OF PLACENTA AND SEROTONIN TRANSPORTER

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Serotonin (5-HT) and its specific transporter, SERT, play important roles in pregnancy. In order to investigate their roles in placental function in detail we performed the histopathologic analysis of the 18 d placentas dissected from mice lacking the gene encoding SERT, SERT-knock out (KO) and from mice lacking tryptophan hydroxylase-1 (TPH1), the rate-limiting enzyme in the synthesis of peripheral 5-HT, TPH1-KO, and from the wild-type (WT) counterparts.

Placentas of SERT-KO mice show an abnormal thick band of fibrosis and necrosis under the giant cell layer, which are moderately present in the placentas of TPH1-KO and minimally present in WT. The etiology of these findings was tested with TUNEL assays. The placentas from SERT-KO and TPH1-KO showed 49- and 8-fold increase in apoptosis without a concurrent change in the DNA repair or cell proliferation as measured by quantitative immunohistochemistry for Ki67 compared to WT placentas. Interestingly, the majority of the SERT-related changes were located at the junctional zone of the placenta. However the proliferation rate in the embryos of TPH1-KO mouse was 16-fold lower than gestational age matched embryos of WT or SERT-KO mice. These findings explored the novel roles of SERT in protecting placental cells against apoptosis and perivillous fibrin formation via regulating the local, intervillous/retroplacental 5-HT ratio which influence the cell proliferation in embryos.

GABAERGIC SENSITIZATION OF THE SEROTONIN SYSTEM IN STRESS-INDUCED OPIOID RELAPSE

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Opioids are effective analgesics but also carry a high abuse liability. Even after prolonged abstinence, former addicts can remain vulnerable to relapse, particularly under stressful conditions. The 5-hydroxytryptamine (5-HT) system is poised to integrate opioid and stress responses, and yet its roles in opioid addiction and relapse remain unknown and understudied. Electrophysiology studies in our laboratory have examined the cellular mechanisms of opioid-5-HT interactions and cellular substrates underlying opioid addiction and relapse. We first showed that stressors/stress neurohormones (corticotropin-releasing factor) and opioids (morphine) regulate 5-HT dorsal raphe nucleus (DRN) neurons in an opposing manner. This finding supports the hypothesis that stress precipitates relapse in abstinent subjects with a prior history of opioid addiction because it regulates 5-HT DRN neurons in a 'drug-opposite' manner. Next we showed that 5-HT DRN neurons from animals exposed to a stress-induced opioid relapse model are sensitized to GABAergic inhibition. This sensitization is not seen in subjects exposed only to stress or only to opioids, but instead results from the interaction between stress and opioid history. Additionally, site-directed injections of $GABA_A$ agonists and antagonists indicate that GABAergic signaling in the DRN is both necessary and sufficient for stress-induced opioid reinstatement. We hypothesize that GABAergic sensitization of the 5-HT DRN system results in serotonergic hypofunction and consequent dysphoric mood states which confer vulnerability to stress-induced relapse. Current studies are continuing to pursue the role of DRN GABA signaling with the use of conditional gene deletion strategies to produce mice with DRN- or 5-HT- specific deletion of $GABA_A$ receptors and examination of their stress-induced opioid reinstatement phenotype in both conditioned place-preference and self-administration models. Underappreciated in the drug abuse literature, we propose a novel role for the 5-HT system in drug addiction, particularly as it may contribute to the negative mood states that motivate drug-seeking behaviors.

COMPLEX EFFECT OF ASCENDING MIDBRAIN RAPHE PROJECTIONS ON OSCILLATORY SYNCHRONIZATION IN THE HIPPOCAMPUS: BEYOND THETA SUPPRESSION

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Hippocampus is well-known for its involvement in learning and memory as well as in controlling emotions. Theta rhythm is critical for hippocampal function and represents a key signal establishing transient coupling between hippocampus and other structures. For example, coherent theta rhythm links hippocampus with prefrontal cortex in cognitive tasks and with amygdala during processing emotional information. Drugs affecting cognitive and emotional processing are known to differentially modulate theta; most procognitive compounds increase theta power whereas anxiolytics decrease theta frequency. Two major structures in the brainstem, nucleus pontis oralis (RPO) and median raphe (MR) are involved in controlling hippocampal theta oscillations through serotonin, GABA, and glutamate inputs to medial septum and hippocampus. While RPO is considered the primary glutamatergic theta-synchronizing structure, and MR a major 5-HT desynchronizing structure, this traditional model has been challenged by current understanding of the complexity of the raphe projection which, besides the slow modulation, allows fast acting regulation using different types of serotonergic neurons, parallel glutamatergic and serotonergic raphe-limbic projections, and co-release of the two neurotransmitters by serotonergic neurons. In this study we investigated the effect of stimulation of MR and RPO on hippocampal theta synchronization. We confirmed earlier data suggesting that stimulation of RPO increases theta frequency and power, dependent on stimulus intensity. Stimulation of MR lead to a decline in theta power (R^2=0.48+0.07, slope=-1.25+0.45) but at the same time increased the frequency at which theta appeared (from 5.14+0.25 to 6.74+0.32 Hz; R^2=0.69+0.09). The data on suppression of theta power by MR stimulation are consistent with previous results, but the mechanism of parallel increase in theta frequency is not known and may involve fast acting raphe neurotransmitter mechanisms. Given the opposite effect of anxiolytics on theta frequency (i.e. theta slowing) these data are also consistent with recent reports of anxiogenic consequences of MR activation.

MODIFICATION OF THE ABUSE-RELATED EFFECTS OF COCAINE BY LORCASERIN IN NON-HUMAN PRIMATES

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The results of previous studies have suggested that the 5-HT2 family of receptors may be a promising target for novel medications to modulate the abuse-related effects of cocaine. The recent approval of the 5-HT_{2C} agonist lorcaserin (LOR) for chronic weight management by the FDA suggests that, if effective in selectively decreasing cocaine self-administration in preclinical studies, LOR could rapidly move into clinical trials for the management of cocaine use disorder. The present study evaluated the ability of LOR to reduce the reinforcing and discriminative stimulus effects of cocaine in non-human primates. Self-administration studies were designed to evaluate the effects of acute and chronic treatment with LOR on cocaine (0.01 mg/kg/inj)- and/or food-maintained responding. Adult rhesus monkeys (N=4) responded under a 30-response fixed-ratio (FR): time-out 60-sec schedule of food or IV cocaine reinforcement during daily 120-min sessions. In acute tests, LOR (0.1-1.0 mg/kg, IM) was administered 15-min prior to the beginning of behavioral sessions. Chronic treatment with LOR (0.032-0.32 mg/kg/hr, IV) was administered for 5 consecutive days and doses were presented in an ascending order. In drug discrimination studies, adult squirrel monkeys (N=4) were trained to discriminate (0.1 mg/kg, IM) methamphetamine from saline under a 10-response FR schedule of stimulus-termination. Results show that in cocaine self-administration studies, acute and chronic LOR dose-dependently decreased cocaine self-administration with complete suppression of cocaine intake at the highest dosage tested. Food-maintained responding was moderately decreased by LOR administration in acute studies but unaffected in chronic studies. In discrimination studies, LOR (0.32 mg/kg, IM) shifted the cocaine dose-response curve approximately 3-fold to the right but did not alter methamphetamine's discriminative stimulus. These results suggest that LOR reduces the reinforcing and discriminative stimulus effects of cocaine but did not as consistently alter methamphetamine's effects. Further evaluation of LOR as a candidate pharmacotherapy for cocaine dependence is warranted.

SEROTONIN AND ANXIETY IN ADOLESCENT RATS

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Risk taking behavior peaks during adolescence. Our research suggests that enhanced release of dopamine to stimulate motivated behavior and inadequate serotonergic modulation of motivated behavior contribute to adolescent risk taking behavior. Deficits in serotonergic function are associated with disinhibition, impulsive aggression, and risk taking, but few studies have investigated the contribution of serotonin to adolescent risk taking. Findings presented here will show that the lack of serotronergic inhibition of behavior culd have significant consequences for risks as diverse as impulsive self-directed aggression during initiation of SSRI's and vulnerability to substance abuse.

We will present data showing that serotonergic agonists including the indirect agonist fluoxetine, the direct 5HT1A agonist 8 OHDPAT and the 5HT2 agonist meta-chlorophenylpiperazine (mCPP) do not inhibit behavior as assessed in the light/dark test and novelty-induced hypophagia models. The behavioral findings showed that serotonergic modulation of behavior in anxiety models (which entails risk assessment and behavioral inhibition in response to threat) is less in adolescents than adults. Immaturity of both serotonergic mechanisms and downstream activation by serotonin receptors likely contributes to these behavioral findings. Serotonin content in the frontal cortex has not attained adult levels, and the indirect agonist fenfluramine which increases extracellular 5-Ht does so less in adolescents than adults. However, immaturity of cortical:amygdala interactions may also be immature, as c-Fos activation in amygdala and cortex by 5 HT1a agonist treatment is less in adolescents than adults although receptor number and function were not different. Finally, we show pilot data which demonstrate an additional potential risk presented by this immaturity of serotonergically-mediated behavioral inhibition. The entactogen/stimulant methylenedioxymethamphetamine which increases synaptic serotonin and dopamine is not self-administered robustly by adult rats, an effect thought to reflect serotonergic inhibition of the reinforcing effects of dopamine released by the drug. Pilot studies in adolescent female rats demonstrate that they readily acquire MDMA self-administration, an effect we hypothesize is mediated by a greater DA/5HT ratio in released monoamines in the adolescent rats compared to adults.

Overall, these results suggest that serotonergic innervation of frontal cortex circuits that contribute to behavioral inhibition and suppression of impulsivity are relatively immature in adolescent compared to adult rats. This immaturity contributes to less behavioral inhibition in conditions of threat, and could contribute to adolescent risk taking. This state has significant implications for adolescent risk of impulsive self harm during initiation of SSRI treatment for depression as well as for adolescent abuse of mixed DA/5-HT releasing stimulants. Supported by DA 019114

SEROTONIN AND CORTICAL DISINHIBITION: A NOVEL SYNERGY BETWEEN 5-HT$_{1A}$ AND 5-HT$_{2A}$ RECEPTORS IN PREFRONTAL CORTEX

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In medial prefrontal cortex, layer 6 pyramidal neurons play a critical role in attention and express receptors for serotonin (5-HT), a neuromodulator implicated in many psychiatric disorders with attention deficits. Yet, little is known about the direct electrophysiological consequences of 5-HT on layer 6 neurons themselves and on their regulation of local cortical activity. Whole cell recording and pharmacological manipulations were performed in acute brain slices from transgenic mice expressing either eGFP or a fusion protein of eGFP-channelrhodopsin in prefrontal layer 6 pyramidal neurons. Optogenetic excitation was used to test the effects of 5-HT on the inter-laminar excitatory circuit between layer 6 pyramidal neurons and fast-spiking interneurons in layer 5, which are important mediators of cortical inhibition. Here, we demonstrate that prefrontal layer 6 pyramidal neurons are strongly inhibited by 5-HT through activation of 5-HT$_{1A}$ and, surprisingly, 5-HT$_{2A}$ receptors. This direct serotonergic suppression of neuronal excitability in layer 6 is complex, with the inhibition mediated by 5-HT$_{2A}$ receptors strongest during conditions that would otherwise lead to layer 6 spiking. Optogenetic investigation of the circuit between layer 6 pyramidal neurons and fast-spiking interneurons in layer 5 shows that this connection is suppressed by 5-HT through a synergistic effect of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors. These findings reveal a novel modulatory role of 5-HT on cortical excitability through strong inhibitory effects on layer 6 and its feedforward projections. Disturbances of normal serotonin signalling in deep cortex would thus have unexpectedly broad consequences for prefrontal cortex activation and signal-to-noise ratios in circuits important to attention.

EXTRAEMBRYONIC REGIONALIZATION OF SEROTONIN REGULATION: IMPLICATIONS FOR BRAIN DEVELOPMENT

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Serotonin (5-HT) serves as a neuromodulator in dynamic circuit regulation, but mounting evidence indicates that early 5-HT can mediate early neurodevelopmental events, particularly in the forebrain. Our previous studies demonstrated that the placenta is a major source of forebrain 5-HT during early forebrain development, but the processes of how 5-HT production, metabolism, and transport from placenta to fetus are regulated are unknown. Studies were undertaken of the expression patterns of genes critical for 5-HT system function in mouse extraembryonic tissues using multiplex fluorescent in situ hybridization methods in defined cell types. Trypotphan hydroxylase 1(*Tph1*) and dopa decarboxylase (*Ddc*) are found in the syncytiotrophoblast I (SynT-I) and sinusoidal trophoblast giant cells (S-TGC), whereas monamine oxidase a (*Maoa*) is expressed in SynT-I, syncytiotrophoblast II (SynT-II) and S-TGC. Solute carrier family 22 member 3 (*Oct3*) expression is observed in the SynT-II only, while the vesicular monoamine transporter 2 (*Vmat2*) is mainly expressed in S-TGC. Surprisingly, there was very robust expression of *Tph1*, *Ddc*, and *Maoa* in the yolk sac visceral endoderm, with synthesis capacity of 5-HT. Conditional disruption of Maoa gene expression in SyntT-I cells leads to increased extraembryonic and fetal forebrain accumulation of 5-HT. This may have substantial effects on 5-HT axon growth, as explant culture experiments reveal that 5-HT can modulate the growth of 5-HT axons originating from the fetal raphe complex. The findings raise the possibility of a more complex regulation of 5-HT access to the fetal brain through the differential roles of trophoblasts that surround maternal and fetal blood space and of yolk sac endoderm.

ENTERIC SEROTONIN, SERT AND THE 5-HT4 RECEPTOR AND DEVELOPMENTAL SSRI EXPOSURE

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Although most research done on serotonin (5-HT) focuses on the brain, 95% of 5-HT in the body is found in the intestine where 5-HT serves critical functions in enteric nervous system (ENS) development, motility, and epithelial homeostasis. After 5-HT is released from enterochromaffin cells or enteric neurons and acts, it must be taken up by the 5-HT reuptake transporter (SERT) in order to be inactivated. If SERT is inhibited, however, 5-HT is inactivated inefficiently and defects in intestinal development and function ensue.

Depression occurs in ≈20% of pregnant women. Selective serotonin reuptake inhibitors (SSRIs) are frequently used to treat this depression. SSRIs inhibit SERT thus causing the amount and duration of 5-HT at receptors to increase in the brain and intestine. SSRI exposure during pregnancy causes long-term changes in CNS connectivity. Relatively little is known about the long-term effects that SSRI exposure exerts on ENS formation and gut function. A clinical study, however, showed that children exposed to anti-depressants *in utero* were ten-fold more likely to require laxatives for constipation. This finding is consistent with the idea that SSRIs cause long-lasting changes in ENS development and motility. This hypothesis was tested.

We found that significant abnormalities in ENS development and gastrointestinal function occurred in mice exposed during development to the SSRI, fluoxetine. The ENS abnormalities include a neuronal hyperplasia with a selective overabundance of neuronal subsets thought to develop under serotonergic influence. We also observed that motility and intestinal epithelial proliferation were abnormal. If 5-HT_4 receptors ($5\text{-HT}_4\text{R}$) were antagonized simultaneously with fluoxetine treatment the abnormalities were prevented. These observations suggest that 5-HT, SERT, and $5\text{-HT}_4\text{R}$ play important roles in ENS development and intestinal function. Abnormalities in SERT function may also underlie some conditions that affect the brain-gut axis; moreover, drugs that regulate $5\text{-HT}_4\text{Rs}$ may be helpful in treating these conditions.

REGULATION OF 5-HT6 RECEPTOR CONSTITUTIVE ACTIVITY BY INTERACTING PARTNERS

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Active G protein-coupled receptor (GPCR) conformations are not only promoted by agonists, but also occur in their absence, leading to constitutive activity. The serotonin 5-HT6 receptor is a Gs-coupled receptor exhibiting high constitutive activity, which has emerged as a promising target for the treatment of cognitive deficits associated with several neuropsychiatric disorders, including Alzheimer's disease and schizophrenia. This receptor also controls key neuro-developmental processes such as neuronal migration and differentiation. Using a proteomic strategy, we identified more than 60 receptor interacting proteins. These include neurofibromin, a Ras-GTPase activating protein encoded by the *Nf1* gene, the mutation of which causes Neurofibromatosis type 1 (NF1), a genetic disorder characterized by multiple benign and malignant nervous system tumors and cognitive deficits, and Cyclin-dependent kinase (Cdk)5, a key regulator of neuronal migration, neurite growth and dendritic spine morphogenesis. Functional studies showed that agonist-independent activation of Gs signaling by 5-HT6 receptor depends on its physical interaction with neurofibromin. Accordingly, mutations identified in NF1 patients that disrupt 5-HT6 receptor/neurofibromin interaction strongly inhibit agonist-independent receptor-operated Gs signaling, which is also impaired in a mouse model of NF1, suggesting that this interaction might contribute to neuronal abnormalities and cognitive impairment in NF1. 5-HT6 receptor likewise constitutively activates Cdk5 signaling to promote neurite growth and migration of cortical neurons, two processes perturbed in neurodevelopmental disorders such as schizophrenia. Moreover, engagement of Cdk5 signaling requires receptor phosphorylation at Ser^{350} by associated Cdk5, indicating a reciprocal interplay between $5\text{-}HT_6$ receptor and Cdk5, whereby the receptor stimulates Cdk5 activity and is itself a Cdk5 substrate. Collectively, these findings demonstrate that agonist-independent 5-HT6 receptor-operated signaling is critically dependent of its association with interacting proteins and offer a unique opportunity to develop new drugs to control 5-HT6 receptor activity in neurodevelopmental disorders and the associated cognitive deficits.

5-HT$_{1A}$ RECEPTOR AGONISTS FOR THE TREATMENT OF L-DOPA-INDUCED DYSKINESIA IN PARKINSON'S DISEASE: BIASED AND MIXED AGONISM

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Parkinson's disease is characterized by the emergence of the cardinal motor and non-motor symptoms. The primary nigrostriatal dopaminergic deficit can be managed by treatment with dopamine receptor agonists in the initial disease phases. As the disease course progresses, however, the need for intervention with the dopamine precursor levodopa (L-DOPA), recognized as the gold-standard therapy, is inevitable. A common side effect of L-DOPA treatment though is the emergence of debilitating dyskinesia caused by aberrant uncontrollable *"false neurotransmitter"* release of dopamine, following the conversion of L-DOPA within serotonergic neuronal terminals. Reducing serotonergic tone by the application of (5-hydroxytryptamine; 5-HT)$_{1A}$ receptor agonists, or other serotonergic strategies, may help in the alleviation of these effects. In particular, 5-HT$_{1A}$ receptor agonists have been shown to reduce the expression of established dyskinesia in the non-clinical and clinical setting, but are often associated with a reduction in the antiparkinsonian efficacy of L-DOPA.

Here, we review our recent data exploring the efficacy of the of 5-HT$_{1A}$ receptor (biased) agonists in reducing L-DOPA-induced dyskinetic-like behavior and impacting striatal microdialysis measures following either acute or chronic treatment regimes. We include exciting new developments with compounds possessing much greater potency and selectivity at 5-HT$_{1A}$ receptors including, for example, F13714, F15599 and Befiradol (NLX-112).

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PLACENTAL SEROTONIN SIGNALINGIS DISRUPTED IN HUMAN FETAL GROWTH RESTRICTION.

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Fetal growth restriction (FGR) is one of the major problems of pregnancy. FGR is associated with a 5- to 20-fold increase in perinatal mortality, in addition, 40–50% of FGR babies require neonatal intensivecare. While there are many causes of FGR, the underlying pathology is a failure of adequate placental transfer of nutrients from mother to fetus. FGR is often associated with placental insufficiency. An important placental function is the uptake of the amino acid tryptophan, and its metabolism to serotonin and kynurenine metabolites, which are essentialfor growth and development of the fetus and for fetal neuronal and immune protection. However, changes in tryptophan metabolism and its metabolites in FGR are largely unknown. Therefore, we hypothesised that placental tryptophan metabolism is altered in FGR and contributes to abnormal serotonin signalling in FGR.

Using placentae collected from third trimester idiopathic FGR (n=20) and gestation-matched control pregnancies (n=15) relative mRNA expression of tryptophan metabolic pathway genes including serotonin transporters and receptors was assessed using Fluidigm single-cell DNAseq and TaqMan chemistry (Thermo Fisher Scientific). Tryptophan metabolising enzymes, IDO-1 , IDO-2 and TDO-2; tryptophan hydroxylase enzyme, TPH-1; serotonin transporter SERT-1 and 2; and serotonin receptors HTR5A and HTRB5 mRNA were detected in all human placental samples. The expression levels of tryptophan metabolising enzymes IDO-1, TPH-1 and the transporter, SERT-1 were significantly decreased in FGR placentae ($p<0.05$), while serotonin receptor, HTRB5 mRNA was significantly increased in FGR compared to control ($p<0.01$).

This is the first study to report that the gene expression for tryptophan metabolising enzymes and serotonin signalling molecules are altered in FGR placentae. Our findings highlight that placental serotonin signalling is disrupted in FGR and may contribute to the pathogenesis of FGR.

DYSREGULATION OF 5-HT1B RECEPTORS AND ITS IMPLICATIONS FOR NOVEL TREATMENTS IN DRUG DEPENDENCE

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Early research on 5-HT_{1B} receptor (5-HT_{1B}R) agonists found that the drugs facilitated cocaine self-administration in rats whereas 5-HT_{1B}R antagonists had no effect. These findings suggested that 5-HT_{1B}R agonists would be counter-indicated for cocaine use disorders. However, our lab found that after a period of abstinence from cocaine self-administration, 5-HT_{1B}R agonists decreased cocaine self-administration on both low ratio and progressive ratio schedules of reinforcement and decreased cue and cocaine-primed reinstatement of extinguished cocaine-seeking behavior. These findings suggest that a neural circuitry involving 5-HT_{1B}Rs is dysregulated as a result of cocaine self-administration such that the functional effects of 5-HT_{1B}R agonists change from facilitating to inhibiting cocaine abuse-related behaviour during the course of abstinence. Recently we have found that the effects of 5-HT_{1B}R agonists show a similar pattern of effects on locomotor activity and cocaine-conditioned place preference in mice. More importantly, we have found that 5-HT_{1B}R agonists inhibit methamphetamine self-administration and seeking behaviour regardless of whether or not rats undergo a period of abstinence. Collectively, these findings suggest that 5-HT_{1B}R agonists may have efficacy for treating psychostimulant use disorders.

