Serotonin Club Satellite Meeting
Posters

SCP001

Phasic nociceptive responses in dorsal raphe serotonin neurons

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Serotonin neurons play a central role in a variety of behavioural and cognitive functions, particularly in processing aversive stimuli. It has been suggested that serotonergic dorsal raphe neurons might encode a prediction-error rule for aversive stimuli (Daw *et al.*, 2002). This hypothesis makes the prediction that serotonin neurons should be activated by noxious stimuli that are aversive or punishing. We, therefore, examined the hypothesis that noxious footshocks will phasically activate neurochemically-identified serotonin neurons in the dorsal raphe nuclei. Serotonin neurons are typically identified by their electrophysiological properties (a slow clock-like firing pattern and broad action potentials). Recent studies using juxtacellular labelling show that not all of these presumed serotonin neurons are actually 5HT-positive (Allers and Sharp, 2003). Therefore, we have combined electrophysiological recording in the dorsal raphe of anaesthetised rats (Sprague–Dawley, male, weight range from 220–480 g) and the juxtacellular labelling method to identify these neurons. During the recording nociceptive footshocks were administered, and then the individual neurons were labelled juxtacellularly, and identified using immunohistochemistry. We find that neurons can be grouped based on their combined electrophysiological and histological properties. 5HT-positive cells with characteristic clock-like firing were typically phasically activated by the footshocks. However, a second group of 5HT-positive neurons were either inhibited or showed no reaction to the footshocks – interestingly the majority of these neurons showed stereotypic bursting activity. Lastly 5HT-negative neurons (with clock-like activity and broad action potentials) showed a range of responses. These preliminary results suggest that some dorsal raphe serotonin neurons are phasically activated by nociceptive stimuli.

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SCP002

Haplotypes analyses of the serotonin transporter gene (SLC6A4) provide new interpretation for the gene *x* environment model of depression

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Since the first report of the significant interaction of gene *x* environment on depression published previously; literature is considerably contradictory in this field. To clarify this question we analyzed the interaction between serotonin transporter gene (SLC6A4) and threatening life events (TLE) on depressive phenotype. Five markers tagging the whole SLC6A4 gene (5-*HTTLPR* and 4 SNPs) were genotyped in 567 non-clinical individuals. Consistent with the Hungarian average population, life time and one year prevalence of depression in our sample were 18.9 and 7.2%, respectively. Phenotype was measured by Zung Self-rating Depression Scale and List of Threatening Life Events (TLE). We used generalized linear models to analyze single marker associations while likelihood ratio tests and score tests for haplotype analysis. Haplotype analysis revealed a significant global effect of haplotypes on ZSDS score in high TLE subgroup ($P = 0.008$) but not in the low TLE subgroup ($P = 0.117$). In the high TLE subgroup the most common haplotype was significantly associated to the highest ZSDS score ($P < 0.001$), while two less frequent haplotypes to the lowest ZSDS. These findings suggest that subjects carrying 'A' allele of rs140 700 scored lower on ZSDS independently of 5-*HTTLPR* carrier status. These data indicate a potential additional effect of the middle region of the SLC6A4 gene tagged by rs140 700 on gene function besides the promoter region that may influence the depressive phenotype. Our results demonstrate heterogeneity of individuals carrying 'S' alleles of 5-*HTTLPR* in association with high TLE providing possible explanation for the inconsistency of previous studies. We report the first evidence for the significant effects of haplotypes of the SLC6A4 gene and threatening life events on depressive phenotype. These studies were supported by the Sixth Framework Programme of the EU, LSHM-CT-2004-503474, HRF T03298/2000 and the PhD Fellowship Program of Semmelweis University, Ministry of Culture and Education, Hungary.

SCP003

5-HT in the peripheral vasculature

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The role of 5-HT as a neurotransmitter in the central nervous system is well established, as drugs that affect 5-HT concentration (e.g. Prozac®) are widely used to treat conditions such as depression, anxiety and obesity. However, the role of 5-HT in the peripheral non-pulmonary cardiovascular system has been a subject of controversy (Watts, 2005). In the periphery, platelets are the largest 5-HT store site and may function as a buffer, keeping the free circulating 5-HT in low levels (Nilsson *et al.*, 1985; Vanhoutte, 1991; Brenner *et al.*, 2007). Indeed, platelet 5-HT uptake is decreased with age and in hypertension. SERT is the main mechanism for platelet regulation of extracellular and intracellular 5-HT concentrations (Amstein *et al.*, 1991; Brenner *et al.*, 2007). Our laboratory first demonstrated that peripheral arteries have a functional SERT and, therefore are also able to control intracellular and extracellular concentrations of 5-HT (Ni *et al.*, 2004). Compared to arteries, less attention is given to the role of veins in the regulation of vascular tone. We hypothesized that veins, as arteries, have the ability to synthesize, uptake and metabolize 5-HT. Surprisingly, measurements by high liquid performance chromatography (HPLC) revealed that after exposure to extracellular 5-HT, 5-HT was accumulated in a larger scale in rat vena cava (16.4 ± 1.1 ng/mg protein, $n = 10$) than in rat aorta (6.4 ± 0.6 ng/mg protein, $n = 24$). Whereas 5-HT uptake in arteries was SERT-dependent, it was SERT-independent in veins, since fluvoxamine did not inhibit 5-HT accumulation in vena cava (14.5 ± 1.4 ng/mg protein, $n = 6$, $P > 0.05$) as it did in aorta (3.9 ± 0.3 ng/mg protein, $n = 6$, $P < 0.05$). The 5-HT intermediary 5-hydroxytryptophan (5-HTP) was detected by HPLC in vena cava and in aorta after incubation with the precursor tryptophan. Inhibition of monoamine oxidase, the enzyme responsible for 5-HT degradation significantly decreased the levels of the 5-HT metabolite 5-hydroxyindole acetic acid (5-HIAA) accumulation in vena cava (4.3 ± 0.5 ng/mg protein, $n = 7$ to 0.3 ± 0.06 ng/mg protein, $n = 5$ and in aorta (from 3.6 0± 0.4 ng/mg protein, $n = 12$ to 0.5 ± 0.1 ng/mg protein, $n = 16$). MAO and SERT were expressed in aorta and vena cava. These data indicate that veins and arteries are able to synthesize, uptake and metabolize 5-HT. Our data also suggest that in addition to platelets, arteries and veins may function as a reservoir for 5-HT in the cardiovascular system. It has been shown that in pulmonary arteries intracellular 5-HT mediates smooth muscle cell proliferation (Marcos *et al.*, 2003), suggesting that once inside the cell, 5-HT function is not terminated and may control the activity of other proteins. These unique findings showing the differences in 5-HT handling between veins and arteries may represent alternative avenues for targeting the 5-HT system in the peripheral circulation for controlling vascular tone.

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SCP004

The brain serotonin 1A receptors in hibernation

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Serotonin is the central neurotransmitter that is implicated in the regulation of hibernation; the striking adaptive behavior represents the combination of reversible prolonged sleep-like state and deep hypothermia. The brain serotonin 1A receptors (5-HT_{1A}) play the key role in the autoregulation of serotonin neurons activity and neurotransmission. However there was no data, neither on the structure of gene encoding 5-HT_{1A} receptor in the brain of hibernating animals nor on the expression of this gene in diverse stages of the sleep-awake cycle. We have determined the sequence of the ground squirrel (*Spermophilus undulatus*) 5-HT_{1A} receptor gene fragment and it is compared it with the known sequences of rat, mouse and human 5-HT_{1A} receptor genes and other serotonin receptors. The significant percent of homology between the obtained sequence and the sequences of 5-HT_{1A} receptor gene of other species was revealed. However the insertion of three nucleotides non-hibernators 5-HT_1A receptor genes was found in the area encoding third intracellular loop of the ground squirrel 5-HT_{1A} receptor gene. We have also found significant structure-specific changes in 5-HT_{1A} mRNA level in the diverse stages of the sleep-awake cycle. The increase of 5-HT_{1A} receptor gene expression that was revealed in the hippocampus occurs directly before entering into hibernation and notwithstanding drastically decreased body temperature in hibernating animals, 5-HT_{1A} receptor mRNA level in all examined brain regions remained relatively high. These data prove the essential role of this serotonin receptor subtype in the regulation of hibernation.

SCP005

5-HTTLPR s allele is not associated with coping styles inspite of its association with subclinical depression

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The 5-HTTLPR polymorphism has been found to be associated with both threshold and subthreshold forms of depression. However, the role of the individual psychological factors on the association of the s allele with the emergence of depression has been only scarcely studied. Coping with stress and stress resiliency is well known to play an important role in the emergence of affective disorders, and it has been demonstrated previously that 5-HTTLPR mediates the effects of stressful life events in the development of major depression. Therefore it can be expected that the 5-HTTLPR polymorphism of the serotonin transporter gene is associated with ways of coping with stressful life events. The aim of our study was to investigate the association of the 5-HTTLPR s allele and coping styles in a psychiatrically healthy population. 169 psychiatrically healthy females participated in our study. All participants completed the Zung self-rating depression scale (ZSDS) and the preferential ways of coping with anxiety scale, an instrument with eight subscales to assess characteristic styles of coping with stress and anxiety provoking life events. Subjects carrying the s allele had a significantly higher score on the ZSDS compared with subjects not carrying the s allele. However, there was no significant difference between the two groups on any of the eight subscales of the coping scale. Despite its

association with depressive traits, 5-HTTLPR seems to be independent of coping styles according to the results of our study. This suggests that the role of the 5-HTTLPR polymorphism in the manifestation of depression and depressive traits is not modulated by an influence on coping styles and processes.
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SCP006

Functional investigations of tegaserod in human isolated proximal and distal coronary arteries

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The 5-HT_4 receptor agonist, tegaserod, a gastrointestinal prokinetic agent, is known to also display binding affinity for human recombinant 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors (Beattie *et al.*, 2004). In addition, tegaserod may act as a competitive antagonist at non-human primate and human vascular 5-HT_{1B} receptors (Weber *et al.*, 2006). In view of concerns about clinical coronary ischemic events in patients with cardiovascular risk factors who were treated with tegaserod, we assessed the potential effect of tegaserod on healthy and diseased isolated human coronary arteries, and compared the results with those obtained with sumatriptan. Segments of the right epicardial and distal coronary artery were obtained from 20 heart-beating organ donors (10 males, 10 females, 17–63 years) who died of non-cardiac causes less than 24 hours before the tissue was taken to the laboratory. Segments of the proximal and distal coronary artery were mounted in 15 ml organ baths and Mulvany myographs, respectively. Proximal segments were divided between healthy and diseased according to the relaxant response to substance P (1 nM) after precontraction with 1 μM $PGF_{2\alpha}$, in distal segments such a division was not made in view of the reproducibly high response to substance P in this preparation. To assess contractile properties of tegaserod, concentration-response curves to tegaserod and sumatriptan were constructed. Further, concentration-response curves to sumatriptan in the absence or presence of tegaserod were obtained to assess potential antagonism of the contractions to sumatriptan by tegaserod. In proximal coronary artery segments, sumatriptan induced a concentration-dependent contraction (E_{max} 17 ± 9% of contraction to 100 mM KCl, pEC_{50} 5.58 ± 0.23), while tegaserod induced a contraction only at concentrations of 10 μM or higher (2 ± 1% at 10 μM, 20 ± 4% at 100 μM). There was no difference between results obtained in healthy or diseased vessel segments. In distal coronary artery segments, sumatriptan induced greater contraction than in proximal coronary arteries (E_{max} 58 ± 14%, pEC_{50} 6.38 ± 0.09), while the contractions to tegaserod were significantly lower than in proximal coronary arteries (2 ±2% at 10 μM, 0.1 ± 2% at 100 μM). In antagonist experiments, tegaserod (1 μM) did not induce a significant shift of the concentration-response curve to sumatriptan in either proximal or distal coronary artery segments. In summary, sumatriptan induced concentration-dependent contractions in human isolated coronary arteries, which were similar to our previous observations (MaassenVanDenBrink *et al.*, 1998). Tegaserod contracted healthy or diseased human proximal isolated coronary arteries only at concentrations of 10 μM (1000 times C_{max} of the 6 mg b.i.d. therapeutic dose) or higher. In distal coronary arteries this contractile activity was generally absent. At a concentration of 1 μM, tegaserod did not display any antagonist properties at 5-HT_{1B} receptors.

