

Serotonin Club Satellite Meeting

Symposia Presentations

SC1.1

Imaging animal models of 5-HT function

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It is well established that the neurotransmitter 5-hydroxytryptamine (5-HT) is important in key brain functions such as mood regulation, cognition, sleep and pain processing. Furthermore, dysfunction of the 5-HT system has been implicated in the pathophysiology and treatment of a range of psychiatric disorders including major depression. However, animal studies of 5-HT function rely on invasive techniques and thus are non-transferable to human studies. Functional MRI offers the potential to investigate the effect of pharmacological manipulations on brain function in a non-invasive manner that is clinically translatable. We have demonstrated that pharmacological MRI (phMRI) can be used to detect the action of fenfluramine, a 5-HT releasing agent, on neuronal activity in rat brain. We have demonstrated significant effects of enhanced 5-HT activity on the phMRI response throughout the 5-HT axis of the brain, including the dorsal raphe nucleus (DRN), nucleus accumbens, hippocampus, motor cortex and prefrontal cortex. Pre-treatment with the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine, attenuated the response to fenfluramine in some regions (nucleus accumbens, prefrontal cortex), but not others (motor cortex). Further, we have demonstrated that the phMRI response to fenfluramine is predominantly mediated by the 5-HT_{2A} receptors, although a contribution of the 5-HT_{2C} receptors in some regions is likely. Finally, we show that induction of a systemic inflammatory response can alter the phMRI response to increased 5-HT levels in the rat brain, such that in some regions (DRN, motor cortex and nucleus accumbens) the effect of fenfluramine is entirely abolished. These findings have important implications for the management of individuals at risk of mood disorders such as major depression.

SC1.3

Human models of 5-HT pharmacological fMRI

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Pharmacological MRI (phMRI) provides a way of investigating both the direct and modulatory effects of drugs in the human brain and using drugs with pharmacological specificity may provide information about the regional neurochemistry. Challenge phMRI involves drug infusion and is constrained by the availability of suitable, well tolerated, drugs which can safely be given intravenously over a few minutes. Changes over time in blood oxygenation level dependent (BOLD) signal corresponding to drug effects can be assessed by applying regressors (psychological or pharmacokinetic) or by data-driven methods. In modulatory phMRI the participant is pretreated with the drug of interest and its effects on task-related activations compared with those after placebo pretreatment. We have used infusion of *m*-chlorophenylpiperazine (mCPP) to probe 5-HT_{2C} receptor function and have shown activation of 5-HT_{2C} receptor-rich areas in the brain, an effect attenuated by mirtazapine, an antidepressant with potent 5-HT_{2C} antagonist properties. We have used mCPP challenge phMRI to investigate 5-HT_{2C} function in participants with antisocial personality disorder and preliminary results show alterations in prefrontal areas compared with controls. Infusion of citalopram, a selective serotonin reuptake inhibitor, increases BOLD signal in cingulate cortex, amygdala and hippocampus and studies are underway using this as a measure of presynaptic 5-HT function in remitted and currently depressed patients, and after SSRI treatment in normal volunteers. We have carried modulatory phMRI using mCPP and citalopram and shown that both drugs increase right lateral orbitofrontal activation to a Go-NoGo task implicating 5-HT in behavioural inhibition. In addition acute citalopram pretreatment attenuates right amygdala activation to aversive faces suggesting that antidepressants may act by a direct effect on emotional processing. Further studies are underway to investigate 5-HT modulation of emotional processing in remitted and currently depressed patients and in relationship to response to antidepressant treatment. phMRI is a promising tool for the investigation of brain 5-HT function but further validation of specific challenges and results from investigations in patient population are required.

SC1.4

Effects of citalopram on cortical glutamate and glutamine in healthy

volunteers are localised: a proton magnetic resonance spectroscopic study

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Magnetic Resonance Spectroscopy (MRS) is a non-invasive imaging technique that can provide localised measures of brain chemistry *in vivo*. In particular, levels of glutamatergic markers, glutamate and glutamine, can be measured. Thus when combined with appropriate pharmacological challenges, MRS provides a tool for investigating the interactions between serotonergic and glutamatergic systems that are a focus of increasing research interest. Recently we demonstrated that healthy volunteers receiving the selective serotonin reuptake inhibitor, citalopram, daily for 1 week had higher levels of glutamate and glutamine (Glx) in occipital cortex than those receiving placebo. However, it was unclear if this was a global effect on cortical function that could be expected to generalise to other regions, or a specific local change. Twenty-one healthy volunteers were randomised to receive either citalopram 20 mg or a placebo capsule daily for 7–10 days and were scanned using a 3T VARIAN INOVA system. Standard short-TE PRESS (TE = 26 ms) and PRESS-J spectra were acquired from a single 8 cm³ voxel in a frontal region incorporating anterior cingulate cortex. Both glutamate (Glu) and total glutamate and glutamine (Glx) levels were quantified relative to creatine (Cr) as an internal control. The two treatment groups did not differ in Glx/Cr levels at the end of the study (mean ± SEM citalopram 1.90 ± 0.05 vs. placebo 1.84 ± 0.07, *P* > 0.4). Similarly, Glu/Cr levels were unaffected (*P* > 0.4). These data suggest that the effects of serotonin reuptake inhibition of cortical glutamatergic MRS measures are regionally localised. This supports the potential for MRS in assessing neuroanatomically-specific serotonin-glutamate interactions.

SC2.1

5-HT₃ receptors in the human gut; distinct molecular targets

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The human 5-HT₃ receptor is an established target for the symptomatic relief of emesis and irritable bowel syndrome. It is now appreciated that the 5-HT₃ receptor results from the products of at least five separate genes (h5-HT3A-E). It appears that the prerequisite 'alpha' subunit is the h5-HT3A subunit, which forms efficiently into functional homomeric h5-HT3A receptor complexes. Of the remaining subunits (h5-HT3B-E), most information is known about the h5-HT3B subunit that for instance confers an increase in single channel conductance upon the heteromeric h5-HT3AB receptor relative to the homomeric h5-HT3A receptor, whilst retaining a comparable pharmacology at the 5-HT binding site. Early evidence suggests that the heteromeric h5-HT3A3C, h5-HT3A3D and h5-HT3A3E receptors also display a comparable pharmacology. However, the relatively distinct structures of the N-termini of the h5-HT3C-E subunits, which may contribute to the ligand binding domain, suggests that pharmacological discrimination between different h5-HT₃ receptor isoforms may be possible. The potential significance of which is highlighted by the differential organ expression of the different h5-HT₃ receptor subunits; particularly the limited expression of the h5-HT3D and h5-HT3E subunits, which are present in the gut yet appear absent within the CNS. Hence the ability to pharmacologically target h5-HT₃ receptors incorporating either h5-HT3D and/or 5-HT3E subunits may offer gut selectivity. Alternatively, clinical investigation of 5-HT₃ receptor ligands that avoid interaction with isoforms incorporating either h5-HT3D and/or 5-HT3E subunits would be of interest given some of the side-effects associated with 5-HT₃ receptor antagonists [e.g. constipation, ischaemic colitis (although rare)]. Such potential provides optimism that isoform-selective 5-HT₃ receptor ligands may offer clinical benefit above existing compounds.

SC2.2

Serotonergic control of gastrointestinal function

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5-Hydroxytryptamine (5-HT) is a major transmitter molecule within the gastrointestinal (GI) tract. It is contained in enterochromaffin (EC) cells within the mucosal epithelium and neurones within the enteric nervous system. It is present in mucosal mast cells in some species which may be particularly important in inflammation when mast cell numbers are increased. 5-HT receptors are widely distributed within the gut wall, including on the terminals of enteric and extrinsic sensory neurones suggesting involvement of 5-HT in the transduction of visceral stimuli and reflex responses affecting motor and secretory function and blood flow. Physiologically, 5-HT release from these sources may result in the orchestration of reflexes responsible for transit of material along the bowel at a rate that is appropriate for digestion and absorption of nutrients. However, there is evidence that under pathophysiological conditions, 5-HT acting together with other inflammatory mediators may cause inappropriate intestinal secretomotor activity and/or initiate sensations such as nausea or discomfort/pain. The bioavailability of 5-HT within the gut wall has been shown to change in a number of post-inflammatory models of gut dysfunction with increased numbers of EC cells and mast cells with increased 5-HT content in proximity to sensory nerve endings, and decreased serotonin reuptake mechanisms. These changes, together with altered neuronal excitability and transmitter release in the CNS may result in enhanced nociceptive transmission within the CNS leading to visceral hypersensitivity which is a hallmark of conditions like irritable bowel syndrome.

SC2.3

Serotonin and GI clinical disorders

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The gut contains abundant serotonin, mostly stored in enterochromaffin cell granules which release it in response to bacterial toxins (e.g. cholera toxin), and mucosal pressure. 5-HT released in the mucosa stimulates mucosal primary afferent neurones (IPANs) which initiate local secretomotor reflexes causing secretion and propulsive motility. Locally release 5HT stimulates vagal afferents via 5HT₃ receptors, stimulating motility and in excess inducing nausea and vomiting. Serotonin's action is limited by rapid uptake by the serotonin transporter, (SERT). Increased 5-HT release is found in a range of gastrointestinal disorders including chemotherapy-induced nausea and vomiting, carcinoid syndrome, coeliac disease, inflammatory bowel disease and irritable bowel syndrome (IBS) with diarrhoea (IBS-D), especially that developing following enteric infection (post infective IBS). Animal models show inflammation increases 5HT content and decreases SERT mRNA. The associated increased afferent nerve firing induced by distension can be blocked by 5HT₃ antagonists. Human studies also show that sensitivity to rectal distension is correlated with mucosal 5HT- containing enteroendocrine cell numbers. IBS-D duodenal biopsies have reduced mRNA for SERT while platelets show impaired 5HT uptake which might account for the increase in 5-HT availability in IBS-D. 5-HT₃ receptor antagonists inhibit chemotherapy-induced nausea and diarrhoea associated with both carcinoid syndrome and IBS-D. By contrast IBS with constipation (IBS-C) is associated with impaired 5-HT response and may benefit from 5-HT₄ agonists such as Prucalopride and 5-HT₄ partial agonists such as Tegaserod.

SC2.4**A functional variant in the miR-510 target site of the serotonin receptor type 3E gene is associated with diarrhea predominant irritable bowel syndrome in females**

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Serotonin type 3 (5-HT₃) receptor antagonists are beneficial in some but not all patients with irritable bowel syndrome and diarrhoea (IBS-D). As cis-regulatory variants can play a role in the etiology of complex conditions by affecting efficiency of translation, we investigated the 5' and 3' untranslated region (UTR) of the 5-HT_{3A} and 5-HT_{3E} subunit genes. Mutation analysis was carried out in a pilot sample of 200 patients with irritable bowel syndrome and 100 healthy controls from the UK. We found the HTR3A 5'UTR variant c.-42C>T ($P = 0.020$, OR = 2.01) and the novel HTR3E 3'UTR variant c.*76G>A ($P = 0.033$, OR = 8.53) associated with the IBS-D subtype. We confirmed the association of c.*76G>A with female IBS-D in an independent German cohort of 119 IBS-D patients and 195 controls ($P = 0.0046$, OR = 4.92). Pooled analysis for c.*76G>A in females resulted in highly significant association ($P = 0.0003$, OR = 5.53). Functional studies showed that c.-42C>T and c.*76G>A both lead to significant upregulation of subunit expression on translational level. The HTR3E variant c.*76G>A affects the microRNA binding site hsa-miR-510 and leads to a higher luciferase reporter gene expression. Both, HTR3E and hsa-miR-510, co-localize in enterocytes of the mucosal cell layer of the gut epithelium as shown by *in situ* hybridization and RT-PCR. This is the first example indicating expression regulation of a serotonin receptor gene by a microRNA and the first description of a cis-regulatory variant affecting this regulation and appearing to be associated with female IBS-D. We suggest that the increased expression of 5-HT_{3A} and 5-HT_{3E} subunits might result in a change in 5-HT₃ receptor composition and/or density of 5-HT₃ receptors in the epithelial cell layer of the mucosa and neurons of the enteric and central nervous system and could therefore contribute to the pathophysiology of IBS-D.