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5-HT$_6$ RECEPTORS IN STRIATUM: LOCATION AND FUNCTION.

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Using virally mediated gene transfer in rat striatum, we have found that 5-HT$_6$ receptors (5-HT$_6$Rs) can alter learning and habit behaviors, and drug-reward mechanisms. Interestingly, we found that increased 5-HT$_6$ expression in indirect pathway medium spiny neurons in ventral striatum reduced cocaine self-administration by increasing the sensitivity to rewarding and reinforcing properties of low dose cocaine. The 5-HT$_6$R is heavily expressed in striatum, but is also the only serotonin receptor that is expressed in primary neuronal cilia, an organelle that contains a discrete set of signaling proteins that allow sensing of the extracellular environment. This means that 5-HT$_6$Rs are exposed to extrasynaptic 5-HT; their role in regulating mature striatal neuron morphology and excitability is not well understood. We observe that 5-HT$_6$Rs localize predominantly to primary neuronal cilia both in primary culture and in brain tissue. Using mouse primary culture from wild-type and 5-HT$_6$ knockout striatal neurons, we found that 5-HT$_6$ antagonists reduced cilia length in wild-type but not 5-HT$_6$ knockout neurons; added agonist has limited effects. Mutations of the putative cilia targeting sequence greatly reduce but do not totally prevent trafficking of 5-HT$_6$Rs to cilia. Increasing the amount of transfected DNA increases nonciliary localization of 5-HT$_6$Rs, suggesting that high levels of expression saturate trafficking regulation that otherwise restricts 5-HT$_6$Rs to primary cilia; this may also be relevant to the extent of constitutive activity observed when heterologous expression is used. We are currently examining how loss of Gs vs fyn signaling alters 5-HT$_6$-mediated regulation of cilia morphology.

PSYCHEDELICS ARE POTENT ANTI-INFLAMMATORIES WITH THERAPEUTIC EFFICACY AGAINST SPECIFIC DISEASES INCLUDING ASTHMA

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We previously discovered and reported that serotonin 5-HT_{2A} receptor activation with psychedelics have potent novel anti-inflammatory activity in both cell culture and whole animal models. Although all psychedelics tested in vitro have anti-inflammatory properties, the small molecule agonist (*R*) 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane [*R*-DOI] is significantly more potent than even similar structural analogs with an IC_{50} of ~20 pico molar. In a generalized mouse model of inflammation involving systemic administration of TNF-α, powerful anti-inflammatory effects of systemic (R)-DOI were observed in several tissues, but most pronounced in the small intestine. In a mouse model of allergic asthma, (R)-DOI potently prevents the development of airways hyperresponsiveness, mucus over production, peribronchial inflammation, and eosinophilia. In the ApoE-/- high fat diet mouse model of atherosclerosis and metabolic disorder, (R)-DOI blocks vascular inflammation, attenuates the increase in total cholesterol, and normalizes glucose homeostasis. Our data indicate that the effects of (*R*)-DOI are not due to a generalized anti-inflamatory process, but that selective inflammatory pathways are targeted and inhibited. Significantly, the levels of (R)-DOI necessary to produce profound anti-inflammatory effects are orders of magnitude less than what are required for alterations of behaviors. The promise of a 5-HT_{2A} receptor agonist anti-inflammatory is an effective and highly bioavailable small molecule treatment of a variety of inflammatory conditions by a novel anti-inflammatory mechanism that is steroid sparing.

LIPID RAFT TRAFFICKING OF 5HT6R AND GSA: POSSIBLE INSIGHTS TO THE BIOLOGY OF DEPRESSION

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5HT6R and their cognate G protein, Gs□, shuttle between lipid raft and non-raft domains on the plasma membrane and into the cellular interior. The latter function is facilitated by SNS14, a protein closely related to SNX13 (aka RGSPX1), which has been reported to have RGS activity for Gs□. While SNX14 does not activate the Gs□ GTPase, it does bind specifically to Gs□, and not other G proteins as well as to 5HT6 but not other Gs-coupled receptors. Given the linkage between 5HT6R and mood disorders, we elected to use the trafficking of Gs□ within membrane compartments to develop a possible biosignature for depression and antidepressant response. A series of antidepressant drugs appear to localize within lipid rafts and translocate Gs□ into non-raft fractions, where it binds to Adenylyl Cyclase 6. This requires about 3 days treatment in a cellular model system. Ketamine, reported to have rapid acting antidepressant properties, requires only 15 minute exposure. Other NMDA antagonists do not share these effects. However, ketamine and monoaminergic antidepressants result in a sustained cAMP increase as well as downstream sequellae of increased cAMP. Initial results suggest that this same phenomenon exists in certain blood cells and might represent a biomarker for both Major Depressive Disorder and an early harbinger for antidepressant response. It is also suggested that fluorescent versions of Gs□ might be used to develop a high-content screen that will indicate antidepressant activity of a given compound and

THE SEROTONIN-BDNF LINK: DEVELOPMENTAL AND EPIGENETIC MECHANISMS

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A key regulatory element for serotonin (5-HT) transmission is represented by the 5-HT transporter (5-HTT or SERT), which terminates the action of 5-HT by re-uptaking it into the presynaptic terminals. This gene is characterized by a functional polymorphism that confers enhanced risk for major depression upon exposure to adverse life events. On these bases we have used SERT knockout rats in order to investigate mechanisms that may contribute to the enhanced vulnerability for mood disorders, namely the expression of brain-derived neurotrophic factor (*Bdnf*), a key player in neuronal plasticity, which has been implicated in the etiology and treatment of depression. We found that *Bdnf* levels were significantly reduced in the hippocampus and prefrontal cortex of SERT knockout rats, through transcriptional changes that affect different neurotrophin isoforms. The reduction of *Bdnf* gene expression observed in prefrontal cortex is due, at least in part, to epigenetic changes that affect the promoter regions of exons IV and VI. Interestingly the impairment in neuronal plasticity in prefrontal cortex and hippocampus originates early in development. Indeed, we found that *Bdnf* expression was already reduced during the first week of life and the magnitude of these alterations became more pronounced from PND21. These changes are sustained by reduced expression of specific transcription factors, including Npas4 and CaRF, which can be preceded by epigenetic mechanisms, including increased DNA methylation at *Bdnf* promoters as well as reduced expression of *Gadd45β*, an enzyme involved in activity–dependent modulation of the neurotrophin.

These early changes may increase stress susceptibility during critical windows of brain maturation, which may eventually lead to the heightened predisposition to mood disorders. Moreover, these results suggest that interventions aimed at modulating epigenetic mechanisms may hold the promise to improve the dysfunction that may be associated with reduced expression of the serotonin transporter.

SEROTONIN: DEPRESSION AND COGNITION

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FUNCTIONAL CROSSTALK OF THE 5-HT$_{2C}$ RECEPTOR IN FOOD INTAKE AND REWARD

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The serotonergic system, specifically the serotonin receptor 2c (5-HT$_{2C}$), has shown to be of critical importance in the regulation of appetite and satiety and to be involved in the rewarding aspects of food and drugs of abuse. Lorcaserin, a specific 5-HT$_{2C}$ receptor agonist, has recently been identified as novel anti-obesity drug, shown to reduce dopaminergic firing in the mesolimbic circuitry and suggested as a novel therapeutic to treat substance abuse. We have recently shown that lorcaserin attenuates ghrelin-mediated food intake and sucrose preference in mice via a dimerization with the GHS-R1a receptor, another key receptor implicated in the homeostatic control of appetite and reward. In addition, we also show compelling evidence for a novel dimerization of the 5-HT$_{2C}$ receptor with the dopamine D1 receptor, which inhibits D1-mediated cAMP signaling, suggesting a potential mechanism for 5-HT-mediated attenuation of DA signaling via the 5-HT2C receptor. Using multi-array electrophysiology in hippocampal rodent slices we show that acute 5-HT$_{2C}$ activation blocks GHS-R1a-induced potentiation of field potentials and modulates D1 receptor mediated neuronal transmission, suggesting a key role for 5-HT$_{2C}$ receptor crosstalk in short term synaptic plasticity. These results encourage further studies investigating the functional consequences of the interaction between the 5-HT$_{2C}$ receptor and the dopaminergic and ghrelinergic signaling systems in the regulation of appetite, food reward and addiction.

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NEUROCHEMICAL AND BEHAVIOURAL IMPACT OF COMBINED SEROTONERGIC AND DOPAMINERGIC LESIONS IN THE NHP, A NOVEL MODEL FOR PARKINSON'S DISEASE.

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It is noteworthy that serotonergic neurons degenerate in Parkinson's disease. To determine the role of this 5-HT injury besides the dopaminergic one, we developed a new monkey model exhibiting a double DA/5-HT lesion by using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 3,4-methylenedioxy-N-methamphetamine (MDMA, better known as ecstasy). The effects of both lesions were assessed by positron emission tomography imaging and post-mortem analysis (immunohistochemistry and autoradiography). The behavioral impact of lesions was investigated using different experimental paradigms. A special attention was directed toward non-motor symptoms of Parkinson's disease, such as neuropsychiatric-like manifestations.

LORCASERIN AS A POTENTIAL TREATMENT FOR SMOKING CESSATION AND ASSOCIATED WEIGHT GAIN: RESULTS OF A RANDOMIZED, 12-WEEK, PHASE 2 TRIAL

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Lorcaserin is a selective 5-HT_{2C} receptor agonist approved by the US FDA for weight management. Preclinical evidence suggests that 5-HT_{2C} receptor agonism also inhibits dopaminergic neurons in the ventral tegmental area which are important for drug reinforcement.

A randomized, placebo-controlled study was conducted at 30 U.S. sites to assess lorcaserin, against a background of standardized counseling, as an aid to smoking cessation. 603 smokers (averaging 26 years of smoking, 19 cigarettes/day, 4 prior quit attempts - 50% failing pharmacotherapy) were randomized to receive 12 weeks of lorcaserin 10 mg once daily (LOR q.d.), 10 mg twice daily (LOR b.i.d.), or placebo (PBO); 458 (76%) completed the study.

Continuous Abstinence Rates for the last 4 weeks of treatment (CAR), confirmed by weekly exhaled carbon monoxide, were 5.6% (PBO), 8.7% (LOR q.d.) and 15.3% (LOR b.i.d.), with abstinence rates for LOR b.i.d. significantly greater than PBO (3.02; 95% confidence interval [CI], 1.47, 6.22; p=0.0027) and LOR q.d. (1.89; 95% CI, 1.01, 3.56; p=0.0477).

7-day point-prevalence of abstinence at Week 12 was achieved by 20.4% in the LOR b.i.d. group and 11.8% on PBO (OR 1.92; 95% CI, 1.10, 3.35; p=0.0219). At Week 12, the LOR b.i.d. group achieved greater weight loss from baseline than participants on PBO (-0.98 kg vs. -0.01 kg, respectively; p=0.0004). In participants exhibiting CAR, the LOR b.i.d. group lost weight at Week 12 relative to baseline (-0.41 kg), as compared to both LOR q.d. (+0.76 kg) and PBO (+0.73 kg) who gained weight. Lorcaserin was well tolerated, with an adverse event profile consistent with the Phase 3 weight management experience. No serious AEs occurred that were considered related to treatment.

In this Phase 2 study, lorcaserin 10 mg twice daily was an efficacious aid for smoking cessation and prevention of associated weight gain in a population of long-term smokers.

IMPACT OF MATERNAL SEROTONIN TRANSPORTER GENOTYPE ON PLACENTAL SEROTONIN, FETAL FOREBRAIN SEROTONIN, AND NEURODEVELOPMENT

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Elevated blood serotonin levels are found in 30% of children with autism spectrum disorder. The serotonin transporter (SERT) gene is associated with blood serotonin levels in males but not females. Genetic linkage points to rare SERT variants in families of males with autism spectrum disorder (ASD). Male knock-in mice expressing the most common of these rare variants, SERT Ala56, have increased 5-HT clearance, receptor hypersensitivity, and altered social and repetitive behavior. Having identified changes in behavior in adult animals, we turned our attention to serotonin-sensitive aspects of neurodevelopment. Early in gestation, before midbrain 5-HT projections have reached the cortex, peripheral sources supply 5-HT to the forebrain, suggesting that altered maternal or placenta 5-HT system function could impact the developing embryo. We therefore used different combinations of maternal and embryo SERT Ala56 genotypes to examine effects on blood, placenta and embryo serotonin levels and neurodevelopment at embryonic day E14.5, when peripheral sources of 5-HT predominate, and E18.5, when midbrain 5-HT projections have reached the forebrain. Maternal SERT Ala56 genotype was associated with decreased placenta and embryonic forebrain 5-HT levels at E14.5. Low 5-HT in the placenta persisted, but forebrain levels normalized by E18.5. Maternal SERT Ala56 genotype effects on forebrain 5-HT levels were accompanied by a broadening of 5-HT-sensitive thalamocortical axon projections. In contrast, no effect of embryo genotype was seen in concepti from heterozygous dams. Blood 5-HT levels were dynamic across pregnancy and were increased in SERT Ala56 dams at E14.5. No changes were observed in placenta tryptophan hydroxylase expression or activity. Placenta RNA sequencing data at E14.5 indicated substantial impact of maternal SERT Ala56 genotype, with alterations in immune and metabolic related pathways.

Collectively, these findings indicate that maternal SERT function impacts offspring placental 5-HT levels, forebrain 5-HT levels, and neurodevelopment.

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PSILOCYBIN ENHANCES EMPATHY AND REDUCES SOCIAL PAIN IN HEALTHY SUBJECTS: IMPLICATION FOR MOOD DISORDERS

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Social cognition is a crucial factor influencing development, progress, and treatment of psychiatric disorders. Particularly, depressed patients show an increased negative reaction to social exclusion and deficits in empathy. The 5-HT2A/1A receptor agonist psilocybin has been reported to reduce the neural response to negative stimuli (Kraehenmann et al. 2016). However, it is not known if this extends to negative social interaction and whether 5-HT2A/1A receptor stimulation affects empathy. Given the clear need for improved treatment of socio-cognitive functioning in psychiatric disorders, it is important to better understand the neuronal and neuromodulatory substrates of social cognition.

This study assessed the neural response to ostracism after the acute administration of psilocybin (0.215mg/kg) and placebo in 21 healthy volunteers using functional magnetic resonance imaging. Furthermore, we assessed cognitive and emotional empathy using the Multifaceted Empathy Test. A double-blind, randomized, cross-over design was applied with volunteers counterbalanced to receive psilocybin and placebo in two sessions at least 10 days apart.

The neural response to social exclusion was reduced in the dorsal anterior cingulated cortex (peak: $x=6,y=26,z=22,p<0.05$,FWE) after psilocybin administration versus placebo. Emotional empathy was increased after psilocybin administration ($F(1,31)=7.09,p<0.01$), while no significant differences were found in cognitive empathy ($F(1,31)=1.28,p>0.27$).

These results indicate that the 5-HT2A/1A receptor subtypes play an important role in the modulation of socio-cognitive functioning and therefore may be relevant for the treatment of social cognition deficits in psychiatric disorders. In particular, they may be important for the normalization of empathy deficits and increased negative reaction to social exclusion in depressed patients.

KYNURENINE METABOLITES ACROSS PREGNANCY AND AT PARTURITION IN PREGNANT WOMEN, AND THE EFFECTS OF LOW OXYGENATION ON TRYPTOPHAN METABOLISM IN THE HUMAN PLACENTA.

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Alternate to serotonin production, tryptophan catabolism occurs via the kynurenine pathway. The first aim of this study was to determine kynurenine metabolite levels across pregnancy in women. Maternal serum samples were collected at 7-10, 14-17, 25-27 weeks gestation, and at 37-41 weeks where delivery was either by caesarean (c-) section or spontaneous vaginal birth. Tryptophan, kynurenine, kynurenic acid, 3- hydroxyanthranilic acid were measured by HPLC, and picolinic and quinolinic acids by GC-MS. Kynurenine, 3- hydroxyanthranilic, kynurenic, quinolinic and picolinic acids were present in maternal blood throughout pregnancy and in umbilical cord blood collected at delivery. Maternal levels of tryptophan, kynurenine, 3-hydroxyanthranilic and picolinic acids decreased, while kynurenic and quinolinic acids increased with advancing gestation. Vaginal delivery after labour was associated with significantly higher concentrations of kynurenine and 3-hydroxyanthranilic acid in maternal and fetal blood compared to c-section delivery.

The second aim of this study was to determine if low oxygenation affects placental mRNA expression and activity of kynurenine pathway enzymes in early and late pregnancy. First trimester and term placental explants (n=8, 15 respectively) were incubated in 5-8% or 20% O_2 for 24 or 48 h. Reduced oxygen delivery (5-8%) was associated with significantly decreased mRNA expression for IDO, TDO, KYN-OHase, and 3HAO in both 1st trimester and term placental explants compared to explants incubated with 20% O_2, with significantly decreased production of kynurenine and quinolinic acid by 1st trimester and term placenta also observed.

In summary, kynurenine metabolites are present in maternal serum throughout pregnancy, and may compete with the serotonin pathway for availability of tryptophan. mRNA expression and kynurenine enzyme activities in the placenta are regulated by oxygen availability. These results may explain some of the changes of tryptophan catabolism at the maternal- fetal interface that occur with advancing gestation and in pregnancies complicated by preeclampsia, fetal growth restriction, and infection.

"ROLE OF MEDIAL PREFRONTAL CORTEX SEROTONIN 2A RECEPTORS IN THE CONTROL OF RECOGNITION MEMORY RETRIEVAL IN RODENTS."

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Episodic memories contain information about our personal experiences. But memories would be useless if we could not retrieve them. Memory retrieval requires the correct selection of a particular trace to be expressed. However, many memories share cues, so how does the brain control interference between similar memories during retrieval? A system including the medial Prefrontal Cortex (mPFC) has been proposed to mediate response selection and control interference.

Serotonin is an important modulator of mPFC function, however it is not clear the role that the serotonergic system in general and the serotonin 2a receptors (5-HT2aR) in particular play in memory interference processes. We employed a recognition memory paradigm in rats to address this question. The object-in-context (OIC) task requires the animals to recognize the incongruence between the context and one of the objects presented during the retrieval phase. This task involves multiple brain regions including the hippocampus and perirhinal cortex (PRH). We found that infusion of MDL 11,939, a 5-HT2aR specific antagonist, in the mPFC before retrieval affects the ability of this structure to control memory interference during the OIC task. Modulation of mPFC activity by 5-HT2aR also regulates the reconsolidation of the memory traces. Infusion of a protein synthesis inhibitor like emetine in the PRH after the retrieval blocked reconsolidation of only one of the object memories. However, infusion of 5-HT2aR antagonist in mPFC allowed labilization of both memory traces during the retrieval making them both susceptible to emetine. These results suggest that 5-HT2a receptors in mPFC control memory reactivation allowing the expression and reconsolidation of the most relevant memory trace in the PRH.

CHROMATIN REGULATION BY DOPAMINE AND SEROTONIN

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Systemic administration of psychostimulants or antidepressants induces long-lasting changes in behavior that are driven by adaptations in the function of neurons within corticolimbic brain regions. We have been studying the chromatin regulatory processes induced in neurons by chronic exposure to these drugs with the goal of understanding how modifications of the epigenome contribute to the regulation of addictive- and depressive-like behaviors. For example, we have found that signaling through both dopamine and serotonin receptors can induce the phosphorylation of the methyl-DNA binding protein MeCP2 (pMeCP2) in select populations of neurons within distinct mesolimbocortical brain regions. Consistent with a functional role for pMeCP2 in neuronal adaptations to these drugs, we find that mice bearing a phosphorylation site mutation knocked into the *Mecp2* gene show enhanced expression of addictive-like behaviors following chronic amphetamine or cocaine and a failure to respond to chronic antidepressant treatment in the social defeat stress paradigm. I will discuss how these findings have led to ongoing work in the lab in which we are purifying neuronal populations to detect cell-type specific regulation of transcription and studying the chromatin mechanisms that underlie the behavioral phenotypes in our mutant mice.

EPIGENETICS, SYNAPTIC PLASTICITY, AND MEMORY

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It has long been appreciated that long-term memory formation requires gene expression. However, we are only beginning to understand the role of epigenetic mechanisms that modulate gene expression required for memory. Epigenetic mechanisms are known to establish long-lasting changes in cell function, and in neurons, that may lead to stable changes in neuronal plasticity underlying persistent changes in behavior. One major epigenetic mechanism is histone acetylation carried out by the opposing enzymes histone acetyltransferases (HATs) and histone deacetylases (HDACs). In general HATs acetylate lysine residues on relax chromatin structure to facilitate gene expression and HDACs perform the opposite function. We have been investigating the role of HDAC3, the most abundant HDAC in the brain, in synaptic plasticity, learning, and memory. We have found from a number of different studies that HDAC3 is a powerful negative regulator of synaptic plasticity, memory formation, and information processing in the young and aging brain.

TRANSLATIONAL RESEARCH FINDINGS ON THE ROLE OF SEROTONIN IN IMPULSIVE AND DISINHIBITED BEHAVIORS

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Tryptophan is an essential amino acid that serves as the physiological precursor for the neurotransmitter serotonin (5-HT), and changes in central nervous 5-HT synthesis have been linked to the underlying neurobiology of impulsive and disinhibited behaviours. Changing the physiological availability of tryptophan via different strategies can impact substrate availability for central nervous synthesis of 5-HT, and acute tryptophan depletion (ATD) as achieved by administering a tryptophan free diet is a physiological and neurodietary research method to lower brain 5-HT synthesis in humans for a short period of time. In this presentation, the underlying principles of ATD as a translational research method to challenge the central nervous 5-HT-system will be outlined and discussed. Moreover, different research findings on ATD-induced changes of behavioural and fMRI-related data obtained in humans will be presented and discussed. This will be done with a particular focus on impulsive and disinhibited behaviours. Understanding the complex role of 5-HT in the context of such behaviours is of scientific and clinical importance to allow for new strategies for interventions in vulnerable populations.