References:

Beattie DT *et al.* Br J Pharmacol. 2004; 143: 549–560.
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SCP007

Distribution of 5-HT receptors and interacting proteins in the human colonic mucosa, longitudinal and circular muscle layers

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The aim of this study was to examine the distribution of 5-HT receptors in the human colon. 5-HT induces desensitization of the circular muscle and since this is facilitated by GPCR receptor kinases (GRKs) and other proteins, we also examined their distribution. Five different human sigmoid colon samples were dissected into three separate layers (mucosa, longitudinal and circular muscles). 5-HT induced concentration-related relaxations of human colonic circular muscle with an EC_{50} value of 136 ± 24 nM (n = 5). RNA was extracted only from patients with functionally viable circular muscle samples. The RNA samples were amplified by RT-PCR and qualitative expression was determined via agarose gel electrophoresis. Transcripts of the 5-HT receptors were detected in all layers. All 5-HT_7 receptor splice variants were expressed in all tissues as was the 5-HT_{2B} receptor. 5-HT_4a,b,c and n splice variants were also expressed in all tissues. 5-HT_{4d} was in all tissues in three out of five samples and lower amounts of 5-HT_{4g} and 5-HT_{4i} were expressed (less intense bands) in comparison to the other splice variants. The 5-HT_{2A} receptor was seen predominantly in the circular muscle of the colon (four out of five samples). Surprisingly, we detected only one transcript of SERT in the muscle layers which suggests that the role of GRKs in the colon may be significant. Variation was seen in GRK expression with GRK2 and three predominantly expressed in the mucosa, and circular muscle while GRK5 (this was also common in the circular muscle) and six were found more commonly in the longitudinal muscle. Several PDZ proteins (e.g. SNX27, NERF, LIN7A,B and C) that may be involved in 5-HT receptor trafficking were also detected in all regions of the sigmoid colon. The 5-HT_{3A} subunit was expressed in all tissues whereas the 5-HT_{3E} subunit was mainly found in the mucosa layer while the 5-HT_{3B} subunit was more commonly found in the muscle layers. RIC-3 which is involved in transporting 5-HT_3 receptor subunits is expressed less in the mucosa compared to the muscle layers. These results show that there is variation in 5-HT receptor distribution within the sigmoid colon and form the basis for future immunohistochemical studies to study 5-HT receptor distribution at a protein level.

SCP008

Modulation of 5-HT responses through porcine atrial and ventricular 5-HT_4 receptors by phosphodiesterases PDE3 and PDE4; plausible ontogenic changes in ventricle

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Responses to 5-HT, mediated through 5-HT_4 receptors, are facilitated by phosphodiesterase (PDE) inhibition in human and porcine myocardium (Kaumann and Levy, 2006). 5-HT-evoked increases in human atrial contractility tend to fade (Saunders and Kaumann, 1992) and non-selective inhibitor 3-isobutyl-1- methylxanthine abolishes fade of 5-HT-evoked inotropic response in porcine atrial trabeculae (DeMaeyer *et al.*, 2006) but the PDE isoenzymes involved have not been characterized. We investigated the effects of the the PDE3-selective inhibitor cilostamide (300 nM) and PDE4 inhibitor rolipram (1 μM) on the fade to the inotropic responses to 5-HT (10 μM) in left atrial trabeculae, as well as the ability of these PDE inhibitors to uncover inotropic 5-HT responses in right ventricular trabeculae, obtained from adolescent pigs (2 months either sex). Pigs were anaesthetised with pentobarbital (70 mg/kg), the hearts rapidly removed and tissues dissected and paced at 1 Hz at 37°C. Atrial 5-HT responses faded continuously and disappeared completely (n = 6) after 30 min. Fade of the 5-HT response was completely prevented by concomitant cilostamide + rolipram (104 ± 13% n = 5). Cilostamide and rolipram preserved the 5-HT response by 52 ± 13% (n = 6) and 27 ± 4% (n = 5) per cent respectively by the 30th minute. Similar results were obtained from left atria of newborn piglets. Cilostamide but not rolipram tended to uncover ventricular 5-HT responses from newborn piglets (P = 0.045 n = 9). Neither cilostamide nor rolipram revealed 5-HT responses in adolescents. Concomittant cilostamide + rolipram unconcealed 5-HT responses in both piglets (P = 0.025 n = 8) and adolescent (P = 0.006 n = 7). We conclude that both PDE3 and PDE4 blunt atrial 5-HT responses. In newborn piglets PDE3 appears to selectively prevent 5-HT responses while in the ventricle of adolescents PDE3 and PDE4, acting in concert, abolish the 5-HT response.

References:

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SCP009

5-HT turnover – the effects of 5-HT_{1A} and 5-HT transporter genotypes

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There is substantial inter-individual variability in the response to anti-psychotic drug treatment. This is likely to have a genetic component; we have demonstrated associations of both the 5-HT_{1A} C-1019G polymorphism and the 5-HTT short/long (S/L) polymorphism on negative symptom response to anti-psychotic treatment of first-episode patients. Therefore these polymorphisms are highly functional in clinical response as well as in the activity of their respective gene products, which is already well established. We postulate that variability in symptom response is mediated by differences in neurotransmitter turnover brought about by these genotypes. We have genotyped a striatal cohort of 26 individuals and a cortical cohort of 60 individuals for both the 5-HT_{1A} and the 5-HTT polymorphisms and determined the association between genotype and rate of 5-HT turnover in both brain regions. We found no significant effect of the 5-HT_{1A} polymorphism on changes in 5-HT turnover for either cohort. A significant association of the 5-HTT S/L promoter polymorphism was found in both the striatal and the cortical samples. SS individuals showed the highest turnover for the striatal cohort, while conversely SS individuals from the cortical cohort showed the lowest turnover. Cofactor analyses and stepwise linear regressions showed no significant effect of sex, age, post-mortem delay, disease status or treatment status in either cohort. These results demonstrate a significant effect of the 5-HTT S/L promoter polymorphism on the rate of 5-HT turnover in both the striatum and the cortex. However, the direction of the effect differs between the regions. The striatal effect can be interpreted as greater turnover of transmitter being a consequence of lower uptake activity associated with the S allele in the synapse. The cortical result may be a consequence of the lower density of transporter in the cortex, resulting in turnover reflecting neuronal activity under the control of somatodendritic transporter in the raphe nucleus.

SCP010

Ribosomal S6 kinase 2 regulates 5-hydroxytryptamine 2A receptor signaling via phosphorylation of serine 314 within the third intracellular loop

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Given the pivotal role G protein-coupled receptors (GPCR) play in physiological responses, various mechanisms to ensure the tight regulation of GPCR signaling have evolved. Classically, agonist exposure attenuates receptor responsiveness (i.e., desensitization) through multiple mechanisms, including receptor phosphorylation by second messenger-activated (PKA and PKC) and/or G protein-coupled receptor (GRKs) protein kinases, receptor internalization, and receptor down-regulation. We recently discovered that ribosomal S6 kinase 2 (RSK2), a downstream effector of the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade, interacts with the 5-hydroxytryptamine 2A (5-HT_{2A}) receptor and exerts a 'tonic brake' on agonist-mediated receptor signaling (Sheffler *et al.*, 2006). Importantly, genetic deletion of RSK2 potentiates 5-HT_{2A} signaling in fibroblasts without affecting its sub-cellular distribution, global G protein function, and expression of serotonergic pathway genes. Furthermore, re-introduction of wild-type RSK2 – but not 'kinase-dead' RSK2 (K451A) – into fibroblasts restores 5-HT_{2A} signaling. Therefore we hypothesized that RSK2 regulates 5-HT_{2A} receptor signaling through direct receptor phosphorylation. Initially, we used *in vitro* kinase

experiments incorporating either purified, full-length receptor or third intracellular loop (i3 loop) peptides to demonstrate that RSK2 directly phosphorylates the 5-HT_{2A} receptor within the i3 loop. Subsequent mass spectrometry, site-directed mutagenesis, and phospho-specific antibody immunoblotting studies using either purified full-length receptors or i3 loop peptides confirmed that RSK2 phosphorylates the 5-HT_{2A} receptor at serine 314 (S314) within the i3 loop. We next sought to address whether RSK2 requires S314 phosphorylation for regulating 5-HT_{2A} receptor signaling in cultured fibroblasts. Towards this end, we stimulated phosphorylation-deficient 5-HT_{2A} (5-HT_{2A}-S314A) receptors with multiple agonists and measured agonist efficacy via second messenger-based assays. In fibroblasts expressing 5-HT_{2A}-S314A receptors, agonist activation elicited significantly greater levels of inositol phosphates and intracellular Ca^{2+} when compared to wild-type 5-HT_{2A} receptor-expressing fibroblasts. Importantly, these studies were performed in the presence of endogenous RSK2, suggesting that the S314A mutation renders the 5-HT_{2A} receptor RSK2-insensitive. Taken together, these data demonstrate for the first time that RSK2 regulates 5-HT_{2A} receptor signaling via phosphorylation of S314 within the third intracellular loop. These findings are significant because they provide direct evidence for a novel GPCR regulatory pathway wherein downstream members of the ERK/MAPK cascade directly phosphorylate a GPCR (Supported by RO1MH57635, RO1MH61887, the Case Cancer Center, and the NIMH Psychoactive Drug Screening Program to BLR).

Reference:

Sheffler DJ *et al.* PNAS 2006; 103 (12): 4717–4722.

SCP011

Cortico-striatal cAMP-PDE4 signaling in stereotypic deer mice and response to chronic fluoxetine versus desipramine treatment

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Deer mice (*Peromyscus maniculatus bairdii*) develop distinct stereotypic behaviours that respond selectively to serotonin (5HT, eg. fluoxetine) but not noradrenergic reuptake inhibitors (e.g. desipramine; Korff *et al.*, 2008). Obsessive compulsive disorder (OCD) involves changes in cortical-striatal circuits (Whiteside *et al.*, 2004), while cyclic adenosine monophosphate (cAMP) signaling has been implicated in the disorder (Perez *et al.*, 2000). Phosphodiesterase-4 (PDE4), which catalyzes the hydrolysis of cAMP, is also implicated in depression- and anxiety-like behaviours (Hudson *et al.*, 1993). We separated deer mice (either sex, 8 weeks of age or older) into high (n = 19), low (n = 18) and non-stereotypic (n = 12) phenotypes. The degree of stereotypy was correlated with basal levels of cAMP and PDE4 activity in the prefrontal cortex and striatum, as determined by radiometric assay. This correlation was then also analysed in the presence and absence of either fluoxetine or desipramine treatment (both 20 mg/kg ip x 3 weeks; n = 6–10). All data were analysed using one-way ANOVA and Tukey post-hoc tests. Striatal and cortical cAMP was significantly elevated in stereotypic compared to non-stereotypic mice. Higher stereotypy was significantly associated with higher cAMP and lower PDE4 activity. In both brain regions, data concurred that PDE4 activity diminishes as stereotypy increases. Fluoxetine or desipramine treatment significantly decreased cortical cAMP in both low and high stereotypic mice, together with a paradoxical further decrease in PDE4 activity. Data in striatal tissue proved less definitive. We conclude that, although a selective response to fluoxetine was not demonstrated, altered cortico-striatal cAMP-PDE4 signaling is evident in stereotypic deer mice and as such may represent therapeutic targets for anti-depressant treatment of disorders characterised by heightened stereotypy, such as OCD.

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Perez *et al.* Am J Psychiatry 2000; 157: 284–286.
Whiteside *et al.* Psychiatry Res Neuroimaging 2004; 132: 69–79.