SC3.1**TPH1 knock-out mouse as a model to define the role of peripheral serotonin during foetal and adult life**

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The rate-limiting enzyme tryptophan hydroxylase (TPH), responsible for serotonin synthesis (5-HT) is found only in 5-HT-producing cells and exists in two forms: TPH2 is present in the central nervous system while TPH1 is found in peripheral tissues. To define the *in vivo* action of peripheral 5-HT, we disrupted the TPH1 gene by homologous recombination. Initial studies indicated important roles for 5-HT in cardiac functions as the mutant mice developed progressive loss of heart contractility leading to heart failure. More recently, with these TPH1 mutant mice, we have shown that maternal 5-HT was required for normal development based on observed embryonic abnormalities arising in embryos whose gestation had taken place in 5-HT deficient females. Latest results established that severity of the cardiac phenotype, in TPH1^{-/-} adult mice, is inversely correlated with the plasma 5-HT concentration but not the whole-blood 5-HT level and that the plasma 5-HT concentration, is influenced by the maternal serotonergic status. Current work indicates that reduced platelet counts, impaired platelet function and/or structure in adult TPH1^{-/-} mice, might trigger the cardiac phenotype. These data support a model whereby 5-HT is a peripheral factor directly implicated in platelet production, and possibly sustains their maturation. The TPH1 knock-out mouse line is therefore a unique model to address the unsuspected roles of peripheral 5-HT which have been neglected in favour of central serotonin.

SC3.3**Transcriptional regulators and functional polymorphisms in the 5-HT1A receptor gene: implications in mental illness**

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The serotonin system is implicated in mental illnesses including depression, and suicide and its activity is regulated by presynaptic 5-HT_{1A} autoreceptors located on serotonin neurons of the raphe nuclei. We previously identified a functional C(-1019)G 5-HT_{1A} promoter polymorphism that was associated with depression, suicide, and treatment response. We review the evidence that the C(-1019)G polymorphism is associated with mental illness, and discuss its regulation by transcription factors. The C(-1019) allele is within a 26-bp DNA palindrome element that binds transcription factors Deaf-1/NUDR and Hes-5; while these factors fail to bind to the G(-1019) allele. Deaf-1 represses the expression of the 5-HT_{1A} receptor in raphe cells, hence subjects with the G(-1019) allele should have elevated 5-HT_{1A} receptor expression, as observed in post-mortem brain tissue. In PET studies of depressed subjects, the G/G genotype was associated with significantly increased 5-HT_{1A} receptor density in the raphe area. Interestingly, Deaf-1 enhances 5-HT_{1A} transcription in certain post-synaptic neuronal cells; hence, in subjects with the G(-1019) allele, a decrease in 5-HT_{1A} receptor expression in certain 5-HT target such as cortical or hippocampal regions is predicted, and has been observed in depressed subjects. Hes family proteins (Hes-1 and Hes-5) are expressed in neural progenitors and repress neuronal genes and become down-regulated upon neural differentiation. In addition to Hes-5, Hes-1 also represses the 5-HT_{1A} receptor gene, but is more resistant to the C(-1019)G change. In Hes-1^{-/-} mice, we recently found that 5-HT_{1A} receptor expression is dramatically up-regulated throughout the brain, but particularly in the raphe. We hypothesize that the G(-1019) allele affects not only Deaf-1 regulation of

5-HT_{1A} expression in adult, but also Hes-5 (and possibly Hes-1) repression during early development, which may influence lifelong behavioural phenotype. In agreement with this, DNA methylation of the Hes and Deaf-1 sites is increased in the frontal cortex of schizophrenics, and greatly increase 5-HT_{1A} transcription. Hence, both early developmental and persistent alterations in regulation at the C(-1019)G site may influence 5-HT_{1A} expression and development of mental illness. By targeted alteration of transcriptional regulatory or DNA methylation processes it may be possible to reverse early changes that predispose to mental illness.

SC3.4**Maternal serotonin is essential for normal mouse development and survival**

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Serotonin (5-HT) is a biogenic amine produced in the brain by cells originating in the raphe nuclei of the brain stem and released by serotonergic endings. 5-HT is also synthesized in large quantities in gut enterochromaffin cells. Tryptophan hydroxylase (TPH), the rate-limiting enzyme of 5-HT synthesis, is found only in 5-HT-producing cells, and exists in two forms, one of which is predominantly in neuronal cells (TPH2) while the other is non-neuronal (TPH1). The early appearance of 5-HT, its receptors, and transporter (SERT) during mouse embryonic development, together with the ability of 5-HT-specific pharmacological agents to interfere with development, strongly suggest that 5-HT functions as a humoral morphogen before it becomes a neurotransmitter. Along this line, our recent data showed that maternal 5-HT is a key factor for normal embryonic development in mice. We observed that at embryonic day 12.5 (E12.5) tph1^{-/-} embryos from tph1 null mothers either developed normally, suffered growth retardation or died *in utero*. To reveal the developmental time point at which differences emerge between mutant and wild-type embryos, a series of experiments were conducted in which the various staged null embryos (tph1^{-/-}) were recovered at different time points, from E7.5 to E18.5 and compared to control embryos obtained from wild-type females. We performed histological, immuno-histochemical and *in situ* hybridization studies. Most interestingly, hematoxylin and eosin staining of the tph1^{-/-} embryos from 5-HT deficient females, demonstrated abnormal morphogenesis of organs including brain and liver. Gross anatomical analysis revealed that beginning at E12.5 and apparent by E13.5, 5-HT deficient embryos were pale and their overall liver architecture was significantly impaired as compared to wild-type embryos. Moreover, the livers of E13.5 mutant embryos displayed a lacy appearance and spaces devoid of cells were also observed. At the brain level, in addition to morphological abnormalities, the results demonstrate that maternal 5-HT seems to modulate the expression of SERT. Results will be discussed in terms of health issues as maintenance of a proper level of 5-HT may be critical for normal development, for example, although the pathophysiology of autism is unknown, autism is thought to be a 'disease of development'.

SC4.1**5-HT_{2C} mRNA editing and ligand-dependent and -independent signaling**

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The post-transcriptional process of mRNA editing changes up to three amino acids in the second intracellular domain of the serotonin_{2C} (5-HT_{2C}) receptor, a region known to play a role in signal transduction. It has been well-established that 5-HT_{2C} ligands can selectively regulate different signaling pathways coupled to the 5-HT_{2C} receptor (i.e. functional selectivity). However, agonist functional selectivity is lost at the fully edited 5-HT_{2C}-VSV and 5-HT_{2C}-VGV receptors for both phospholipase C (PLC) and phospholipase A2 (PLA2) signaling whereas for a partially edited isoform, 5-HT_{2C}-VNI, only the capacity for preferential PLA2 activation is lost. In addition, ligand-independent (i.e. constitutive) receptor activity toward PLC for edited receptors (5-HT_{2C}-VNI, 5-HT_{2C}-VSV and 5-HT_{2C}-VGV) is markedly reduced as compared to the highly constitutively active 5-HT_{2C}-INI receptor. Interestingly, there is no difference in the thermal stability between these edited receptors and the non-edited isoform, which suggests that mRNA editing does not alter the capacity of receptors to adopt active conformations. Although it has been suggested that RNA-editing reduces the coupling efficiency of the receptor to its cognate G proteins, maximal PLC responses elicited by 5-HT (full agonist) and DOI (partial agonist) at edited receptors are not different from 5-HT_{2C}-INI receptors, suggesting that the capacity of the agonist-occupied receptor to couple to Gq/11 proteins is not different. These data indicate that RNA editing can produce profound changes in receptor function that differ depending upon whether the receptor is unoccupied, or occupied by agonist.

SC4.2**Brain regional differences in 5-HT_{2C} receptor modulation of dopamine release in the rat nucleus accumbens**

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Central serotonin_{2C} receptors (5-HT_{2C}Rs) are known to exert tonic and phasic inhibitory controls on the mesoaccumbens dopamine (DA) pathway *in vivo*. Control of DA release in the nucleus accumbens (NAc) involves endogenous 5-HT and 5-HT_{2C}R constitutive activity (CA), and distinct regional regulations by 5-HT_{2C}Rs. Specifically, studies comparing the effects of 5-HT_{2C}R agonist and inverse agonists, led to the conclusion that both VTA and NAc 5-HT_{2C}Rs participate in the overall inhibitory control of NAc DA release, whereas only the NAc serves as a primary site

for the effect of 5-HT_{2C} CA. Intracranial microinjection studies, assessing the influence of 5-HT_{2C} compounds on DA-dependent behaviours, have recently suggested that 5-HT_{2C}Rs in the prefrontal cortex (PFC) may also control DA release in the NAc. Thus, the present study was aimed at assessing this hypothesis. Experiments were performed using *in vivo* microdialysis coupled with HPLC-ECD in halothane-anesthetized rats (male Sprague Dawley rats, 340–360 g), allowing the simultaneous implantation of a dialysis cannula in the NAc and an injection cannula in the ipsilateral PFC. The increase in NAc DA outflow induced by the intraperitoneal (i.p.) administration of 10 mg/kg cocaine was significantly decreased and increased by the intra-PFC injection of selective 5-HT_{2C}R antagonists (SB242084 and SB243213, 0.5 µg/0.2 µl) or agonist (Ro60-0175, 5 µg/kg) respectively. Also, intra-PFC injection of SB242084 decreased NAc DA outflow induced by morphine (10 mg/kg, s.c.), but failed to alter the increase in NAc DA outflow induced by the 5-HT_{2C}R inverse agonist SB 206553 (5 mg/kg, i.p.). Finally, at variance with their systemic effects, intra-PFC injection of SB242084, SB243213 or SB 206553 (0.5–1 µg/0.2 µl) has no influence on basal NAc DA outflow. These findings demonstrate that PFC 5-HT_{2C}Rs exert excitatory control on activated DA outflow in the NAc, and suggest that the PFC does not serve as a site of action for the 5-HT_{2C} CA.

SC4.3 Regulation of the serotonin 5-HT_{2C} receptor (5-HT_{2C}R) in addictive processes

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Neuroadaptations in 5-HT_{2C}R function may contribute to cocaine dependence, withdrawal and/or relapse. Cocaine-evoked 5-HT_{2C}R neuroplasticity may occur at the level of the mRNA (RNA editing), the protein (intracellular trafficking) or the downstream signaling web in regions [ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC)] implicated in addiction. Employing behavioral sensitization and self-administration models, we are investigating cocaine-associated 5-HT_{2C}R neuroadaptations that track with behavioral 5-HT_{2C}R subsensitivity. At day 3 of withdrawal from a sensitizing-regimen of cocaine (15 mg/kg, 2X/day for 7 day), total 5-HT_{2C}R protein expression in homogenates or in the postsynaptic density (PSD; a specialized structure which transduces synaptic signals) of rat PFC was unaltered. However, after 21 days of self-administration (2 h/day, 0.75 mg/kg/0.1 ml, i.v.), increased 5-HT_{2C}R protein expression was observed in membrane-enriched fractions of medial PFC at 1 day of forced abstinence, with a return to baseline seen on day 30. Thus, functional 5-HT_{2C}R subsensitivity seen following experimenter-delivered cocaine depends neither on 5-HT_{2C}R protein degradation (unchanged in homogenate) nor 5-HT_{2C}R synaptic internalization (unchanged in PSD) in PFC. In contrast, augmented 5-HT_{2C}R protein at the membrane during acute withdrawal from active cocaine self-administration is consistent with a role for 5-HT_{2C}R in mediating withdrawal-related sequelae and highlights the importance of contingency in cellular plasticity. Ongoing studies are focused on modifications in 5-HT_{2C}R trafficking in PSD of other limbic-cortico-striatal regions, intracellular signaling molecules, and patterns of edited 5-HT_{2C}R isoform expression in these regions. These studies are moving toward a greater understanding of the behavioral implications of 5-HT_{2C}R regulatory processes in addiction.

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SC4.4 Dopaminergic lesion of the substantia nigra pars compacta dramatically enhances oral dyskinesia induced by 5-HT_{2C} receptor stimulation: possible involvement of entopeduncular nucleus

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Parkinson's disease is characterised by the destruction of dopaminergic (DA) neurons of the substantia nigra pars compacta (SNc). It is associated with numerous adaptive changes of neurochemical systems in basal ganglia, including the serotonergic (5-HT) system. In rats having a unilateral lesion of dopamine neurons, it has been reported that oral dyskinesia elicited by the serotonin agonist m-CPP are enhanced. This effect was paralleled by an increase in the protooncogene c-Fos in the entopeduncular nucleus (EPN), the equivalent of the internal globus pallidus in primates. Here, using the 5-HT_{2C} agonist Ro-60-0175, we have addressed the possibility that the potentiation of adverse motor effects elicited by a 5-HT agonist in hemiparkinsonian rats is related to 5-HT_{2C} receptors and the EPN. Experiments were performed in male Sprague Dawley rats (250–300 g). DA-lesioned or sham-lesioned rats were obtained by administering 6-hydroxydopamine (6-OHDA, 8 µg/4 µl) or its vehicle in the left SNc, respectively, about 3 weeks before behavioural experiments. In some experiments, a guide-cannula was implanted above the left EPN during the surgery and fixed to the skull to permit the lowering of an injector in the EPN 2–4 weeks after the lesion. Bouts of oral movements (chewing, jaw tremor and tongue darting occurring without any evident physical support) were measured during 1-h after drugs injection. When coadministered, the 5-HT_{2C} antagonist SB-243213 (1 mg/kg, i.p.) was injected 1-h before Ro-60-0175. Statistical analysis of oral bouts was performed using ANOVAS followed, when significant ($P < 0.05$), by the protected least significant difference test. The results show that the number of oral bouts induced by Ro-60-0175 (3 mg/kg, i.p.) in sham-lesioned rats was dramatically enhanced (+100%) in 6-OHDA-lesioned rats. The 5-HT_{2C} antagonist SB-243213, without effect by itself, abolished the potentiating effect of Ro-60-0175 in DA-lesioned rats. When administered in the left EPN, Ro-60-0175 (0.3 and 1 µg/0.1 µl), without effect in sham-lesioned rats, elicited a robust increase in oral bouts with respect to the vehicle in DA-lesioned rats. These results demonstrate that DA lesions of the SNc dramatically enhance oral dyskinesia mediated by 5-HT_{2C} receptors in part through a sensitisation of 5-HT-mediated responses in the EPN.