IDENTIFICATION OF FUNCTIONAL HETEROGENEITY WITHIN SEROTONIN NEURON POPULATIONS

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Serotonin has been implicated in the regulation of homeostatic and behavioral processes as diverse as breathing, heartrate, sleep, anxiety, and affiliative and aggressive social behavior. The functional diversity along with a rich body of literature indicating unique patterns of innervation originating in different serotonergic nuclei and regional differences differences in characteristics of neuronal excitability suggest a large degree of heterogeneity within the overall population of serotonin-producing neurons. Ultimately, to understand the diverse functional role of serotonin at the organismic level, we must decode the heterogeneity of function at the cellular level as well. Here we address the question: Do serotonin neurons from distinct embryonic lineages differ in their biophysical properties? Guiding our electrophysiological studies are transcriptome profiling data demonstrating differential gene expression of serotonin neurons sorted by developmental lineage (rhombomere (R) of origin) that highlight differentially expressed ion channels and G-protein coupled receptors, whcih could affect neuronal excitability and pharmacological profiles. To precisely target neurons for whole cell patch clamp recordings, we used a duel recombinase strategy in which mice carrying Pet1-Flpe (targets serotonin neurons) and a rhombomere specific cre driver (differing by rhombomere targeted) were crossed with mice carrying an intersectional allele to express GFP only in cells in which cre and Flpe expression overlapped. In general, we observed lineage specific differences in parameters of excitability and identified cell-type specific receptor responses. For example, R2-derived serotonin neurons were on average more excitable than adjacent neurons from separate lineages, and, within the R2-lineage, serotonin neurons separated based on their response to ligands for the oxytocin and tachykinin 3 receptors. Our findings indicate functional heterogeneity even within anatomically grouped serotonin neurons. Differences at the cell level likely underlay functional differences at circuit and behavioral levels and suggest the potential for subset specific targeting (i.e., functional targeting) in therapeutic applications.

HIPPOCAMPAL 5-HT INPUT REGULATES MEMORY STORAGE AND SCHAFFER COLLATERAL EXCITATION

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Activity-dependent long-term homosynaptic plasticity provides a well-characterized substrate for hippocampal-dependent memory. Efficacy and duration of memory storage is regulated by heterosynaptic modulatory transmitter actions. While serotonin plays an important role in such modulation in the invertebrate *Aplysia*, its function in the vertebrate hippocampus is less clear. Specifically, the consequences elicited by the spatio-temporal gradient of endogenous 5-HT release are not known. Here we applied optogenetic techniques in mice to gain insight into this fundamental biological process. We find that activation of serotonergic terminals in the hippocampal CA1 region both potentiates excitatory transmission at CA3-to-CA1 synapses and enhances spatial memory. Conversely, optogenetic silencing of CA1 serotonin terminals inhibits memory storage. We furthermore find that synaptic potentiation is mediated by 5-HT4 receptors, and that systemic modulation of 5-HT4 receptor function can bidirectionally impact memory formation. Collectively, these data reveal powerful modulatory influence of serotonergic synaptic input on hippocampal function and memory storage.

Interestingly elevated levels of 5-HT signaling during development result in blunted 5-HTergic function in adulthood. At the level of the hippocampus we find that early life 5-HTT blockade leads to reduced 5-HTergic innervation and a diminished ability of optogenetic 5-HTergic activation to potentiate excitatory transmission at CA3-to-CA1 synapse. Furthermore, developmental 5-HTT blockade also leads to impaired performance in spatial memory tasks. Altogether these data demonstrate critical developmental sensitivity of the 5-HT system with robust consequences on hippocampal physiology and function.

RECIPROCAL CONTROL OF CORTICOFUGAL OUTPUT BY SEROTONIN AND ACETYLCHOLINE

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Pyramidal neurons in the mouse prefrontal cortex comprise two broad subclasses of neurons defined by their long-distance axonal projections: commissural/callosal (COM) neurons, and corticopontine (CPn) neurons. By projecting to different cortical and subcortical targets, these two populations represent distinct cortical output channels that play specific roles in decision making and behavior. In addition to having distinct morphological and physiological properties, growing evidence indicates that COM and CPn neurons are differentially regulated by modulatory neurotransmitters, including serotonin (5-HT) and acetylcholine (ACh).

We have shown that COM and CPn neurons in the mouse medial prefrontal cortex (mPFC) exhibit different responses to transient exposure to 5-HT. While CPn neurons are universally inhibited via $G_{i/o}$-coupled 5-HT_{1A} (1A) receptors, COM neurons are excited via G_q-coupled 5-HT_{2A} (2A) receptors. Although both neuron subpopulations express G_q-coupled M1 muscarinic ACh receptors, we report that endogenous ACh selectively enhances the excitability of CPn neurons. This suggests that 5-HT and ACh exert opposing influences on corticofugal output to the brainstem. Consistent with this hypothesis, in cortical tissue from mice expressing channelrhodopsin-2 in cholinergic neurons, single flashes of blue light selectively and persistently increased action potential output in CPn neurons (for up to 60 s), and this output was halted by subsequent serotonergic signaling at 1A receptors. To test whether 5-HT and ACh differentially affect synaptic transmission in cortical circuits, we are using optogenetic approaches to selectively activate COM afferents onto COM and CPn target neurons. Preliminary results suggest 5-HT may preferentially suppresses glutamate release at COM synapses on CPn neurons. We are continuing to explore the selectivity of cholinergic and serotonergic regulation of synaptic transmission in cortical circuits.

Our findings suggest that 5-HT and ACh may exert reciprocal control over corticofugal output to the brainstem, and suggest a circuit-based mechanism by which these transmitters may differentially contribute to behavior.

DUODENUM-DERIVED SEROTONIN REGULATES LIVER HEPCIDIN EXPRESSION AND IRON METABOLISM IN RESPONSE TO HYPOXIA

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Serotonin (5-hydroxytryptamine, 5-HT) is mainly produced by enterochromaffin cells in the intestine and has pleiotropic functions. Its role in the regulation of erythropoiesis and red blood cells survival has been demonstrated in our lab (*Amireault 2011, 2013*). Iron metabolism is tightly regulated by liver hepcidin, which controls iron bioavailability (*Nemeth 2004*). In case of hypoxia and need of iron for erythropoiesis, liver hepcidin is downregulated. Our hypothesis was that gut-derived 5-HT was involved in the regulation of iron metabolism in the liver, and in response to hypoxia.

We studied the effect of 5-HT on human and murine hepatocytes. To discriminate between bone marrow-derived and gut-derived 5-HT contributions in the regulation of liver hepcidin in response to hypoxia, we performed bone marrow adoptive transfer experiments. WT versus Tph1$^{-/-}$ bone marrow have been transferred in WT or Tph1$^{-/-}$ recipient mice, placed under hypoxia conditions.

In vitro experiments show that adding 5-HT or 5-HT$_{2B}$ agonists on hepatocytes reduce by more than 50% hepcidin expression. In 5-HT deficient mice, liver hepcidin expression is increased. In contrast, low plasma 5-HT was correlated with an increased in iron levels. In transfer experiments, only the wild-type recipients (i.e. with a normal secretion of gut-derived 5-HT), receiving bone marrow cells from either 5-HT deficient or WT, were able to trigger a response to hypoxia, characterized by a significant rise in Tph1 mRNA, in addition to a specific duodenum 5-HT synthesis and a concomitant decrease of liver hepcidin mRNA. Conversely, Tph1$^{-/-}$ mice (with an aborted secretion of gut-derived 5-HT) could not regulate liver hepcidin.

We show that 5-HT synthesis by enterochromaffin cells in the intestine is necessary for the downregulation of hepcidin in the liver and in response to hypoxia. These results may offer new therapeutic options for patients suffering from iron metabolism disorders.

HOW CAN WE KNOW WHO WILL BENEFIT FROM SSRIS?

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Serotonin-selective reuptake inhibitors (SSRIs) are first-choice treatments for major depressive disorder (MDD) and some anxiety disorders, yet significant numbers of patients do not show acceptable improvement after an initial drug/dose or long trials involving dosage increases and/or SSRI switching. We hypothesized that platelet serotonin transporter (SERT) function varies across individuals such that higher pretreatment SERT capacity is correlated with better SSRI responses. Subjects were recruited from the UCLA BRITE-MD study, an 8-week antidepressant intervention trial. Male and female patients (N=32) with a DSM-IV diagnosis of MDD were selected for SERT functional measurements. Male and female healthy volunteers (N=40) were also recruited from a UCLA study on neuroinflammation. Patients received escitalopram (ESC; 10 mg/kg/day). After one week, some patients were randomly switched to bupropion (BUP; 100 mg/kg/day). All subjects then continued on the same antidepressant for seven additional weeks. Blood was sampled at weeks 0, 1, 2, and 8. Platelet SERT capacity was assessed in MDD patients and healthy volunteers by high-speed chronoamperometry and correlated with therapeutic effects in patients receiving 8-weeks of ESC. SERT capacities varied across healthy and depressed individuals, being lower overall in MDD ($P<0.01$). In a small sample (N=12) of MDD patients completing 8-weeks of ESC, remitters had the highest pretreatment SERT capacities and downward trends over the first two treatment weeks. Patients with partial responses to ESC had similar pretreatment SERT capacities but only showed decreases in uptake rates during the first week of ESC. Nonresponders had the lowest pretreatment SERT capacities. Differences in pretreatment SERT capacity were not correlated with SLC6A4 noncoding polymorphisms. Determining peripheral SERT capacity prior to and during early treatment of MDD patients may have predictive value for deciding whether to continue with SSRIs or to switch sooner, rather than later, to an antidepressant with a different mechanism of action.

ABNORMALITIES IN SLEEP AND THE RESPONSE TO SEDATING ANTIPSYCHOTIC MEDICATIONS UNDERSCORE A LINK BETWEEN THE IMMEDIATE EARLY GENE *EGR3* AND THE 5-HT$_{2A}$ RECEPTOR

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The schizophrenia risk-associated gene *Egr3* is an immediate early gene transcription factor activated in response to environmental stimuli and required for memory formation and synaptic plasticity. We have previously reported that *Egr3*-/- display schizophrenia-like phenotypes including resistance to the sedating effects of clozapine and other second-generation antipsychotics, a feature that parallels the heightened tolerance of schizophrenia patients to side effects of these medications. This response is mediated by 5-HT$_{2A}$ receptors, which show a 70% decrease in Egr3-/- mice. The fact that 5-HT$_{2A}$R-/- mice display the same resistance to sedation by clozapine suggests a molecular interaction between *Egr3* and the 5-HT$_{2A}$R gene *Htr2a*, itself a schizophrenia-associated gene.

We will present results of our studies to further investigate the mechanisms underlying this pharmacologic response and an additional schizophrenia-like phenotype, which underscore the shared functions of *Egr3* and *Htr2a*. First, we used electroencephalography (EEG) to define the electrophysiologic signature of clozapine on WT and *Egr3*-/- mice. These results demonstrate that clozapine induces a previously undescribed “dissociated state” (low amplitude, low frequency EEG alpha rhythm paired with low muscle tone) that lasts up to 2 hours in WT mice. This response was completely absent in *Egr3*-/- mice. In addition, a selective 5-HT$_{2A}$ antagonist, alone or in combination with a selective 5-HT$_{2BC}$ antagonist, produced EEG slowing coincident with behavioral quiescence in WT mice but not in *Egr3*-deficient mice. Second, we have identified that *Egr3*-/- mice display sleep abnormalities suggestive of the deficits seen in patients with schizophrenia. Intriguingly, 5-HT$_{2A}$R-/- mice show an identical sleep phenotype, suggesting that *Egr3* may modulate sleep via its regulation of the 5-HT$_{2A}$ receptor. Together these findings suggest that *Egr3* influences sedation and sleep through regulation of the 5-HT$_{2A}$ receptor. These findings suggest a mechanism whereby dysfunction in *Egr3* may explain the 5-HT$_{2A}$ receptor deficits consistently reported in schizophrenia patient studies.

DISSOCIATING THE PROCOGNITIVE AND ANXIOLYTIC-LIKE EFFECTS OF EXERCISE AND ENVIRONMENTAL ENRICHMENT IN 5-HT1A RECEPTOR KNOCK-OUT MICE

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Clinical evidence indicates that serotonin-1A receptor (5-HT_{1A}R) gene polymorphisms are associated with anxiety disorders and deficits in cognition. In animal models, exercise (Ex) and environmental enrichment (EE) can change emotionality-related behaviours as well as enhance some aspects of cognition and hippocampal neurogenesis. We investigated the effects of Ex and EE (which does not include running-wheels) on cognition and anxiety-like behaviours in wild-type (WT) and 5-HT_{1A}R knock-out (KO) mice. Using an algorithm-based classification of search strategies in the Morris water maze, we report for the first time that exercise increased the odds for mice to select more hippocampal-dependent strategies. In the retention probe test, Ex (but not EE) corrected long-term spatial memory deficits displayed by KO mice. In agreement with these findings, only Ex increased hippocampal cell survival and BDNF protein levels. However, only EE (but not Ex) modified anxiety-like behaviours, demonstrating dissociation between improvements in cognition and innate anxiety. Interestingly, the EE-induced anxiolytic-like effect was associated with increased hippocampal 5-HT_{2C} gene expression in both genotypes. In contrast, EE enhanced hippocampal cell proliferation in WT mice only, suggesting a crucial role for intact serotonergic signalling in mediating this effect. Together, these results demonstrate differential effects of exercise versus EE in a mouse model of anxiety with cognitive impairment. Overall, the 5-HT_{1A}R does not seem to be critical for those behavioural effects to occur. These findings will have implications for our understanding of how exercise and environmental enrichment enhance experience-dependent plasticity, as well as their differential impacts on anxiety and cognition.

5-HT$_{1A}$ AGONIST, 8-OH-DPAT, and 5-HT$_{2A}$ ANTAGONISTS, KETANSERIN AND SARPOGRELATE, PROTECT THE RETINA FROM LIGHT-INDUCED RETINOPATHY.

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Recent studies have provided evidence that serotonin receptors are located in the mammalian retina and that targeting these receptors can elicit neuroprotective effects. We assessed the neuroprotective effects of 8-OH-DPAT, a 5-HT$_{1A}$ agonist, as well as ketanserin and sarpogrelate, 5-HT$_{2A}$ antagonists, in a rodent light-induced retinopathy model.

To explore neuroprotective effects, albino BALB/c mice were injected intraperitoneally with either vehicle or varying doses of each drug. Two time courses were examined. The five-day time course consisted of five daily injections and on the third day of injections, mice were exposed to bright light (~10,000 lux). For the one-day time course, injections were administered once immediately prior to bright light exposure. Seven days after bright light exposure, retinal structure and function were assessed using spectral domain optical coherence tomography and electroretinography. To confirm the role of the 5-HT$_{1A}$ receptor in protection, WAY 100635, a 5-HT$_{1A}$ antagonist, was used to block the action of 8-OH-DPAT in BALB/C mice. As an additional control, 5-HT$_{1A}$ knockout mice were injected with 8-OH-DPAT. To explore cellular mechanisms responsible for the observed neuroprotection, retinas were harvested at different times post-injection and post-bright light exposure. Kinase pathway analysis was performed using phospho-specific antibodies and quantitative western blots.

8-OH-DPAT, ketanserin and sarpogrelate protected retinas from light-induced retinopathy. Both a five-day time course and a one-day time course of 8-OH-DPAT, ketanserin and sarpogrelate significantly preserved outer retinal structure and function. Neuroprotective effects observed with 8-OH-DPAT required activation of the 5-HT$_{1A}$ receptor. When WAY 100635 was injected prior to 8-OH-DPAT, neuroprotective effects were reduced. In addition, 8-OH-DPAT did not elicit neuroprotective effects in 5-HT$_{1A}$ knockout mice. Injections of 8-OH-DPAT, ketanserin and sarpogrelate temporally shifted and increased ERK1/2 activation that occurs due to bright light exposure.

Ongoing studies are focused on understanding the cellular mechanism of retinal neuroprotection and utilizing serotonin agonists and antagonists for the treatment of outer retinal degeneration.

OPTOGENETIC INVESTIGATION OF THE SEROTONERGIC CONTROL OF THE BASOLATERAL AMYGDALA MICROCIRCUITRY

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Serotonergic projections from the dorsal raphe (DRN) are important regulators of microcircuits of the amygdala, and particularly in the basolateral nucleus (BLA), which are critical for emotional learning. The microcircuitry of the BLA is a complex combination of pyramidal (output) neurons (PNs), local GABAergic interneurons (INs), and a serotonin input onto 5-HT receptors of different types. One challenge facing investigations of the influence of 5-HT on this circuitry is need for methods to selectively stimulate the 5-HT neuron input. Here we investigated the influence of the 5-HT innervation on the BLA microcircuit using a 5-HT-targeted optogenetic strategy.

Channelrhodopsin (ChR2) was delivered by a viral vector to the DRN of SERT Cre-mice, and shown to elicit 5-HT neuron-specific expression. Acute slices were prepared from ChR2-injected SERT-Cre mice, whole-cell patch clamp recordings were obtained from electrophysiologically characterised BLA INs and PNs, and 5-HT axons were activated optically at varying frequencies.

Optical activation evoked marked effects on BLA INs; 53% of INs displayed a slow excitation that was abolished by the 5-HT_{2A} receptor antagonist MDL100907 (150 nM), while 37% of INs showed a slow inhibition that was abolished by the 5-HT_{1A} receptor antagonist WAY100635 (1 µM). Interestingly, 55% of INs exhibited a fast excitatory response that was abolished by glutamate receptor blockers (10µM NBQX and 50µM APV). The data suggested that glutamate- and 5-HT- mediated responses exhibited different sensitivity to stimulation frequency and may be targeted at INs with different electrophysiological properties. In comparison to INs, BLA PNs responded to optical activation with a slow inhibition that was blocked by WAY100635. Optical activation also evoked an increase in GABAergic inhibitory inputs onto PNs, as evidenced by an increase in sIPSCs.

These data suggest that the 5-HT input to the BLA targets INs through 5-HT_{2A} and 5-HT_{1A} receptors, and PNs via 5-HT_{1A}. Intriguingly, our data provide the first evidence for the modulation of BLA INs by the co-release of glutamate from 5-HT neurons. Experiments are ongoing to establish the role of 5-HT and co-released glutamate in the BLA microcircuitry and emotional learning.

ALTERED 5-HT4 RECEPTOR SIGNALLING IN IRRITABLE BOWEL SYNDROME MAY BE CAUSED BY IMPAIRED MIRNA REGULATION

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Irritable bowel syndrome (IBS) is a complex gastrointestinal (GI) disorder in which disturbed motility is thought to play a role. 5-HT4 receptors regulate motor function and are targeted therapeutically in the treatments of IBS with constipation (IBS-C) and functional constipation. We hypothesize that disturbed 5-HT4 receptor regulation or functional defects in the 5-HT4 receptor gene (*HTR4*) may be involved in the motor dysfunction seen in these patients.
As 3' untranslated regions (3'UTRs) are a major site of posttranscriptional regulation, we screened different *HTR4* isoforms in a small IBS pilot cohort for variants in their 3'UTRs. In this cohort, we identified the rare SNP (rs201253747) *HTR4b* c.*61T>C to be exclusively present in IBS with diarrhoea (IBS-D) patients (2/98 in IBS-D, 0/100 in IBS-C and 0/92 in controls). In a subsequent replication study, we confirmed the polymorphism as significantly enriched in IBS-D patients compared with healthy controls (p=0.033; OR=3.09; 2185 healthy controls, 613 IBS-C and 829 IBS-D patients were tested in total).
The SNP locates in a putative miR-16 family binding site. We show that *HTR4* is specifically downregulated by this miRNA family via multiple target sites. Furthermore we describe a novel isoform of *HTR4b* with an alternatively spliced 3'UTR. This isoform escapes miR-16 regulation in reporter assays when the *HTR4b* c.*61C allele is present, suggesting an increase of 5-HT4 receptor expression in *HTR4b* c.*61C carriers.
In conclusion, we have shown for the first time that *HTR4* is susceptible to miRNA regulation and that the c.*61T>C polymorphism impairs the regulation of *HTR4* via the miR-16 family. Thus we postulate that people carrying this SNP may have a higher 5-HT4 receptor activity and therefore higher risk of developing IBS-D. In order to prove this hypothesis we are currently exploring the miRNAs' and *HTR4b* isoforms' expression in patient tissue and in *in vitro* studies.

HOW DOES THE BRAIN IMPLEMENT DECISION-MAKING TO EAT? IMPLICATION OF THE 5-HT_4 RECEPTORS

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Adaptive decision-making to eat is crucial for survival but in anorexia nervosa, the brain persistently supports reduced food intake despite a growing need for energy. How the brain persists in reducing food intake to the point of death despite the evolution of mechanisms to ensure survival by governing adaptive eating behaviors remains just as mysterious as the switch from anorexia to bulimia. Neural substrates belong to the reward-habit system and could differ from overeating-induced obesity. The contribution of serotonin receptors in eating is critical in humans and animal models. One possibility is that restrictive food intake critically engages decision-making (goal-directed) systems where the serotonin 4 receptors play a pivotal action in two critical brain structure of the reward system; The nucleus accumbens and the medial prefrontal cortex. These studies introduce the view that a persistent food restriction might mimic some aspects of addiction to drugs of abuse. Furthermore, novel molecular mechanisms influenced by the constitutive activity of the serotonin 4 receptors , in the nucleus accumbens, could underlie the switch from under- to overeating in animal models.

EMOTIONAL INSTABILITY AND BEHAVIORAL TRANSITION IN SEROTONIN DEPLETED MICE

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During their lives, organisms have to face disturbing forces that upset their homeostasis. These forces, called stressors, trigger a stress response, an innate adaptive reaction whose task is restoring normal balance in the organism, essential for survival. In vertebrates the brain is central in perception and adaptation to stress, and in organization of appropriate counteraction. However, due to a combination of genetic and environmental factors, stress response can be maladaptive thus destabilizing homeostasis and increasing the risk of neuropsychiatric disorder onset.
Serotonin has a central role in normal brain function modulating several physiological processes including mood regulation and emotional behavior, and it has been implicated in both adaptive and maladaptive stress response. However, the precise role of serotonin in the modulation of emotional behavior in health and disease needs to be further elucidated.
We used Tph2 knockout mice to evaluate the consequences of serotonin depletion on the emotional behavior. Tph2 mutant mice displayed reduced depression-like behaviors in the forced swim test, tail suspension test, in the novelty-suppressed feeding test and also slower habituation in a novel environment, suggesting that serotonin depletion results in hyperarousal.
We then asked how exposure to unpredictable Chronic Mild Stress (uCMS), an effective paradigm for inducing depression-like symptoms in rodents, could impact the behavioral state of Tph2 mutants. Results showed that uCMS induced depressive-like behaviors in the forced swim test in both Tph2 knockouts and control littermates. However, uCMS produced a greater increase in immobility between non-stressed and stressed mutant mice than between non-stressed and stressed wild-type animals, thus acting as a precipitating factor for the exaggerated behavioral switch exhibited by mutant mice. Molecular and biochemical results in both non-stressed and stressed Tph2 mutants account for the observed behavioral data, shedding new lights on the complex role of serotonin in emotional regulation.