SCP012

Gastrointestinal rhythmic movement regulates human mood

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Patients with depression or other mental illness are always accompanied with gastrointestinal rhythmic disorder. 2% serotonin (5-hydroxytryptamine: 5-HT) is distributed in central nervous system (CNS), while over 90% is distributed in the enteric nervous system (ENS). We have reported that endogenous 5-HT receptors are involved in pacemaker activity of mouse gastrointestinal tract. Through a 10-year trace investigation on healthy people in Japan, in which 30 000 suicides were reported annually, we now show that certain Chinese traditional exercise, which was reported to regulate GI tract rhythmic movement, is helpful in reducing stress, anxiety and in improving mood. Since 5-HT is implicated in numerous CNS rhythms as well as in mood control, up-regulation of 5-HT is supposed to be a possible mechanism. Our results suggest a novel approach on brain-gut axis as a supplemental therapy for patients as well as precaution for healthy people against depression, irritable bowel syndrome or other mental disorders.

Reference:

Fukudo *et al.* Gut. 2006; 55: 146–148.

SCP013

Inflammation-induced increases in the release and uptake of serotonin in mouse colon

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Serotonin (5-HT) containing enterochromaffin (EC) cells of the intestine detect chemical and mechanical stimuli in the lumen and respond by releasing 5-HT on to afferent nerve terminals. Recent electrochemical studies in healthy mucosa have shown that the real-time release of 5-HT is a dynamic and highly regulated process, but how this might change in disease is unknown. Our aim was to characterize real-time uptake and release of 5-HT in a mouse model of colitis and compare it with ELISA measurements of 5-HT. Real-time electrochemical methods were coupled with an ELISA assay to determine the effect on 5-HT availability of a mouse model of inflammation (5% w/v dextran sodium sulphate (DSS) induced colitis). Peak and steady state (SS) 5-HT concentrations (calculated from the oxidation current at +400 mV; amperometry mode) were measured with our without the serotonin reuptake transporter blocker fluoxetine (1 μM) in control and DSS-treated mice. Paired and unpaired data were compared with a one way ANOVA ($P < 0.05$). In mouse colon, SS release of 5-HT was 1.9 ± 0.6 μM (n = 9) and compression-evoked release was 7.1 ± 2.5 μM (n = 9). In DSS treated mice, the release of 5-HT was significantly increased (SS: 3.4 ± 0.6 μM; peak: 14.7 ± 3.0 μM; n = 11). In control mice, fluoxetine significantly increased peak (9.9 ± μM) but not SS release (2.6 ± 0.4 μM), while in DSS mice both were significantly increased (SS: 7.3 ± 1.2 μM; peak: 23.4 ± 4.1 μM). The effects of fluoxetine in DSS mice were greater than in control. ELISA assays supported these data, showing an increase in 5-HT release detected from inflamed colon (n = 5) compared to control (n = 5) in unstimulated or mechanically stimulated preparations and with or without fluoxetine. The release and uptake of 5-HT from the EC cells of the mouse colon are increased during DSS colitis. Our electrochemical data show that both peak and steady state levels are increased and these changes are mirrored by the ELISA data. In addition, the localised 5-HT concentrations at the site of release, measured using amperometry, are significantly higher than those reported using ELISA techniques. Overall, these data show that during colitis, 5-HT availability will be increased. These raised concentrations may substantially alter the activation or desensitisation of serotonin receptors on afferent nerve terminals.

SCP014

Are 5-HT evoked arrhythmias, mediated through a small 5-HT4 receptor population, facilitated by tight coupling to L-type Ca^{2+} channels?

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5-HT, noradrenaline and adrenaline cause arrhythmias through human atrial 5-HT4 receptors, β_1 and β_2-adrenoceptors (β_1AR, β_2AR), possibly related to the initiation of atrial fibrillation (AF). Although the density of human atrial 5-HT4 receptors is at least 10-fold and 5-fold lower than the density of β_1AR and β_2AR, they mediate a maximal increase of L-type Ca^{2+} current ($I_{Ca\text{-}L}$) equivalent to 90% of the maximum effects through either β_1AR or β_2AR. This evidence suggests that activation of 5-HT4 receptors sends considerably more efficient and tighter signals to Ca^{2+} channels than activation of β_1AR or β_2AR. AF reduces the $I_{Ca\text{-}L}$ response through the three receptors and forskolin by 1/3. To compare the dependence of Ca^{2+} channel activity on receptor-mediated cAMP → PKA activities we titrated the agonist-induced increase in $I_{Ca\text{-}L}$ as a function of PKA by using the PKA inhibitor Rp-8-Br-cAMP. $I_{Ca\text{-}L}$ was maximally increased by 100 μM of each (-)-noradrenaline through β_1AR, (-)-adrenaline through β_2AR and 5-HT through 5-HT4 receptors. Rp-8-Br-cAMP antagonized the $I_{Ca\text{-}L}$ responses of the three agonists. The concentrations of Rp-8-Br-cAMP to half inhibit agonist-induced E_{max} of the $I_{Ca\text{-}L}$ response were ~500-fold and higher for (-)-noradrenaline- and (-)-adrenaline responses than for 5-HT responses, suggesting that three orders of magnitude lower active PKA levels suffice to phosphorylate Ca^{2+} channels through 5-HT4 receptors compared to β_1AR and β_2AR receptors. In atrial myocytes obtained from AF patients ~1000-fold lower PKA concentrations sufficed to produce matching increases of $I_{Ca\text{-}L}$ by 5-HT compared to (-)-noradrenaline and (-)-adrenaline. We suggest that in order to induce matching $I_{Ca\text{-}L}$ responses, stimulation of 5-HT4 receptors leads to activation of ~1000-fold less PKA than PKA activated after stimulation of β_1AR and β_2AR and that this relationship is hardly changed in AF. Our results suggest a remarkably tight coupling of 5-HT4 receptors to Ca^{2+} channels via PKA-catalysed phosphorylation compared to β_1AR and β_2AR even in AF, which may facilitate the initiation of arrhythmias despite the low 5-HT4 receptor density.

SCP015

Organic cation transporter-3: a target for alcohol?

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Alcoholism is a widespread but poorly understood psychiatric disorder. Emotional and behavioral responses to alcohol have been attributed to ethanol increasing the concentration of serotonin (5HT) in brain, which could be explained by an ability of alcohol to block the serotonin transporter (SERT). However, our evidence that ethanol potently inhibits 5HT clearance in hippocampus of SERT-knockout mice suggests that this is not the case (Daws *et al.*, 2006). A role for the neuronal organic cation transporter (OCT3) as an alternative 5HT uptake mechanism has recently been described. Using SERT knockout mice, which lack (−/−), and heterozygotes, that have 50% fewer SERTs (+/−) than wildtype (+/+) mice, we showed that OCT3 mRNA and protein expression is increased in SERT mutant mice. Moreover, this corresponded to greater inhibition of 5HT clearance after blockade of OCT with corticosterone in SERT +/− and −/− mice (Baganz *et al.*, 2007). Because the effects of corticosterone and ethanol on 5HT clearance were similar in these SERT genotypes, we reasoned that OCT3 could be a site of action for ethanol. Our finding in wildtype, but not SERT-deficient, mice that co-administration of the OCT blocker, corticosterone, and the selective serotonin reuptake inhibitor (SSRI), fluvoxamine, more robustly inhibited 5HT clearance than either drug alone supports this premise. Consistent with corticosterone and ethanol having a common action at OCT3, in no genotype did corticosterone given with ethanol produce a greater net ability to inhibit 5HT clearance compared to either drug given separately. Collectively, these data suggest that OCT3 mediates, at least in part, the effect of ethanol to inhibit 5HT clearance. These findings are intriguing given evidence that humans expressing fewer SERTs are predisposed to alcoholism and are resistant to SSRI treatment. OCT3 may therefore be a novel target for treatment of alcoholism and addiction-related disorders, especially for individuals who respond poorly to currently available therapies.

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SCP016

Sleep effects of citalopram in control and MDMA-pretreated rats

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Selective serotonin reuptake inhibitors (SSRIs) such as citalopram, are extensively used for the treatment of depression and anxiety disorders. 3,4-methylenedioxy-methamphetamine (MDMA), the active ingredient of ecstasy may cause long term neurotoxic effects specific to the central serotonergic systems. The aim of this study was to investigate the effects of citalopram using sleep analysis after serotonergic damage caused by MDMA treatment. Seven-week-old male Dark Agouti rats, weighing 230–300 g, were randomly divided into two groups for pre-treatment with MDMA (15 mg/kg, i.p.) or vehicle (i.p. injection of 0.9% NaCl). Fifteen days before the acute challenge, animals were chronically equipped with EEG and electromyogram (EMG) electrodes. Twenty-one days after MDMA or saline treatment animals of both groups received randomly citalopram (citalopram hydrobromide) or vehicle (saline) and EEG, EMG and motor activity were recorded for 24 h after treatment performed at light onset. Active- and passive wake (AW, PW), light and deep slow wave sleep (SWS-1, SWS-2) and REM were studied. Cosinor analysis was used for investigation of the effects of MDMA pretreatment and citalopram treatment on circadian rhythm. The serotonergic damage caused by MDMA pretreatment was demonstrated by changes of serotonin transporter (5-HTT) fiber densities in different brain areas studied by immunohistochemistry. Our results show that 5-HTT densities were significantly decreased in several brain areas. MDMA pre-treatment significantly increased the amount of REM sleep together with the reduction of REM latency in the first two hours of the passive phase (lights on). Citalopram decreased REM sleep amounts within the first two hours after administration and increased REM sleep latency in both vehicle- and MDMA-pre-treated animals. In the third hour, citalopram decreased REM sleep in control pre-treated rats, but this effect was diminished by MDMA-pre-treatment. Cosinor analysis showed a significant decrease in the mesor of REM sleep after citalopram in both vehicle- and MDMA-pre-treated rats. Other sleep stages were not or only slightly affected by the drugs. We can conclude that, the SSRI citalopram significantly reduced REM sleep even if the serotonergic system was partially damaged, but the effects of citalopram were reduced by MDMA pre-treatment.

SCP017

Mechanism of action of triple-monoamine uptake inhibitors on the serotonergic system

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The effects of triple-acting reuptake inhibitors on 5-HT levels were measured by microdialysis in the rat prefrontal cortex (PFC) and hippocampus (Hipp) following the administration of various constructed dual and triple acting reuptake inhibitors. The citalopram (5 mg/kg) induced increase in 5-HT levels in the PFC was markedly reduced by a pre-treatment with desipramine, methylphenidate or GBR12 909. However, the desipramine pre-treatment did not significantly modulate the low or medium 1 and 2.5 mg/kg doses of citalopram. The citalopram enhancement of 5-HT levels in the Hipp was not significantly influenced by desipramine and only moderately enhanced by adjunctive methylphenidate treatment. The combination of venlafaxine with a GBR12 909 pretreatment caused also a marked reduction of PFC 5-HT levels compared to the effects induced by venlafaxine (10 mg/kg) alone. Desipramine, methylphenidate or GBR12 909 did not *per se* influence the levels of 5-HT in the Hipp or the PFC. The extracellular levels of PFC NA were only marginally affected by treatments with the combined reuptake inhibitors compared to the effects induced by methylphenidate or venlafaxine alone. The α_2 antagonist idaxozan produced a minor reduction of the desipramine suppression on 5-HT levels indicating that α_2 adrenoceptors are less important for mediating the desipramine effect. However, the effects of combined administration of the DA/NA reuptake inhibitors with the 5-HT reuptake inhibitors (citalopram and venlafaxine) on attenuation of 5-HT efflux were completely reversed by a pre-treatment with the 5-HT_{1A} receptor antagonist WAY 100 635 (0.1 mg/kg). The hippocampal and PFC 5-HT levels are thus controlled by different mechanisms of action.