SC5.1 Serotonergic strategies to prevent antipsychotic induced weight gain

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The aim of this study was to explore the ability of the 5-HT₆ receptor ligands, (e.g. E-6837 an 5-HT₆ agonist), to reduce/prevent olanzapine-induced weight gain in high-fat fed, female Sprague-Dawley rats and determine whether a fixed-dose combination therapy comprising an antipsychotic drug with a 5-HT₆ receptor ligand would be a therapeutically beneficial approach for the treatment of schizophrenia and related psychiatric disorders. Overall, E-6837 not only prevented the weight-gain evoked by this atypical antipsychotic (+5.6% vs. vehicle), but also evoked substantial weight-loss in these animals. The weight reduction observed in the group of rats receiving olanzapine plus E-6837 was as great as those produced by the compound when given alone (-6.6 & -7.2% vs. vehicle), indicating that their anti-obesity action was not in any way reduced when given together with the antipsychotic. In defining the pharmacological mechanisms responsible for these effects, it is evident that the weight-gain observed with olanzapine treatment was, at least in part, due to an increase in food consumption. The prevention of weight-gain by E-6837, and in fact, the weight-loss, seems to be largely due to a reduction in food intake to a level that was significantly lower than that of the vehicle-treated control group. In short, these observations are in agreement with a clinically acceptable mechanism for the prevention of antipsychotic-induced weight-gain and weight-loss.

SC5.2 Potential role for 5-HT_{1A} receptor agonism in ameliorating negative and cognitive symptoms in schizophrenia

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The 5-HT_{2A} receptor antagonist component of the dopamine D₂ or D₂ + D₁ antagonist of the 2nd generation antipsychotics has significantly improved their tolerability compared with the 1st generation of drugs, e.g. haloperidol, by increasing the therapeutic window between efficacy and the incidence of extrapyramidal side-effects (EPS) and tardive dyskinesia (TDs). Despite these undoubted benefits, there are significant aspects of schizophrenia that are still poorly served by 1st and 2nd generation antipsychotics: chief amongst them are cognitive deficits and negative symptoms, including the associated domains of anxiety and depression. In the search for novel antipsychotics with a good therapeutic window, and in addition, a broader spectrum of efficacy in the treatment of schizophrenia, various 5-HT receptor targets either have been or are being actively explored. This presentation will focus on 5-HT_{1A} agonism as an adjunctive pharmacological mechanism to D₂ receptor antagonism or partial agonism to help improve cognitive function and alleviate important aspects of negative symptomatology, especially anxiety and depression. Antipsychotics with 5-HT_{1A} agonist properties include approved drugs, ie ziprasidone and aripiprazole, and others that are still in development, e.g. bifeprunox and SSR181507 (both D₂/5-HT_{1A} partial agonists) and SLV-313 (D₂ antagonist/5-HT_{1A} full agonist). Results emerging from preclinical testing indicate that including 5-HT_{1A} agonism in the pharmacological profile of novel antipsychotic drugs is likely to confer efficacy in several important areas of unmet clinical need in schizophrenia, including social withdrawal, impaired cognitive functioning and in the alleviation of anxiety and/or depression that are often associated with negative symptomatology. These data will be reviewed and presented.

SC5.3 Serotonergic approaches in the development of novel atypical antipsychotics

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Schizophrenia is a chronic, debilitating neuropsychological disease characterised by positive (hallucinations, delusions), negative (anhedonia, avolition, emotional blunting), cognitive (attention, working memory) symptoms and affective disorders. Selective D₂ receptor antagonists or typical antipsychotics (e.g. haloperidol or chlorpromazine) are severely limited in efficacy as they treat only the positive symptoms of schizophrenia and do so at the risk of severely debilitating and often treatment-limiting neurological or motoric side-effects (extra-pyramidal side-effects; EPS). The development of second generation or so called atypical antipsychotics (e.g. clozapine and olanzapine), which possess a much 'richer' pharmacological activity, has resulted in improved treatment efficacy across both positive and negative symptomatology but with diminished EPS. However, other side effect sequelae have been demonstrated such as weight gain and agranulocytosis. Thus, new focussed treatment strategies are being developed based upon more selective receptor activity profiles in the hope that treatment efficacy can be increased without inducing the side-effect profiles seen with present therapies. While it has been suggested that 5-HT_{2A} receptor antagonism is an important adjunct to D₂ receptor antagonism, many antipsychotics possess (partial) agonist activity at 5-HT_{1A} receptors (including clozapine, ziprasidone and aripiprazole), combined with D₂ receptor antagonism. It is thought that combined 5-HT_{1A} (partial) agonism alleviates EPS associated with D₂ receptor antagonism and may confer additional beneficial effects for all symptom domains of schizophrenia. Whilst 5-HT_{2A} receptor antagonist and 5-HT_{1A} receptor agonist modalities have demonstrated benefits, therapeutic efficacy may be achieved by other novel methods of serotonergic system manipulation. One such strategy involving the addition of selective serotonin reuptake inhibitor (SSRI) functionality combined with D₂ receptor antagonism has shown early promise in the treatment of the sequelae of schizophrenia. Other serotonergic targets may also be of importance, namely 5-HT₆ receptor antagonism and effects at the 5-HT₇ receptor. The goal of

this presentation, however, is to serve as a review of recent developments exemplifying the putative benefits of both the 5-HT_{1A} agonist and SSRI combination strategies in addition to D2 receptor antagonism for the treatment of the gamut of schizophrenic symptoms.

SC5.4

The 5-HT_{1A} paradox: why 5-HT inhibits and 5-HT_{1A} agonists activate pyramidal neurons in prefrontal cortex through 5-HT_{1A} receptors?

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Prefrontal cortex (PFC) 5-HT_{1A} receptors are important for the pro-cognitive action of several drug classes, including atypical antipsychotics. This receptor is expressed by 50–60% pyramidal and 20–30% GABAergic neurons in rat PFC (Santana *et al.*, 2004). Endogenous 5-HT inhibits PFC pyramidal neurons via 5-HT_{1A} receptors (Amargós-Bosch *et al.*, 2004) whilst systemic administration of selective 5-HT_{1A} agonists paradoxically increases their activity *in vivo* (Díaz-Mataix *et al.*, 2005). In an attempt to clarify this discrepancy, we examined whether 1) 5-HT_{1A} agonists may activate 5-HT_{1A} receptors located on PFC GABAergic interneurons to disinhibit pyramidal neurons, and 2) the increase in pyramidal activity is due to presynaptic 5-HT_{1A} receptor activation in 5-HT cell bodies (autoreceptors), which subsequently removes the 5-HT tone on other postsynaptic 5-HT receptors. We analysed the effects of two 5-HT_{1A} agonists (8-OH-DPAT and F15599) on pyramidal cell activity and local field potentials (LFP) in the mPFC of anaesthetised male Wistar rats (250–275g). 1) 8-OH-DPAT produced mostly biphasic responses (excitations followed by inhibitions at higher doses) whose proportion was significantly reduced by bicuculline (GABA_A antagonist) in the recording pipette. 2) The marked LFP reduction induced by a low 8-OH-DPAT dose (0.75 µg/kg i.v.) occurred with a minor reduction (~20%) of 5-HT release in PFC, suggesting a predominant postsynaptic effect at this 8-OH-DPAT dose. Furthermore, the selective postsynaptic 5-HT_{1A} agonist F15599 increased pyramidal firing rate at doses (0.2–1 µg/kg i.v.) that do not inhibit 5-HT cell firing. Thus, postsynaptic 5-HT_{1A} receptors in GABAergic interneurons may participate in the persistent increase of activity induced by 5-HT_{1A} agonists in PFC, an effect possibly related to the anti-deficit actions of F15599 in models of cognition (Auclair *et al.*, 2007). Supported by grants from FIS PI 060204 and Pierre Fabre.

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SC6.1

Cardiac 5-HT₄ receptors: is 5-HT-evoked Ca²⁺-induced Ca²⁺ release reduced in atrial fibrillation?

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5-HT increases contractility and causes arrhythmias in human atrium. Evidence suggests that these effects occur through the cascade 5-HT₄ receptors → (Gs protein → (adenylyl cyclase → cAMP → (activation of cAMP protein kinase (PKA) → phosphorylation of L-type Ca²⁺ channels (I_{Ca-L}) → (increase in I_{Ca-L}, presumably followed by Ca²⁺-induced Ca²⁺ release and accompanied by PKA-catalysed phosphorylation of phospholamban and perhaps of ryanodine RyR2 channels. The resultant increase in sarcoplasmic Ca²⁺ not only activates contractile proteins but could also cause changes in ionic currents leading to arrhythmias and initiate atrial fibrillation (AF). Recent work of the author and his colleagues and others has shown that 5-HT-evoked increases in I_{Ca-L} are reduced by ~1/3 in atrial myocytes from AF patients, compared to patients with normal sinoatrial rhythm (SR). The maximum I_{Ca-L} response to 5-HT is similar to the maximum I_{Ca-L} responses to catecholamines. As with 5-HT, the I_{Ca-L} responses to (-)-noradrenaline and (-)-adrenaline, mediated through β₁- and β₂-adrenoceptors (β₁AR, β₂AR) respectively, were also reduced by ~1/3 in AF. Maximum contractile responses (Emax) to (-)-noradrenaline and (-)-adrenaline are reduced by ~1/3 but responses to 5-HT are nearly abolished in atrial trabeculae of AF, compared to SR. The loss of contractile response to 5-HT in AF, despite the partially persistent I_{Ca-L} response, suggests an impairment of Ca²⁺-induced Ca²⁺ release. The marked effects of 5-HT on I_{Ca-L} occur despite the 10-fold and 5-fold lower 5-HT₄ receptor density, compared to β₁AR and β₂AR densities respectively, apparently due to a ~1000-fold tighter coupling of I_{Ca-L} to 5-HT₄ receptors than to β₁AR and β₂AR.

SC6.2

Role of myocardial 5-HT₄ and 5-HT_{2A} receptors in heart failure

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In heart failure, cardiac responsiveness to neurohumoral stimulation is changed. We recently found functional 5-HT₄ receptors coupled to a positive inotropic response in porcine and human cardiac ventricles, with increased 5-HT₄ mRNA in heart failure. Whereas the normal rat cardiac ventricle does not respond to serotonin, functional 5-HT₄ receptors are expressed after myocardial infarction and in chronic heart failure and mediate a positive inotropic response to serotonin. In acute heart failure, the rat cardiac ventricle expresses both 5-HT₄ and 5-HT_{2A} receptors, which mediate inotropic responses through different mechanisms. The 5-HT₄ receptor mediates a positive inotropic as well as a lusitropic response dependent on cAMP, comparable to stimulation of beta-adrenoceptors. The 5-HT_{2A} receptor mediates a positive inotropic response without a lusitropic response, comparable to stimulation of alpha-1-adrenoceptors. This inotropic response involves phosphorylation of myosin light chain, probably resulting in sensitisation of the myofilaments to Ca²⁺. Based on the similarity of 5-HT₄- and beta-adrenoceptor-mediated effects in the failing cardiac ventricle and the beneficial effects of beta-adrenoceptor blockade in

heart failure we examined the effects of treatment with a 5-HT₄ receptor antagonist in rats with chronic post-infarction heart failure. We observed reduced cardiac remodelling, consistent with a beneficial effect of treatment with a 5-HT₄ antagonist in heart failure. These results have been followed up by a randomised clinical trial, evaluating the safety and efficacy of treatment with the 5-HT₄ receptor antagonist piboserod in patients with heart failure.