ATOMIC-LEVEL RESOLUTION OF SEROTONIN RECEPTOR SIGNALING

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Ergolines such as LSD and ergotamine are endowed with medicinal properties by manifesting their pharmacological action at serotonin G protein-coupled receptors. Ergolines, however, suffer from extreme side-effects by acting at 5-HT_{2B} receptors to produce cardiac valvulopathy. To address this issue, we previously solved the structure of the 5-HT_{2B} receptor in complex with ergotamine, and discovered that ergolines exert enhanced potency for arrestin recruitment leading to β-arrestin-bias. To understand the molecular basis of arrestin-bias and that LSD's actions, we have now solved the 5-HT_{2B} receptor in complex with LSD and investigated the kinetics of LSD binding as it pertains to arrestin recruitment. Compared to all serotonin receptors, we find that LSD exhibits an underappreciated and extremely slow on- and off-rate at the 5-HT_{2B} receptor, with the slowest off-rate exhibited at the 5-HT_{2A} receptor- LSD's major target for psychedelic experiences. Furthermore, when LSD's on- and off-rate are accelerated by mutation of extracellular loop 2 (EL2), LSD loses arrestin-bias. LSD's slow kinetics and its interaction with EL2 at serotonin receptors are key to manifest potent arrestin recruitment.

Furthermore, the design of drugs devoid of 5-HT_{2B} receptor agonist activity remains a challenge. To examine the molecular basis for ergoline efficacy, we also solved the 5-HT_{2B} in complex with methyl ergonovine, the active metabolite of the antagonist, methysergide. We find that the N1-methyl of methysergide contacts residue Ala(5.46) leading to antagonism, and glycine mutation of Ala(5.46) leads to unexpected agonist activity by methysergide, reinforcing the fact that residue 5.46 acts as an activation switch in the 5-HT_{2B} receptor. Taken together, crystallographic and pharmacological evidence are converging to uncover that ergolines have unique ligand binding poses and kinetics, which lead to either biased agonism or antagonism. These discoveries will likely explain their medicinal or psychedelic properties and lead to novel drugs targeting serotonin receptors.

SEROTONIN 5-HT$_{2C}$ RECEPTOR ANTAGONISM PARADOXICALLY IMPROVES DECISION-MAKING IN A RODENT GAMBLING TASK IN THE PRESENCE OF WIN-PAIRED CUES

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A preclinical analogue of the Iowa Gambling Task, the rodent gambling task (rGT), was developed to investigate the neurobiology of cost/benefit decision-making and how it becomes maladaptive in clinical populations. In the rGT, rats choose between 4 options with varying frequencies and magnitudes of reward and punishment time-outs. Most rats learn to favour the more advantageous options (i.e. low-risk/low-reward: “P1”, “P2”) to gain maximal amounts of food rewards. Pairing rewarded trials (“wins”) with audiovisual cues biases animals’ choice preference towards more disadvantageous options (i.e. high-risk/high-reward: “P3”, “P4”), resembling the effects of salient cues in driving addiction-like behaviours. Since 5-HT$_{2A/C}$ receptor signalling may modulate the influence of cues, we assessed the effects of the following compounds on male rats’ performance in the uncued (n=21) and cued (n=16) rGT: the 5-HT$_{2C}$ agonist, Ro-60-0175; the 5-HT$_{2A}$ antagonist, M100,907; and the 5-HT$_{2C}$ antagonist, SB-242084. Across both tasks, the expected dose-dependent effects on premature responding were seen, with Ro-60-0175 and M100,907 decreasing, and SB-242084 administration increasing, this form of motor impulsivity. Similarly, Ro-60-0175 and M100,907 enhanced, while SB-242084 reduced, latencies to choose; however, numbers of omitted or total trials were unaffected and only Ro-60-0175 and SB-242084 administration altered reward collection latencies. Patterns of choice preference remained unchanged with the striking exception of SB-242084, which dose-dependently increased choice of the most advantageous option, P2, on the cued, but not uncued, task. Given that this effect occurred concurrently to enhanced impulsivity and speed of responding—associated with disadvantageous choice preference at the population level in the uncued rGT—these findings highlight a seemingly paradoxical role for 5-HT$_{2C}$ receptors in modulation of cost/benefit decision-making in the presence of win-paired cues. Elucidating the mechanism by which 5-HT$_{2C}$ receptor antagonism facilitates optimal decision-making in this context may assist in developing targeted therapeutics to modulate maladaptive choice in clinical populations.

ADAPTATIONS OF THE DORSAL RAPHE IN A RAT MODEL OF DEPRESSION AND FOLLOWING ANTIDEPRESSANT TREATMENT.

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Serotonin (5-hydroxytryptamine; 5-HT) is strongly implicated in mood disorders such as depression. It is poorly understood, however, how 5-HT neurons dysfunction in depression, or if depression-related changes are topographically organized within the raphe. Furthermore, antidepressants such as SSRIs are typically studied in normal animals, but it is unclear if and how they might act to ameliorate dysfunction of 5-HT neurons in a depressed brain. Therefore, the goal of this study was to identify if raphe neuron function is altered in an animal model of depression (maternal separation), and whether this dysfunction is reversed by antidepressant treatment. Adult rats that underwent either maternal separation or were left undisturbed (control) for the first two weeks of life were given 14 daily injections of the SSRI fluoxetine or vehicle injections. Activated 5-HT neurons in the raphe in response to an acute swim stress were then quantified via immunolabeling of Fos and tryptophan hydroxylase proteins. Unexpectedly, maternally separated and control rats had similar numbers of serotonin neurons with activated Fos. However, fluoxetine dramatically suppressed activation of 5-HT neurons throughout the raphe in all rats. Acute injection of the 5-HT1A receptor antagonist WAY-100635 before stress reversed the effect of fluoxetine alone, and returned activation of 5-HT neurons to the level of vehicle-treated control rats. Maternally separated rats appeared more sensitive to WAY treatment following antidepressant administration, which resulted in 5-HT hyperactivation, particularly in the rostral dorsal raphe. The number of 5-HT1A receptor binding sites in the raphe as measured using receptor autoradiography was not affected by early life experience, suggesting the 5-HT1A receptor is not desensitized in maternally separated animals. These data suggest that 5-HT1A receptors exert enhanced feedback inhibition on 5-HT neurons after two weeks of fluoxetine treatment, and that unique adaptations in 5-HT1A regulation may be associated with this model of depression.

POSTER #2

COGNITIVE DEFICITS DUE TO CHRONIC CONSUMPTION OF Δ9-TETRAHYDROCANNABINOL DURING ADOLESCENCE: ROLE OF THE 5-HT_6/MTOR PATHWAY

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Cognitive deficits of schizophrenia severely compromise the quality of life of patients and are poorly controlled by currently available treatments. They are largely reproduced in cannabis abusers suggesting common pathological mechanisms. Recently, we demonstrated that the activation of mTOR induced by 5-HT_6 receptors in the prefrontal cortex (PFC) underlies the cognitive impairments in developmental models of schizophrenia. Further supporting a critical role of mTOR in cognitive deficits, a previous study has shown that acute treatment with Δ9-tetrahydrocannabinol (THC), the main psychoactive compound of cannabis, induces an activation of mTOR leading to memory impairment and cognitive deficits. In line with these findings, our study aimed to explore the perturbations of synaptic transmission in the PFC in a model of chronic-THC consumption during adolescence and to determine whether cannabis-elicited mTOR activation can be controlled by 5-HT_6 receptor ligands.

Adolescent mice have been treated daily with THC and left undisturbed until their adulthood for electrophysiological, biochemical and behavioral analyses. First, we showed an increased mTOR activity in PFC, which persists at adulthood, in this model. Moreover, administration of 5-HT_6 antagonist during adolescence rescued this non-physiological mTOR activation at the adult stage. The frequency of mIPSCs is also decreased in THC-treated animals compared to controls. Again, co-administration of 5-HT_6 antagonist during the adolescence rescued a normal mIPSC frequency. Then, we demonstrated that chronic THC-treated mice exhibit a deficit in the novel object recognition test, compared to controls. This deficit is completely abolished by the early treatment with 5-HT_6 antagonist.

Here, we demonstrated that altering the normal PFC maturation by chronic THC consumption during adolescence induces a non-physiological mTOR activation, an alteration of inhibitory transmission and cognitive impairments at adult stage. Collectively, our data suggest that the blockade of the 5-HT_6/mTOR pathway during the adolescence prevent these alterations at adult stage.

ELEVATED 5-HT ACTIVITY FOLLOWING CONSTITUTIVE SERT-KO AND ACUTE CITALOPRAM TREATMENT BROADLY REDUCES INCENTIVE MOTIVATION

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Rationale: Many lines of evidence suggest that serotonin (5-hydroxytryptamine; 5-HT) decreases incentive motivation – the psychological process by which motivationally significant stimuli elicit appetitive behaviours. Much of this literature is based on findings that acute, pharmacological elevations of 5-HT activity reduce instrumental responding for primary reinforcers including food, drugs, and brain-stimulation reward. Additionally, Sanders et al. (2009) have recently shown that chronically elevated 5-HT produced by serotonin transporter knockout (SERT-KO) also decreases responding for food. However, incentive motivational processes are not specific to primary reinforcers. Other types of stimuli have the ability to elicit behaviour, including stimuli associated with reward acquisition (conditioned reinforcers) and sensory stimuli themselves (sensory reinforcers). Such stimuli are thought to engage motivational circuitry and influence goal-directed behaviour, which is particularly evident in the ability of drug-associated cues to precipitate relapse in human addicts. The role of 5-HT in modulating the motivational significance of non-primary incentive stimuli is not well understood. The present experiments determined whether the influence of 5-HT was specific to primary reinforcers or generalized to other incentive stimuli. Here, acute and chronic elevations of 5-HT on responding for primary, conditioned, and sensory reinforcers were examined.

Methods: In the chronically elevated 5-HT condition, responding for all three reinforcers was compared between SERT-KO mice and wild-type (WT) littermates. In the acutely elevated 5-HT condition, responding for all three reinforcers was examined following treatment with 10 or 20 mg/kg citalopram. Separate groups of mice (n=10-12 in each) underwent tests of responding for a primary reinforcer (0.2 ml of 0.2% saccharin), a conditioned reinforcer (conditioned stimulus (CS) previously paired with saccharin delivery) or a sensory reinforcer (lights+sound). In tests of primary reinforcement, mice were trained to lever press for saccharin on a fixed-ratio (FR)1 schedule of reinforcement and then shifted to random ratio (RR)2 and RR4 schedules for the duration of testing. In tests of responding for a conditioned reinforcer, mice first underwent Pavlovian conditioning sessions wherein the delivery of saccharin was paired with a CS. Subsequently, in an operant conditioning phase, lever pressing for the CS alone serving as a conditioned reinforcer was examined on RR2 or RR4 schedules of reinforcement. In tests of responding for a sensory reinforcer, mice acquired lever pressing for the stimulus on a FR1 schedule of reinforcement and were then shifted to a RR2 schedule for the remainder of testing.

Results: Compared to WT littermates, SERT-KO mice made fewer responses for a primary reinforcer, a conditioned reinforcer, and a sensory reinforcer. Similarly, both 10 and 20 mg/kg doses of citalopram decreased responding for all three reinforcers.

Conclusions: These results demonstrate that both chronic and acute increases in 5-HT neurotransmission decrease responding for three different types of reinforcers. This provides strong evidence for a broad, inhibitory role of 5-HT in the control of incentive motivational processes. Future studies will use optogenetic approaches to address the circuit mechanisms by which 5-HT modulates incentive motivation.

EPIGENETIC ROLE OF 5-HT2ARECEPTOR-DEPENDENT SIGNALING IN SCHIZOPHRENIA TREATMENT

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Antipsychotic drugs, including both typical such as haloperidol and atypical such as clozapine and risperidone, remain the current standard for schizophrenia treatment. Nevertheless, about 30% of schizophrenia patients are considered treatment resistant, and will continue to experience psychotic and other symptoms despite the optimal use of antipsychotic medication. Recent preclinical and clinical studies suggest that histone deacetylase (HDAC) inhibitors are efficacious when given chronically in combination with atypical antipsychotics. HDACs remove acetyl groups from lysine residues in the N-terminal tails of core histones, which shifts the balance toward chromatin condensation and thereby silences gene expression. We have intriguing data in mouse and human suggesting that a serotonin 5-HT2A receptor (5HT2A)-dependent increase in cortical pyramidal HDAC2 promoter activity by chronic treatment with atypical antipsychotics may restrain the therapeutic effects of these agents, and that inhibition of HDAC2 represents a promising new approach to augment the treatment of schizophrenia. Chronic administration of atypical, but not typical, antipsychotics markedly up-regulates HDAC2 expression in the frontal cortex, an effect that is associated with decreased 5HT2A density and 5HT2A-dependent modulation of HDAC2 transcription. Using viral-mediated gene transfer, we demonstrate that HDAC2 over-expression in mouse frontal cortex induces behavioral changes indicative of a schizophrenia-like phenotype, such as models of psychosis, deficits in sensory motor gating, and impairments in working memory. Conversely, both peripheral and intra-frontal cortex administration of HDAC inhibitors increase the expression of genes implicated in spine formation and plasticity, and augment the therapeutic-like behavioral effects of atypical antipsychotics. Mechanistically, we find that the transcription factor NF-κB is potentially involved in the effect of chronic treatment with atypical antipsychotics on 5HT2A-dependent up-regulation of HDAC2. Together, these data support the hypothesis that inhibition of HDAC2 may serve as a new pharmacological approach to improve the currently limited therapeutic range of chronic treatment with atypical antipsychotics.

ANTIDEPRESSANT-LIKE EFFECTS OF THE "UPTAKE-2" BLOCKER, DECYNIUM 22 (D22) IN THE FLINDERS SENSITIVE LINE RAT MODEL OF DEPRESSION: AN ASSESSMENT OF ACUTE VS. CHRONIC ADMINISTRATION

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Selective serotonin reuptake inhibitors (SSRIs) are widely prescribed yet many patients report low to no relief of depressive symptom with SSRI treatment. We found that activity of low-affinity, high-capacity (uptake-2) transporters for biogenic amines, in particular organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT), may limit therapeutic efficacy of SSRIs. In C56BL/6 mice, the non-selective uptake 2 blocker, D22, produced antidepressant-like effects when serotonin transporter was pharmacologically or genetically compromised. These studies point to D22 sensitive transporters as potential targets for antidepressant development.

Here, we examine the utility of D22 in a genetic animal model of depression, the Flinders Sensitive Line (FSL) rat. Like humans, FSL rats show reduced depressive behaviors after repeated but not acute antidepressant administration and can be used to identify novel drugs or combinations with faster-acting antidepressant-like activity and improved efficacy. Behaviors were assessed in adult Sprague Dawley (SD) and FSL rats using the forced swim test (FST), 30 min after single drug injections (i.p.).

Expectedly, FSL rats displayed higher basal immobility in the FST indicating higher depressive behavior compared with SD control rats. Also, as expected, acute injection of the SSRI fluvoxamine (10 mg/kg) reduced immobility time in SD but not FSL rats. Conversely, acute D22 (0.1 mg/kg) administration significantly lowered immobility time in FSL rats, but was not effective in controls. FSL rats also had elevated total protein expression of hippocampal OCT3 and PMAT as measured by western blotting.

The reduction in immobility time observed after single, low dose D22 in FSL rats suggests that uptake-2 transporters may be targets for rapidly acting antidepressant drugs, and underscores the utility of animal models for depression. Studies are underway to assess chronic effects of D22 treatment with and without SSRI administration.

POSTER #6

SEROTONIN INPUTS TO THE BNST REDUCE ANXIETY IN A 5-HT$_{1A}$ RECEPTOR DEPENDENT MANNER

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Identifying circuits that underlie anxiety is of utmost importance for treating anxiety disorders. Dorsal raphe nucleus (DRN) neurons release serotonin (5-HT) throughout the forebrain to regulate anxiety but its specific effects on its downstream targets have not been fully elucidated. In particular, the bed nucleus of the stria terminalis (BNST) and the central amygdala (CeA) that together comprise the extended amygdala, have been proposed to be sites of neuroadaptations underlying anxiety-like behaviors. Studies in rodents suggest that anxiety but not fear can be modulated by 5- HT inputs to the BNST. Similarly, studies in humans using behavioral paradigms that parallel the rodent experiments suggest that the same may be true. However, the mechanisms by which 5-HT modulates the extended amygdala in vivo and the consequent effect on anxiety-like behaviors remain unclear.

Here, by using tracers we demonstrate that the 5-HT projection to the BNST and the CeA, emanate from individual DRN neurons. Remarkably, optogenetic stimulation of 5-HT inputs to the dorsal BNST (dBNST), but not the CeA, decreases anxiety. These data suggest that DRN-dBNST synapses modulate anxiety by providing inhibitory input to the dBNST, but that input from those same DRN neurons to the CeA is without effect. Next, by combining electrophysiology, optogenetics and pharmacology we further demonstrate that 5-HT release in the dBNST results in hyperpolarizing responses that can be blocked by a 5-HT$_{1A}$ antagonist and that 5-HT$_{1A}$ receptor activation is necessary for the anxiolytic effect. Finally, preliminary studies indicate that photostimulation of 5-HT inputs in the dBNST increases CeA firing rates indicating that each region contributes to this state in a functionally different way. Our results suggest a mechanism whereby DRN inputs inhibit dBNST neurons, via activation of 5-HT$_{1A}$ receptors, which by extrapolation acts to disinhibit target sites, such as the CeA, leading to a reduction in anxiety.

POSTER #7

BRAIN SEROTONIN DEFICIENCY AFFECTS FEMALE SEXUAL ACTIVITY

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Recent studies in women undergoing *in vitro* fertilization treatment with donated oocytes revealed serotonin-related gene polymorphisms to influence clinical outcomes. A strong correlation was found between early pregnancy loss and genetic variants of serotonin-related genes carried by recipients. To study the impact of neurotransmitter serotonin on reproduction, we used Tph2-deficient ($TPH2^{-/-}$) mice lacking serotonin exclusively in the central nervous system. Regular breeding of $TPH2^{-/-}$ animals revealed a lower pregnancy rate when compared to wildtype (WT) animals, consistent with the human data. Further studies displayed no difference between $TPH2^{-/-}$ and WT females in the progression of the estrus cycle, *in vitro* development of preimplantation embryos from zygote to blastocyst, and implantation rates and fetus development after transfer of WT embryos into pseudopregnant $TPH2^{-/-}$ and WT females arguing for a normal reproductive physiology in the absence of central serotonin. Decreased pregnancy rates can also originate from the disturbance of centrally regulated processes, such as lower sexual activity or inability to adapt sexual motivation to reproductive state. Interestingly, while analysis of vaginal plugs revealed similar rates of mating success between genotypes, 61% of TPH2-/- females exhibited mating plugs during non-receptive stages of the estrus cycle (mostly in metestrus), compared to only 6% of WT females. These data argue for an increase in sexual activity of TPH2-/- females during non-receptive stages of the estrus cycle and suggest a role of serotonin in the adaption of sexual behavior to physiological state.

A new drug for treatment of Hypoactive Sexual Desire Disorder (HSDD), that decreases serotonin in the prefrontal cortex, was approved recently in the US, demonstrating the clinical relevance of studying brain serotonin effects on sexual activity.

THE SEXUAL DIMORPHIC ROLE OF A SMALL POTASSIUM CURRENT IN SEROTONIN NEURONS IN BINGE EATING, ANXIETY AND DEPRESSION

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Dysfunctions of serotonin neurons are implicated in multiple psychological disorders, including binge eating disorder, anxiety and depression. However, whether and how altered serotonin neural activity may affect these complex behaviours remain unclear. We found that the vast majority of serotonin neurons express a small conductance Ca^{2+}-activated K^{+}-3 channel (SK3, encoded by the Kcnn3 gene). To examine the physiological role of SK3 in serotonin neurons, we generated a conditional knockout mouse model, in which SK3 was deleted selectively in mature serotonin neurons ($Kcnn3^{f/f}$/Tph2-CreER). We found that serotonin neurons in these mutant mice showed diminished SK3-mediated outward potassium currents, decreased after-hyperpolarization, increased firing frequency and elevated resting membrane potential. In addition, both male and female mutant mice were resistant to development of binge-like eating compared to their littermate controls ($Kcnn3^{f/f}$). Interestingly, surgical depletion of ovarian hormones restored the binge-like eating behavior in mutant females. Further we found that female mutant mice showed decreased anxiety in the open field test, the light-dark test and the elevated plus maze test; these female mutants also showed decreased depression in the forced swim test. Surprisingly, these anxiolytic and anti-depressant phenotypes were not observed in male mutants. In summary, we demonstrated that SK3 is required to maintain normal firing activity of serotonin neurons, and we further suggest that enhanced SK3 functions in serotonin neurons may be involved in the development of binge eating, anxiety and depression.

IN UTERO EXPOSURE TO VENLAFAXINE ALTERS PLACENTAL AND FETAL HEART SEROTONIN SYSTEMS INDUCING FETAL HEART DEFECTS IN THE RAT

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During pregnancy 6.2% of women use selective serotonin and norepinephrine reuptake inhibitors (SS/NRIs), and this may be associated with increased risk of congenital heart defects, the leading causes of infant mortality. However, these effects are controversial and the underlying molecular pathway remains unclear. The placenta serves as an early source of serotonin, which is critical in fetal programming and development. We hypothesized that treatment with venlafaxine (the most prescribed SS/NRI) during pregnancy alters placental and fetal heart serotonin system and thus increases the risk of fetal heart defects. In our animal study, 78 timed-pregnant Sprague Dawley rats were gavaged with venlafaxine (0, 3, 10, 30 or 100 mg/kg/day) starting from gestation day 8 (GD 8). On GD 21, fetuses were examined for external and internal malformations, and placentas and fetal hearts were collected for gene expression analysis. Venlafaxine treatment significantly increased the placental index (fetal body to placental weight ratio; 11-12%) and doubled the incidence of fetal cardiac anomalies. Interestingly, ventricular septal defect (VSD) were found predominantly in male fetuses. Venlafaxine exposure decreased placental expression of the serotonin transporter (SERT) (mRNA: 30% and protein: 65%). In fetal heart, venlafaxine increased SERT expression in female (mRNA: 3-fold and protein: 20-fold), but not in male. The levels of serotonin 2B receptor mRNA (2-fold) and fibroblast growth factor 8, a gene involved in the establishment of the left/right asymmetry during embryogenesis (1.5-fold), were induced in fetal hearts from the 3 mg/kg/day group. Together, results showed that treatment with venlafaxine increased risks of fetal cardiac anomalies and altered placental and fetal heart serotonin systems in rats. Thereby, disturbances in serotonin signaling during an early window of times of development could altered fetal programming and thus induced not only congenital defects but also long term effects in childhood and adulthood.