SCP018

Peripheral serotonin and the control of food intake

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Brain serotonin (5-HT) is involved in the regulation of emotions, circadian rhythm and food intake. Pharmacological studies have also suggested a satiating effect of peripheral 5-HT. Recently, two isoforms of the tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT synthesis, were detected (Walther *et al.*, 2003). TPH2 seems to be pre-dominantly expressed in the brainstem, whereas TPH1 appears in the gut, pineal gland, spleen, and thymus. In TPH1 deficient mice (*Tph1*-/-) relatively normal 5-HT levels were detected in the brain, whereas in the periphery low 5-HT levels were found (Walther *et al.*, 2003). Therefore, these mice seem to be a useful tool to further elucidate the role of peripheral 5-HT in satiety. Initially, food intake, body weight and activity of *Tph1*-/- mice were recorded. In order to distinguish between central and peripheral effects on food intake 5-HT was administered systemically, since it is unable to pass the blood-brain-barrier. Fenfluramine (i.p.), a 5-HT_{2C} receptor agonist and 5-HT releaser, was used as a positive control because of its primarily centrally mediated satiating effect. *Tph1*-/- mice exhibited higher body weight, probably caused by increased food intake but not due to lower activity. The satiating effect of systemically administered 5-HT was more prominent in *Tph1*-/- mice, whereas fenfluramine induced hypophagia likewise in *Tph1*-/- and in control mice. The increase in food intake and body weight of *Tph1*-/- mice underlines the relevance of peripheral serotonin satiety mechanisms. Moreover, the pronounced effect of 5-HT in food intake of *Tph1*-/- mice argues for adaptive changes of the peripheral 5-HT receptors. The data further suggest a relatively independent action of peripheral and central 5-HT with regard to the control of feeding and satiety.

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SCP019

Hazards in the identification of serotonin: historical note

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Serotonin first made its appearance at the Cleveland Clinic in January, 1948 as a crystalline complex. The elemental analysis (C H N) of the crystals indicated five nitrogen and two oxygen atoms, one of the oxygen attributable to 'water of crystallization' that was required to rationalize the analytical values. This degree of complexity did not allow identification of structure with the limited quantity of material at hand. In a later study, at Columbia in New York City, I showed that the crystals contained. creatinine sulfate, leaving the pharmacologically active substance with two nitrogen and two oxygen. Good evidence was available for assigning one oxygen to the five position of the indole moiety. The other oxygen seemed most likely to be located in the side chain as found in epinephrine and norepinephrine. Alternatively it could also be attributed to the 'water of crystallization'. Furthermore, there was strong evidence that the side chain was not a primary amine because the reaction with ninhydrin-sodium acetate was negative whereas a purple or dark red color was generally obtained with primary amines. When serotonin picrate was isolated, the elemental analysis could only be rationalized by again postulating a molecule of 'water of crystallization' despite the effort to dry the sample thoroughly. Overcoming these ambiguities was hazardous: an incorrect assignment of structure could not be corrected. Fortuneately, the proposed structure, 5-hydroxytryptamine, proved to be correct and this quickly led to organic synthesis and ready availability of material for pharmacological and other biological studies.

SCP020

Effects of a 5-HT_6 receptor antagonist, SB-271046, on fear motivated learning and memory

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Serotonin$_6$ (5-HT_6) receptors are primarily located in central nervous system where they are implicated in cognition, psychosis and possibly nociception (Woolley *et al.* 2004). The role of 5-HT_6 receptors in cognition is supported by their high abundance in brain areas involved in learning and memory such as the hippocampus, nucleus accumbens and striatum. Furthermore, selective 5-HT_6 receptor antagonists, such as SB-271 046, enhance cognition in several behavioural paradigms, such as novel object discrimination and Morris water maze (Woolley *et al.*, 2004). This study determined the role of 5-HT_6 receptors in a hippocampal-dependent cognitive task by testing the effects of SB-271 046 on acquisition and consolidation in contextual fear conditioning (CFC). Adult male Lister Hooded rats (250–300 g) were placed in the light side of a two chamber CFC apparatus (30s) and received either 0 or 3 mild footshocks (0.4 mA, 1s, US) immediately after a 5s light and tone (89 dB) cue (CS) on entry into the dark compartment. SB-271046 (10 mg/kg, i.p.) or vehicle (3 ml/kg, i.p.) was administered 30 min prior to or immediately after CFC training. Rats were re-placed directly in dark chamber (5 min) on the next four consecutive days and the time spent freezing recorded. SB-271046 only attenuated the CFC-induced freezing behaviour seen 24 h after the US-CS trial (by 53% from that in vehicle treated controls $P < 0.05$ Tukeys test after ANOVA) when given before CFC training but not when administered post-training (24% NS). The data suggest that pre-administration of SB-271 046 caused a dissociation between the context and the aversive stimuli in CFC, as it was not observed following post-training administration. This effect could result from an anti-nociceptive or anxiolytic effect of 5-HT_6 receptor blockade (Finn *et al.*, 2007) during CFC training rather than any direct impairment of memory in this paradigm. Future studies need to determine the effects of SB-271 046 on a pharmacological-induced memory deficit in this behavioural paradigm.

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SCP021

5-HT7 receptor activation inhibits mechanical allodynia secondary to capsaicin sensitization in mice

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Subplantar capsaicin injection produced delayed secondary allodynia resulting from sensitization of spinal cord neurons. On this basis, capsaicin sensitization represents a useful model for correlative studies in pain conditions involving central sensitization, such as neuropathic pain. Serotonin (5-HT) has been demonstrated to play a role in the modulation of nociceptive processes, but the complete repertoire of receptors involved in such an analgesic effect has not been fully investigated. This work aimed to evaluate the potential role of 5-HT_7 receptor in the capsaicin model. First, we examined the effect of 5-HT_7 receptor agonists in modulating capsaicin-

induced mechanical allodynia in mice. The binding profile and intrinsic efficacy *in vitro* of 5-HT$_7$ receptor agonists used were also evaluated. Our results revealed that systemic administration of 5-HT$_7$ receptor agonists exerted a clear-cut dose-dependent anti-allodynic effect that was reversed by 5-HT$_7$ receptor antagonists. In contrast, a dose-dependent promotion of mechanical allodynia was observed when 5-HT$_7$ receptor antagonists were administered. The order of anti-allodynic efficacy of agonists correlated with their *in vitro* efficacy stimulating cAMP formation in cells stably expressing the human 5-HT$_7$ receptor. These findings suggest that activation of 5-HT$_7$ receptors exerts an inhibitory role in the control of nociception involving sensitizing stimuli and point to a new potential therapeutic use of 5-HT$_7$ receptor agonists in the field of analgesia.

SCP022

5-HT7 receptor agonists inhibit neuropathic pain in mice

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Patients with neuropathic pain often suffer from spontaneous pain, allodynia (pain response to normally innocuous stimuli) and hyperalgesia (exaggerated pain evoked by noxious stimuli). Serotonin (5-HT) has been demonstrated to play a role in the modulation of nociceptive transmission, depending on the receptor involved and on their divergent neuronal localization. Anti-depressants acting as 5-HT reuptake inhibitors, administered alone or as adjuvants, have proven to be effective in the management of certain types of pain, but the repertoire of 5-HT receptors involved in such an analgesic effect elicited by 5-HT needs to be fully investigated. One of the most recently identified subtypes of 5-HT receptors that could play a role in nociceptive processing is the 5-HT$_7$, but only scarce attempts have been made to study the involvement of this receptor in pain, due at least in part to the lack of selective 5-HT$_7$ ligands until recent time. In the present study we pharmacologically examined the role of the 5-HT$_7$ receptor in modulating nerve injury-evoked mechanical allodynia and thermal hyperalgesia employing recent highly-selective ligands. Our results using the partial sciatic nerve ligation model of neuropathic pain in mice revealed that activation of 5-HT$_7$ receptors by systemic administration of 5-HT$_7$ receptor agonists exerts clear-cut dose-dependent anti-allodynic and anti-hyperalgesic effects that were reversed by using a selective 5-HT$_7$ receptor antagonist. These findings reinforce the involvement of the 5-HT$_7$ receptor subtype in the control of pain and point to a new potential therapeutic use of 5-HT$_7$ receptor agonists as analgesics.

SCP023

5-HT6 receptor mediated modulation of glutamatergic effects on recognition memory

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The 5-hydroxytryptamine$_6$ (5-HT$_6$) receptor appears to be exclusively expressed in the CNS where increasing evidence suggests that it modulates cognitive function (Mitchell and Neumaier, 2005). The acute pro-cognitive activity of several 5-HT$_6$ receptor antagonists has been described (Woolley *et al.*, 2003; King *et al.*, 2004). In this work, the effects of two novel highly selective 5-HT$_6$ receptor antagonists, E-8694 E-53856, alone or in combination with the NMDA receptor antagonist memantine, and the blockade of MK-801-induced impairment were studied. The two trial novel object discrimination paradigm was utilised. Briefly, after an habituation procedure, two consecutive 3 min trials separated by an inter-trial interval (ITI) of 4 h were performed. A 1 min. ITI was used for the MK-801 experiments. During the first trial (T_1), rats were exposed to two identical objects. In the second trial (T_2) one of the objects was replaced by a novel one. Exploration of the objects was recorded. Adult male Lister Hooded rats (Charles River, UK) weighing 240–350 g at the start of the experiment were used. ANOVA was used to study the effects on discrimination ratio and *post hoc* comparisons were performed using Bonferroni's multiple comparison test, where appropriate. Data are presented as mean (± s.e.m.), and statistical significance was set at $P < 0.05$. Vehicle-treated animals did not discriminate between objects. Memantine and the two 5-HT$_6$ receptor antagonists dose-dependently induced object discrimination. In synergism studies, memantine together with E-8694 or E-53856, at non-active doses when administered alone, induced object discrimination when used in combination. Moreover, the two antagonists were able to block the MK-801-induced impairment. The synergism with memantine and the blockade of MK-801-induced impairment suggest that the 5-HT$_6$ receptor may modulate the glutamatergic system involved in memory processes.

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SCP024

Dorsal raphe dopamine neurons: an *in vitro* comparative electrophysiological and pharmacological study in mice

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The dorsal raphe nucleus (DRN) is well known to contain a small population of dopaminergic neurons scattered amongst the principal serotonergic cells. Morphological and anatomical characterisation have provided a detailed description of their major targets (Hasue and Shammah-Lagnado, 2002), however there are no reports concerning either their electrophysiological or pharmacological characteristics. To resolve this issue we have used a combination of fluorescence-guided whole-cell recordings and immunohistochemistry in acutely prepared brain slices from transgenic mice (male, 2–3 months-old) expressing GFP (green fluorescent protein) under the promoter region of the transcription factor pitx3, recently identified to be selectively expressed in midbrain dopamine neurons (Zhao *et al.*, 2004). All breeding and experimental procedures were conducted in accordance with the Animals Scientific Procedures Act of 1986. Results presented herein come from analysis of 120 GFP-pitx3 positive cells and 20 non-GFP, 5-HT immunopositive DRN neurons. TH positive, GFP-pitx3 positive DRN cells exhibited strikingly different electrical behaviour compared to non-GFP, 5-HT immunoreactive neurons in the DRN. DRN dopamine neurons had high input resistances (Rin range 0.5–2.0 GΩ), broad action potentials (duration range 1.7–2.5 ms at base), prominent pacemaker activity (frequency range 4–10 Hz), a hyperpolarisation-activated cation current (I_h) and a transient potassium current (I_A) mediated post-inhibitory delayed repolarisation. By contrast, 5-HT immunopositive cells exhibited smaller Rin (0.2–0.5 GΩ), long duration action potentials characterised by a hump deflection (duration range of 3–6 ms at base), lowerThhHTbjdw pacemaker activity discharges (frequency range 3–6 Hz), and a low threshold calcium current mediated post-inhibitory excitation. The majority of the TH positive DRN dopamine neurons were inhibited by the dopamine-2 receptor (D2) agonist quinpirole (1–10 μM, $n = 13$) and by the 5-HT1A receptor agonist, 8-OH-DPAT (0.3–1 μM, $n = 9$), whereas DRN serotonin neurons were excited by quinpirole ($n = 8$) and inhibited by 8-OH-DPAT ($n = 10$) at the same range of concentrations. In conclusion DRN dopamine neurons have clearly distinct properties from the DRN serotonin neurons. Understanding how these two groups of neurons interact will be a crucial issue for future research.