SC6.3

Critical dual role of angiotensin AT₁ and serotonin 5-HT_{2B} receptors in non-cardiomyocyte for beta-adrenergic agonist induced left-ventricular hypertrophy

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The epistatic relationships between beta-adrenergic, angiotensin AT-1 and serotonin 5-HT_{2B} receptors in cardiomyocytes vs. non-cardiomyocytes for pathological heart hypertrophy *in vivo* remains poorly addressed. By mimicking sympathetic stimulation *in vivo*, we previously reported that mice lacking serotonin 5-HT_{2B} receptors did not present isoproterenol-induced left ventricular hypertrophy. We will provide evidence that mice with restricted rescue of 5-HT_{2B} receptors into cardiomyocytes still did not respond to isoproterenol as assessed by cardiac echography or measurements of plasma hypertrophic cytokines. Using angiotensinogen mutant mice, we also found that the isoproterenol-dependent cardiac hypertrophy is dependent on local angiotensin II production. The resulting autocrine stimulation of AT-1 receptors required 5-HT_{2B} receptors expression for hypertrophic cytokine production by cardiac fibroblasts. Symmetrically, AT-1 receptor activity was necessary for serotonin-dependent cytokine release. Either serotonin- or angiotensin II-dependent cytokine production occurred via transactivation of epidermal growth factor receptors in cardiac fibroblasts. Importantly, this work highlights a transinhibition mechanism between 5-HT_{2B} and AT-1 receptors that may involve heterodimeric receptor complex. These findings in mice are further validated by a significant correlation observed between 5-HT_{2B} receptor ventricular overexpression, plasma cytokine concentrations, and sympathetic activity in patients with congestive heart failure. Collectively, these results demonstrate that interactions between AT-1 and 5-HT_{2B} receptors co-expressed by non-cardiomyocyte cells are limiting key events in adrenergic agonist induced angiotensin-dependent pathological cardiac hypertrophy. 5-HT_{2B} receptor antagonists may thus represent novel effective therapeutics for sympathetic overstimulation-dependent heart failure.

SC6.4

Role of plasmatic serotonin and implication of maternal serotonin in the regulation of the cardiac function in adult mouse

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Serotonin (5-HT) is not only the messenger of some thousands of neurons in the nervous system, but is a ubiquitous substance in peripheral tissues and fluids. Circulating 5-HT, mainly stored in platelets, regulates hemostasis, vascular tone and cardiac function. We generated a mouse line defective for the Tph1 gene and, thus, defective for peripheral 5-HT synthesis. In these Tph1-mutant mice, 5-HT concentration in blood is 3–15% of that in wild-type mice. We addressed the issue of the influence of the circulating 5-HT deficiency on the cardiac structure and function, by testing the hypothesis that there is a correlation between cardiac dysfunction and individual low circulating 5-HT level. Through cardiac functional exploration (electrocardiography, echocardiography and conductance catheterization), we show that 5 months old Tph1^{-/-} male mice, on a C57BL/6 background, display a significant decrease of cardiac contractility and cardiac output, indicative of an impaired left ventricular function. They exhibit progressive dilated cardiomyopathy (DCM), normal valves, and are unable to adapt appropriately to a pharmacological stress. The Tph1 mutant's DCM is heterogeneous in its severity and in the time-course of its progression. Analysis of the circulating 5-HT level in the platelet and plasma compartments of the Tph1^{-/-} mice reveals that the severity of the DCM is inversely correlated with the plasma 5-HT concentration but not the whole-blood 5-HT concentration. Moreover, we show that the cardiopathy is more severe in adult Tph1^{-/-} mice born to homozygous mothers than to heterozygous mothers. These findings show that cardiac function, through the plasma 5-HT concentration, is influenced by the maternal serotonergic status. This work demonstrates the importance of the fine regulation of the circulating 5-HT levels for the cardiac function. The regulation of the plasma 5-HT levels, probably determined during the embryonic development, may depend on modification of the structure and/or function of blood platelets.

SC7.1

DOI reduces low frequency oscillations in rat prefrontal cortex. Reversal by antipsychotic drugs

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Perceptual, psychic and cognitive alterations are fundamental elements of schizophrenia, a pathology associated with an abnormal macro- and micro-circuitry of several brain areas including the prefrontal cortex (PFC). Hence, alterations in the information processing in PFC may underlie schizophrenic symptoms. Using single unit and local field potential (LFP) extracellular recordings

we assessed the effects of DOI, a 5-HT_{2A/2C} agonist with LSD-like hallucinogenic properties, on mPFC activity in anesthetized rats. DOI markedly disrupts cellular and network activity in rat PFC. DOI altered pyramidal discharge in mPFC (39% excited, 27% inhibited, 34% unaffected; $n = 51$). In all instances, DOI concurrently reduced low frequency oscillations (0.3–4 Hz; power spectrum: 0.25 ± 0.02 and $0.14 \pm 0.01 \mu\text{V}^2$ in basal conditions and after 50–300 $\mu\text{g}/\text{kg}$ i.v. DOI, respectively; $n = 51$). DOI also disrupted the temporal association between active phase of LFP and pyramidal discharge. Both effects were reversed by M100907 (5-HT_{2A} receptor antagonist) and were not attenuated by thalamic lesions, supporting an intracortical origin of DOI effects. The reduction in low frequency oscillations induced by DOI was significantly reversed by the antipsychotic drugs haloperidol (0.1–0.2 mg/kg i.v.) and clozapine (1 mg/kg i.v.). Thus, DOI disorganizes network activity in PFC, reducing low frequency oscillations and desynchronizing pyramidal discharge from active phases of LFP in a manner similar to that of phencyclidine. These alterations may underlie the hallucinogenic properties of 5-HT_{2A} receptor agonists and non-competitive NDMA receptor antagonists. Also, the reversal by clozapine and haloperidol suggests that antipsychotic drugs may act by normalizing an altered PFC function, irrespectively of different pharmacological and/or cellular targets. Work supported by grants SAF 2007-62378 and FIS 060264. Support from the Spanish Ministry of Health, Instituto de Salud Carlos III (CIBER Salud Mental) and from SENY Fundació is also acknowledged.

SC7.2

Temporal sequence of behavioral effects of LSD in rats: Freedman revisited?

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The clinical effects of LSD as occur in two temporal phases; a 'psychedelic experience' in the early phase, and a second phase that is 'clearly a paranoid state.' The effects of LSD in rats also occur in two temporal phases, with the initial effects mediated by activation of 5-HT_{2A} receptors, whereas the later temporal phase is mediated by dopamine D₂-like receptors. Our recent evidence suggests that the dopamine D₄ receptor mediates the cue when rats are tested 90 min after LSD administration. We have used two-lever drug discrimination in rats trained to discriminate saline from LSD with a 30 min preinjection time (LSD-30) and LSD (0.16 mg/kg, i.p.) with a 90 min preinjection time (LSD-90). Testing a large number of agonists and antagonists belonging to distinct pharmacological classes suggested that the delayed effects of LSD were mediated by a dopamine D₂-like receptor. We then carried out substitution and combination tests with dopamine D₄ agonists and several antipsychotic agents. D₄ antagonists produced 80% inhibition of the LSD-90 cue but only 45% inhibition of the LSD-30 cue. In parallel with these studies, the effects of LSD in heterologous cellular systems expressing D₂, D₃, and D₄ receptors demonstrated that LSD was a potent D₄ agonist, with potency similar to quinpirole. These results, as well as several ancillary studies, show that chronic administration of LSD (0.16 mg/kg) to rats produces persistent behavioral disruption that appears to involve dopamine D₄ receptor sensitization.

SC7.3

Effects of serotonergic hallucinogens on perception and patterns of altanserin displacement in humans

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The preferential serotonin-2A agonist psilocybin is known for its ability to induce a psychotic state including visual hallucinations that resemble some of the signs of insipient schizophrenia. Recent studies of visual processing in schizophrenia have indicated disturbances in object completion associated with alterations in the N170 component. To further investigate the role of 5-HT_{2A} receptors in visual processing the effect of psilocybin (115 $\mu\text{g}/\text{kg}$ and 215 $\mu\text{g}/\text{kg}$ vs. placebo) on object completion has been assessed using the Kanizsa figures and EEG/ERP technique in normal volunteers ($n = 16$). In a parallel study, cortical 5-HT_{2A} receptor occupancy during psilocybin administration (215 $\mu\text{g}/\text{kg}$) was assessed using F18-Altanserin-PET ($n = 10$). We found that psilocybin dose-dependently impaired object completion associated with a preferential reduction of the N170 amplitude to the Kanizsa relative to non-Kanizsa stimuli in occipital cortex (V1/V2 and/or LOC). This finding was supported by an additional behavioural visual detection task. Moreover, the overall reduction of the N170 amplitude in the Kanizsa and the non-Kanizsa condition correlated with the degree of psilocybin-induced visual hallucinations. Finally, we found that the high dose of psilocybin (215 $\mu\text{g}/\text{kg}$) resulted in 30–40% occupancy of 5-HT_{2A} receptors in visual cortex. Given that similar findings were found in schizophrenic patients the present results suggest that a disruption of the serotonergic neurotransmission, particularly at the level of the 5-HT_{2A} receptor, may underlay the aberrant object completion found in schizophrenia.

SC7.4

Behavioural effects of 5-methoxy-N,N-dimethyltryptamine combined with inhibitors of MAO in rats: Pharmahuasca

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The hallucinogenic tea known as Ayahuasca is made from a combination of psychoactive plants that contribute the active components N,N-dimethyltryptamine (DMT) and 5-methoxy-DMT (5-MeO-DMT), as well as the monoamine oxidase (MAO) inhibitors (MAOIs) harmaline and harmaline for oral activity. The present study examined the effects of 5-MeO-DMT in combination with MAOIs in rats using the Behavioral Pattern Monitor (BPM), which enables analyses of patterns of locomotor activity and exploration. 5-MeO-DMT (0.01, 0.1, and 1.0 mg/kg) decreased locomotor activity and investigatory behavior. In rats pretreated with a behaviorally inactive dose of harmaline (0.1 mg/kg), 1.0 mg/kg 5-MeO-DMT had biphasic effects on locomotor activity, initially reducing locomotion and then increasing activity as time progressed. This behavioral profile resembles that produced by the complex hallucinogen LSD, and is not induced by higher doses of 5-MeO-DMT in saline-pretreated animals. The ability of harmaline to shift 5-MeO-DMT

to a biphasic locomotor pattern was shared by the selective MAO-A inhibitor clorgyline. The late hyperactivity induced by 5-MeO-DMT in combination with a MAO-A inhibitor is blocked by pretreatment with the 5-HT_{2A} receptor antagonist MDL 11,939. Pretreatment with the 5-HT_{1A} receptor antagonist WAY-100635 failed to attenuate either the early hypoactivity or the late hyperactivity. It is concluded that the ability of harmaline to modify the behavioral effects of 5-MeO-DMT is mediated by inhibition of MAO-A. Further, 5-HT_{2A} receptors are responsible for the late hyperactivity induced by 5-MeO-DMT in the presence of MAOIs. Additional studies are currently in progress to further elucidate the mechanism by which MAO-A inhibition shifts 5-MeO-DMT to a LSD-like behavioral profile.

SC8.1

Human tryptophan hydroxylases: molecular properties and clinical implications

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Tryptophan hydroxylase 1 and 2 (TPH1 and TPH2, respectively) are tetrahydrobiopterin and iron dependent enzymes which catalyze the rate limiting step in the biosynthesis of serotonin. TPH1 is mainly found in peripheral tissues and the pineal gland, while TPH2 is found in the brain and peripheral serotonergic neurons. Human TPH1 and TPH2 proteins have a sequence identity of 71%, mainly in their catalytic and C-terminal oligomerization domains. The structure of the TPH1 catalytic domain has been determined using X-ray crystallography and the structures of the intact tetrameric enzymes have been predicted using homology and *ab initio* molecular modelling. Protein kinase A (PKA) and calcium-calmodulin dependent protein kinase II phosphorylate human TPH2 *in vitro* on Ser19 and PKA also phosphorylates the enzyme at Ser104. TPH2 is further activated and stabilized by binding to 14-3-3 proteins (Cichon *et al.*, 2008). TPH1 is also phosphorylated *in vitro*, but the physiological significance of the phosphorylation of either of the enzymes has not yet been established. TPH1 and TPH2 may be involved in several human disorders. Both enzymes are targeted by rare autoimmune disorders and epitope mapping have revealed both shared and unique epitopes in TPH1 and TPH2 (Ekwall *et al.*, 1998). Common and rare variants of the human TPH1 and TPH2 genes are associated with neuropsychiatric disorders which have been linked to disturbances of the serotonin system, such as depression, bipolar disorder (Winge *et al.*, 2008) and attention-deficit/hyperactivity disorder. We have characterized the molecular properties of several rare missense variants of human TPH1 and TPH2, using three different expression systems. Some of the mutant enzymes have severely reduced activity and stability, indicating that patients will have decreased production of serotonin in the brain and that they are causally involved in these complex disorders. As more variants of TPH1 and TPH2 are being discovered, it will be important to develop robust assay systems to evaluate their possible pathogenic role in human disease.