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NEW PSYCHOACTIVE SUBSTANCES AND THEIR IMPACT ON THE SEROTONERGIC SYSTEM

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Monoaminergic systems require delicate regulation to maintain homeostasis. Various compounds affect the monoaminergic homeostasis by targeting monoaminergic receptors and transporters in the central nervous system. Of particular interest are monoamine transporters (MATs) for serotonin (SERT), dopamine (DAT) and norepinephrine (NET). MATs retrieve extracellular monoamines and terminate their availability and thereby their activity in the synaptic cleft. As a consequence, MATs serve as targets to treat a variety of disorders, like the selective serotonin reuptake inhibitors that alleviate the symptoms of major depression or compounds of the family of the amphetamines that help in the treatment of attention deficit hyperactivity disorder. However, MATs are also targeted by illicit psychostimulants, such as cocaine or 3,4-methylenedioxymethamphetamine. Currently, new psychoactive substances (NPS) are edging into the market and provide legal alternatives to scheduled substances. A plethora of NPS impact on the serotonergic system. NPS may target (i) 5-HT receptors, like the 2,5-dimethoxy-4-bromoamphetamine derivatives sold as “bromo dragonflys”; or (ii) MATs with profound activities at SERT, like the cathinone- derivative mephedrone. Knowledge about the molecular mechanisms of action of NPS is of tremendous importance. We sought to characterize a variety of NPS and tested them for their abilities to disrupt MAT-function. We identified several NPS that target SERT in an amphetamine-like fashion with notable activities at vesicular monoamine transporters. Thus, these findings identified NPS with increased potential as neurotoxic agents and highlight the major role of SERT as a target for novel designer drugs.

INVESTIGATING A PUTATIVE PROTEIN:PROTEIN INTERACTION BETWEEN THE SEROTONIN (5-HT) 5-HT_{2A} RECEPTOR (5-HT_{2A}R) AND 5-HT_{2C}R

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The serotonin (5-HT) 5-HT_{2A} receptor (5-HT_{2A}R) and 5-HT_{2C}R play important roles in behavior and physiology. We have recently demonstrated that knockdown of 5-HT_{2C}R following microinjection of 5-HT_{2C}R shRNA into rat medial prefrontal cortex (mPFC) evokes a behavioral phenotype characterized by increased motor impulsivity and elevated reactivity to cues associated with cocaine self-administration. The 5-HT_{2C}R knockdown in mPFC also resulted in upregulation of 5-HT_{2A}R protein in the mPFC and a leftward shift in potency of systemic M100907 to suppress motor impulsivity, suggesting functional disruption of local 5-HT_{2A}R:5-HT_{2C}R balance. Furthermore, co-immunoprecipitation studies suggested that a protein:protein interaction exists between 5-HT_{2A}R and 5-HT_{2C}R in mPFC. Here, we tested the hypothesis that a protein:protein interaction occurs between 5-HT_{2A}R and 5-HT_{2C}R. In a proximity ligation assay (Duolink), we found that native, unmodified proteins are in close proximity in mPFC. Next we performed a direct coupling analysis (DCA, which examines the co-evolution of residues in over 100 species) and identified candidate pairs of amino acid residues that are predicted to be in direct functional contact. The luciferase complementation assay (LCA) is being employed to test the hypothesis that the N-terminal domains are the primary points of interaction between the two receptors . In the LCA, two complementary luciferase N- (NLuc) and C-terminus (CLuc) fragments are fused to the two receptor proteins of interest, respectively. In the presence of the substrate D-luciferin, association of the two proteins brings the inactive fragments into close proximity to reconstitute the enzyme activity. We are co-expressing 5-HT_{2A}R-CLuc and 5-HT_{2C}R-NLuc, expressed on the C-terminus in mammalian cells to demonstrate the formation of a protein:protein interaction between 5-HT_{2A}R and 5-HT_{2C}R in live cells. Our findings suggest that 5-HT_{2A}R:5-HT_{2C}R protein interaction may provide a new neurobiological mechanism underlying behavior and a possible target for novel pharmacotherapuetics, such as heterobivalent ligands.

DEVELOPING SEROTONIN-SPECIFIC *IN VIVO* NEUROSENSORS TO LINK NEURAL SIGNALING WITH COMPLEX BEHAVIORS

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The BRAIN Initiative aims to unite nanotechnology and neuroscience to investigate neural circuitry within the integrated central nervous system at the spatial and temporal scales pertinent to information encoding. Altered interneuronal serotonin signaling has been known to be associated with a myriad of neuropathological processes such as depression and anxiety. To investigate how malfunctioned neurocircuitry underlies neuropsychiatric diseases associated with serotonin levels in the brain, chemically specific *in vivo* neurotransmitter sensors that approach the size of nanometer-sized synapses that respond in milliseconds or less are required. To tackle this challenge, we employ aptamers as artificial receptors, which have emerged as superior synthetic alternatives to antibodies for molecular recognition. We have also developed “neurochips” by designing and combining advanced surface chemistries and high-throughput patterning methods to screen for neurotransmitter-specific aptamers in an environment optimized for biorecognition. Decorating with small molecules mimicking cognate neurotransmitters, our neurochips were able to capture and to sort aptamers targeting different small-molecule neurotransmitters. The current project is to use neurochips to test the binding affinity of serotonin-specific aptamers, which can recognize surface-tethered serotonin precursors, L-5-hydroxytryptophan molecules that mimic endogenous serotonin in neural synapses. Once the *in situ* binding affinity is determined, we will immobilize serotonin-specific aptamers onto highly sensitive field-effect transistor-based biosensors to detect subnanomolar concentrations of serotonin in artificial cerebrospinal fluid. Our ultimate aim is to create neurosensors that can correlate chemical signaling events in different regions of the brain with complex behaviors that are not originated from the basal levels.

POSTER #13

TRANSCRIPTOMIC AND FUNCTIONAL DIVERSITY OF SEROTONIN NEURON SUBTYPES

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Serotonergic (5HT) neurons modulate numerous behavioral, cognitive, and physiological processes and are implicated in a variety of clinical disorders. Though often viewed as a single neuron type based on neurochemical identity, phenotypic diversity within the 5HT neuron system has been observed across several parameters – such as neuropeptide expression, electrophysiology, and synaptic connectivity - suggesting the existence of specialized subtypes of 5HT neurons that differentially regulate biological functions. Given that cell phenotypes are strongly determined by cell type-specific gene regulation, global gene expression profiling, or transcriptomics, has emerged as an indispensible tool for classifying neuron subtypes and identifying the molecular underpinnings of their cellular properties. We recently combined intersectional genetic fate mapping, neuron sorting, and genome-wide RNA-Seq to deconstruct the mouse 5HT system at multiple levels of granularity—from anatomy, to genetic sublineages, to single neurons. We found that 5HT neuron transcriptomes cluster into groups defined by a combination of lineage and anatomy, and that 5HT neuron subtypes with distinct transcriptomes display corresponding differences in electrophysiological properties and neuropeptide receptivity, as well as differential involvement in behaviors. These lineage and anatomy defined subtypes represent a first tier of system organization, and we are now further refining these subclassifications through multi-scale intersectional genetic experiments, combining pairwise driver genes suggested by our expansive RNA-seq data set and performing RNA-seq, electrophysiology, and functional mapping experiments. Through these ongoing studies we continue to characterize the transcriptomic and functional diversity of 5HT neurons, and have uncovered previously undescribed 5HT neuron subtypes expressing unique combinations of co-regulated genes, showing differential responses to signaling molecules, and mapping to restricted sets of functions, such as aggression and social interest.

THE 5-HT$_{2B}$ RECEPTOR IS A POSITIVE MODULATOR OF RAPHE NEURONS.

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The serotonin system is involved in mood disorders. In humans and mice, the lack of serotonin 5-HT$_{2B}$ receptor is associated with serotonin-dependent phenotypes, including impulsivity, aggressivity and suicidality. We showed previously that 5-HT$_{2B}$ receptors are necessary for serotonin releasing effects and reinforcing properties of MDMA. We also reported that the genetic or pharmacologic blockade of 5-HT$_{2B}$ receptors eliminates behavioral and biochemical acute effects of serotonin specific reuptake inhibitors (SSRI) antidepressant, which can be mimicked by a 5-HT$_{2B}$ receptor agonist BW723C86. Blockade of 5-HT$_{2B}$ receptors strongly reduce SSRI-induced extracellular serotonin accumulation and serotonin neurons express 5-HT$_{2B}$ receptors. In this work, we tested the hypothesis that 5-HT$_{2B}$ receptors act as a direct modulator of raphe serotonergic neurons. Using a conditional genetic ablation strategy using the *Pet1-Cre* and *Htr$_{2B}$* floxed mice (*Pet1-Cre$^{+/0}$; Htr$_{2B}^{lox/lox}$*) to eliminate *Htr$_{2B}$* gene specifically and exclusively from serotonergic neurons, we first observed that MDMA behavioral and sensitizing effects are eliminated. As well, the acute behavioral and chronic neurogenic effects of fluoxetine are eliminated in these conditional mice. Electrophysiological cell-attached recordings of identified raphe serotonin neurons using slices of *Gt(ROSA)26Sor$^{tm1.1(CAG-EGFP)Fsh}$; Pet1-Cre$^{+/0}$* mice stimulated by subsaturating concentrations of phenylephrine revealed that further stimulation of 5-HT$_{2B}$ receptors by BW723C86 increases the firing frequency of 5-HT neurons. Furthermore, *in vivo* extracellular recordings of anesthetized mice revealed that *Pet1-Cre$^{+/0}$; Htr$_{2B}^{lox/lox}$* mice displayed a decrease in firing rate of serotonin neurons compared to *Pet1-Cre$^{0/0}$; Htr$_{2B}^{lox/lox}$* mice. We will discuss how 5-HT$_{2B}$ receptors may act as a direct positive modulator of serotonin neurons.

STATE-DEPENDENT PROTEIN INERACTIONS STABLIZE HYPERACTIVE CONFORMATIONS OF THE SEROTONIN TRANSPORTER

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Disrupted serotonin (5-HT) signaling is associated with multiple neurological disorders, including major depressive disorder (MDD) and autism spectrum disorder (ASD). The high-affinity 5-HT transporter (SERT) tightly regulates 5-HT clearance at the synapse. Accumulating evidence suggests that SERT is dynamically regulated in distinct activity states as a result of environmental stimuli and disease-associated coding variants. Systemic administration of lipopolysaccharide (LPS) shifts the transporter to a high-affinity state (SERT*), a transition that requires p38α MAPK signaling, and leads to enhanced 5-HT clearance. Our lab has identified an ASD-associated SERT hypermorphic variant, Ala56, that constitutively imposes the SERT* state. The SERT Ala56 model demonstrates that SERT* activity is unable to be further enhanced by p38 MAPK activation, but is normalized by pharmacological p38α MAPK inhibition. We hypothesize that changes in SERT-interacting proteins (SIPs) support the shift of SERT into the SERT* state. Definition of the complexes that induce or stabilize distinct SERT conformational states will afford greater understanding of disorders that feature perturbed 5-HT signaling. Using candidate and proteomic-based approaches, we are profiling SIPS from wildtype, SERT Ala56, and LPS-injected mice. Candidate protein analysis reveal changes in associations of previously reported SIPs, including protein phosphatase 2A and syntaxin 1a comparing SERT Ala56 and wildtype animals. Similar analyses using LPS treatments reveal changes in nitric oxide synthase (NOS) associations. Finally, proteomic analyses revealed the presence of a novel SIP, melanoma antigen E1 (MAGE-E1) that preferentially interacts with SERT Ala56 versus wildtype SERT. As MAGE-D1 associates with SERT (Mouri et al. 2012) via a MAGE homology domain (MHD) shared with MAGE-E1, we speculate that the two proteins may differentially support basal vs SERT* states. Our ongoing studies aim to determine the distinct modes of MAGE-D1 versus MAGE-E1 interactions and to determine their contributions to different activity states of SERT and linkage to neuropsychiatric disorders.

CHRONIC INHIBITION OF P38A MAPK NORMALIZES SEROTONIN CLEARANCE, SEROTONIN RECEPTOR HYPERSENSITIVITY AND SOCIAL BEHAVIOR DEFICITS IN THE SERT ALA56 GENETIC MODEL OF AUTISM SPECTRUM DISORDER

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder exhibiting language and communication deficits, perturbations in social interactions, and repetitive behaviors. No FDA approved pharmacotherapies are currently available that mitigate the core symptoms of ASD. Interestingly, hyperserotonemia, or elevated blood 5-HT levels, are a well-replicated ASD biomarker and hypermorphic variants in the *SLC6A4* gene encoding the serotonin (5-HT) transporter (SERT) have been linked to ASD. Transgenic mice expressing one of these ASD-associated SERT coding variants, Gly56Ala, exhibit hyperserotonemia, elevated *in vivo* 5-HT clearance, a hypersensitivity of 5-HT_{1A} and 5-HT_{2A} receptors and multiple ASD-like behavioral phenotypes including deficits in social interactions. Further, expression of SERT Ala56 results in the p38 MAPK-dependent hyperphosphorylation of SERT, a state linked to increased activity of the transporter. We hypothesized that treatment with a selective, CNS penetrant p38α MAPK inhibitor may attenuate one or more ASD-like phenotypes in the SERT Ala56 model. Chronic treatment with the novel p38α MAPK inhibitor MW150 (5 mg/kg, QD x 1 week) normalized SERT Ala56-mediated increases in hippocampal 5-HT clearance and *in vivo* hypersensitivity of 5-HT_{1A} and 5-HT_{2A} receptors. Furthermore, chronic MW150 treatment normalized SERT Ala56-mediated ASD-like social deficits in the tube test. Chronic treatment with a similar p38α MAPK inhibitor, MW108, paralleled effects found with MW150. These effects arose without concomitant changes in SERT protein levels or changes in tissue levels of monoamine neurotransmitters or their metabolites. Acute MW150 treatment was without effect on receptor and behavioral indices. The efficacy of these treatments in adulthood points to the continual influence of p38α MAPK in enhanced SERT activity, altered 5-HT signaling and specific ASD-like behavioral perturbations evident in SERT Ala56 mice. These studies document the pharmacologic reversal of ASD-like phenotypes via the selective inhibition of p38α MAPK signaling and warrant the further evaluation of p38α MAPK-based treatments as potential therapeutics for ASD.

CHRONIC ISOLATION STRESS ALTERS THE EXCITABILITY AND THE MODULATION OF DORSAL RAPHE SEROTONIN (5-HT) NEURONS

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The dorsal raphe nucleus (DRN) is a critical site for modulation of emotional responses. Dysfunction of serotonergic (5-hydroxytryptamine, 5-HT) neurons in the DRN has been implicated in the pathophysiology underlying anxiety disorders and depression. These 5-HT neurons are also the main targets of antidepressant action. Using a validated chronic social isolation stress paradigm followed by the whole cell electrophysiological recording in DRN slices of adult mice, we investigated how chronic social isolation stress affects the activity and the modulation of 5-HT neurons. We find that the intrinsic excitability of 5-HT neurons to optogenetic and electrophysiological stimuli is significantly reduced upon chronic social isolation, compared to those in DRN slices obtained from group-housed controls. Importantly, the firing activity of 5-HT neurons induced by their endogenous excitatory neuromodulators is also significantly decreased by chronic isolation stress. These alterations in the activity of 5-HT neurons upon chronic stress are accompanied by changes in the magnitude of their afterhyperpolarization (AHP) potentials and can be normalized by blockade of voltage-gated calcium and small conductance calcium-activated potassium (SK) channels. After calcium dynamics were examined with multiphoton imaging, further experiments probed the balance of SK2 and SK3 channels involved in regulating neuronal excitability. Here, we demonstrate that the slowing of 5-HT neurons upon chronic social isolation stress arises from a striking change in the molecular configuration of the channels mediating the AHP.

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SEROTONIN AND AFFECT REGULATION IN HUMANS: A COMBINED 5-HT1A [11C]CUMI-101 PET AND FUNCTIONAL MRI STUDY

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Background:

Acute selective serotonin reuptake inhibitors (SSRI) administration is associated with altered fear related behavioral responses (1, 2) and shown to alter amygdala reactivity to aversive emotions in a functional magnetic resonance imaging (fMRI) paradigm (3). In a previous Positron Emission Tomography (PET) study using novel 5-HT_{1A} receptor tracer [^{11}C]-CUMI-101, we showed that intravenous citalopram increased the [^{11}C]-CUMI-101 availability in the postsynaptic cortical areas in healthy volunteers (4). In this study, we aim to investigate the effect of intravenous citalopram on neural processing of aversive stimuli and its relationship with 5-HT_{1A} changes as measured with [^{11}C]-CUMI-101 in human subjects.

Methods:

A total of 13 (mean age 47.95±9.2 years) healthy male subjects received either normal saline or a citalopram infusion intravenously (10 mg over 30 minutes) on two separate occasions with a single blind, random order, and cross-over design. On each occasion, participants then underwent a fMRI face-emotion processing task (block design featured happy, sad and neutral faces known to activate the amygdala (6)). Out of 13, ten subjects had previously taken part in the citalopram/saline infusion and [^{11}C]CUMI-101 PET study and therefore have measures of brain 5-HT_{1A} availability.

Results:

Contrast estimates were extracted from left amygdala voxel that was significantly activated to faces vs baseline contrast (MNI coordinates: [x=-24 y=-4 z= -7], Z=4.31, P<0.001, SV-FWE). We found that citalopram increased amygdala response to fearful (relative to neutral) faces, at trend level (placebo mean (SD) -0.20 (0.72); citalopram mean (SD) 0.59 (1.06); t(12)=2.02p=0.06). Left amygdala response to fearful (relative to neutral) faces at this voxel (x=-24 y=-4 z= -7) was negatively correlated with citalopram induced cortical [^{11}C]CUMI-101 change (N=10; r=-0.70, r2=-0.49, P<0.03).

Conclusions:

Our preliminary findings suggests that citalopram might modulate cortical 5-HT levels (i.e. higher [^{11}C]CUMI-101 response to citalopram) leading to the measured changes in amygdala neural activity in

responses to fearful facial stimuli.

REQUIREMENT OF SERT IN PREFRONTAL CORTICAL NEURONS TO CONTROL SYNAPTIC MATURATION IN SUBCORTICAL TARGETS.

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The high affinity plasma membrane serotonin transporter (SERT) is the main molecular target of antidepressants and mediates the reuptake of serotonin from extracellular space. In the adult brain SERT is exclusively expressed by serotonin raphe neurons, however during postnatal development there is a transient expression of SERT in non-serotonin neurons in several subcortical regions where SERT plays a role in circuit refinement. Much less is known about the function of the transient expression of SERT in the developing prefrontal cortex (PFC). In mice SERT is expressed from P0 to P10 in a subpopulation of glutamatergic projection neurons of layers 5-6. Using viral tracing and genetic tools we determined that they correspond to a specific category of subcerebrally-projecting neurons. We established that the period of PFC SERT expression overlaps with the invasion of PFC axons to their targets. Using high-resolution array tomography to examine the synaptic anatomy, as well as electrophysiological recordings, we found that SERT invalidation causes a marked increase in the density of PFC synaptic innervations within target regions including the thalamus and dorsal raphe nucleus. Similar results were obtained when SERT expression was selectively invalidated in cortical neurons, indicating a role of cortical SERT in the refinement of PFC descending synaptic circuits. Transcriptome profiling showed that gene networks involved in axon and synapse development are altered in PFC neurons when SERT expression is invalidated. This study shows unique features of developing PFC descending pathways during postnatal life, giving novel insights into how developmental perturbations on serotonin transmission could alter the assembly of corticofugal circuits.

CHRONIC SSRI TREATMENT PROMOTES INHIBITORY SEROTONERGIC SIGNALING IN RAT PREFRONTAL CORTEX

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The selective serotonin reuptake inhibitor (SSRI) fluoxetine is one of the most widely prescribed drugs for treatment of depression and anxiety. While it is known that SSRIs directly increase the amount of serotonin (5-HT) in the brain by blocking 5-HT transporter proteins, little is known about how elevated levels of 5-HT contribute to the therapeutic efficacy of SSRIs. Here we present findings that demonstrate that chronic treatment with the most commonly prescribed SSRI, fluoxetine, alters 5-HT responsivity in pyramidal neurons in the rat prefrontal cortex (PFC). Previously, our lab has shown that serotonergic responses in mouse PFC are determined by axonal projection target, with neurons projecting axons across the corpus callosum to the contralateral hemisphere (COM neurons), showing robust 5-HT_{2A} receptor (2A)-mediated excitatory responses. We further showed a separate non-overlapping population of neurons projecting to the pons (CPn neurons) exclusively exhibited 5-HT_{1A} receptor (1A)-mediated inhibition. Here we measured 5-HT responses in COM and CPn neurons in the rat PFC. As found in the mouse, COM neurons in the rat PFC were excited by 5-HT via 2A activation (61%; n = 32/52), while only 6% (n = 3/52) of cells were inhibited by 5-HT. Surprisingly, rat CPn neurons were more variable in their response to 5-HT, with 68% (n = 40/59) of CPn neurons excited and 19% (n = 11/59) inhibited by 5-HT. However, chronic treatment with the SSRI fluoxetine (7 mg/kg/day) for 21 days via a subcutaneously implanted osmotic pump, shifted serotonergic responsivity of CPn neurons, such that the majority (56%; n = 44/79) of CPn neurons were *inhibited* via 1A receptor activation, while only 34% (n = 27/79) exhibited serotonergic excitation ($p < 0.01$; Fisher's Exact Test). The effects of chronic fluoxetine were selective to CPn neurons, as 5-HT responses in COM neurons from fluoxetine-treated animals remained virtually unchanged from control (61%; n = 36/59 excited and 3%; n = 2/59 inhibited). These data reveal a novel form of plasticity that exists in the rat PFC. Importantly, these data potentially serve as the substrate for the delayed therapeutic action of SSRIs.