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SCP025

Pharmacological characterization of the inhibition produced by dihydroergotamine and methysergide on the cardioaccelerator sympathetic outflow in pithed rats

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The ergot derivatives dihydroergotamine and methysergide inhibit the tachycardic responses to preganglionic (C_7-T_1) sympathetic stimulation in pithed rats without affecting those produced by intravenous (i.v.) bolus injections of exogenous noradrenaline. The present study has identified the pharmacological profile of the receptors/mechanisms involved in the above inhibition. Male Wistar rats were pithed and prepared to selectively stimulate the preganglionic (C_7–T_1) cardiac sympathetic outflow. Then, the effects of several antagonists (given i.v.) were determined on the cardiac sympatho-inhibition induced by i.v. continuous infusions of dihydroergotamine and methysergide. I.v. continuous infusions of dihydroergotamine (1.8, 3.1 and 5.6 μg/kg/min) or methysergide (100, 300 and 1000 μg/kg/min) dose-dependently inhibited the tachycardic responses produced by sympathetic stimulation. The inhibition produced by 3.1 μg/kg/min dihydroergotamine and 300 μg/kg/min methysergide was the maximum response. The cardiac sympatho-inhibition to either dihydroergotamine (3.1 μg/kg/min) or methysergide (300 μg/kg/min) was: (1) unaffected by saline (1 mL/kg); (2) partially blocked by rauwolscine (300 μg/kg; α_2) or GR127935 (300 μg/kg; 5-HT$_{1B/1D}$); and (3) completely antagonised by rauwolscine plus GR127935. These antagonists, at doses high enough to completely block their respective receptors, failed to modify the sympathetically-induced tachycardic responses *per se*. Our results suggest that the cardiac sympatho-inhibition produced by either dihydroergotamine (3.1 μg/kg/min) or methysergide (300 μg/kg/min) involves the activation of α_2-adrenoceptors and 5-HT$_{1B/1D}$ receptors.

SCP026

5-Hydroxytryptamine-induced vasoconstriction in the isolated canine external carotid artery is mediated by 5-HT$_{2A}$ receptors and, to a lesser extent, by 5-HT$_{1B/1D}$ receptors

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Previous studies have shown that 5-hydroxytryptamine (5-HT) produces constriction in the isolated canine external carotid artery, but the mechanisms involved have not been elucidated (Villalón and Centurión, 2007). Thus, the present study was carried out to investigate the receptors/mechanisms involved in the 5-HT-induced contractions in this blood vessel. For this purpose, ring segments of endothelium-denuded canine external carotid arteries were suspended in organ bath chambers for measurement of isometric force. Then, the effects produced by 5-HT or the agonists (±) DOI (5-HT$_2$), 5-carboxamidotryptamine (5-CT; 5-HT$_7$, 5-HT$_1$, 5-ht$_5$) and sumatriptan (5-HT$_{1B/1D}$) were analyzed. Furthermore, the contractile responses to 5-HT were analyzed before and after: (i) the antagonists ketanserin (5-HT$_{2A}$; 3–30 nM), GR127935 (5-HT$_{1B/1D}$; 1–10 nM), tropisetron (5-HT$_{3/4}$; 0.3–3 mM) or prazosin (α_1; 3–30 nM); and (ii) ketanserin, in rings pretreated with GR127935. 5-HT, 5-CT and (±) DOI produced concentration-dependent contractions, with an apparent rank order of agonist potency of 5-HT > 5-CT = (±) DOI. In contrast, sumatriptan failed to contract this blood vessel. On the other hand, the antagonists ketanserin (5-HT$_{2A}$), GR127935 (5-HT$_{1B/1D}$), tropisetron (5-HT$_{3/4}$) or prazosin (α_1) failed to block the 5-HT-induced contractile responses. Interestingly, in rings pre-treated with GR127935 (3 nM), ketanserin, produced a rightward shift of the contractile-responses to 5-HT with an

apparent pA_2 = 9.0. The above findings suggest that the contractile response to 5-HT in the isolated canine external carotid artery is mainly mediated by 5-HT_{2A} receptors and, to a lesser extent, if any, by 5-$HT_{1B/1D}$ receptors.

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SCP027

Pharmacological and electrophysiological characterization of the naturally occurring Arg^{344}His variant of the human 5-HT_{3A} receptor

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The 5-HT_3 receptor, a ligand-gated ion channel, is involved in, e. g., emesis, perception of pain and pathogenesis of psychiatric diseases. The aim of the present study was to examine the pharmacological and electrophysiological properties of the naturally occurring Arg^{344}His variant of the human 5-HT_{3A} receptor, identified in a schizophrenic patient. In intact HEK293 cells expressing the wild-type receptor (WTR) or the variant receptor (VR), the agonist-induced Ca^{2+} inward current through the 5-HT_{3A} receptor channel was determined by an aequorin luminescence-based Ca^{2+} assay. In excised outside-out cell membrane patches, cation currents were determined by electrophysiological techniques. The pharmacological properties were analyzed by [^{3}H]GR65630 binding to cell membrane fragments and intact cells. The density of [^{3}H]GR65630 binding sites in cells expressing the VR was reduced to 55% of that in cells expressing the WTR, whereas the maximum Ca^{2+} influx through the receptor channels induced by 5-HT remained unchanged. This discrepancy may be due to an increased mean open time of single VR compared to single WTR channels; the latter could be measured as a tendency in preliminary experiments on single channel 5-HT_{3A} receptors in which three arginine residues were replaced (R432Q, R436D, R440A). In contrast to the tendency towards prolongation of the mean open time of the VR single channels, the amplitude of single channel current influx did not differ between VR and WTR. Radioligand competition and Ca^{2+} influx measurements revealed that the affinity and the potency of five 5-HT_{3A} receptor agonists and four antagonists at the VR did not differ from that at the WTR. In conclusion, the expression of the Arg^{344}His VR at the surface of HEK293 cells is reduced, whereas the mean VR single channel open time seems to be increased, resulting in a lack of change in overall ion influx through the VR compared to the WTR channels in intact HEK293 cells.

SCP028

Changes in activity and temperature fail to correlate with 5-HT release following repeated MDMA administration in rats

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Human binge use of repeated low doses of 3,4-methylenedioxymethamphetamine (MDMA/ecstasy) may maintain the euphoric state while reducing tolerance (Parrott, 2005). However there is limited information from animal studies whether analogous repeated MDMA causes behavioural effects and long-term serotonergic neurotoxicity. In this study we determined the acute effects of repeated low doses of MDMA (3 and 6 mg/kg i.p.) on body temperature, activity and 5-HT release in the rat by combining telemetry and microdialysis. Male Lister hooded rats (100–130 g) were individually housed after i.p. implantation of a telemetry device. Two weeks later a microdialysis probe was implanted into the hippocampus. The following day either MDMA (3 or 6 mg/kg) or saline were given i.p. three times every 2 h and microdialysis samples collected with activity and body temperature monitored simultaneously using telemetry. MDMA (3 mg/kg) significantly decreased body temperature after each injection and this returned to normal 3 h after the last injection. The higher dose of MDMA (6 mg/kg) reduced body temperature after the first injection but then increased temperature to a maximum of +1°C 2 h after the last injection. There was no significant difference in activity between saline and MDMA (3 mg/kg) treated animals. In contrast the higher dose of MDMA (6 mg/kg) induced hyperactivity after each injection with a prolonged increase after the final dose. Both doses of MDMA increased extraneuronal hippocampal 5-HT with a maximum release 1 h after each injection. The higher dose of MDMA (6 mg/kg) produced greater release of 5-HT following the first injection (+556% from predrug basal) than the lower dose (+127% from predrug basal). After the second and the third injections increases in 5-HT release were similar for both doses (approximately +300% from basal). The results indicate that repeated administration of the higher dose of MDMA causes hyperthermia and hyperactivity while the lower dose results in hypothermia and no effect on activity. Moreover there appears to be no correlation between changes in extracellular 5-HT and either activity or temperature following repeated low dose MDMA administration. The results indicate that factors other than 5-HT release (possibly dopamine release) are involved in the behavioural and physiological effects acute low doses of MDMA.

Reference:

Parrott. J Psychopharmacol. 2005; 19(1): 71–83.

SCP029

Citalopram inhibition of light-induced phase shifts in hamster circadian activity rhythms: modulation by 5-HT receptor antagonists

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Circadian wheel running activity rhythms in the Syrian hamster can be advanced or delayed by light pulses given at subjective dawn and dusk, respectively. Previously we reported that citalopram and other SSRIs inhibit light-induced phase advances in hamster circadian activity rhythms (Gannon and Millan, 2007). However, the 5-HT receptor subtype(s) mediating the inhibitory effect of citalopram has not been identified, and many are potentially implicated in the 5-HT inhibition of light-induced phase advances in the hamster. Therefore, in this report we tested antagonists at several candidates, 5-HT_{1A}, 5-HT_{5A}, 5-HT_6, and 5-HT_7 receptors, for their ability to modulate both light-induced phase advances and citalopram-inhibition of phase advances in the hamster. Young male Syrian hamsters were individually housed in cages equipped with a running wheel and maintained in conditions of constant darkness with food and water provided *ad libitum*. Wheel-running revolutions were continuously recorded for a period of three weeks. On day ten of the experiment, hamsters were removed from their cage and exposed to a phase-advancing 10 min pulse of dim light, then returned to their home cages. Vehicle/drugs were injected i.p. 45 min prior to the light pulse. On day 21, hamsters were removed from constant darkness and phase advances in wheel running rhythms calculated. Citalopram (10 mg/kg) inhibited light induced phase advances by 60%. The 5-HT_{1A} antagonist, WAY 100 635 (0.5 mg/kg), had no effect on phase advances when administered alone, and significantly potentiated the action of citalopram to nearly 90%. In a separate experiment, the 5-HT_{5A} antagonist, A843,277, had no effect on light-induced phase shifts at doses up to 10 mg/kg. The 5-HT_6 antagonist SB 399885 likewise had no effect on light induced phase shifts at doses as high as 10 mg/kg, and at a dose of 5 mg/kg failed to modulate the inhibitory effect of 10 mg/kg citalopram. Finally, the 5-HT_7 antagonist SB 269970 at doses of 1–5 mg/kg had no effect on light-induced phase shifts, nor did it affect the inhibition of 10 mg/kg citalopram. Therefore, to date, only the 5-HT_{1A} receptor antagonist WAY 100 635 has been found to interact with (facilitate actions of) citalopram in the hamster. [Supported by IOB 0549980 (RLG)].

Reference:

Gannon and Millan, Psychopharmacology. 2007; 195: 325–332.

SCP030

Systematic spatio-temporal analysis of differential tryptophan hydroxylase isoform expression in murine and human brain and other control tissues: implications for neuropsychiatric disorders

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Dys-regulation of the brain serotonin (5-HT) system, including dysfunction based on genetic variation in tryptophan hydroxylase (TPH) -dependent 5-HT synthesis, has long been implicated in the etiopathogenesis of a wide variety of neuropsychiatric disorders, although there is presently considerable controversy regarding the differential expression pattern and the specific role of the two isoforms, TPH1 and TPH2. Here, we performed a systematic spatio-temporal analysis of Tph1 and Tph2 expression in mouse brain (C57BL/6) during pre- and post-natal development (from E9.5 to P23) and in murine and human adult brain. To assess expression at both the transcriptional and translational level in brain as well as in other TPH-expressing tissues such as mouse pineal gland and human jejunum small intestine serving as internal control, we used complementary methods permitting isoform-specific detection of mRNA (quantitative real time PCR and *in situ* hybridization) and protein (immunohistochemistry and Western blot). Effects of potential confounding factors on brain TPH isoform content conveyed by non-neural circulating cells were also evaluated. Commencing before E11 during murine development, TPH2 expression was found in the raphe nuclei of both species, as well as in fibers in the deep pineal gland and in the small intestine. In contrast, none of the techniques used identified significant TPH1 expression, neither during murine brain development, nor in mouse and human adult brain. Physiologically meaningful TPH1 expression was nevertheless detected in the pineal gland and small intestine. Our findings are relevant to the interpretation of the TPH isoforms' role in brain development and function as well as in the molecular neurobiology of complex behavior and psychopathology.