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SC8.2

Interaction of steroids, stress sensitivity and citalopram on gene expression of tryptophan hydroxylase 2 in the dorsal raphe nucleus of female macaques

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Upon the discovery of tryptophan hydroxylase-2 (TPH2), we questioned (i) whether it was regulated by ovarian steroids or (ii) different between individuals with differential sensitivity to stress and (iii) whether it was altered by antidepressant treatment. A monkey specific TPH2 cDNA was constructed for *in situ* hybridization (ISH) followed by autoradiography. Ovariectomized (Ovx) rhesus monkeys were administered placebo, estrogen (E), progesterone (P), or E+P for 1 month ($n = 4$ /treatment) and the midbrain was harvested for TPH2 ISH. All of the hormone treatments caused a significant and similar increase in TPH2 mRNA over placebo controls. TPH2 mRNA expression was then examined in intact female cynomolgus macaques that were characterized as stress sensitive (SS) or stress resilient (SR) and administered placebo or citalopram for 17 weeks. SS animals have lower endogenous production of ovarian steroids than SR animals. TPH2 mRNA was significantly lower in SS animals than in SR animals treated with placebo. Citalopram treatment significantly increased E and P concentrations in SS animals, but significantly reduced TPH2 mRNA compared to SS animals treated with placebo. Citalopram treatment had no effect in HSR animals. Preliminary data suggest that citalopram increased serotonin immunostaining in SS animals. In summary, ovarian steroids, either exogenously administered or endogenously robust, increase TPH2 mRNA. Paradoxically, citalopram treatment increased ovarian steroid production and increased serotonin detection, but decreased TPH2 mRNA expression in SS monkeys. These data support the stimulatory effect of E and P on TPH2 gene expression and further indicate a complex effect of citalopram on the serotonin system. Supported by NIH MH62677, HD18185 and RR00163.

SC8.3

Tryptophan hydroxylase 2 polymorphisms: behavioral and therapeutic implications

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The brain serotonin (5HT) system plays a critical role many neuronal functions and dysregulation of its homeostasis contributes to many psychiatric disorders. Raising extracellular brain 5HT using selective serotonin re-uptake inhibitors (SSRI), can effectively treat numerous conditions, such as major depression and anxiety. Tryptophan hydroxylase (TPH2), the rate-limiting enzyme for 5HT synthesis, is

expressed in neurons while TPH1 is expressed peripherally. Our initially identification of single nucleotide polymorphism (SNP) in the mouse Tph2 gene that results in a ~50% decrease in the *in vitro* activity of the enzyme and a similar reduction in brain 5HT synthesis established the relevance of Tph2. A distinct but closely located mutation in the human TPH2 (R441H) was then identified in a cohort of treatment-resistant unipolar depressed patients, which decreased the activity of the enzyme by >80%. To study the physiological implications of this loss-of-function mutation, a knockin mouse carrying a (R439H) mutation in mTph2 (Tph2KI), equivalent to the (R441H) mutation in humans was generated that recapitulates the >80% predicted decrease in brain 5HT and produces behavioural abnormalities in 5HT related emotional states and anxiety of the animals. Treatment of Tph2KI with 5HTP, the product of Tph2, replenishes normal brain 5HT. While Tph2KI respond to acute SSRI treatment, they are much less responsive to chronic interventions. However, pharmacological or genetic inactivation of GSK3beta signalling corrects the low 5HT-associated behavioural phenotypes suggesting an involvement of this signalling pathway in behavioural consequences of reduced brain 5HT. We have recently characterized an additional SNP, which results in a truncated inactive/dominant negative Tph2 that is more common in depression. Thus, Tph2 is important for maintenance of 5HT homeostasis and could contribute to 5HT-related disorders.

SC8.4

Subregional variation in the dorsal raphe: implications for function

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The dorsal raphe nucleus (DRN), which provides considerable serotonergic input to structures important in regulating mood and anxiety, displays a complex organization across its anteroposterior extent. The DRN can be divided into two main subregions: ventromedial and dorsomedial, with distinct dorsolateral wings present in the mid-regions. Although many past studies have modeled the DRN as a single unit; multiple studies have suggested that these subdivisions differ in cellular and anatomic morphology, connectivity, expression of tryptophan hydroxylase-2 and other genes, as well as response to stress and pharmacologic agents. I will discuss recent findings regarding differential function within DRN subregions and their implications for regulation of mood, anxiety, and stress.

SC9.1

From genes to drugs: SERT-mediated regulation of BDNF, neurogenesis and anxiety

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Reduction in the recapture of serotonin from the extracellular space is a powerful adaptive force. The long-term effects of drugs that inhibit the serotonin transporter (SERT) are believed to underlie the mechanism of many antidepressants. Additionally, lifelong decreases in serotonin reuptake resulting from constitutive reductions in SERT expression in mice and possibly humans expressing a common SERT gene variant, the serotonin transporter-linked polymorphic region, have been associated with altered anxiety and stress responsiveness. However, changes in emotionally related behavior in response to these two different forms of reduced uptake are dichotomous. We are investigating the consequences of SERT gene deficiency on the expression of brain-derived neurotrophic factor (BDNF), the proliferation and survival of adult born neurons in the dentate gyrus, and anxiety-related behavior. Furthermore, we are contrasting this scenario to the effects of chronic antidepressant administration in adult animals on these same parameters. We hypothesize that 5-HT exerts control over the expression of specific BDNF transcripts differently in response to constitutive versus adult pharmacologic reductions in serotonin reuptake. Moreover, while SRIs increase neurogenesis in adult animals, we are finding that constitutive reductions in SERT are associated with decreased adult hippocampal neurogenesis. This is consistent with the contextually dependent effects of constitutive versus adult-mediated reductions in serotonin reuptake to produce opposing effects on anxiety-related behavior. Since genetic and environmental factors work together to shape behavior and susceptibility to complex psychiatric disorders, we are also examining how chronic mild stress and aging alter adaptive responses to different forms of reduced serotonin reuptake.

SC9.2

Early molecular aging and increased emotion-related behaviour in aging mice with altered serotonin homeostasis

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The role of serotonin in the etiology and pharmacological treatment of major depression, combined with serotonergic dysregulation during normal aging, suggests a potential causative role for this neurotransmitter in age-related processes. Specifically, altered serotonin availability and signaling may support some of the age-related cognitive symptoms and may be partly responsible for the increased vulnerability to develop mood disorders in older subjects. However, no causative role for serotonin has yet been identified in age-related processes. Mice lacking the *Htr1B* gene, which codes for a pre-synaptic regulatory receptor, display altered serotonin homeostasis. We now show that *Htr1B*^{KO} display an early profile of age-related increased and decreased gene expression in the brain and a significant reduction in longevity. Indeed, aging is characterized by a predictable set of changes in gene expression in the brain. In *Htr1B*^{KO} mice, this 'molecular signature' of aging started early and peaked at around 18 months of age, corresponding to the time of early death events in the KO group, and resulting in a 15–20% average reduction in lifespan. Subsequent preliminary age-related findings in mice lacking the serotonin transporter and on the translational consequences of the *Htr1B* findings for human brain aging will also be presented. Together, these results provide new evidence for a role for serotonin in aging, while suggesting a pathogenic pathway linking serotonin, aging, and potentially mood disorders.

SC9.3

Consequences of altered brain-derived neurotrophic factor protein levels on hippocampal serotonin neurotransmission and behavior

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Antidepressants such as Selective Serotonin Reuptake Inhibitors (SSRI) act as indirect agonists of monoamine receptors. While these drugs produce a rapid blockade of serotonin (5-HT) transporters (SERT) *in vitro*, the onset of clinical benefits takes several weeks to occur. This paradox has not been solved yet. Recent studies have identified adaptations of intracellular signaling proteins and target genes that could contribute to antidepressant-like activity of SSRI (e.g. increases in neurogenesis and BDNF protein levels in adult hippocampus following chronic SSRI treatment). These data suggest a positive regulation of 5-HT on the expression of the gene coding for BDNF. Are there reciprocal effects of BDNF on brain 5-HT neurotransmission? To answer this question, a dual experimental strategy was developed by inducing either a decrease (1) or an increase (2) in BDNF. In order to study neurochemical and behavioral consequences in male mice. (i) We studied SSRI responses in heterozygous BDNF +/- mice, in which brain BDNF protein levels are decreased by half. These constitutive mutants develop behavioral abnormalities known to correlate with 5-HT dysfunction (enhanced inter-male aggressiveness, hyperphagia associated with weight gain). We found that constitutive reductions in BDNF decreased *in vitro* and *in vivo* SERT capacity to reuptake 5-HT in adult hippocampus. Thus, BDNF +/- mice can be viewed as an animal model of genetic resistance to serotonergic antidepressant drugs. (ii) We increased BDNF after its local intra-hippocampal injection. BDNF decreased extracellular 5-HT levels in the hippocampus, as measured by using microdialysis in wild-type mice. BDNF also decreased KCl-evoked release of 5-HT, an effect being blocked by the TrkB receptor antagonist, K252a. BDNF also induced antidepressant-like activity by increasing swimming, but not climbing, behavior in the forced swim test. Interestingly, intra-hippocampal BDNF injection induced a rapid and transient potentiation of paroxetine effects on dialysate 5-HT and swimming behavior. Thus, BDNF and SSRI may lead to faster neurochemical and behavioral benefits when combined in an antidepressant polytherapy. All together, these data provide a better knowledge of the complex relationship of BDNF and 5-HT neurotransmission, which help to understand better the physiopathology and subsequent treatment of depression.

SC9.4

Creation and phenotypes of TPH1, TPH2 AND TPH1/TPH2 DKO deficient mice

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The serotonin (5-HT) system is involved in many behavioural and physiological functions in both the peripheral and central nervous systems. 5-HT is synthesized by tryptophan hydroxylase that exists in two isoforms: tryptophan hydroxylase-1 (TPH1), which controls most peripheral 5-HT synthesis; and tryptophan hydroxylase-2 (TPH2), which is neuron specific. In our study, we used a gene-targeting approach to generate mice with selective and complete elimination of the two known TPH subtypes. The TPH1, TPH2 knockout and TPH1/TPH2 double knockout (DKO) mice are viable and no abnormalities were noted in the behavioural observation battery and in assays for cardio-vascular, immune, endocrine, and ophthalmic functions. In TPH2 mice immobility was decreased and struggling increased in the forced swim test while immobility was increased in the tail suspension test. Marble burying was increased in TPH2 mice. These effects were potentiated in DKO mice. Notably, all behavioural alterations took place in assays sensitive to detection of 5-HT uptake inhibitors, without any non-specific effects. Levels of brain 5-HT and its metabolite 5-HIAA were decreased in TPH2 mice and virtually absent in DKO mice.

SC10.1

Opposing regulation of early embryogenesis by serotonin and retinoic acid: evidence from mice and sea urchins

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Studies in mice and sea urchins provide evidence that 5-HT via 5-HT2B receptors, and retinoic acid (RA) via RAR, exert opposing influences on early embryogenesis. In mice, 5-HT2B, CRABP1 and RALDH2 immunoreactivity (IR) are co-distributed in the craniofacial region, limb buds and heart. *In vitro* studies show that cell differentiation (chondrogenesis; Bhasin *et al.*, 2004a), and cell proliferation (Bhasin *et al.*, 2004b) are promoted by 5-HT and the 5-HT2B agonist, BW723C86, suggesting that 5-HT2B receptors mediate these effects. RA inhibits the same ontogenetic processes, effects that are blocked by the pan-RAR antagonist, BMS-189453 (BMS), suggesting mediation by RAR. Opposite effects of 5-HT and RA, together with co-distribution of associated molecules, raise the possibility that these two signaling pathways work together to exert opposing actions on important developmental pathways. Co-localization of 5-HT2B and RARalpha IR in cells of the embryonic mouse craniofacial region and limb bud supports this view. In sea urchins, where embryos were exposed to drugs at the 2 cell stage, RA arrested development at the blastula stage. As expected, these effects were prevented by BMS, but were also inhibited by the 5-HT2 agonist, DOI, and completely blocked by BW, suggesting a compensatory role for 5-HT2B. Taken together, these findings suggest that RA and 5-HT may regulate early embryogenesis by exerting opposing actions on common pathways. Grant support: NIDA, CAAT (JML, GAB); Russian Federation (GAB).