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POSTER #22

SEROTONINERGIC SIGNALING IN A TRANSGENIC MOUSE MODEL OF HUNTINGTON'S DISEASE

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Affective disorders, such as anxiety and depression, commonly manifest in individuals with Huntington's disease (HD). Of particular note, these disorders precede the onset of motor symptoms. In HD, anxiety and depression predominantly are treated with pharmacological agents that enhance serotonin (5-HT) neurotransmission. 5-HT is not the principal mediator of these behaviors, but rather acts as a neuromodulator of the primary behavioral effects of other neurotransmitters. It is this postsynaptic modulation that is the therapeutic target of 5-HT compounds utilized for the treatment of anxiety and depression. Therefore, we hypothesize that the neuromodulatory input from the serotonergic system to target structures involved in affective control is altered in HD. In order to study whether serotonergic signaling is altered in HD, we quantified 5-HT neurotransmission using *in vivo* microdialysis in 8 week old BACHD transgenic mice to measure extracellular 5-HT efflux in the ventral hippocampus. This region has a well-documented involvement in the etiology of affective behaviors. We previously have shown that the BACHD model exhibits anxiety-like and depressive-like symptoms at 8 weeks old, and that these symptoms manifest prior to motor deficits assessed via rotarod testing. At this age, we found that 5-HT efflux is decreased in the ventral hippocampus of BACHD mice. These findings support our hypothesis that serotonergic synaptic signaling is disrupted as a result of the HD mutation, and that this pathology may be critically involved in driving the observed behavioral phenotype in BACHD mice. Given our findings, as well as evidence of altered serotonergic neurotransmission in HD patients, we are investigating whether altered reuptake or metabolic processes of the 5-HT system may explain our results. Such an approach may provide insight into the altered synaptic mechanisms of 5-HT signaling that we hypothesize to result in impaired 5-HT neurotransmission and changes in affective circuits in humans with HD.

STEROID RECEPTORS AND ACTIONS IN THE LOCUS COERULEUS OF MALE MACAQUES: REGULATION OF SEROTONIN?

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We found that serotonin-related gene expression is stimulated by androgens, but serotonin neurons lack nuclear androgen receptors (AR). This study determined whether noradrenergic (NE) neurons of the locus coeruleus (LC) mediate the stimulatory action of androgens on serotonin-related gene expression in male macaques. Male Japanese macaques (*Macaca fuscata*) were castrated for 5-7 months and then treated for 3 months with [1] placebo, [2] T (testosterone), [3] DHT (dihydrotestosterone; non-aromatizable androgen) plus ATD (steroidal aromatase inhibitor), or [4] FLUT (flutamide; androgen antagonist) plus ATD (n=5/group). The noradrenergic (NE) innervation of the raphe wasdetermined with immunolabeling of axons using an antibody to dopamine-□-hydroxylase (DBH). Immunolabeling of tyrosine hydroxylase (TH) dendrites and corticotropin releasing hormone (CRH) axons in the LC was executed. Androgen receptors (AR), estrogen receptor alpha (ER□□□ and estrogen receptor beta (ER□□□ immunostaining was accomplished. Double-label for each receptor plus TH determined co-localization. Image analysis followed, Androgens ± aromatase activity stimulated DBH axon density in the raphe (ANOVA, p=0.006), and LC dendritic TH (ANOVA, p<0.0001). There were more AR-positive neurons inT- and DHT+ATD-treated groups compared to placebo or FLUT+ATD-treated groups (ANOVA, p= 0.0014). There was no difference in the number of neurons stained for ER□□ or ER□□ between treatment groups. The CRH axon density in the LC was significantly reduced with aromatase inhibition, suggesting that CRH depends on estrogen, not androgens (ANOVA, p = 0.0023). NE neurons did not contain AR. Rather, AR-positive nuclei were found in neighboring neurons. However, greater than 80% of LC NE neurons contained ER□□ or ER□□□ Therefore, the LC NE neurons may transduce the stimulatory effect of androgens on serotonin-related gene expression. Since LC NE neurons lack AR, the androgenic stimulation of dendritic TH and axonal DBH may be indirectly mediated by other neurons. Estrogen was necessary for robust CRH innervation of the LC, which differs from female macaques.

THE ANTIDEPRESSANT-LIKE EFFECT OF KETAMINE IN SERT MUTANT MICE

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Twenty percent of adults are diagnosed with major depressive disorder and are typically treated with selective serotonin reuptake inhibitors (SSRIs). However, this type of medication takes approximately six weeks to produce a therapeutic effect. Recently, low doses of ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, have been shown to produce fast-acting and long-lasting antidepressant effects. Studies have begun to examine the antidepressant mechanism of action, yet these studies have focused on intracellular changes mediated by the NMDA and/or AMPA receptors. However, the traditional view of the biology of depression involves the need for an increase in extracellular serotonin to regulate mood. Surprisingly, there has not been much research into the effect of ketamine on serotonin uptake. One study found an increase in extracellular serotonin while another found an inhibition of serotonin uptake. Taken together, these studies show a role for serotonin in the antidepressant-like effects of ketamine, and putatively one involving the serotonin transporter (SERT). In order to further elucidate ketamine's effect, we examined the role of SERT on the antidepressant-like effects of ketamine using SERT mutant mice. SERT knockout, heterozygous, and wildtype adult mice were treated with 0.0, 3.0, 10.0, or 30.0 mg/kg ketamine and tested either 24 hours or 7 days later in the tail suspension test, a validated model for antidepressant activity. It is hypothesized that SERT knockout mice will not exhibit antidepressant activity 24 hours or 7 days after treatment with ketamine in the tail suspension test indicating a role for SERT in the antidepressant-like effect of ketamine. Results from these studies will rule in, or out, a role for SERT in the mechanism of action of ketamine's antidepressant-like effect.

A NOVEL CONDITIONAL KNOCKOUT MOUSE TO ELUCIDATE THE ROLE OF SERT (SLC6A4) IN THE SYMPATHOADRENAL STRESS RESPONSE

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Global genetic deletion ($SERT^{-/-}$ mice) or pharmacological block (SSRIs) of serotonin transporter (SERT) activity leads to an exaggerated sympathoadrenal stress response (enhanced plasma epinephrine) in rodents and humans. However, it is unclear if this reflects loss of SERT in the CNS and/or in the periphery. Adrenal chromaffin cells, the neuroendocrine component of the sympathetic nervous system, secrete epinephrine, norepinephrine, and neuropeptides to coordinate the physiological response to stressors. Chromaffin cells do not synthesize 5-HT but prominently express SERT which accumulates small amounts of 5-HT (~750 fold lower than epinephrine) in the cells. We have used carbon fiber amperometry to investigate the functional impact of SERT on stimulus-secretion coupling in isolated chromaffin cells. Our data support the idea that adrenal SERT regulates catecholamine secretion from chromaffin cells during the sympathoadrenal stress response. First, the charge of amperometric spikes (amount of transmitter released from a single vesicle) was ~35% smaller in cells from $SERT^{-/-}$ compared to wild-type mice, even though the adrenal gland catecholamine content was unaltered. This effect was recapitulated in wild-type chromaffin cells by *in vitro* (~48hrs in culture) pharmacological block of SERT with an SSRI (escitalopram). SERT also regulated the number of spikes (vesicular fusion events) by a second, distinct mechanism involving interaction with 5-HT_{1A} receptors. To further investigate SERT function in the sympathoadrenal system we generated a conditional knockout model ($SERT^{\Delta TH}$) by crossing floxed SERT ($SERT^{fl/fl}$) with tyrosine hydroxylase Cre mice. SERT expression was absent in the adrenal gland but intact in the CNS of $SERT^{\Delta TH}$ mice. Adrenal gland 5-HT content was reduced in $SERT^{\Delta TH}$ mice but catecholamine content was unaltered. Ongoing studies will exploit this novel conditional knockout to elucidate the mechanisms by which adrenal SERT and serotonergic signaling control the sympathoadrenal stress response.

STRESS ACTIVATION OF KOR TRANSLOCATES SERT TO THE PLASMA MEMBRANE IN AXON TERMINALS OF TPH2-ir NEURONS PROJECTING TO VENTRAL STRIATUM

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We have previously shown that behavioral stress exposure causes translocation of the serotonin transporter (SERT) from the endosomal compartment to the plasma membrane via a p38 MAPK-dependent mechanism. In this study, we have investigated the structural features of the transporter involved in translocation by generating a series of SERT/DAT chimeras and SERT point mutations of intracellular residues that have previously been implicated in either p38 regulation of SERT or p38 phosphorylation sites. AAV Virally-packaged constructs of SERT mutants were injected into the dorsal raphe nucleus of SERT knockout mice to determine the effect of structural changes in the transporter on expression and stress-induced translocation. Synaptosomes were isolated from the ventral striatum of these animals and surface expression was assessed by western blots of biotin labeled proteins and 5HT transport kinetics using in vitro rotating disk electrode voltammetry (RDEV).

Pharmacological activation of the kappa opioid receptor (KOR) with agonist U50,488 increases surface expression and activity of SERT in synaptosomes harvested from ventral striatum as measured by surface protein biotinylation and RDEV. We have successfully measured uptake using RDEV in other regions including the dorsal striatum, dorsal raphe, and ventral tegmental area and plan to examine the effects of U50,488 administration in those regions as well. Further, we have successfully expressed YFP-tagged hSERT and hDAT constructs as well as SERT/DAT chimeras in HEK cells and in vivo to confirm functional translocation to the membrane. These constructs will be essential in elucidating the structural features responsible for regulating stress-induced SERT translocation to the plasma membrane.

DIFFERENTIAL MODULATION OF SEROTONERGIC NEURAL ACTMTY BY MELATONIN MT1 AND MT2 RECEPTORS. AN IN-VIVO ELECTROPHYSIOLOGICAL STUDY IN MT1, MT2 AND MT1-MT2 RECEPTOR KNOCKOUT MICE

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Melatonin (MLT) is a neurohormone synthesized from serotonin (5-HT) in the pineal gland during the dark phase. MLT is implicated in several physiological processes including circadian rhythms, sleep, mood, and cardiac functioning. The effects of MLT are mainly mediated by two high-affinity G protein coupled receptors, MT1 and MT2. Our ongoing research is demonstrating that the two MLT receptors can control mood in a different and/or opposite way, but it is not yet characterized how and if MLT modulates 5-HT, one of the main neurotransmitters controlling mood. We thus examined the impact of the genetic inactivation of MLT receptors on dorsal raphe nucleus (DRN) 5-HT neurons using in-vivo electrophysiology. DRN 5-HT firing activity was recorded in wild-type (WT) controls and in MT1 receptors (MT1KO), MT2 receptors (MT2KO) and MT1-MT2 receptors (MT1-MT2KO) knockout mice during both light and dark phases. No differences between genotypes were found in the spontaneous DRN 5-HT firing activity, but the 5-HT burst-firing activity, which is related to the release of the neurotransmitter, was reduced in all three-knockouts during both light and dark phases. We also found that while in WT the there was an increase in the activity of 5-HT neurons in the high-firing subgroup during the dark phase (+52%; $p>0.001$), no phase difference was observed in all three-knockouts. In WT, the high- and low-firing subgroups during the dark phase accounted for 25% and 75% of 5-HT neurons, respectively. Interestingly, Chi-square analysis indicated that compared to WT, this distribution was altered in MT1KO (40%-60%, $p=0.035$) and in MT1/MT2KO (45%-55%, $p=0.005$) but not in MT2KO (30%-70%). These results demonstrate that MLT receptors modulate the release of 5-HT and the circadian rhythmicity of DRN 5-HT neurons, and thus confirm that MLT receptors deserve further investigation as possible targets in psychopharmacology, especially for those diseases involving mood circadian dysfunctions.

A NOVEL ROLE FOR THE SEROTONIN TRANSPORTER: CONTROLLING STIMULUS-SECRETION COUPLING IN SYMPATHOADRENAL CHROMAFFIN CELLS

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Adrenal chromaffin cells comprise the neuroendocrine arm of the sympathetic nervous system and upon stimulation release a cocktail of catecholamines and neuropeptides to mediate the physiological response to stress. Curiously, adrenal chromaffin cells prominently express the serotonin transporter (SERT) even though they do not synthesize 5-HT. It has been reported previously that SERT knockout mice ($SERT^{-/-}$) mice display an exaggerated sympathoadrenal response to restraint stress (increase in plasma adrenaline) compared to wild- type. Similarly, selective serotonin reuptake inhibitors (SSRIs) enhanced the sympathoadrenal response to hypoglycemia in rodents and humans. These data suggest that serotonergic signaling helps control the sympathetic stress response, but it remains unclear if this is due to local serotonergic signaling within the adrenal gland and/or altered SERT function in the CNS.

We show for the first time SERT and 5-HT receptors interact to control stimulus-secretion coupling in mouse adrenal chromaffin cells. 5-HT reduced the number of vesicular fusion events detected by carbon fiber amperometry (amperometric spikes). This 5-HT mediated inhibition was only apparent when SERT function was blocked pharmacologically, or in cells isolated from $SERT^{-/-}$ mice. The inhibition of secretion was blocked by the selective $5HT_{1A}$ receptor antagonist WAY100635, and recapitulated by the $5HT_{1A}$ receptor agonist 8-OH-DPAT, but not by the $5HT_{1B}$ receptor agonist CP93129. There was no effect of 5-HT or 8-OH-DPAT on voltage-gated Ca^{2+} channels, K^{+} channels, or intracellular [Ca^{2+}] handling, suggesting an atypical downstream target mediates the inhibition. Together, our data reveal a novel role for SERT, and support the idea that adrenal chromaffin cells are a previously unrecognized hub for serotonergic control of the sympathetic stress response. In ongoing work we are further testing this hypothesis by developing conditional knockout mice with selective excision of SERT in the peripheral sympathetic nervous system but not the CNS.

PHARMACOLOGIC INHIBITION OF SEROTONIN PROTECTS AGAINST THE DEVELOPMENT OF BRONCHOPULMONARY DYSPLASIA AND PULMONARY HYPERTENSION IN MICE.

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Background: The association of bronchopulmonary dysplasia (BPD) complicated by pulmonary hypertension (PH) worsens clinical outcomes in former preterm infants. We previously showed that serotonin (5-HT) signaling is increased in a neonatal murine model of BPD and PH. Whether blockade of serotonin signaling protects against the development of BPD and PH is uncertain.

Objective: Determine whether pharmacologic inhibition of the 5-HT 2A receptor with ketanserin protects against the development of BPD and PH in a neonatal bleomycin model of BPD and PH.

Methods: , Wild-type mice were injected with intraperitoneal (IP) PBS, Bleomycin (3u/kg) dissolved in PBS, or Bleomycin with Ketanserin 3 times/wk beginning on day 1-2 of life for 3wks. Mice were euthanized for tissue harvesting at 3 wks of age. To evaluate alveolar development, inflation fixed lungs were analyzed for radial alveolar counts (RAC). PH was evaluated by assessing right ventricular hypertrophy (RVH). To identify total and muscularized small vessels (<30 microns) lung sections were immunostained with Factor VIII or □-SMA. Total or % muscularized vessels were expressed per high-powered field under 20x magnification. Lung protein from 3 week old mice was analyzed by Western blot for Tph1, 5-HT_{2A} R. Data were analyzed by 1-way ANOVA with Bonferroni post-hoc analysis using Prism. Significance defined as $P<0.05$.

Results: Bleomycin increased lung protein expression of Tph1 (0.39 +/-0.03 – 0.94 +/-0.12, $P<0.005$) and 5-HT_{2A} R (1.01+/- 0.14 – 1.98 +/- 0.27, $P< 0.01$). Ketanserin protects against bleomycin-induced disruption in alveolar development. Ketanserin protects against the development of RVH (Fulton index: PBS 0.28 +/-0.017, Bleomycin 0.36+/-0.022, Bleomycin/Ketanserin 0.29 +/- 0.01, $p <0.05$). Bleomycin induced pulmonary vascular remodeling in prevented with ketanserin.

Conclusion: Treatment of neonatal mice with ketanserin protects against bleomycin-induced BPD and PH. These findings suggest that 5-HT contributes to alveolar simplification and pulmonary vascular disease.

EFFECTS OF A 5-HT$_{1B}$ RECEPTOR AGONIST ON LOCOMOTION AND REINSTATEMENT OF COCAINE-CONDITIONED PLACE PREFERENCE AFTER ABSTINENCE FROM REPEATED INJECTIONS IN MICE

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We previously reported that the 5-HT$_{1B}$ receptor (5-HT$_{1B}$R) agonist CP94253 enhances cocaine self-administration and seeking in rats tested during daily self-administration sessions, but inhibits these behaviors when tested after a period of forced abstinence. This study investigated whether a similar pattern of effects occurs in mice given 21 daily cocaine (15 mg/kg, IP) injections followed by testing for effects of CP94253 on locomotion and reinstatement of cocaine conditioned place preference (CPP) either 1 or 21 days after the last injection. In the CPP experiment, mice underwent conditioning procedures while receiving their daily injections of cocaine or saline either during or ≥2 h after CPP procedures. The procedural timeline consisted of baseline preference testing (days 12-13 of the chronic regimen), conditioning (days 14-19, 2 daily 30-min sessions separated by 5 h), CPP test (day 21), extinction (days 22-39), CPP extinction test (day 40), and reinstatement test (day 41). On test day, mice were pretreated with either saline or CP94253 (10 mg/kg, IP) and 30 min later they were primed with either saline or cocaine (15 mg/kg, IP) and then immediately tested. We found that CP94253 initially increased locomotion in mice receiving repeated administrations of either saline or cocaine, but after the 21-day abstinence period from the repeated injections, CP94253 had no effect on spontaneous locomotion but did attenuate expression of cocaine sensitized locomotion. In noninjected, drug-naïve mice, CP94253 had no effect on locomotion. Mice reinstated cocaine-CPP when given a cocaine prime and showed a trend toward CP94253 attenuation of reinstatement. We are adding n/condition to further examine this trend. The findings suggest that CP94253 attenuates effects of cocaine after a period of abstinence from a chronic administration regimen, consistent with its effects in rats. This study supports the idea that 5-HT$_{1B}$R agonists may be useful for treating cocaine dependence.

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DISRUPTED CAV1.2 L-TYPE CALCIUM CHANNEL FUNCTION AND EXPRESSION ALTERS BEHAVIOR AND ASCENDING SEROTONIN SYSTEM ACTIVITY

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Human genetic variation in the gene *CACNA1C* altering expression of the Cav1.2 L-type calcium channel (LTCC) has been strongly associated with enhanced risk for the development of neuropsychiatric disorders including major depression, bipolar and schizophrenia. Furthermore, a missense mutation (G406R) of the Cav1.2 LTCC that results in reduced channel inactivation associated with Timothy Syndrome has been modeled in the TS2-neo mouse model of autism spectrum disorders (ASD). Here, we ask whether alterations in Cav1.2 LTCC function and expression influence a common neural circuit in the ascending serotonin (5HT) system. First, we assessed 5HT system abnormalities in the TS2-neo mouse. Following an acute stressor (forced-swim), behavioral analyses and immunofluorescent co-labeling of Tph2 and C-Fos reveals that TS2-neo mice exhibit enhanced active coping behavior, enhanced 5HT neuron activity, and altered 5HT1A-dependent feedback inhibition in both caudal and rostral subregions of the dorsal raphe nucleus (DRN). These alterations are accompanied by enhanced 5HT and 5HIAA content in forebrain regions including the orbitofrontal cortex, dorsal striatum and dorsal hippocampus. Next, we sought to determine whether temporally controlled knock-out of 5HT neuron Cav1.2 LTCCs also produces behavioral alteration and changes in 5HT neuron activity. To this end, we crossed Tph2-icre/ERT2 mice with Cav1.2-loxP/Ai14-TdTomato mice (Tph2-Cav1.2KO). Following tamoxifen treatment, preliminary results suggest that Tph2-Cav1.2KO mice display altered behavior on the forced-swim and open-field paradigms. Additional behavioral characterization and analysis of 5HT neuron activity within the DRN is ongoing. Collectively, our results suggest that disruptions of Cav1.2 LTCC function in the TS2-neo mouse and Cav1.2 LTCC expression within 5HT neurons may alter a common neural circuit in the ascending 5HT system. This provides a potential neurological mechanism through which alterations in the gene *CACNA1C* may enhance risk for development of a broad range of psychiatric and developmental disorders.

POSTSYNAPTIC 5-HT$_{1A}$ RECEPTORS AND REGULATION OF BODY TEMPERATURE IN MICE.

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5-HT$_{1A}$ receptors are generally involved in the control of body temperature. Activating 5-HT$_{1A}$ receptors by 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) leads to reduced body temperature. Unlike rats and humans, it is still a matter of debate whether the 5-HT$_{1A}$ receptor-mediated regulation also takes place on postsynaptic level in mice. In our group, within phenotyping a transgenic mouse line permanently overexpressing the 5-HT$_{1A}$ receptor in serotonergic projection areas (OE mice), Bert et al. (2008, PMID: 18396339) revealed exaggerated 8-OH-DPAT-provoked hypothermic response. Thus, the present study aimed at assigning clear thermoregulatory function to the postsynaptic 5-HT$_{1A}$ receptor in mice.

We used radio telemetry technique to monitor basal body temperature and hypothermic effects of 8-OH-DPAT (0.1 mg/kg – 4 mg/kg i. p.) in male OE mice in comparison to NMRI wild-type (WT) males. Additionally, we investigated whether reduction of serotonergic activity by pretreatment with the 5-HT synthesis inhibitor para-chlorophenylalanine (PCPA; 100 mg/kg, i. p. on four consecutive days) would alter the effects of 8-OH-DPAT on body temperature.

OE mice revealed lower levels of basal body temperature (36.15 °C) than wild types (37.18 °C). In both genotypes, systemic administration of 8-OH-DPAT dose-dependently decreased body temperature, being significantly more pronounced in mutant mice (-2.55 °C compared to -1.4 °C in WT mice). Dose-response curves of 8-OH-DPAT revealed ED_{50} = 0.77 mg/kg in OE and ED_{50} = 1.16 mg/kg in WT mice. PCPA pretreatment did not alter the hypothermic response to 8-OH-DPAT in mice.