SCP031

Regulation of the human tryptophan hydroxylase-2 gene promoter activity in immortalized serotonergic RN46A cells

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Promoter polymorphism of the human tryptophan hydroxylase-2 (hTPH2) gene coding for the rate-limiting enzyme of serotonin (5-HT) synthesis in the brain is thought to be related to neuropsychiatric diseases as well as personality traits. Elucidation of mechanisms involved in the transcriptional regulation of the hTPH2 gene will assist our understanding of how the expression of the hTPH2 gene could be altered by various pathologic and pharmacologic states. To address these questions more directly, we tested the hTPH2 gene promoter activity and found critical elements necessary for the hTPH2 gene expression. An 8.7 kb fragment upstream to the transcription start site was cloned into pGL4-Basic and a series of 5'-deletion mutants were constructed. Promoter activities were assessed by transient transfections into immortalized rat serotonergic RN46A cells (a generous gift from Dr. Whittemore SR., University of Louisville, KY). RT-PCR analysis confirmed the expression of serotonergic marker genes, transcription factors Mash1, Nkx2.2, Lmx1b, GATA2, GATA3, Pet-1, and functional proteins 5-HTT and 5-HT1A receptor in RN46A cells. RN46A cells do not express Phox2b, a central repressor of serotonergic fate. Unexpectedly, RN46A cells express rTPH1 but not rTPH2. Accordingly, hTPH2 gene promoter activities could not be measured. A bipartite NRSE-like sequence (Patel *et al.*, 2007) of the hTPH2 gene was found at the vicinity of the transcription start site. Either by introducing mutations into this element or treating RN46A cells with histone deacetylase (HDAC) inhibitors (trichostatin A, sodium butyrate or valproic acid), hTPH2 gene promoter activities was able to be measured. As expected, endogenous expression levels of TPH2 mRNA were increased by the treatment with HDAC inhibitors in RN46A cells. These results

suggest that the expression of the TPH2 gene is strictly controlled through the repressor-mediated regulatory mechanisms in RN46A cells. The neuronal activity-dependent modulation of the repressor system may contribute to the transcriptional regulation of the TPH2 gene in the brain.
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Reference:
Patel *et al.* J Biol Chem. 2005; 282: 26717–24.

SCP032
5-HT_{1A} receptor mRNA distribution in chemically identified neurons of the mouse rostral brainstem: Implications for the role of serotonin in the regulation of sleep and emotional behaviours
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The brain monoamine serotonin (5-HT) effects on the regulation of sleep and mood are mainly mediated by 5-HT_{1A} receptors (5-HT_{1A}R) whose complex distribution at pre- and post-synaptic level confuses the attribution of agonistic effects to behavioural outcomes. Our aim was to identify the neuronal phenotypes/pathways involved in 5-HT_{1A}R-mediated effects at the level of the rostral brainstem in the adult mouse. For the extensive and detailed mapping of chemically identified neurons expressing 5-HT_{1A}R mRNA, we used double *in situ* hybridization histochemistry (ISHH) or immunohistochemistry combined with ISHH. In the dorsal (DR) and median (MnR) raphe nuclei, we confirmed that most of 5-HT neurons expressed 5-HT_{1A}R and, reciprocally, 5-HT_{1A}R was mainly found in 5-HT neurons. However, we observed that 5-HT_{1A}R expression was not strictly confined to 5-HT neurons. In the rostral DR, we characterized an individualized group of GABAergic neurons endowed with 5-HT_{1A}R mRNA. Throughout the MnR, numerous non-5-HT neurons that were not identified with any markers used in this study, also expressed 5-HT_{1A}R. Altogether these results emphasize differential 5-HT regulation of the DR and the MnR which may underlie distinct functional roles. Outside raphe nuclei, 5-HT_{1A}R were selectively present in GABAergic and glutamatergic neurons of structures related to the sleep/wake cycle and emotion circuits, such as the interpeduncular complex, dorsal nucleus of Gudden, pontine reticular formation and parabrachial internal nucleus. Our findings bring new insights to reconsider the exclusive autoreceptor role of 5-HT_{1A}R in the raphe nuclei and also provide a neuroanatomical basis for revisiting 5-HT_{1A}R-mediated control of sleep/wake mechanisms and emotional behaviours.

SCP033
5-HT_{2C}R pre-RNA editing, alternate splicing and function in a mouse model of Prader–Willi syndrome
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Prader–Willi syndrome (PWS) is a complex genetic disorder caused by the loss of paternal gene expression from chromosome 15q11-q13. In addition to a number of coding genes, there are several imprinted small nucleolar (sno)RNAs present in the PWS cluster. Recent research has demonstrated that one of these, HBII-52, has a regulatory function in that it reduces alternate splicing of the RNA-editing region of the serotonin 2C receptor (5-HT_{2C}R) pre-RNA, and that loss of expression of this snoRNA may lead to abnormal molecular processing of 5-HT_{2C}R in the PWS brain. In turn, these molecular modifications of 5-HT_{2C}R pre-RNA lead to a much less functional receptor. Using a mouse model of PWS (PWS-$IC^{+/del}$) we are investigating the consequences of the loss of mbii-52 (mouse homologue of HBII-52) for 5-HT_{2C}R functioning at a molecular, neurochemical and behavioural level. To achieve this we have examined the brains of adult PWS-$IC^{+/del}$ mice and found altered levels of expression and alternate splicing of 5-HT_{2C}R. We are now examining RNA editing at five key sites of the 5-HT_{2C}R pre-RNA. Behavioural studies, using a cohort of 64 mixed gender mice (28 PWS-IC $^{+/del}$, 36 Wild Type (WT), weight range 19–65 g) are focussing on those known to be influenced by serotonin. These include spontaneous behaviours, such as marble burying and locomotor activity, and complex cognitive processes. PWS-$IC^{+/del}$ mice are hypoactive, but were no different to WT mice in the marble burying test. Importantly, marble burying behaviour was also not to be affected by dosing with the 5-HT2CR antagonist SB242084, demonstrating a degree of specificity of any 5-HT deficits in the PWS-$IC^{+/del}$ mice. We are now using the 5-choice serial reaction time test (5-csrtt) to examine aspects of attention and impulse control. The specificity of any deficits in the PWS-$IC^{+/del}$ mice and will be tested by examining the effects of pharmacological manipulations with 5-HT2CR and 5-HT2AR drugs. Our findings suggest that abnormalities in 5-HT_{2C}R functioning may underlie aspects of the behavioural phenotype seen in PWS, and that the imprinted snoRNA mbii-52 plays an important role in sculpting brain and behaviour.

SCP034
The first serotonin receptor allelic variant database
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Serotonin (5-hydroxytryptamine, 5-HT) controls a variety of physiological functions. 5-HT receptor subtypes mediating serotonin action can be divided into seven main classes (5-HT_1R to 5-HT_7R). A multitude of candidate gene screenings has been published during the last years. We have started to structure this information in the first serotonin receptor allelic variant database using LOVD (Leiden Open source Variation Database). Up to now, the database comprises data of 5-HT_3 receptor subunits. To date, five different human subunits are known (5-HT_{3A-E}), which are encoded by the serotonin receptor genes *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D* and *HTR3E*. Different receptor subtypes seem to be involved in chemotherapy induced nausea and vomiting (CINV), irritable bowel syndrome (IBS) and psychiatric disorders. During the last years *HTR3* case-control and pharmacogenetic studies indicated that *HTR3A* and *HTR3B* polymorphisms may contribute to the etiology of psychiatric disorders and may predict CINV and medical treatment of psychiatric patients. Currently, the database is subdivided into five sub-databases, referring to the serotonin receptor genes. This database will successively be extended by data of additional serotonin receptor genes. Within each sub-database we are collecting mutations, polymorphisms, demographic information as well as pharmacogenetic data. Every sub-database includes general information about the respective gene and is linked to other resources such as OMIM, GDB, HGMD and HAPMAP. The remote user is able to search the data and to submit new data into the database. This central information pool should help clinicians as well as scientists to evaluate their findings and to use the information for subsequent studies. Data about functional consequences of variants will be integrated in future as well to enable specific drug design in the therapy of respective conditions.
References:
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Niesler B *et al.* PGBase Focus of Experts, Pharmacogenomics. in press, 2008.
Niesler B *et al.* Human Mutation. 2007; 28(10): 933–938.

SCP035
5-HT_{2B}-mediated serotonin signalling participates in retinal and cranio-facial morphogenesis during *Xenopus laevis* development
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Serotonin (5-HT) is a neurotransmitter that mediates a wide variety of effects in the central and peripheral nervous systems. Experimental evidence demonstrated that serotonin even has an important role as growth and differentiating factor for neuronal and non-neuronal cells by controlling proliferation, migration and apoptosis during development. All the biological actions of 5-HT are mediated by G-coupled receptors and, among these, the 5-HT2B receptor is expressed during CNS, heart and craniofacial development. By using *Xenopus laevis* as a model system, we demonstrated that 5-HT2B receptor loss of function determines a decrease in the proliferation rate of retinoblasts and increases the apoptosis of retinal cells thus resulting in abnormal eye morphology (De Lucchini *et al.*, 2005). In order to further investigate the 5-HT2B role during development, we performed complementary experiments of gene gain of function. The 5HT2B overexpression, leads to the formation of eyes with irregular form, position and orientation and showing defects in the optic fissure closure and in the pigmented epithelium formation. A detailed molecular analysis revealed a disorganization of the typical laminar retinal structure and the presence of differentiated retinal cells in ectopic position. As pharmacological treatments with 5-HT2 antagonists elicited in mice craniofacial alterations, we are now studying the formation of craniofacial skeletal elements and their associated muscles in 5-HT2B gain and loss of function experiments. In particular, 5-HT2B gene gain of function results in altered formation of the jaw and hyoid cartilages and correlated muscles. We showed that 5-HT, *via* 5-HT2B receptor, is among the key extracellular signals that control *Xenopus* retinal histogenesis and eye morphogenesis. Moreover, our results suggest for the first time a direct involvement 5-HT2B receptors in mediating the serotonin action on craniofacial morphogenesis by influencing the formation of skeletal elements and that of the connected muscles.
Reference:
De Lucchini *et al.*, Mol Cell Neurosci. 2005; 29(2): 299–312.

SCP036
Continuous controls exerted by serotonin$_{2C}$ receptors in rat basal ganglia involve both serotonergic tone and its constitutive activity: regional distribution on c-Fos expression and behavioral aspects
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Serotonin$_{2C}$ receptors (R5-HT_{2C}) exert continuous controls on neuronal excitability in basal ganglia, a group of subcortical cells involved in motor control. Because the constitutive activity of the R5-HT_{2C}, an activity occurring without a ligand and blocked by inverse agonists, endogenously impacts on cellular activities, it may coexist with the tonic control exerted by endogenous 5-HT on the receptor. To determine whether these continuous controls are distinct functionally and regionally, we have studied the effect elicited by the 5-HT_{2C} antagonist SB-243213 or the inverse agonist SB-206553 on orofacial activity and on the expression of the proto-oncogene c-Fos, a marker of change of neuronal activity, in rat basal ganglia. Experiments were performed in male Sprague–Dawley rats (320–380 g). Oral bouts were measured during 1 h after drugs injection. In case of coadministration, the 5-HT_{2C} antagonist was injected 1 h before the non-selective 5-HT_{2C} agonist *m*-CPP or the 5-HT_{2C} inverse agonist SB-206553. Intracardiac perfusion of paraformaldehyde for Fos immunochemistry (number of Fos-immunoreactive cells) was performed 2 h after SB-206533 or *m*-CPP injection and 3 h after SB-243213 injection. Statistical analysis was performed using ANOVAs followed by the protected least significant difference test. Results show that SB-206553 (1–20 mg/kg, i.p.), but not SB-243213 (1–10 mg/kg, i.p.), elicited a dose-dependent increase in oral bouts. In a parallel experiment, the agonist *m*-CPP (1 mg/kg, i.p.) enhanced oral bouts. Pre-treatment by SB-243213 abolished oral bouts induced by both *m*-CPP and SB-206553 (3–10 mg/kg). SB206553 (10 mg/kg) stimulated Fos expression in striatum, and, modestly in entopeduncular nucleus (EPN), subthalamic nucleus (STN) and substantia nigra. SB-243213 (1, 10 mg/kg) increased Fos expression in STN and striatum only. *m*-CPP (1 mg/kg) enhanced Fos expression in STN, EPN and medial striatum. None of these drugs enhanced Fos expression in globus pallidus. These results show that constitutive activity of R5-HT_{2C} regulates oral activity in awake animals and suggest that constitutive activity of R-5HT_{2C} differentially affects cellular activities in basal ganglia with respect to both tonic and phasic controls.