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SC10.2

Serotonin-specific invalidation of the vesicular monoamine transporter 2, a new model to study the developmental role of serotonin during the postnatal period

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Because serotonin has pleiotropic effects on development, specific models are needed to address the role of serotonin depletion in different regions and at different periods in life. We produced and characterized a new mouse model in which the vesicular monoamine transporter type 2 is selectively ablated in the neurons expressing 5-HTT on a permanent or transient basis. This includes the raphe and thalamocortical neurones. Recombined mice show a delayed body growth and maturation of the upper layers of the cerebral cortex is retarded. Conversely, other cortical layers develop normally and layer 4 neurons form normal clusters in the barrel cortex. Delayed growth defects can be rescued with inhibitors of monoamine oxydase A, arguing for a causal role of central serotonin depletion in the observed phenotypes.

SC10.3

Stimulation of 5-HT_{2B} and 5-HT₄ receptors enables serotonin to regulate the development and maintenance of the enteric nervous system

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5-HT is best known as a CNS neurotransmitter; nevertheless, the gut contains much more 5-HT than the CNS. Enteric 5-HT initiates peristaltic and secretory reflexes, is a neurotransmitter within the enteric nervous system (ENS), and donates 5-HT to blood and platelets, enabling 5-HT to be a hormone effecting distant organs. 5-HT also participates in ENS development and maintenance. A variety of 5-HT receptors mediate enteric functions of 5-HT. Of these, 5-HT_{2B} and 5-HT₄ are of developmental significance. The 5-HT_{2B} receptor arises during ENS development. In the mouse, its initial appearance follows that of serotonergic neurons, but precedes that of the enterochromaffin cells that contain the bulk of enteric 5-HT. 5-HT_{2B} receptors promote the development of enteric neurons. 5-HT_{2B} knockout impedes development/survival of enteric serotonergic neurons, but does not prevent the appearance of a functional ENS. 5-HT₄ receptors are presynaptic and enhance prokinetic neurotransmission by enhancing the release of acetylcholine, which in the ENS, is primarily responsible for fast excitatory neurotransmission. 5-HT₄ receptors are also neuroprotective and promote the generation of new neurons from dividing stem cells that are retained in the adult ENS. Knockout of 5-HT₄ receptors, slows, but does not stop intestinal motility. It also causes the normal age-related loss of enteric neurons to accelerate, suggesting that 5-HT plays a role in maintaining the ENS by protecting old neurons and/or promoting the generation of new ones; moreover, these effects of 5-HT are 5-HT₄-mediated. These observations suggest that the developmental/trophic actions of 5-HT are important and may be useful in therapy. In contrast, caution should be employed before embarking on the long-term administration of compounds that antagonize 5-HT_{2B} and 5-HT₄ receptors.

SC10.4

Redefining the central serotonergic system based on genetic lineage

P Jensen^a, AF Farago^a, RB Awatramani^b, SM Dymecki^c *^aHarvard Medical School, Boston, MA, USA; ^bNorthwestern University Feinberg Medical School, Chicago, IL, USA* Abnormalities in serotonin (5-HT)-producing neurons and/or neurotransmission are increasingly implicated in a broad spectrum of human disorders, such as sudden infant death syndrome, fetal alcoholism and autism spectrum disorder, to adult psychiatric disorders of mind and mood. Each of these disorders differs in clinical feature, and mounting evidence suggests that different 5-HT neuron subtypes are selectively affected. Heterogeneity within the 5-HT neuron population is further demonstrated by differences in anatomical distribution, axonal morphology and trajectory, neurotoxin sensitivity and physiological properties. Mechanisms that determine these differences are largely unknown and presently few molecular markers have been identified which are capable of distinguishing individual 5-HT neuron subtypes. Such knowledge is central to understanding etiological differences among 5-HT neuron disorders and for gaining genetic access to select 5-HT neuron subgroups for experimental study. We have generated a novel set of site-specific recombinase-based intersectional and subtractive genetic fate-mapping tools to test the hypothesis that 5-HT progenitor cell position and gene expression history in the embryonic hindbrain is predictive of mature 5-HT neuron subtype identity – neuron subtype reflected by anatomical position, axon projection pattern and/or function. Application of these tools has uncovered unique subsets of mature 5-HT neurons based on their rhombomeric origin and associated gene expression profile. We will present progress on the application of these tools along with ongoing studies designed to address the physiological relevance of this subdivision of the serotonergic system based on genetic lineage. Support: NIH grants P01 HD036379, R01 DK 067826, and R01 DA023643 to S.M.D., a Charles H. Hood Foundation (Boston, MA) grant to P.J., and a grant from the Charles King Trust Foundation to R.B.A.

SC11.1

The 5-HT transporter in brain and periphery

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One key regulator of the serotonin (5-HT) system is the plasma membrane transporter (5-HTT), which regulates 5-HT levels within extracellular space, in contact with multiple 5-HT receptor types. Only one gene (of the SLC6 gene family) encodes 5-HTT in both the CNS and peripheral tissues, but regulatory mechanisms can affect differently 5-HT transport in neurons versus other cell types. Long term inactivation of 5-HTT either by pharmacological means (with antidepressants of the Selective Serotonin Reuptake Inhibitor – SSRI – class) or 5-HTT gene knock-out induces adaptive changes of the 5-HT system mainly at receptor level. In the CNS,

5-HTT inactivation down regulates 5-HT_{1A} and 5-HT_{1B} autoreceptors, and also 5-HT_{2A} and 5-HT_{2C} receptors, implying functional consequences of particular relevance with regard to psychiatric disorders such as depression and addiction. At the periphery, 5-HTT has been shown to play key roles notably in the control of gastrointestinal motility and smooth muscle cell proliferation in lung. In particular, hypoxia-induced pulmonary hypertension (HPH) was shown to be causally associated with 5-HTT gene induction in pulmonary artery smooth muscle cells. Thus, 5-HTT inactivation reduced HPH, whereas 5-HTT overexpression exacerbated the disease. Recent studies of the effects of SSRIs at the 5-HTT protein level led to the hypothesis that these drugs bind at two different sites, one responsible for 5-HTT blockade and the other involved in allosteric modulation of the transporter. This offers new opportunities for the development of 5-HTT modulators of great potential therapeutic interest to prevent/reverse 5-HT dysfunctions in brain and/or periphery.

SC11.2

Neurobiological effects of increased expression of the 5-HT transporter

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The 5-HT transporter (SERT) plays a key role in regulating transmission at central 5-HT synapses. Brain levels of SERT vary up to three fold between the individuals, and the source of this variation may include one or more commonly occurring SERT gene polymorphisms. Some SERT polymorphisms are associated with altered anxiety phenotypes in human populations but the role of altered SERT expression in these genotype-phenotype associations is uncertain. To address this issue, we developed a genetic mouse model with a 2–3 fold increase in SERT expression. Here we review the neurochemical and behavioural phenotypes of this model. In a range of anxiety tests including elevated plus maze and fear conditioning, SERT overexpressing (OE) mice demonstrated reduced anxiety levels. SERT OE mice also had a depressive-like phenotype (forced swim test) but did not display anhedonia (sucrose consumption) or cognitive deficits (eg. water maze). SERT OE mice had a low body weight but normal food intake and satiety sequence. In neurochemical experiments (eg. microdialysis, voltammetry), SERT OE mice demonstrated low extracellular 5-HT and a deficit in 5-HT release/storage mechanisms. The low anxiety phenotype and 5-HT deficit may be linked as both were reversed by administration of a 5-HT reuptake inhibitor. However, our data also suggest the presence of a 5-HT deficiency independent of increased SERT expression. Overall, SERT OE mice demonstrate striking behavioural and neurochemical phenotypes, many of which are opposite to those reported for SERT knock out mice. Collectively, data from these mouse models support reported SERT genotype-phenotype associations.

SC11.4

Organic cation transporter 3: a novel target for antidepressant medications

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Dysfunction of serotonin (5HT) neurotransmission is strongly implicated in neuropsychiatric illnesses, such as depression. Inconsistent with current thinking that hyposerotonergic states are linked to depression, individuals carrying a low-expressing variant the 5HT transporter (SERT) gene, who would be predicted to have higher extracellular levels of 5HT, are more prone to these disorders. Moreover, these individuals are less responsive to treatment with selective serotonin reuptake inhibitors (SSRIs). These observations could be explained by a compensatory upregulation of an alternative 5HT uptake mechanism. The neuronal organic cation transporter (OCT) can take up 5HT. Using SERT mutant mice, which lack (-/-) or have 50% fewer SERTs (+/-) than wildtype (+/+) mice, we showed that OCT3 mRNA and protein is increased in the hippocampus of SERT mutants. Functionally, this corresponded to greater inhibition of 5HT clearance after blockade of OCT3 with decynium-22 (D-22) in SERT +/- and -/- mice. Thus, the OCT3 appears to be a viable candidate transporter for 5HT when SERT expression is low. Given the apparent reciprocal relationship between SERT and OCT expression and function, we reasoned that D-22 would be more effective in tests of antidepressant efficacy such as the tail suspension test (TST). Consistent with an antidepressant action, we found that after blockade of OCT3 with D-22 both +/- and -/- mice spent significantly less time immobile than +/+ mice in the TST. Preliminary data suggest that combined blockade of OCT3 and SERT reduce time spent immobile in the TST more so than blockade of each transporter separately, but only in SERT +/- mice. These findings suggest that blockers of OCT3 may be novel therapeutics for patients resistant to current frontline treatments with SSRIs (e.g. low expressing variants of the SERT) and are especially intriguing given recent reports that SSRIs are most effective in severely depressed individuals.

SC12.1

Serotonin–dopamine interactions in cognitive dysfunction: improving cognition in schizophrenia via serotonergic targets

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Multiple serotonin (5-HT) receptors have been implicated in cognitive function. This is, in part, via their influence on dopaminergic function directly or indirectly, but effects on the cholinergic system to enhance acetylcholine efflux are also important. These actions may be mediated by effects on glutamatergic and GABAergic neurons. Most but not all studies find that atypical antipsychotic drugs (APDs) such as clozapine are able to improve cognitive function in schizophrenia more so than

typical APDs. These drugs are more potent 5-HT_{2A} than D₂ antagonists, a combination which we have shown can enhance cortical and hippocampal dopamine (DA) efflux acutely and chronically. We have now found an important role for 5-HT_{2A} receptor antagonism in the ability of atypical APDs to reverse a deficit in novel object recognition produced by subchronic administration of phencyclidine (PCP). There is consensus that 5-HT_{1A} receptors also play a key role in cognition but there are data consistent with both worsening and improvement of cognitive function in response to 5-HT_{1A} receptor stimulation or antagonism. We have found that 5-HT_{1A} partial agonists, tandospirone and buspirone, have modest ability to improve some domains of cognition in schizophrenia. Microdialysis studies show these agents enhance cortical DA efflux. 5-HT_{2C} agonists have recently been shown to be antipsychotic and to be active in some models which suggest the ability to improve cognition but the relationship to DA has not been studied. Taken together, there is much reason to conclude that 5-HT has an important role in cognitive function and that there are multiple drug targets related to 5-HT to improve cognition.

SC12.3

Role of animal models in understanding the importance of serotonin in cognitive dysfunction

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As the 5-hydroxytryptamine 6 (5-HT₆) receptor is in areas associated with learning and memory, many studies have examined its effect on cognitive function in the rodent. In healthy adult rats, 5-HT₆ antagonists enhance retention in the Morris water maze, improve consolidation in autoshaping tasks and reverse natural forgetting in object recognition. 5-HT₆ antagonists facilitate both cholinergic and glutamatergic neurotransmission, reversing scopolamine- and NMDA receptor antagonist-induced impairment in object and social recognition. Some pro-cognitive effects of 5-HT₆ antagonists have translational relevance to deficits seen in schizophrenia, including reversal of impaired object and social recognition and improvement in attentional set shifting. However, whether 5-HT₆ antagonists reverse cognitive deficits in animal models of the disorder, such as rearing rats in social isolation from weaning, requires demonstration. The 5-HT₆ antagonist, PRX-07034, normalises the hyperactivity seen in a novel arena and restores impairment of novel object recognition produced in isolation reared rats. The 5-HT₆ receptor antagonist Ro-046790 improves reversal learning in isolates in the Morris water maze. Ro-046790 also improves object discrimination both in 18-month-old rats and adult rats that received chronic intermittent phencyclidine. These results support the potential role of 5-HT₆ antagonists to improve cognitive deficits seen in schizophrenia. More recently several selective high affinity 5-HT₆ agonists such as E-6801 have been developed. Surprisingly these compounds also improve object discrimination impaired by either time delay or scopolamine. Furthermore, E-6801 at a dose that is ineffective when given alone acts in a synergistic manner with the anticholinesterase, donepezil, or the low affinity NMDA receptor antagonist, memantine, restoring object discrimination. Thus both 5-HT₆ receptor agonists and antagonists show promise as pro-cognitive agents in preclinical studies but the explanation for their paradoxical analogous effect is currently unclear.