The dose-response curves indicate a higher potency of 8-OH-DPAT in OE mice. The exaggerated hypothermic response to 8-OH-DPAT in mutant mice implies that postsynaptic 5-HT$_{1A}$ receptors could be involved in thermoregulatory function in mice. This assumption is further confirmed by the fact that 8-OH-DPAT-evoked thermal responses were not influenced by pretreatment with PCPA, most notably in transgenic mice overexpressing 5-HT$_{1A}$ receptors postsynaptically.

THE 5-HT$_{1B}$ RECEPTOR AGONIST, CP 94,253, ATTENUATES THE REINFORCING AND MOTIVATIONAL EFFECTS OF METHAMPHETAMINE.

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The selective 5-HT$_{1B}$ receptor (5-HT$_{1B}$R) agonist, CP 94,253, enhances cocaine self-administration (SA) during initial training but attenuates SA when resumed after 3 weeks of forced abstinence. Here we examined if CP 94,253 produces a similar abstinence-dependent effect on methamphetamine SA. Male rats were tested for the effects of CP 94,253 on methamphetamine reinforcement rates on both low (VR and FR 5) and high (progressive) effort ratio schedules. We found the typical inverted U-shaped methamphetamine dose-response function when rats were pretreated with vehicle on both low ratio schedules. Prior to abstinence CP 94,253 produced a downward shift of the descending limb of the DR function. Post-abstinence, CP 94,253 produced a downward shift of the entire DR function. Similar results were found for CP 94,253 effects on the progressive ratio schedule where reinforcers obtained and break-points were attenuated after agonist pretreatment compared to vehicle pretreatment regardless of whether the rats went through forced abstinence. Importantly, administration of SB 224,289, a selective 5-HT$_{1B}$ receptor antagonist, blocked the attenuating effects of CP 94,253 on methamphetamine SA at doses that had no effect on locomotor activity. Thus, unlike the abstinence-dependent modulatory effect of CP 94,253 on cocaine SA, this study found that CP 94,253 had an inhibitory effect on methamphetamine SA both pre- and post-abstinence. The results suggest that 5-HT$_{1B}$R agonists may differentially modulate cocaine and methamphetamine SA initially, but that after a period of abstinence, the agonist inhibits the reinforcing effects of both psychostimulants. These findings suggest that 5-HT$_{1B}$R agonists may have clinical efficacy as treatments for psychostimulant use disorders.

ANOREXIA INCREASES SENSITIVITY TO COCAINE-INDUCED LOCOMOTION: EVIDENCE FOR DYSREGULATION OF SEROTONIN AND DOPAMINE TRANSPORTERS IN EATING DISORDERS

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Eating disorders, including anorexia, are major public health concerns, compounded by a lack of effective treatments. These complex disorders are most common in females and often manifest during adolescence. Although their etiology remains unclear, dysregulation of serotonin (5-HT) and dopamine (DA) neurotransmission is a consistent finding. The 5-HT and DA transporters (SERT and DAT, respectively) terminate 5-HT and DA neurotransmission by high-affinity uptake of these neurotransmitters into neurons and because of this are primary regulators of the strength and duration of 5-HT and DA signaling. Given their critical function, together with strong evidence supporting dysregulation of 5-HT and DA neurotransmission in eating disorders, it is likely that function of these transporters is aberrant in individuals with these illnesses. Surprisingly, however, few studies have investigated SERT and DAT activity in eating disorders. To this end, we used the "activity-based anorexia" (ABA) model in rats to investigate a role for the cocaine-sensitive SERT and DAT in anorexia. This model recapitulates key characteristics of anorexia, including hyperactivity and reduced food intake. As an index of SERT and DAT function *in vivo*, we examined cocaine-induced locomotion. We found that "anorexic" adolescent female rats were more sensitive to cocaine than control rats as evidenced by a leftward shift in the ascending and descending limbs of the dose-response curve. Moreover, ABA females were more sensitive to the effects of cocaine on locomotion than ABA males. Preliminary studies using high-speed chronoamperometry to measure transporter function *in vivo*, suggest that DAT activity is increased as a result of ABA, but that this effect is restricted to female rats. Ongoing studies are investigating SERT activity *in vivo*. Together, these data support the hypothesis that during adolescence, SERT and/or DAT function is especially sensitive to reduced food intake and hyperactivity, which may contribute to the development and progression of anorexia.

STRIATAL 5-HT$_6$ RECEPTORS REGULATE COCAINE REINFORCEMENT IN A PATHWAY-SELECTIVE MANNER

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Drug addiction is a disease that affects millions of people throughout the world and contributes heavily to healthcare costs. The nucleus accumbens (NAc) in the ventral striatum plays a vital role in addiction, particularly in motivation for drug and reward-seeking behavior. Serotonin (5-HT) neurotransmission has also been implicated in addiction, and 5-HT$_6$ receptors are strongly expressed in the neurons that constitute the direct and indirect pathways, the two main outputs from NAc. While there is evidence linking these receptors to drug reward, the exact mechanism by which they influence drug-associated behavior is unknown. We hypothesized that 5-HT$_6$ receptors function in a pathway specific manner to influence cocaine self-administration by regulating the reinforcing properties of the drug.

We used viral vectors that target the direct or indirect pathway neurons selectively to increase expression of 5-HT$_6$ receptors or GFP in rat NAc. The rats then underwent cocaine self-administration using fixed ratio, progressive ratio, and dose-response operant reinforcement sessions. The number of cocaine infusions per session was taken as a measurement of reinforcement by and motivation for cocaine; parameters that are closely related to the addictive properties of drugs. Rats with increased 5-HT$_6$ receptor expression in direct pathway neurons showed no differences when compared to GFP controls; however, rats with increased 5-HT$_6$ receptor expression in indirect pathway neurons self-administered significantly less cocaine than control rats during fixed ratio sessions at medium and low doses, but not progressive ratio sessions. These rats also demonstrated longer times to initial cocaine infusion, titrated around lower estimated brain concentrations of cocaine, and developed a conditioned place preference for cocaine at a low dose that did not support CPP in control rats. We conclude that 5-HT$_6$ receptors in indirect but not direct pathway neurons increase the sensitivity to the rewarding properties of cocaine, particularly at low doses, while leaving motivation for the drug unaffected.

POSTER #36

PROMOTING SEROTONERGIC NEUROTRANSMISSION TO ENHANCE SOCIABILITY

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Impaired social interaction is the most prominent and drug treatment-resistent of core autism symptoms. Clinical findings and rodent studies demonstrate serotonin transmission is often disrupted in the socially-deficient brain. We have employed three-chamber sociability preference tests for over five years and have made several discoveries cosistent with key clinical findings. For example, we saw that pharmacological depletion by para-chlorophenylalanine (PCPA) or dietary depletion of the 5-HT precursor tryptophan (TRP) impairs social interactions in mice, just like it worsened behavioral symptoms of autism in a clinical study, while TRP supplementation improved sociability in some mouse models. Also we found that drugs such as buspirone, pargyline and vortioxetine that mimic some postsynaptic effects of serotonin are able to enhance murine social behavior within a limited dose range or time frame. We and others have published our discovery that the selective serotonin reuptake inhibitor (SSRIs) Prozac (fluoxetine) enhances sociability in black and tan brachyury tufted BTBRT+tf/J (BTBR) mice. Unfortunately, fluoxetine only does so for limited subpopulations of patients with autism. This could be because SSRI efficacy is diminished if 5-HT transporter (SERT) function is compromised by common or rare gene polymorphisms. Aside from SERT, auxilliary transporters of 5-HT in the brain include organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT) collectively known as “uptake 2”. Uptake 2 transporters remove serotonin from extracellular fluid with greater capacity but lower affinity than SERT. Our hypothesis was that if uptake 2 is blocked, impaired sociability may improve in a broader population of individuals with autism than presently benefit from SSRI treatment. This hypothesis was tested in two socially impaired mouse models, the BTBR strain and SERT knock out mice. We found that blockade of uptake 2 transporters by systemically-administered decynium-22 promoted social behavior in these mice, with either acute or chronic treatment.

Biography

Georgianna earned her PhD in Biology in 2001 from Syracuse University, and completed postdoctoral studies in pharmacology at The University of Texas Health Science Center at San Antonio (UTHSCSA) under the mentorship of Dr. Alan Frazer in 2007. She was an assistant professor of physiology at William Paterson University from 2007-2008. Then she joined the research faculty at UTHSCSA in 2008 to collaborate with Dr. Lyn Daws on novel drug treatments for social behavior impairments in autism. She has published more than 40 peer reviewed articles and book chapters. She involves high school and undergraduate students in her research.

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RECIPROCAL CONTROL OF CORTICOFUGAL OUTPUT BY SEROTONIN AND ACETYLCHOLINE

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Pyramidal neurons in the mouse prefrontal cortex comprise two broad subclasses of neurons defined by their long-distance axonal projections: commissural/callosal (COM) neurons, and corticopontine (CPn) neurons. By projecting to different cortical and subcortical targets, these two populations represent distinct cortical output channels that play specific roles in decision making and behavior. In addition to having distinct morphological and physiological properties, growing evidence indicates that COM and CPn neurons are differentially regulated by modulatory neurotransmitters, including serotonin (5-HT) and acetylcholine (ACh).

We have shown that COM and CPn neurons in the mouse medial prefrontal cortex (mPFC) exhibit different responses to transient exposure to 5-HT. While CPn neurons are universally inhibited via $G_{i/o}$-coupled 5-HT_{1A} (1A) receptors, COM neurons are excited via G_q-coupled 5-HT_{2A} (2A) receptors. Although both neuron subpopulations express G_q-coupled M1 muscarinic ACh receptors, we report that endogenous ACh selectively enhances the excitability of CPn neurons. This suggests that 5-HT and ACh exert opposing influences on corticofugal output to the brainstem. Consistent with this hypothesis, in cortical tissue from mice expressing channelrhodopsin-2 in cholinergic neurons, single flashes of blue light selectively and persistently increased action potential output in CPn neurons (for up to 60 s), and this output was halted by subsequent serotonergic signaling at 1A receptors. To test whether 5-HT and ACh differentially affect synaptic transmission in cortical circuits, we are using optogenetic approaches to selectively activate COM afferents onto COM and CPn target neurons. Preliminary results suggest 5-HT may preferentially suppresses glutamate release at COM synapses on CPn neurons. We are continuing to explore the selectivity of cholinergic and serotonergic regulation of synaptic transmission in cortical circuits.

Our findings suggest that 5-HT and ACh may exert reciprocal control over corticofugal output to the brainstem, and suggest a circuit-based mechanism by which these transmitters may differentially contribute to behavior.

FUNCTIONAL SIGNIFICANCE OF A TRUNCATED SEROTONIN 2C RECEPTOR

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Pre-mRNA transcripts encoding the 2C subtype of serotonin receptor ($5HT_{2C}$) can be differentially edited by RNA-specific adenosine deaminases at five positions. 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, highly edited $5HT_{2C}$ isoforms (e.g. $5HT_{2C\text{-}VSV}$ and $5HT_{2C\text{-}VGV}$) exhibit markedly reduced constitutive activity and altered subcellular localization in comparison to the genomically-encoded isoform ($5HT_{2C\text{-}INI}$). Consequently, RNA editing of the $5HT_{2C}$ receptor has been proposed as a mechanism to dynamically regulate the tonicity of discrete serotonergic circuits. $5HT_{2C}$ transcripts are also alternatively spliced to generate at least two additional splice variants that encode truncated receptors. Both of the truncated isoforms of the receptor have been shown to lack serotonergic ligand binding and phosphoinositide (PI) hydrolysis activity. One of these truncated splice variants ($5HT_{2C\text{-}TR}$) is a highly abundant transcript, accounting for 30-60% of total $5HT_{2C}$ mRNAs in discrete brain regions. Recent studies using heterologous expression systems have shown that expression of $5HT_{2C\text{-}TR}$ decreases specific radioligand binding to the full-length $5HT_{2C}$ receptor ($5HT_{2C\text{-}FL}$) via sequestration of heterodimers in the endoplasmic reticulum, a phenotype that has been further validated through confocal imagining of GFP/YFP-tagged fusion receptors. Our lab has also confirmed that co-expression of various edited $5HT_{2C\text{-}FL}$ isoforms with $5HT_{2C\text{-}TR}$ in heterologous cells causes a decrease in basal and agonist-stimulated PI hydrolysis, thus demonstrating a physiological consequence for this ER retention phenotype. Many *in vitro* studies have shown a similar dominant-negative function for highly truncated GPCRs; however, these findings have not been validated *in vivo*. To this end, we have developed and are currently characterizing an inducible mouse model that will enable overexpression of $5HT_{2C\text{-}TR}$. Our findings may identify the $5HT_{2C\text{-}FI}$/$5HT_{2C\text{-}TR}$ heterodimer as a novel pharmacological target.

SEROTONIN PRODUCTION IN PANCREATIC ISLET OF OLD FEMALE MOUSE AND HUMAN

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It has been known that serotonin is present in β cell and plays several physiological roles. In adulthood, a few amount of serotonin is in β cell and regulates insulin secretion. In gestational period, an excessive amount of serotonin is produced and increases β cell proliferation and insulin secretion. However, the presence of serotonin in pancreatic β cell has been confirmed by immunofluorescence staining only in pregnant mice. Here, we report serotonin synthesis up to the range of detection by immunofluorescence staining in pancreatic islets of old female mice and humans. We conducted an immunofluorescence staining against serotonin on islets of mice aged from 24 weeks to 104 weeks and observed serotonin positive cells in pancreatic islets of old female mice aged over 52 weeks. However, serotonin positive cells were not observed in pancreatic islets of male mice. We also observed serotonin positive cell in pancreatic islets of old female human. Further study is needed to find out whether serotonin is produced in islets of young female mice mimicking old female physiology consisting of ovariectomy-induced-menopause state. Survey on progeria mice model might also be helpful.

EFFECT OF FLUOXETINE AND KETAMINE ON STRESS-RELATED BEHAVIOUR AND HIPPOCAMPAL NEUROGENESIS IN A RAT NEURODEVELOPMENTAL MODEL FOR DEPRESSION

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Social adversity and early-life stress are risk factors for depression and schizophrenia. Post-weaning social isolation of rats produces lasting behavioural and neurochemical alterations relevant to both, and antipsychotics reverse some components. Since few studies have investigated antidepressant sensitivity, we examined stress-related behaviours, cognition and hippocampal neurogenesis following chronic fluoxetine or repeated ketamine.

Two cohorts of male Lister-hooded rats were housed individually or 3 per group from weaning (PND 21). Half of cohort 1 received fluoxetine (10mg/kg/day via drinking water) from PND 42. From PND 54 open field exploration, locomotor activity and novel object discrimination (NOD), elevated plus maze (EPM) exploration, conditioned freezing responses (CFR) and restraint stress-induced hyperthermia (SIH) were assessed at one-week intervals; cohort 2 received vehicle or ketamine (5mg/kg i.p.) 24h prior to each. Plasma was collected after SIH for corticosterone analysis and brains fixed for doublecortin (neurogenesis marker) immunohistochemistry in the dentate gyrus.

Only subtle alterations in stress-induced behaviour occurred; in the open field isolation and fluoxetine increased distance, velocity and central area time while ketamine did little, and isolates showed few changes on the EPM or in SIH/corticosterone, neither of which were altered by either compound. Cognitive changes were more noticeable; isolation-induced NOD deficits were reversed by fluoxetine ($P<0.01$) and just failed to reach significance with ketamine, but both compounds reversed isolation-induced CFR deficits 24 and 48h post-conditioning. There were main effects of housing ($P<0.001$) and fluoxetine ($P<0.01$), but not ketamine, on doublecortin-positive cell counts, which fluoxetine increased under both housing conditions.

Post-weaning social isolation is a useful neurodevelopmental model to examine pathophysiological and therapeutic mechanisms underlying schizophrenia, but may not replicate stress-related alterations relevant to depression. Differential effects of fluoxetine and ketamine on neurogenesis suggest this process alone does not explain pro-cognitive effects that may be relevant to their antidepressant activity.

SELECTIVE 5-HT7 RECEPTOR ANTAGONIST EFFECT ON REVERSAL LEARNING IN THE RAT ATTENTIONAL SET SHIFTING TEST

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Previous work has demonstrated that serotonin 7 receptor (5-HT7) antagonism ameliorates impairments in selective cognitive domains in animal models of schizophrenia induced by NMDA receptor blockade, particularly in novel object recognition and attentional set shifting tests (ASST), but not in prepulse inhibition. Rodent ASST allows for the identification of several types of cognitive rigidity, such as failure on reversals (when the +/- cues are switched), and intradimensional (ID; when a new pair of cues of the same dimension is introduced) and extradimensional set-shifting (ED; when the rat has to switch between cue dimensions). Importantly, failure on the reversals versus on the ID/ED shift were shown to develop after lesioning, respectively, the medial (mPFC) and orbital (OFC) regions of the prefrontal cortex. Since 5-HT7 receptors show stronger OFC expression compared with mPFC, we hypothesized that it will differentially affect these types of cognitive rigidity. Sprague-Dawley rats were tested in the ASST, several times each, after subcutaneous administration of saline, MK-801 (NMDAR antagonist, 0.2 mg/kg), SB-269970 (5-HT7R antagonist, 1 mg/kg), and co-administration of the two compounds. SB-269970 was administered following two protocols: 1) shortly before MK-801 or 2) shortly before MK-801 and ~2 hrs later (i.e. immediately before starting the ASST). We found that MK-801 impaired performance on all discrimination phases of ASST whereas administration of SB-269970 alone had no effect. When co-administered with MK-801 however, SB-269970 reversed the effect of NMDA receptor blockade selectively on the reversal phases of ASST. When an additional dose of the 5-HT7 antagonist was given ~2 hrs after MK-801 injection (i.e. at the start of ASST) it also restored performance on the ED shift. We conclude that 5-HT7 receptor mechanisms may provide a specific contribution to the complex of cognitive deficits in psychiatric diseases, including schizophrenia and anorexia nervosa, which express different forms of cognitive inflexibility.

DISTINCT SIGNALING CASCADES AND RECEPTOR LOCALIZATION UNDERLIE 5-HT$_6$ RECEPTOR REGULATION OF NEURONAL MORPHOLOGY.

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5-HT$_6$ serotonin receptors have been identified as targets for a variety of cognitive phenotypes and processes, including anxiety, depression, habitual behaviors and reward learning. Interestingly, endogenous expression of 5-HT$_6$ receptors is restricted almost exclusively to the primary neuronal cilia of the neurons, a small sensory organelle stemming from the cell body of the neuron that is thought to act like an antenna to receive extracellular signals from other cells and the surrounding environment. Primary neuronal cilia have become therapeutic targets, as they play a critical role in a many disorders, including Huntington's and Alzheimer's disease, making the study of 5-HT6 signaling through primary cilia an intriguing line of investigation. 5-HT$_6$ receptor is known to stimulate two distinct signaling pathways, the first of which is the canonical cAMP-dependent signaling cascade, and the other a non-canonical Fyn kinase/Cdk5 signaling pathway. Recently, 5-HT$_6$ receptor antagonism and inhibition has been shown to decrease cilia length in primary neuronal cultures, and that overexpression of exogenous 5-HT$_6$ receptor overexpression increases receptor targeting outside of the primary cilia compartment. Additionally, exogenous overexpression to a level that the receptor is localized throughout the entire cells surface stimulates neurite outgrowth in NG108-15 cells and immature primary neurons by activating Cdk5-dependent activation of CDC42. In the present study we explore the role of 5-HT$_6$R expression, when primarily restricted to cilia, on signaling in mature neuronal cultures from wild-type and 5-HT$_6$R-KO mice and compare the morphology changes that occur during aberrant receptor targeting. Additionally, we compare the effects of 5-HT$_6$R mutants that are defective in either canonical (cAMP-dependent pathway) or non-canonical (FynKinase/Cdk5 pathway) signaling on dendritic morphology and primary cilia length.

MECHANISMS CONTRIBUTING TO LACK OF ANTIDEPRESSANT EFFICACY IN JUVENILE AND ADOLESCENTS.

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Depression is a major health problem for which most patients are not effectively treated. This problem is compounded in children where only two antidepressants are clinically approved. Both are selective serotonin (5-HT) reuptake inhibitors (SSRIs), which are often less efficacious in young populations compared to adults. We found both the antidepressant-like effect of escitalopram, a common SSRI, and hippocampal expression of the 5-HT transporter (SERT), the target protein of SSRIs, were reduced in juvenile mice compared to adults. Increased extracellular 5-HT is thought to trigger downstream events for SSRI therapeutic response. Lower SERT expression in young mice may limit the ability of SSRIs to increase 5-HT; however, ancillary 5-HT transports, i.e. the organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT), can also limit SSRIs effects. Using [^{3}H]decynium-22 (D22), a blocker of OCTs and PMAT, we found greater [^{3}H]D22 binding in juvenile mice hippocampus compared to adults. Further, [^{3}H]D22 hippocampal binding was lower in mice with genetically ablated OCT3, but not in mice with genetically ablated PMAT, compared to controls. Our data raise the possibility that transporters capable of 5-HT uptake other than SERT, tentatively OCT3, may be preventing extracellular 5-HT from climbing to therapeutically relevant levels after SSRI treatment. To that end, we found D22 (0.1 mg/kg) produced antidepressant-like effects in juvenile mice and enhance the antidepressant-like effect of escitalopram in adolescent and adult mice. *In vivo* chronoamperometry, a technique which measures 5-HT clearance in brain, is being utilized to determine whether the antidepressant-like effects of D22 are related to its ability to inhibit 5-HT clearance. These data suggest the limited therapeutic efficacy of SSRIs in juveniles could be due to greater expression/activity of D22 sensitive transporters relative to SERT. D22 sensitive transporters are promising targets for the development of antidepressants with improved therapeutic efficacy.