SCP037

Stimulation of serotonin$_{2C}$ receptors elicits abnormal oral movements without altering the activity of the sensori-motor pathway in the rat basal ganglia

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Serotonin$_{2C}$ receptors (R5-HT$_{2C}$) are thought to contribute to iatrogenic motor side effects in Parkinson's disease or schizophrenia *via* their influence on the basal ganglia, a group of sub-cortical structures involved in motor control. Whether these abnormal movements are a direct consequence of dysfunctions of the orofacial motor processing is uncertain. Here, we combined behavioral, extracellular single-cell recordings and immunohistochemical approaches in rats to investigate the effect of the 5-HT$_{2C}$ agonist Ro-60-0175 respectively on orofacial dyskinesia, the electrophysiological activity of substantia nigra pars reticulata (SNpr) neuron connected to the orofacial motor cortex (OfMC) and the expression of the marker of neuronal activity c-Fos in basal ganglia. Experiments were performed in male Sprague–Dawley rats (320–380 g). 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 co-administered, the 5-HT$_{2C}$ antagonist was injected 1 h before the Ro-60-0175. Intracardiac perfusion of paraformaldehyde for Fos immunochemistry (number of Fos immunoreactive cells) was performed 2 h after Ro-60-0175 injection. In electrophysiological experiments, a stimulating and a recording electrode were stereotaxically implanted in OfMC and SNr, respectively. Both firing rate of SNpr neurons and peristimulus time histograms were analyzed. The results show that Ro-60-0175 (0.1–3 mg/kg, i.p.) enhanced oral bouts in a dose-dependent manner. The effect elicited by the highest dose was suppressed by the selective 5-HT$_{2C}$ antagonist SB 243213 (1 mg/kg, i.p.). Ro 60-0175 (1–3 mg/kg) did affect neither basal activity of SNpr neurons responding in a triphasic manner to the electrical stimulation of the OfMC, nor the effect of stimulation itself. Finally, Ro-60-0175 induced a dose-dependent enhancement of Fos expression in the medial striatum and a slight increase was observed in the subthalamic nucleus, entopeduncular nucleus and SNr at the highest dose. Fos-immunoreactivity was located medially but not laterally. These results suggest that oral dyskinesia mediated by R-5HT$_{2C}$ are not directly associated to aberrant signalling in the orofacial motor pathway, pointing out limbic and associative territories of basal ganglia.

SCP038

Dose-dependence in the effect of chronic venlafaxine on the sensitivity of 5-HT4 receptors in rat hippocampus

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Venlafaxine is a serotonin and noradrenaline reuptake inhibitor (NSRI) anti-depressant drug for which clinical studies have suggested a high level efficacy and a possible early action onset compared to the classical anti-depressants. It also shows efficacy in treatment-resistant depression. It has been proposed that its therapeutic effects might be due, at least in part, to adaptive changes in serotonergic neurotransmission, through the activation of the different 5-HT receptor subtypes. In this regard, 5-HT$_4$ receptors, mainly located in several areas of central nervous system, mediate neuronal excitability of hippocampal CA1 pyramidal cells. However, the information about the involvement of this subtype in the mechanism of action of venlafaxine is unknown. We have evaluated the effects of a 21 day treatment with venlafaxine at doses of 10 and 40 mg/kg/day (p.o.) in the sensitivity of 5-HT$_4$ receptors in rat hippocampus. The density of 5-HT$_4$ receptors (autoradiography, [^{3}H]GR113808) was significantly decreased in the CA$_1$ field of hippocampus after chronic treatment with 40 mg/kg/day of venlafaxine (41.5 ± 3.9%; $P < 0.05$ unpaired *t* test), but it was unchanged after chronic treatment with 10 mg/kg/day. We also tested the effect of venlafaxine in the neuronal excitability of CA1 pyramidal cells of hippocampus after zacopride application. For the concentration of 10 μM, there was a significant reduction in the zacopride-mediated excitatory effect from rats treated with both doses of the drug, more marked for the 40 mg/kg/day dose (red = 39.5 ± 9.0% and 55.5 ± 12.2%, for 10 and 40 mg/kg/day respectively; $P < 0.05$). In conclusion, our results demonstrate the existence of a dose-dependent, 5-HT$_4$ receptor desensitization following chronic administration of venlafaxine. Taking into account that a high dose is required to obtain a complete desensitization pattern, it is suggested that the noradrenergic component may play a relevant role in the regulation of 5-HT$_4$ receptors by chronic NSRI drugs.

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SCP039

Effect of serotonin on GABAergic interneuron migration in the embryonic cortex

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The discovery that the short allele of the human serotonin transporter gene can influence personality traits and increase the risk for depression in adulthood has led to the hypothesis that a relative increase in the extracellular levels of serotonin (5-HT) during development could be critical for the establishment of brain circuits. Consistent with this idea, a large body of data demonstrates that serotonin is a strong neuro developmental signal that can modulate a wide variety of cellular processes. In humans, serotonergic fibers appear in the developing cortex as early as the 10th gestational week, a period of intense neuronal migration. We hypothesized that an excess of 5-HT could affect embryonic cortical interneuron migration. Using time-lapse videometry to monitor the migration of interneurons in embryonic mouse cortical slices, we discovered that the application of 5-HT decreased interneuron migration in a reversible and dose-dependent manner. Current experiments are designed to identify the serotoninergic receptor(s) implicated in this effect. These results shed new light on the neuro developmental alterations caused by an excess of 5-HT during the embryonic period and contribute to a better understanding of the cellular processes that could be modulated by genetically controlled differences in human 5-HT homeostasis.

SCP040

Chronic fluoxetine regulates both 5-HT$_{1A}$ receptor functionality and hippocampal β-catenin pathway in an animal model of depression

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In the last years, brain neurogenesis has received much attention regarding the molecular mechanisms by which anti-depressants exert their therapeutic effect: in this regard, the β-catenin pathway plays a crucial role in cell proliferation and fate of adult hippocampal stem/progenitor cells (AHPs). On the other hand, 5-HT neuro-transmission, classically involved in anti-depressant-induced responses, appears to play a relevant role in brain plasticity. Because of that, the goal of this study was to analyze both the hippocampal expression of β-catenin (immunoblotting, total cell hippocampal lisates) and the density and functionality of brain 5-HT$_{1A}$ receptors ([^{3}H]OH-DPAT and [^{35}S]GTPγS autoradiography) in an animal model of depression, as well as its regulation by the chronic administration of the serotonin re-uptake inhibitor (SSRI) fluoxetine (10 mg/kg/day, 14 days; s.c.). The animal model of bilateral olfactory bulbectomy in rat (OB) resembles many neurochemical and structural features observed in human depression. Chronic fluoxetine fully attenuated hyperactivity in the 'open-field' test in the OB + fluoxetine group ($P < 0.01$ vs. OB + vehicle) whereas had no effect in sham-operated animals. Regarding Western blot assays, a reduction of β-catenin expression was found in the hippocampal lisates from OB animals. Chronic fluoxetine treatment resulted in a significant increase ($P < 0.05$ vs. non-treated groups) of β-catenin expression in both experimental groups. No changes were found in OB rats in hippocampus either in receptor density or in 8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding in hippocampus and dorsal raphe nucleus (DRN). Chronic fluoxetine significantly reduced the level of 8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding in both sham-operated and OB animals, in areas such as dentate gyrus (169.2, 26.7 vs. 272.5, 45.0 in sham-operated, $P < 0.05$; 128.6, 14.5 vs. 300.7, 84.3 in OB animals, $P < 0.05$) and DRN. These findings support a role for the regulation of neuro-proliferative pathways in the mechanism of action of fluoxetine, raising the question of the involvement of 5-HT mediated mechanisms in these modifications.

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SCP041

Bifeprunox and aripiprazole improve PCP-induced object recognition deficits in rats: Potential role for 5-HT$_{1A}$ receptor agonism in ameliorating cognitive deficit symptoms in schizophrenia

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A subchronic dosing regimen with phencyclidine (PCP) induces cognitive deficits in animal models similar to those seen in patients with schizophrenia. Recent work in our laboratory demonstrated that object recognition deficits are attenuated by treatment with atypical, but not classical, anti-psychotics. To compare the ability of one approved anti-psychotic, aripiprazole and one in development, bifeprunox, both of which have a novel mechanism of action, partial D$_2$ receptor agonism and 5-HT$_{1A}$ receptor agonism, to improve sub-chronic PCP-induced deficits in episodic memory using the novel object recognition paradigm in rats. Female hooded-Lister rats received vehicle or PCP (2 mg/kg i.p. twice daily) for 7 days, followed by 7 days washout. Bifeprunox (0.004–0.25 mg/kg, s.c. 2 h), aripiprazole (0.63–5.0 mg/kg, s.c. 30 min) or vehicles were administered prior to testing: In a 3 min acquisition phase, rats explored two identical objects followed by a 1 min inter-trial interval. In the retention trial, rats explored the familiar and a novel object for 3 min. Exploration time(s) of each object in each trial was recorded. In the retention trial, saline-treated animals explored the novel significantly more than the familiar object ($P < 0.05$). This effect was abolished in PCP-treated rats. The minimal effective dose for attenuation of the effects of PCP was 0.016 mg/kg for bifeprunox and 2.5 mg/kg for aripiprazole and these doses significantly increased novel compared with familiar object exploration ($P < 0.05$–$P < 0.001$). Bifeprunox and aripiprazole attenuate PCP-induced episodic memory deficits, suggesting potential for treatment of cognitive deficits in schizophrenia. The pharmacological mechanism for their efficacy remains to be determined. However based on their unique pharmacological profile, interaction with 5-HT$_{1A}$ receptors is a potential mechanism.

SCP042

PCP induced social withdrawal deficits are reversed by atypical, but not classical anti-psychotics, involvement of 5-HT$_{1A}$ receptors: Implications for treatment of negative symptoms of schizophrenia

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Sub-chronic phencyclidine (PCP) treatment mimics certain aspects of schizophrenia symptomology in rats, in particular cognitive deficits. However, there is a marked lack of validated animal models of negative symptoms. We are working towards the establishment of such a model by investigating the ability of atypical and novel anti-psychotics to reverse PCP-induced social interaction deficits in female rats and exploring the pharmacology of this effect. Adult female hooded-Lister rats received vehicle ($n = 72$) or PCP ($n = 44$; 2 mg/kg i.p.) twice daily for 7 days, followed by 7 days washout. On test days, PCP treated rats were treated acutely with either

haloperidol (0.05 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.) or ziprasidone (2.5 mg/kg, i.p.). In a separate experiment, PCP treated rats received acute treatment with aripiprazole (5 mg/kg, s.c.) or the 5-HT_{1A} receptor antagonist, WAY 100 635 (0.5 mg/kg, i.p.) alone and in combination. All acute treatments were given 30 min prior to testing. For the test, pairs of unfamiliar weight matched rats receiving either acute doses of drugs described above or vehicle were placed in the test arena and social behaviours (following, sniffing, climbing over and under, exploration of inanimate object and avoiding) were recorded on video for subsequent blind scoring. Data were analysed by factorial ANOVA followed by unpaired t-test. Sub-chronic PCP produced a robust and significant reduction in social sniffing and increase in avoiding behaviour ($P < 0.01$–$P < 0.001$). The PCP-induced deficits in social behaviours were significantly attenuated by acute treatment with risperidone, ziprasidone and aripiprazole, but not haloperidol. The pro-social effect of aripiprazole was abolished by pre-treatment with WAY 100 635 which was without effect alone. These findings show that sub-chronic PCP induces robust social interaction deficits in female rats that are improved by novel and atypical, but not classical, anti-psychotic agents. These results suggest that the beneficial effects of drugs such as aripiprazole and ziprasidone on PCP-induced social behaviour deficits, a potential model of negative symptoms of schizophrenia, may be a consequence of modifications of the serotonergic system, in particular through an interaction with 5-HT_{1A} receptors, a hypothesis supported by Bruins Slot and colleagues (2005).