SC12.4

Serotonin depletion of the dorsal hippocampus has no effect on learning and memory in rats

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The neuropathology of schizophrenia may involve abnormalities in the hippocampus and in serotonergic transmission. Rats with serotonin depletion in the dorsal hippocampus (DH), but not ventral hippocampus (VH), display enhanced locomotor hyperactivity following treatment with phencyclidine or ketamine, and disruption of prepulse inhibition – behavioural models of aspects of schizophrenia. This study set out to investigate the effect of DH and VH serotonergic lesions on learning and memory, in an effort to model the cognitive deficits that are characteristic of schizophrenia. Two cohorts of male Sprague-Dawley rats were used, each containing three groups: DH-lesioned, VH-lesioned and sham-operated controls ($n \geq 9$ /group). The first cohort was tested in the Y-maze and Morris water maze to investigate spatial learning; the second cohort was tested for working memory in the Alternating T-maze. Rats (250–300 g) were isoflurane-anaesthetised and stereotaxically microinjected with the serotonergic neurotoxin, 5,7-dihydroxytryptamine, into either the DH or VH; sham-operated controls received vehicle solution. Behavioural testing started two weeks post-surgery and ELISA was used to confirm lesions at the end of the experiments. After 1 and 2 h intervals in the Y-maze, all rats showed intact short-term spatial memory as measured by an increased number of entries and duration of time spent in the novel arm. All groups similarly showed learning after a 4 h interval, when indexed by the number of novel arm entries. However, VH-lesioned rats, unlike sham-operated and DH-lesioned animals, showed a subtle impairment of learning after the 4 h interval, spending less time in the novel arm. In addition, all groups showed intact long-term spatial memory as measured by the Morris water maze. There were no working memory differences between groups in the T-maze cohort following the interposition of 30 and 60 s delays. VH-lesioned rats, however, took significantly longer than sham-operated controls to learn the alternating task. These findings are unexpected, given the role of the dorsal hippocampus in spatial learning and memory, and the salient behavioural change that DH serotonin depletion induces in other models of schizophrenia. Our results suggest a functional redundancy of serotonin in the hippocampus on mechanisms of learning and memory.

SC13.1

Augmentation of SSRI treatment

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Selective serotonin re-uptake inhibitors (SSRIs) are recommended first-line treatment in the pharmacological management of major depression. However, real-world studies such as the STAR*D investigation suggests that only a minority of patients reach symptomatic remission following adequate treatment with an SSRI. There is no clear evidence to guide clinicians on what the next step should be. However, particularly if the patient has had a partial response to SSRI treatment, augmentation with another drug offers a modestly better chance of improvement than switching to another compound. Several augmentation strategies are possible and at present the addition of lithium is a reasonable option though the evidence for thyroid augmentation is less strong. The addition of pindolol (a putative 5-HT_{1A} receptor antagonist) to SSRIs does not seem to be effective. There is growing interest in SSRI augmentation with atypical antipsychotic drugs such as olanzapine and quetiapine. A recent meta-analysis indicated that this approach was reasonably helpful in SSRI non-responders although limited by the adverse effect burden, particularly sedation and weight gain. The way in which atypical antipsychotic drugs might enhance the action of SSRIs is not clear but the 5-HT_{2A/2C} receptor blocking properties of atypical agents might be involved. It is possible that 5-HT_{2A} receptor antagonism might facilitate 5-HT neurotransmission by disabling postsynaptic 5-HT_{2A} receptor mediated feedback to 5-HT cell bodies. Studies with selective 5-HT_{2A} and 5-HT_{2C} receptor ligands will be needed to test this interesting proposal.

SC13.2

Serotonin and emotional processing

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There is growing interest in the effects of antidepressant drug treatment on measures of emotional processing. Such actions may help us understand the role of monoamines in emotional dysfunction in depression and how antidepressant drug treatments work. Recent studies suggest antidepressant drugs increase the processing of positive versus negative emotional information across a variety of paradigms, early in treatment, and in the absence of changes in subjective mood. Such actions can be seen even with acute administration in healthy volunteers and in acutely depressed patients. This increase in positive bias may provide a platform for subsequent cognitive restructuring and learning which contributes to later improvements in mood and symptoms in depression. Consistent with this, we found that early shifts in emotional bias in depressed patients were associated with an improved therapeutic response to treatment after 6 weeks. fMRI studies exploring the neural correlates of these effects in healthy volunteers have found modulation of amygdala responses to negative affective stimuli with both acute and 7 days administration of different antidepressant drug agents. Responses to positive versus negative stimuli within highly interconnected extra-striate visual processing areas were also affected by antidepressant drug administration, suggesting drug-modulation of relatively early stages of processing. Such antidepressant drug effects may also be relevant to screening novel candidate treatments for depression. This was explored by assessing the actions of the neurokinin (NK) 1 antagonist aprepitant previously found to be effective in animal models of depression yet of limited success in the treatment of depression. Consistent with this, aprepitant modulation of emotional processing in healthy volunteers was reduced and with a smaller effect size than seen previously with conventional antidepressant drug treatment. Together these results suggest that antidepressants effects on emotional processing may be important in the therapeutic actions of these treatments in depression and as such may be useful biomarker assessments in drug development programs.

SC13.3

Serotonin and vulnerability to MDD

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The serotonergic (5-HT) system is a key target for antidepressants and may be involved in the pathophysiology of major depression. Using positron emission tomography (PET) it is possible to image various aspects of the 5-HT system directly in the human brain using. In order to avoid confounds of medication or illness, and to study core neurobiological endophenotypic markers, we decided to study only medication free subjects recovered from recurrent depression. Using PET we measured binding of the 5-HT_{1A} ([¹¹C]-WAY100635), 5-HT_{2A} receptor ([¹¹C]-MDL00907), and the 5-HT transporter ([¹¹C]-DASB) in three separate studies. All studies included medication-free, fully recovered, recurrent MDD patients and age and gender matched healthy controls. Regional estimates of binding potential (BP) were obtained using a metabolite corrected plasma input function method (for [¹¹C]-MDL00907 and [¹¹C]-DASB) or the SRTM for [¹¹C]-WAY100635. We found significant global decreases in [¹¹C]-WAY100635 binding in patients compared with controls. We found significant regional increases in 5-HT_{2A} binding using [¹¹C]-MDL00907 in recurrent MDD compared with controls. We found no significant difference in the binding potential of [¹¹C]-DASB between the recovered depressed subjects and controls in any of the brain regions studied.

Discussion: It is clear that there are enduring and persistent deficits at the molecular level in the 5-HT system in patients with recurrent MDD. These abnormalities may represent specific endophenotypic biomarkers to recurrent MDD and further studies will need to validate this assumption.

SC13.4**F15599, a highly selective serotonin 5-HT_{1A} receptor agonist that preferentially targets post-synaptic receptors in the frontal cortex: biochemical and neurochemical profiles**

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Activation of post-synaptic 5-HT_{1A} receptors has been suggested to mediate efficacy of antidepressants. F15599 is a novel agonist with high affinity (pK_i ≈ 8.5 for rat and human receptors), selectivity for and efficacy at 5-HT_{1A} receptors. We characterized its activity on pre- versus post-synaptic 5-HT_{1A} receptors in biochemical and neurochemical models. F15599 and (+)8-OH-DPAT reduced pERK level in hippocampus and increased it in prefrontal cortex (PFC) at similar doses (MED: 0.63 mg/kg). In contrast F13714 (a related high efficacy 5-HT_{1A} agonist) and buspirone modulated pERK levels more potently in the hippocampus (reflecting activation of pre-synaptic 5-HT_{1A} receptors in the raphe, MED's: 0.04 and 2.5 mg/kg, respectively) than in the PFC (MED's: 0.16 and 10 mg/kg, respectively). F15599 stimulated c-fos induction in PFC from 0.16 mg/kg, but did so only weakly in the dorsal raphe at a higher dose (0.63 mg/kg), and was inactive in the median raphe. Following acute administration, F15599 increased extracellular dopamine in the mPFC (ED₅₀ = 0.03 mg/kg), but required higher doses (ED₅₀ = 0.24 mg/kg) to decrease hippocampal 5-HT levels. In contrast, (+)8-OH-DPAT influenced DA and 5-HT levels at similar doses (ED₅₀ = 0.20 and 0.33 mg/kg, respectively). Moreover, upon chronic treatment (14 days subcutaneous minipumps), a high dose (20 mg/kg/day) of F15599 was necessary to desensitize somatodendritic 5-HT_{1A} receptors, as assessed by a lowering of hippocampal 5-HT levels following an acute challenge (day 15) with buspirone (10 mg/kg). Taken together, these data indicate that F15599 preferentially activates post-synaptic 5-HT_{1A} receptors located in PFC, and suggest that it may have faster onset of antidepressant activity, as well as have beneficial influence on cognitive dysfunction.

SC14.1**Mechanisms involved in ergotamine-induced inhibition of the vasodepressor sensory outflow in pithed rats**

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Stimulation of the perivascular sensory outflow in pithed rats produces vasodepressor responses which are: (i) mediated by CGRP release; and (ii) inhibited by alpha2A/2C-adrenoceptors. Moreover, i.v. continuous infusions of ergotamine (0.31–5.6 µg/kg/min) dose-dependently inhibited these vasodepressor responses without affecting those induced by i.v. exogenous CGRP (0.1–1 µg/kg). We have now identified the pharmacological profile of this prejunctional inhibition by ergotamine. I.v. administration of the antagonists rauwolsine (300 µg/kg), haloperidol (300 µg/kg) and GR127935 (30 µg/kg) given separately, as well as the combinations of rauwolsine + haloperidol, rauwolsine + GR127935, haloperidol + GR127935 and rauwolsine + haloperidol + GR127935 (at the doses used above) failed to modify *per se* the electrically induced vasodepressor responses during an i.v. continuous infusion of methoxamine (15–20 µg/kg/min). All of the above compounds (and the corresponding combinations) also failed to significantly block the prejunctional inhibition induced by 3.1 µg/kg/min ergotamine. Interestingly, the prejunctional inhibition induced by 0.31 µg/kg/min ergotamine was abolished by the combination of rauwolsine + haloperidol + GR127935. The above results, taken together, suggest that ergotamine-induced inhibition of the vasodepressor sensory outflow in pithed rats may involve: (i) novel mechanisms which differ from the ones analyzed in the present study when 3.1 µg/kg/min were infused; and (ii) prejunctional activation of alpha2-adrenoceptors, D2-like dopamine receptors and/or 5-HT_{1B/1D} receptors when 0.31 µg/kg/min were infused.

SC14.2**Crosstalk of vascular 5-HT₁ receptors with other receptors: clinical implications**

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5-HT induces vasoconstriction via 5-HT₁ and 5-HT₂ receptors. Vasoconstriction mediated by 5-HT₁ receptors may be augmented and/or potentiated by the presence of other vasoactive substances, such as thromboxane A₂ (Gupta *et al.*, 2006). Whereas some reports suggest that the enhanced vasoconstrictive responses mediated by 5-HT₁ receptors merely depend on an increased pretension induced by other compounds, it seems that this is not the case, since (i) not all compounds that induce an increased tension possess the capability to enhance contractile responses to 5-HT₁ receptor agonists, and (ii) contractile responses to 5-HT₁ receptor agonists may already be augmented by a concentration of another compound that does not elicit a contraction, i.e. a subthreshold concentration. Enhanced vasoconstrictor responses to the 5-HT₁ receptor sumatriptan have extensively been studied in view of its potential relevance in coronary vasospasm after the use of antimigraine drugs. Further, enhanced vasoconstriction mediated by 5-HT₁ receptors seems relevant in the pathophysiology of diseases like variant angina and pulmonary hypertension. A yet only incompletely studied area where an interaction of 5-HT₁ receptors with other receptor systems may be of relevance is pre-eclampsia, where we recently demonstrated that the development of 5-HT₁ receptors is expedited in pregnancies complicated by pre-eclampsia compared to normotensive pregnancies. Future research will hopefully elucidate whether (interference with) the interaction of 5-HT₁ receptors with other receptor systems can be of therapeutic benefit in patients suffering from cardiovascular diseases.