EFFECT OF ACTIVATION OF $G_{i/o}$-COUPLED SIGNALING IN LATERAL HABENULA NEURONS PROJECTING TO THE DORSAL RAPHE ON BEHAVIORAL RESPONSES IN THE FORCED SWIM TEST

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The lateral habenula (LHb), part of the habenular complex in the dorsal diencephalon has recently been implicated as a potential target for anti-depressant therapy. We have previously reported that activation of $G_{i/o}$-coupled DREADD's (hM_4D_i) in LHb neurons produces antidepressant-like effects in the rat forced swim test. Anatomically, the LHb projects to the dorsal raphe and ventral tegmental area among other brain regions. However, the precise contribution of these projections to pro/anti-depressant effects of modulating LHb neuronal activity remains to be determined. Here, we examined the role of LHb output projections to the dorsal raphe in behavioral responses in the forced swim test. Firstly, an anterograde tracer Tamra-Lys-Dextran, injected into rat LHb neurons was efficiently transported anterogradely to the caudal raphe nucleus. Secondly, fluorospheres injected into the caudal raphe were transported retrogradely to the LHb. Finally, rats were injected with a combination of floxed, inverted hM_4D_i into LHb neurons and a canine adenovirus 2 (CAV-2) engineered to express Cre recombinase into the caudal raphe. CAV-2 efficiently infected raphe axon terminals and was retrogradely transported to the LHb, resulting in the expression of hM_4D_i receptors exclusively in LHb neurons projecting to the caudal raphe. Approximately fifteen days after viral infusions a modified Porsolt forced swim test was performed. Preliminary data indicate that rats injected with clozapine-N-oxide (3mg/kg) demonstrate an increase in swim time and a decrease in immobility time, suggesting an antidepressant-like effect of activation of $G_{i/o}$-coupled signaling in LHb neurons projecting to the caudal raphe nucleus.

THE ROLE OF THE SEROTONIN 1B RECEPTOR IN THE MODULATION OF IMPULSIVE BEHAVIOR

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Impulsive behavior is modulated by serotonin but the mechanisms through which these effects occur are largely unknown. Given that polymorphisms in the serotonin 1B receptor (5-HT_{1B}R) are associated with impulsive behavior, and 5-HT_{1B}R knockout mice display increased impulsivity, we generated a transgenic mouse line (floxed tetO-htr1b) which allows for inducible and tissue-specific knockout of 5-HT_{1B}Rs. Using this model, we have begun dissecting the circuits through which 5-HT_{1B}Rs modulate impulsive behavior.

The absence of 5-HT_{1B}R expression increased impulsivity in tests of behavioral inhibition - the differential reinforcement of low-rate responding (DRL) and Go/No-Go operant paradigms. This impulsive behavior this was reversed with adult rescue of the receptor, suggesting an adult mechanism of action. Tissue-specific knockouts interestingly revealed that an absence of 5-HT_{1B} autoreceptors did not significantly alter impulsivity in these tasks. However selective knockout of 5-HT_{1B}Rs from GABAergic cells throughout the brain was sufficient to recapitulate the impulsive phenotype. These results suggest that 5-HT_{1B}Rs affect impulsivity through modulation of inhibitory tone, rather than directly through alterations in serotonin levels.

Furthermore, we explored the extent to which 5-HT_{1B}R signaling influences different dimensions of impulsive behavior beyond behavioral inhibition or impulsive action. Specifically, we assessed impulsive choice, another component of impulsive behavior characterized by intolerance to delay and increased risk-taking. Using delayed discounting and probabilistic discounting tasks, we found no effect of 5-HT_{1B}R on impulsivity in these paradigms. Further analysis using an exploratory factor analysis revealed a good-fitting two-factor model to describe the behavioral data. The latent factors represented impulsive action and impulsive choice tasks as independent components, with 5-HT_{1B}R expression and sex as significant covariates.

Overall, our results point to a role for 5-HT_{1B}R modulation of GABAergic signaling in the regulation of impulsivity, specifically affecting impulsive action rather than impulsive choice.

PHARMACOLOGICAL ASSESSMENT OF SEROTONIN METABOLISM IN MOUSE AND HUMAN HEART

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Serotonin (5-HT) is generated from tryptophan by tryptophan hydroxylase (generating 5-hydroxytryptophan: 5-HTP) and 5-HTP is subsequently decarboxylated to 5-HT. These enzymatic steps are believed to occur in non cardiomyoctes and that 5-HT reaches the heart via the blood. However, we hypothesized that 5-HT might be produced by heart cells within the heart. Indeed, 5-HTP (1-100 µM) exerted a concentration-dependent positive inotropic effect (PIE) and positive chronotropic effect (PCE) in isolated left and right atrial preparations of transgenic mice overexpressing the human 5-HT_4-receptor (TG). 5-HTP was at least 30 fold less potent than 5-HT with regard to the PIE and PCE. Intraperitoneal injection of 100 µl of 10 mM 5-HTP in TG but not in WT increased ejection fraction and heart rate (both measured via echocardiography). In isolated electrically stimulated right atrial strips obtained from patients (n=11) undergoing cardiac surgery, 100 µM 5-HTP exerted a PIE within 30 min (time to pleateau) by about 60 %. This PIE was accompanied by a shortening of time to peak tension (=lusitropic effect). In addition, this positive inotropic and lusitropic effects of 5-HTP were completely antagonized by 10 nM GR125487, a $5HT_4$-receptor antagonist. Interestingly, 100 µM of NSD 1015, an inhibitor of decarboxylation, exerted a PIE of about 25 % in isolated human atrium. This PIE could be washed out and reappeared after a second application of NSD 1015. 100 µM 5-HTP applied in the presence of NSD 1015 was ineffective to increase force of contraction in isolated human atrium. We conclude that 5-HT in amounts sufficient to raise force of contraction can be formed in the mouse and human heart from 5-HTP, probably not only in mast cells but also in cardiomyocytes. The clinical relevance of 5-HT produced in the human heart needs to be elucidated.

THE POTENTIAL IMPACT OF THE POLYMORPHIC SEROTONIN TRANSPORTER GENE *SLC6A4* PROMOTER P2 ON IRRITABLE BOWEL SYNDROME

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The serotonin reuptake transporter (SERT) gene *SLC6A4* represents one of the most promising candidates involved in the aetiology of irritable bowel syndrome (IBS). SERT is responsible for the reuptake of serotonin from the synaptic cleft into the presynaptic neuron and the interstitial space into enterocytes. To date, two distinct promoters of *SLC6A4* have been described, P1 and P2. The short allele of the promoter P1 polymorphism 5-HTTLPR has been found to be associated with IBS in various studies. In addition, its association with depression and anxiety is well established supporting the biopsychosocial model of IBS. , For unravelling the role of P2 driving expression predominantly in the gut in IBS development, we performed sequencing analysis in a discovery sample from the UK. This revealed several single nucleotide polymorphisms (SNPs) to be associated with IBS. Interestingly, haplotype analysis uncovered all SNPs to be in strong LD (>0.9) among each other and to be incorporated in two main haplotypes. The tagging SNP rs2020938 (tagSNP) was determined for validation of the association finding in additional case control samples. It has so far been found to be associated in three case control samples from the UK, USA and Greece. Currently, additional case control collectives from partners in the COST Action BM1106 GENIEUR (The Genes in Irritable Bowel Syndrome Research Network Europe, www.GENIEUR.eu) are being tested for association. Functional follow-up of the risk/protective haplotypes in luciferase reporter assays showed that the risk haplotype leads to enhanced luciferase activity corresponding to increased expression compared to the protective haplotype. The protective allele might consequently lead to decreased expression levels and the risk allele have to opposite effect. We are currently comparing expression in different gut regions, including jejunum, ileum and colon to confirm our findings. We will report on latest replication and expression data.

AN INVESTIGATION OF THE PHARMACOLOGY OF VORTIOXETINE AT HUMAN 5-HT$_3$ RECEPTORS

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Vortioxetine is a novel antidepressant that was approved by the FDA in September 2013 for use in Major Depressive Disorder (MDD). Some of the most common side-effects are emesis and diarrhoea, which may indicate activation of 5-HT$_3$ receptors at therapeutic concentrations. Indeed, vortioxetine displays affinity for a number of 5-HT receptors including the 5-HT$_{1B}$, 5-HT$_3$, and 5-HT$_7$ receptors, and a previous study demonstrated activation of the 5-HT$_3$ receptor but only upon first application of the drug (Bang-Andersen et al., 2011). In the present study we have further explored the interaction of vortioxetine with the 5-HT$_3$ receptor. The 5-HT$_3$ receptor is a ligand-gated ion channel expressed by neurones in the brain (e.g. chemoreceptor trigger zone) as well as in the periphery (e.g. gastrointestinal tract). Two major subtypes of the receptor have been studied in the most detail; the homomeric 5-HT$_3$A receptor and the heteromeric 5-HT$_3$AB receptor. Using HEK293 cell lines stably expressing either the 5-HT$_3$A or 5-HT$_3$AB receptor, vortioxetine behaved as a partial agonist with intrinsic efficacy of 42±3% at 5-HT$_3$A receptors and 37±4% at the 5-HT$_3$AB receptors (mean±SEM, n=4) (see Newman et al., 2013 for methodology) . 5-Chloroindole (5-Cl; 10 µM), a 5-HT$_3$ receptor positive allosteric modulator, increased the efficacy of vortioxetine at both the 5-HT$_3$A receptor (to 80±6% relative to 5-HT) and 5-HT$_3$AB receptor (77±10% relative to 5-HT). The EC$_{50}$ of vortioxetine was similar at 5-HT$_3$A and 5-HT$_3$AB receptors (179±14 nM and 119±9 nM, respectively). Receptor binding experiments demonstrated the affinity of vortioxetine was comparable between 5-HT$_3$A and 5-HT$_3$AB receptors (9±1.4 and 19±2 nM, respectively; mean±SEM, n=3-4). In saturation binding experiments, vortioxetine (10-30 nM) increased the K_d of [^{3}H]-granisetron ($p<0.05$, paired t-test), but had no effect on the density of labelled receptors, indicative of a competitive interaction. Our studies indicate vortioxetine is a relatively high affinity competitive partial agonist at the 5-HT$_3$ receptor. Such actions may be responsible for the emesis and/or diarrhoea experienced by some patients receiving vortioxetine.

SEX- AND SERT-DEPENDENT ANXIETY- AND DEPRESSION-LIKE BEHAVIORS IN MICE

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Changes in serotonin system function are implicated in the etiologies and treatment of mood and anxiety disorders, which manifest differently in men and women and are diagnosed at higher rates in women. Sex-associated differences in behavior are also observed in animal models characterized by differences in anxiety- and depression-like behavior, including mice with constitutive loss of the serotonin transporter (SERT). We recently reported differences in basal and stimulated extracellular serotonin between male and female mice in the context of female estrous phases.[1] Here, we report sex- and SERT-dependent variations in anxiety- and depression-like behavior in mice. For example, in the novelty-suppressed feeding test, which measures anxiety-like behavior, we observed significantly decreased latencies to feed in female mice, compared to male mice, independent of SERT genotype. Genotype-associated differences in behavior are sex-dependent in the forced swim test, where immobilities were significantly different in male SERT wildtype vs. SERT knockout, but not female mice. We will present and discuss interactions between sex and genotype in widely used tests for anxiety- and depression-like behavior in mice and their relevance to human affective and anxiety disorders.

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EXTRACELLULAR SEROTONIN REGULATION IN HEALTHY AND DEPRESSED MOUSE MODELS

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Depression is a debilitating disorder, affecting a significant global population. Because the chemistry underlying depression is not well defined, pharmacological therapies are variable and have low efficacy rates. In addition, because there are limitations in reliable preclinical screening tools for antidepressant efficacy, major pharmaceutical companies have dramatically toned down their drug discovery efforts. Given that the World Health Organization predicts that depression will be the leading cause of disability worldwide by 2030, there is a critical need to address the fundamental chemistry that underpins depression to provide new paths for drug discovers towards more effective therapies. The serotonin system is the primary target for the most widely-prescribed antidepressants; additionally, we and others have evidence to show that serotonin is modulated by other neurotransmitters targeted by antidepressants (*e.g.* dopamine and norepinephrine). It is therefore crucial that a deeper understanding of serotonin's role in depression is elucidated.

In this work, we combine *in vivo* fast-scan cyclic voltammetry (FSCV), fast-scan controlled-adsorption voltammetry (FSCAV), mathematical modeling, and animal behavior to ask a fundamentally important question: "how do serotonin dynamics differ between normal and behaviorally depressed mice?" We employ potent behavioral models of stress to induce depression phenotypes in mice and apply *in vivo* FSCV and FSCAV in the mouse hippocampus to make real-time, quantitative measures of electrically stimulated and ambient serotonin. A twelve differential equation model is applied to experimental data to decipher mechanistic differences in the chemical regulatory mechanisms controlling serotonin between healthy and behaviorally depressed mice. Our work reveals previously unknown, important perturbations of the serotonin system in a mouse depression model that has the potential to better focus therapeutic efforts.

POSTER #51

CHRONIC TREATMENT WITH SARPOGRELATE REVEALS THE ROLE OF 5-HT_{1F} RECEPTORS IN THE INHIBITION OF THE CARDIOACCELERATOR SYMPATHETIC OUTFLOW IN PITHED RATS.

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5-Hydroxytryptamine (5-HT)-induced inhibition of the cardioaccelerator sympathetic outflow in pithed rats involves activation of prejunctional 5-HT_{1B}, 5-HT_{1D} and 5-HT_{5A} receptors. Since chronic sarpogrelate (a 5-HT_{2A} receptor antagonist) is beneficial in the treatment of several cardiac and vascular pathologies, this study has investigated whether chronic oral treatment with sarpogrelate alters the pharmacological profile of this cardiac sympatho-inhibition.

Male normotensive Wistar rats (divided into 28 groups; n=5 each) were pretreated during 2 weeks with sarpogrelate in drinking water (30 mg/kg/day; 20 groups) or equivalent volumes of drinking water (8 control groups). Afterwards, the rats were pithed and prepared for: (i) spinal stimulation (C_7-T_1) of the cardioaccelerator sympathetic outflow (18 sarpogrelate-pretreated and 8 control groups); and (ii) i.v. bolus injections of exogenous noradrenaline (2 sarpogrelate-pretreated groups). These procedures produced frequency-dependent and dose-dependent tachycardic responses, which remained unaltered after physiological saline.

In both sarpogrelate-pretreated and control groups, intravenous (i.v.) continuous infusions of 5-HT induced a dose-dependent cardiac sympathoinhibition, a response that was mimicked by the 5-HT receptor agonists 5-CT (5-$HT_{1/5}$), CP 93,129 (5-HT_{1B}) or PNU 142633 (5-HT_{1D}). In contrast, LY344864 (a 5-HT_{1F} receptor agonist) inhibited the cardioaccelerator sympathetic outflow only in sarpogrelate-pretreated animals. Interestingly, i.v. GR 127935 (0.31 mg/kg; a 5-$HT_{1A/1B/1D/1F}$ receptor antagonist) attenuated 5-CT- and abolished LY344864-induced sympatho-inhibition; while GR 127935 (0.31 mg/kg) plus SB 699551 (1 mg/kg; a 5-HT_{5A} receptor antagonist) abolished 5-CT-induced sympatho-inhibition.

In conclusion, chronic oral treatment with sarpogrelate reveals the role of 5-HT_{1F} receptors in the inhibition of the rat cardioaccelerator sympathetic outflow, while the cardiac sympatho-inhibitory role of 5-HT_{1B}, 5-HT_{1D} and 5-HT_{5A} receptors is confirmed in both sarpogrelate-pretreated and control groups. Therefore, chronic blockade of 5-HT_{2A} receptors could be a potential therapeutic strategy in the treatment of cardiac disorders by potentiating the serotonergic cardiac sympatho-inhibition.

CHARACTERIZATION OF MET RECEPTOR TYROSINE KINASE-EXPRESSING SEROTONERGIC NEURONS

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There is increasing awareness that the brain serotonin system as a unified system is actually far more complex functionally and structurally. Specific subsets of raphe neurons have different connectivity patterns, electrophysiological properties, and molecular profiles. Molecular specification of subpopulations, in functional contexts, is just beginning to be pursued. We discovered that, from late gestation to adulthood in the mouse, the MET receptor tyrosine kinase (MET), an autism risk gene, is specifically expressed in a limited subset of 5-HT neurons within the dorsal raphe nuclei (DRN). Detailed mapping in developing mouse brain reveals that MET is expressed almost exclusively in the caudal part of DRN as a paired nucleus situated just below the aqueduct, within the classically defined B6 subgroup. A small number of MET^{+}-5-HT (5-HT^{MET+}) neurons are also present in the median raphe. To determine the evolutionary conservation of this unique subgroup, we examined the presence of 5-HT^{MET+} neurons in the developing rhesus monkey brainstem. In the 3rd trimester, there was a bilateral, small subset of MET+ neurons in the comparable region of the brainstem raphe. This group of unique 5-HT neurons may thus function in a conserved fashion across species. We set to further characterize this 5-HT^{MET+} subgroup by 1) analyzing its molecular signature using public available database and by 2) determination of their targets in the forebrain. Using the Allen Brain Atlas, we identified the expression of 14 genes, including neurotransmitter/neuropeptide receptors and potassium channels, in the DRN 5-HT^{MET+} region. Initial connectomics experiments revealed that almost all the 5-HT axons innervating the development forebrain in the region of the ventricular/subventricular zones are MET+. Further molecular characterization of this unique subgroup is being evaluated in WT and *Pet-1^{Cre}* x *Met^{fx}* mice. These studies provide a framework for deciphering the unique functional properties of specialized 5-HT neurons in the brain.

INVESTIGATING THE ROLE OF SEROTONIN 1B AND 2C RECEPTORS DURING RESPONDING FOR UNCONDITIONED SENSORY REINFORCEMENT

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Like primary (e.g., food, sex) or pharmacological (e.g., psychostimulants) rewards, sensory stimuli are also reinforcing. While such stimuli often acquire salience through pairing with primary or pharmacological rewards (i.e., a conditioned stimulus), unconditioned sensory stimuli also have reinforcing properties. Determining the neural basis of the motivation to respond for unconditioned sensory reinforcers (USRfs) will provide insight into the neurochemistry of intrinsic motivational salience and how this salience may be altered.

Serotonin (5-HT) is an important modulator of the motivation to obtain reward. The precise role of serotonin in motivation depends on both the type of reinforcer and the receptor subtype involved. 5-HT2C receptor agonists tend to decrease motivation for primary, pharmacological, and conditioned reinforcers. However, activation of 5-HT1B receptors has been reported to either increase or decrease motivation, depending on the reinforcer being obtained.

To determine the effects 5-HT1B and 5-HT2C receptor agonists and antagonists on the motivation to obtain a USRf, male C57BL/6 and CD-1 mice were given the opportunity to press a lever to obtain a USRf (flashing lights and sound stimulus). Compared to an inactive lever, all mice increased responding on only the active lever that delivered the USRf across 15 sessions. Therefore, the USRf had motivational value and functioned as a reinforcer. Although responding was greater in the CD-1 mice compared to the C57BL/6 mice, this difference was not significant. Systemic administration of either a 5-HT1B receptor agonist (CP94253) or antagonist (GR127935) prior to testing did not significantly affect responding for the USRf in either mouse strain. Administration of the 5-HT2C receptor agonist (lorcaserin) significantly decreased responding for the USRf in both strains but to a greater extent in CD-1 mice. These results further demonstrate that 5-HT2C receptor agonists decrease responding for multiple reinforcers, whereas the role of 5-HT1B receptors in motivation may be reinforcer-dependent.

REGULATION OF THE SEROTONIN 2A RECEPTOR GENE (*HTR2A*) IN THE MOUSE PREFRONTAL CORTEX BY EARLY GROWTH REPONSE 3 (EGR3)

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The immediate early gene *EGR3* is associated with schizophrenia in humans and expressed at reduced levels in postmortem patients' brains. We previously found that, in addition to schizophrenia-like behavioral abnormalities, *Egr3*-/- mice have a nearly 70% decrease in prefrontal cortical serotonin 2A receptors ($5HT_{2A}Rs$). This underlies their resistance to sedation by clozapine, a phenomenon that parallels the increased tolerance of schizophrenia patients to antipsychotic side effects. These findings led us to hypothesize that EGR3, a transcription factor, directly regulates the expression of the $5HT_{2A}R$ - encoding gene *Htr2a*.

To test this hypothesis, we used bioinformatics analyses to identify high probability EGR consensus binding sites in the *Htr2a* promoter. We identified a "distal" site located ~2800 bp upstream of the transcription start site, and a "proximal" site ~70 bp upstream of *Htr2a* transcriptional start site. To determine the expression time-course of EGR3 protein we conducted western blot analysis on prefrontal cortex tissue from wide type (WT) mice at baseline, compared with animals sacrificed two hours and three hours after electroconvulsive seizure (ECS), which induces maximal expression of EGR3. These results showed that EGR3 protein levels were highest two hours after ECS. Based on these findings we conducted Chromatin Immunoprecipitation (ChIP) on frontal cortical tissue isolated from WT mice two hours following ECS to evaluate whether EGR3 directly binds to these regions of the Htr2a promoter in *vivo*. ChIP revealed significant binding of EGR3 to the distal region in the *Htr2a* promoter after ECS compare with baseline. To confirm functionality of EGR3 binding to the *Htr2a* promoter, we performed an *in vitro* luciferase-reporter assay. Results of these studies will be presented. These findings provide information about potential regulation of the schizophrenia candidate gene *HTR2A* by the immediate early gene transcription factor EGR3.

PRESYNAPTIC SEROTONIN 2A RECEPTORS MODULATE THALAMOCORTICAL PLASTICITY AND ASSOCIATIVE LEARNING.

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Higher-level cognitive processes strongly depend on a complex interplay between mediodorsal thalamus (MD) nuclei and prefrontal cortex (PFC). Alteration of thalamofrontal connectivity has been involved in cognitive deficits of schizophrenia. Prefrontal serotonin 5-HT2A receptors play an essential role in cortical network activity, but the mechanism underlying their modulation of glutamatergic transmission and plasticity at thalamocortical synapses remains largely unexplored. Here, combining electrophysiological recordings and behavioural studies, we show that 5-HT2A receptor activation facilitates NMDA transmission and gates the induction of temporal-dependent plasticity mediated by NMDA receptors at thalamocortical synapses in acute PFC slices. Expressing 5-HT2A receptors in the MD (presynaptic site) of 5-HT2A receptor-deficient mice, but not in the PFC (postsynaptic site), using a viral gene delivery approach, rescued the otherwise absent potentiation of NMDA transmission, induction of temporal plasticity and deficit in associative memory. These results provide the first physiological evidence of a role of presynaptic 5-HT2A receptors located at thalamocortical synapses in the control of thalamofrontal connectivity and the associated cognitive functions.

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