References:

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 MaassenVanDenBrink A *et al.* *Br J Pharmacol.* 1996; 119: 855–862.
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SC14.3**Pharmacological profile of the contractile responses induced by 5-HT in the isolated canine external carotid artery**

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5-HT produces vasoconstriction in the external carotid bed of vagosympathectomized dogs via 5-HT_{1B} receptors. This study has characterized the 5-HT receptors mediating contractile responses in the isolated canine external carotid artery, in order to compare this profile with that previously obtained *in vivo* in the canine external carotid bed. Rings of endothelium-denuded canine external carotid arteries were suspended in organ bath chambers for measurement of isometric force. The responses to 5-HT were determined in the absence and presence of vehicle or the antagonists GR127935 (1–10 nM), ketanserin (1–10 nM) and the combination of GR127935 (3 nM) plus ketanserin (3 nM). Furthermore, the effects of the agonists 5-carboxamidotryptamine (5-CT; 5-HT₁, 5-HT₂, 5-HT₇), (±)DOI (5-HT₂) and sumatriptan (5-HT_{1B/1D}) were determined. 5-HT, 5-CT and (±)DOI, but not sumatriptan, produced concentration-dependent contractions of the canine external carotid artery with the following rank order of agonist potency: 5-HT > 5-CT = (±)DOI. Interestingly, these responses to 5-HT were: (1) unaffected by ketanserin or GR127935 when given alone; (2) unaffected by GR127935 in rings pre-incubated with ketanserin (3 nM); and (3) antagonised by ketanserin in rings pre-incubated with GR127935 (3 nM). These results suggest that the receptors involved in the contractile responses to 5-HT in isolated canine external carotid artery rings: (i) closely resemble 5-HT₂A receptors; and (ii) are different to those mediating vasoconstriction in the external carotid vascular bed of anaesthetised dogs.

SC15.1**Proteomic analysis of the 5-HT transporter reveals regulation by nNOS and calcineurin, and a signalling pathway initiated by 5-HT reuptake**

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Homeostasis of serotonergic transmission is dependent on the rate of 5-HT reuptake via its plasma membrane transporter (SERT). As for other members of the Na⁺/Cl⁻-dependent transporter family, the functional activity of SERT is tightly regulated by multiple mechanisms including post-translational modifications and physical association with intracellular proteins. Using a proteomic approach, we identified a specific set of proteins that interact with SERT C-terminus. These included proteins known to influence mood and/or to modulate the actions of antidepressants; namely, neuronal nitric oxide synthase (nNOS) and both the catalytic and regulatory subunits of calcineurin. Co-expression of SERT with nNOS in HEK-293 cells reduced plasma membrane insertion of SERT and 5-HT uptake. These effects were expressed independently of NO production and required physical association of nNOS with SERT. Moreover, cerebral 5-HT uptake was increased in nNOS-deficient mice and in mice treated with a membrane-permeant peptide that inhibited the SERT-nNOS interaction suggesting that association with nNOS reduces SERT activity *in vivo*. Reciprocally, exposure of HEK-293 cells co-expressing SERT and nNOS to 5-HT increased NO production. This effect was abolished by citalopram, suggesting that 5-HT uptake leads to functional 'recruitment' of nNOS. Co-expression of a constitutively-active calcineurin mutant with SERT increased 5-HT uptake in HEK-293 cells, whereas 5-HT uptake was decreased in cells co-expressing SERT and a phosphatase-inactivated calcineurin mutant, revealing a functional interaction between calcineurin and SERT. This study exemplifies the use of proteomic approaches for identifying novel SERT regulatory mechanisms of potential pertinence to the induction and control of psychiatric disorders.

SC15.3**Functional modulation of 5-HT_{2A} and 5-HT_{2C} by kinases and scaffolding proteins *in vivo***

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Over the past several years a large number of proteins have been identified as 5-HT_{2A} and 5-HT_{2C} interacting proteins. These include kinases, arrestins, caveolins and PDZ-domain proteins. For these studies PSD-95, caveolin-1 and RSK2 *k/o* mice were obtained and the functional consequences on 5-HT_{2A} and 5-HT_{2C} receptors examined. In PSD-95 *k/o* mice an accelerated turnover of receptors was observed which led to significant reductions in steady-state levels of 5-HT_{2A} and 5-HT_{2C} receptors. Additionally, apical dendritic targeting of 5-HT_{2A} receptors was dramatically attenuated in cortical pyramidal neurons. Induction of head twitch response (HTR) by 5-HT_{2A} agonists was similarly attenuated in PSD-95 *k/o* mice as was 5-HT_{2C}-mediated c-fos induction in hippocampus. In cortical neurons *in vitro* the phenotype was reversed by the reintroduction of PSD-95. Additionally, whole genome microarray studies did not reveal any significant compensatory alterations in mRNA levels for various proteins within the signal transduction cascade for either 5-HT_{2A} or 5-HT_{2C} receptors. We have also found that PSD-95 is required for ligand-induced modulation of 5-HT_{2A} receptor expression *in vivo*. We have recently demonstrated that fibroblasts obtained from RSK2 *k/o* mice show exaggerated signalling by 5-HT_{2A} receptors. Using a variety of proteomic and biochemical studies we have found that RSK2 exerts these effects via a direct phosphorylation of 5-HT_{2A} receptors at a single residue in the 3rd intracellular domain of 5-HT_{2A} receptors. Taken together these findings indicate that scaffolding proteins and kinases exert unexpectedly potent actions on serotonin receptor signalling *in vivo*.

SC15.4**Yif1B, a new protein interacting with the serotonin 5-HT_{1A} receptor, specifies its dendritic localisation in rat neurons**

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The 5-HT_{1A} receptor (5-HT_{1A}R) is a G-Protein-Coupled Receptor, localized at the plasma membrane of somas and dendrites where it plays an important role in the control of serotonergic neuron discharge. In previous studies, we demonstrated that the C-terminal moiety of the 5-HT_{1A}R plays a critical role in its dendritic targeting, but the underlying molecular mechanisms remains unknown. We have now identified Yif1B as a 5-HT_{1A}R C-tail interacting protein in a yeast two-hybrid screen, and investigated its possible contribution to 5-HT_{1A}R trafficking in neurons. 5-HT_{1A}R-Yif1B interaction was confirmed by GST pull-down experiments performed on transfected cells and brain extracts. In the rat, Western blot analysis showed that Yif1B is abundant in the brain, and immunofluorescence experiments have revealed its expression notably in serotonergic neurons of the raphe area whereas the 5-HT_{1A}R acts as a somato-dendritic autoreceptor. Furthermore, in transfected cells, the 5-HT_{1A}R co-localized partially with Yif1B in vesicles co-localized partially with ER and Golgi markers. This localization is in agreement with the known role of its yeast homologue Yif1p, in vesicular trafficking between the endoplasmic reticulum and the Golgi apparatus. Finally, in primary cultures of rat hippocampal neurons transfected with 5-HT_{1A}-eGFP, inhibition of endogenous Yif1B expression by RNA interference (siRNA) specifically prevented the targeting of the receptor to the distal part of dendrites without affecting the traffic of other receptors, such as 5-HT_{3A}, sst2A and P2X₂. All these data support the idea that Yif1B is a new 5-HT_{1A}R-interacting protein playing a key role in the receptor routing and targeting to its final dendritic localisation.

SC16.1**Serotonin pharmacology of behavior in neuregulin-1 mutant mice and BDNF mutant mice: implications for schizophrenia**

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Several candidate genes and modulators have been implicated in schizophrenia and genetically-modified mouse models for these factors have been developed. Our research in these animal models is focused on serotonergic, dopaminergic and glutamatergic regulation of behaviour. Locomotor hyperactivity, a model of psychosis, is measured using automated photocell cages and prepulse inhibition, which is disrupted in schizophrenia, is assessed using automated startle boxes. Neuregulin-1 (Nrg1) is a signalling factor involved in neurodevelopment and synaptic plasticity. Several studies have suggested an involvement of Nrg1 in schizophrenia. In mice heterozygote for the transmembrane domain Nrg1, treatment with apomorphine, amphetamine or MK-801 caused similar disruption of PPI as in wildtype (WT) controls. In one cohort, treatment with 8-OH-DPAT significantly reduced PPI and startle amplitudes in Nrg1 HET mice but not in WT controls. However, in a second cohort, a similar difference could not be replicated. Amphetamine-induced hyperactivity was similar in both groups but the effect of phencyclidine was approximately 50% reduced in Nrg1 HET mice. Brain-derived neurotrophic factor (BDNF) is involved in regulating synaptic plasticity in cortex and hippocampus and reduced BDNF expression has been implicated in schizophrenia. BDNF heterozygote mice tended to show reduced baseline PPI, which became significant after treatment with 8-OH-DPAT. Disruption of PPI after treatment with apomorphine, amphetamine or MK-801 was not significantly altered in BDNF heterozygotes. We are currently investigating locomotor hyperactivity and the effects of corticosteroid stimulation in these mice. These results will be presented.

SC16.2**Mechanisms underlying the behavioral response to chronic antidepressant treatment**

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The therapeutic effects of antidepressants require several weeks of treatment to emerge. We have developed novel animal models in which BALB/cj mice exhibit behavioral responses to chronic, but not subchronic, antidepressant treatment. In

the first model, we have shown that chronic antidepressant treatment reduces baseline anxiety- and depression-related behavior. In the second model, we have shown that chronic SSRI treatment prevents 5-HT_{1B} agonist-induced prepulse inhibition (PPI) deficits and perseverative hyperactivity. Here, we found that chronic administration of the SSRIs fluoxetine (18 mg/kg/day) and citalopram (10–30 mg/kg/day), and the tricyclic antidepressant desipramine (20 mg/kg/day), but not to the antipsychotic haloperidol (1 mg/kg/day), reduce depression-related behavior in the forced swim test (FST). Furthermore, the behavioral response to chronic fluoxetine treatment in the FST and the novelty-induced hypophagia (NIH) test of anxiety was affected neither by ablation of hippocampal progenitor cells, nor by genetic deletion of the 5-HT_{1A} receptor. We also found that the PPI deficits and perseverative hyperlocomotion induced by 5-HT_{1B} receptor activation were prevented by chronic treatment with clomipramine, but not desipramine. Additionally, these effects of 5-HT_{1B} agonists were absent in serotonin transporter knockout mice. In conclusion, we found that BALB/cj mice show a robust behavioral response to chronic SSRI treatment in the FST and NIH tests, which is not mediated by an increase in new neurons in the hippocampus, and does not require the 5-HT_{1A} receptor. We also found that the PPI disruptions and perseverative hyperlocomotion induced by 5-HT_{1B} agonists were blocked by pharmacological or genetic reduction of 5-HTT function.

SC16.3**Corticolimbic abnormalities and executive functions following SERT gene loss in mice**

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Gene variation in the serotonin transporter (SERT) is increasingly implicated in the pathophysiology of affective illness. Paralleling findings from functional human neuroimaging studies, we have recently shown that SERT knockout (KO) mice exhibit abnormalities in the neuronal organization of ventromedial prefrontal cortex (vmPFC) and basolateral amygdala (BLA). These corticolimbic abnormalities provide a candidate mechanism for the impaired stress coping and fear extinction deficits seen in SERT KO, and are hypothesized to be ontogenic in nature given evidence that SERT blockade during development mimics behavioral deficits caused by SERT gene loss. They may have wider implications for understanding the consequences for ontogenic alterations in SERT function for corticolimbic function and behavior, with implications for elucidating the etiology of neuropsychiatric diseases ranging from anxiety disorders to addiction. Research supported by the National Institute on Alcohol Abuse and Alcoholism Intramural Research Program.

SC16.4**Anorexia induced by activation of serotonin 5-HT₄ receptors is mediated by increases in CART in the nucleus accumbens**

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Anorexia nervosa is a growing concern in mental health, often inducing death. The potential neuronal deficits that may underlie abnormal inhibitions of food intake, however, remain largely unexplored. We hypothesized that anorexia may involve altered signaling events within the nucleus accumbens (NAc), a brain structure involved in reward. We found that direct stimulation of serotonin 4 receptors (5-HTR₄) in the NAc, reduces the physiological drive to eat and increases CART (cocaine- and amphetamine-regulated transcript) mRNA levels in fed and food-deprived mice. It further shows that injecting 5-HTR₄ antagonist or siRNA-mediated 5-HTR₄ knock-down into the NAc induced hyperphagia only in fed mice. Results include that 5-HTR₄ control CART mRNA expression, into the NAc, via a cAMP/PKA signaling pathway. Considering that CART may interfere to food- and drug-related rewards, we tested whether the appetite suppressant properties of 3,4-N-methylenedioxymethamphetamine (MDMA, ecstasy) involves the 5-HTR₄. Using 5-HTR₄ knock-out mice, we demonstrate that 5-HTR₄s are required for the anorectic effect of MDMA, as well as for the MDMA-induced enhancement of CART mRNA expression in the NAc. Directly injecting CART peptide or CART siRNA into the NAc reduces or increases food consumption, respectively. Finally, stimulating 5-HTR₄- and MDMA-induced anorexia were both reduced by injecting CART siRNA into the NAc. Collectively, these results demonstrate that 5-HTR₄-mediated upregulation of CART in the NAc triggers the appetite-suppressant effects of ecstasy.