SCP043

Cellular-molecular mechanisms of regulation of myocardium inotropic functions by 5-HT2 and 5-HT4 receptors in children with congenital heart diseases

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Serotonin (5-HT) has morphogenetic actions in the embryonic period of ontogenesis (Ugrumov *et al*, 1994; Nebigil, 2000), and it is involved in the pathogenesis of cardiovascular diseases (Herve, 1995). The influence of serotonin on myocardial contraction in tissue from children with congenital heart disease has not previously been investigated. In this study, we measured serotonin concentrations of plasma and platelets by high-performance liquid chromatography (HPLC). The contraction force of children's atrial strips in response to application of (0,1 μM, 1,0 μM, 10,0 μM) 5-HT agonists selective for 5-HT2 and 5-HT4 receptors was measured using an isometric setup force AD instrument. Strips of children's right atria were obtained from children who had undergone operations for congenital heart disease (CHD). We determined the expression of 5-HT2B and 5-HT4 receptors and the membrane serotonin transporter (SERT) in human right atria strips by immunohistochemistry. For the first time in children with CHD in the age range 2 months – 17 years, the concentration of 5-HT and its core metabolite, 5-HIAA, was determined in blood plasma and in platelets. It was established that the serotoninergic system actively participates in the pathogenesis of pulmonary arterial hypertension (PAH) in children with CHD. Concentrations of 5-HT in the plasma of children with CHD showed an increase in PAH. Concentrations of 5-HIAA in the plasma of children with CHD having increased PAH correlates with the degree of PAH, and thus can serve as a marker for an estimation of efficiency of therapy for these patients. For the first time, the presence of 5HT-2 and 5-HT4 receptors, and also SERT, in the myocardium of children having CHD has been shown, and that this phenomenon depends on a kind of congenital pathology of the heart. For children with CHD from 2 months to 17 years, the role of 5HT2R in atrium contraction is decreased, but the 5HT4R is active during all this period. Thus, these results may be important in relation to decreasing the high mortality in this group of sick children; 5-HT2 blocker which may be very effective are not currently used in the clinic.

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SCP044

Altered sleep homeostasis after restraint stress in 5-HTT-/- knock-out male mice: a role for hypocretins

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Several factors are known to influence sleep homeostasis. Thus, restraint stress (RS) is followed by a rebound of rapid eye movement sleep (REMS) in which serotonin (5-HT) participates. Because hypocretin, a hypothalamic neuropeptide which plays key role in REMS control, is known to interact with 5-HT, we investigated whether hypocretinergic neurotransmission also contributes to stress-altered sleep through such interaction. Studies were performed in both wild-type mice (CD1 background) and 5-HT transporter (5-HTT-/-) knock-out mice. Immunocytochemistry and radioimmunoassay approaches were used to assess the activity of serotonergic and hypocretinergic neurons under basal conditions and after RS. In addition, the effects of specific hypocretinergic receptor 1 (hcrtR1) blockade by SB-334867 (30 mg/kg, i.p.) on sleep were assessed by polysomnographic recordings under the same experimental conditions. At the hypothalamic level, RS produced a long-lasting activation of hypocretin neurons in both strains (as evidenced by the number of double labelled c-Fos and preprohcrt neurons), but this effect was larger in 5-HTT-/- mice. In contrast, RS-induced activation of serotonergic neurons in the anterior raphe area by RS was less in 5-HTT-/- mice. Interestingly, in the later area, hcrt-1 peptide contents were higher in 5-HTT-/- mice than in 5-HTT+/+ mice under basal conditions, and were further increased after restraint stress. On the other hand, polysomnographic recordings showed that RS produced an intense arousal followed by REMS rebound in wild-type mice. In contrast, no REMS rebound was observed in 5-HTT-/- mice. However, pharmacological blockade of the hypocretin receptor type I (hcrtR-I) prior to RS restored in these mutants a delayed increase in REMS that resembled that observed in wild-type animals. In the later mice, SB-334867 treatment did not affect RS-induced REMS changes. Altogether, our data show that an excess of 5-HT at the synaptic cleft such as that showed in 5-HTT-/- mutants has impact on hypocretinergic neurotransmission, which apparently plays key role in sleep homeostasis impairment in these mutants.

SCP045

Neonatal inactivation of the serotonin transporter predicts persistent alterations of sleep patterns and depression-like behaviour

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The most widely used anti-depressants are blockers of the serotonin reuptake (SSRI), and their use during development might lead to persistent anomalies (Cooper *et al.*, 2007). Some of these impairments, such as sleep disorders, may still be ignored, notably because no relevant investigations have yet been performed on a very long term basis, even though SSRIs are extensively prescribed since the 1980s. In mice, the data that we obtained recently indicate that neonatal inactivation of the serotonin reuptake process (either pharmacologically- or genetically-induced) during a 'critical period' results in persistent (life long) alterations of notably sleep regulation and emotional behaviour (Popa *et al.*, 2008). Thus, both mice that underwent transient block of the serotonin transporter (SERT) from post-natal day 5 to 21, and mutant mice that do not express the SERT, exhibit at adult age major sleep alterations (affecting notably REM sleep and the sleep response to acute stress) that evoke a depression-like profile. In parallel, they display reduced anhedonia, as well as behavioural helplessness that can be reversed by chronic, but not acute- treatment with the SSRI anti-depressant, fluoxetine. Conversely, in SERT-/- mutant mice that display enhanced brain levels of serotonin (Fabre *et al.*, 2000), a lasting normalization of sleep patterns and behaviour could be obtained after specific treatment during early post-natal life, but not later on (Alexandre *et al.*, 2006). Such rescue treatment in fact limited the serotonin levels (by synthesis inhibition) or its impact at 5-HT_{1A} receptors (by receptor blockade). Thus, an excess of serotonin levels at 5-HT_{1A} receptors during development in the mouse induces life long alterations of sleep regulations and behavioural traits, thereby producing a relevant model of depression. These results underline the need for studying long term effects on sleep of anti-depressant exposure during development in humans. In addition, they open the way to new strategies for treating depression during pregnancy, or even for preventing possible development of sleep disorders due to genetic causes.

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SCP046

5-HT2A/2C receptors exert opposing effects on locomotor activity in mice

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Behavioral effects of hallucinogenic drugs have been characterized in animal models to better understand the mechanism of action of these compounds in addition to generating models against which to screen anti-psychotic medications. The studies described here using the mouse Behavior Pattern Monitor (mBPM) were designed to test the hypothesis that 5-HT2A and 5-HT2C receptors exert opposing effects on locomotor activity in mice. Our previous studies in C57BL/6J mice indicated that the 5-HT2 agonist DOI produces an inverted U-shaped dose response function on exploratory behavior, increasing distance traveled and investigatory holepokes at lower doses and decreasing these measures at higher doses. To examine the contribution of 5-HT2A receptors to the behavioral profile of DOI, we conducted a dose response of DOI in 5-HT2A wild-type (WT) and knockout (KO) mice on a C57BL/6 background. To examine the effect of 5-HT2C receptor activation on locomotor activity in mice, additional experiments were conducted with 5-HT2C selective compounds. Low doses of DOI (1 mg/kg) increased locomotor activity in 5-HT2A WT mice, an effect that was blocked in 5-HT2A KO mice. Conversely, the decrease in locomotor activity produced by a high dose of DOI (10 mg/kg) was potentiated in 5-HT2A KO mice. We also found that the decrease in locomotor activity produced by a high dose of DOI (10 mg/kg) in C57BL/6 mice was attenuated by the 5-HT2C antagonist SER-082. Along these lines, the 5-HT2C agonist WAY 161 503 decreased locomotor activity and investigatory holepokes in C57BL/6 mice, effects that were blocked by SER-082. These data suggest that the increase in locomotor activity induced by low doses of DOI is mediated by 5-HT2A receptor activation and that the decrease in locomotor activity produced with high doses of DOI is mediated by 5-HT2C receptor activation. Hence, it appears that 5-HT2A and 5-HT2C receptors exert opposing effects on locomotor activity in mice.

SCP047

5-HT_7 receptor stimulation exerts anti-hyperalgesic effects in rats suffering from neuropathic pain via activation of GABAergic interneurons

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Among receptors involved in the serotonin control of *acute* nociception, the 5-HT_7 type is of special interest because it is expressed by both dorsal root ganglion cells and interneurons within the superficial layers of the spinal dorsal horn, where nociceptive messages are relayed. As distinct mechanisms underlie chronic versus acute pain, we investigated herein whether 5-HT_7 receptors may also participate in the control of *chronic* pain in neuropathic rats. Adult male Sprague–Dawley rats underwent unilateral constriction injury to the sciatic nerve (CCI-SN), and the

resulting allodynia/hyperalgesia was assessed by the Randall–Selitto test. Pharmacological treatments were performed two weeks after CCI-SN, when allodynia/hyperalgesia had reached its maximum. Acute administration (i.p.) of 5-HT_7 agonists (AS-19, MSD-5a, E-55888) exerted long lasting anti-hyperalgesic effects, that could be prevented by the 5-HT_7 antagonist SB269970. Because 5-HT_7 receptor stimulation is excitatory, we hypothesized that the anti-hyperalgesic effects of agonists were mediated by activation of inhibitory opioidergic and/or GABAergic interneurons rather than through presynaptic 5-HT_7 receptors on primary afferent fibres. Indeed, pretreatment with bicuculline but not naloxone prevented AS-19-induced anti-hyperalgesic effects. Furthermore, a similar blockade was achieved by injecting bicuculline systemically (i.v.) or intrathecally. These data provided evidence that 5-HT_7 receptor stimulation produces marked anti-hyperalgesic effects, through activation of GABAergic interneurons within the dorsal horn of the spinal cord. in rats suffering from neuropathic pain.

SCP048

Role of serotonin (5-HT)2 receptors in the nicotine-withdrawal evoked depressive-like behavioral effects in rats

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The present study sought to establish whether tonic or pharmacological activation of serotonin (5-HT)$_2$ receptors (5-HT_2R) could affect the behavior in the forced swim test (model of depression-like behavior) in either naive male Wistar rats (220–250 g) or those withdrawn from repeated nicotine treatment. Administration of the selective 5-HT_{2A}R antagonist M100 907 (1–2 mg/kg), but not the non-selective 5-HT_{2A}R agonist DOI (0.1–1 mg/kg) to naive rats decreased immobility; the effect was not related to the changes in the basal locomotor activity. The 5-HT_{2C}R antagonist SB242 084 (0.3–1 mg/kg) reduced immobility time, and this effect paralleled the enhancement in the animals' basal locomotion. Following the 5-HT_{2C}R agonists Ro60-0175 (10 mg/kg) or WAY 163 909 (1.5 and 10 mg/kg), a significant dose-dependent decrease in immobility and attenuation of basal locomotor activity were observed. In rats treated repeatedly (5 days) with nicotine (0.4 mg/kg/day sc) and then withdrawn, significant increase in immobility time the maximum 'pro-depressive' effect was observed on the 3rd day of withdrawal. On that day of nicotine withdrawal, M100 907 (1 mg/kg), SB242 084 (0.3 mg/kg), Ro60-0175 (3 mg/kg) or WAY 163 909 (0.75 and 1.5 mg/kg) produced a decrease in immobility time. Our data demonstrate that 5-HT_2R antagonists and 5-HT_{2C}R agonists, exhibit effects typical of anti-depressant drugs. Furthermore, 5-HT_{2C}R agonists seem to abolish the effects of withdrawal from repeated nicotine treatment in rats.

SCP049

Fast scan cyclic voltammetry evidence that 5-HT transporter overexpression impairs the function of 5-HT nerve terminals

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Increased expression of the 5-HT transporter (SERT) gene is linked to a reduced anxiety phenotype, possibly through decreases in presynaptic 5-HT function (Jennings *et al.*, 2006). The present study examines the influence of genetically increased SERT on the function of 5-HT nerve terminals and somatodendrites using fast-scan cyclic voltammetry at a carbon fibre microelectrode (CFM). Electrically evoked extracellular 5-HT ($[5\text{-HT}]_o$) was measured in brain slices (300 μm) containing terminal (substantia nigra *pars reticulata,* SNr) and somatodendritic (dorsal raphe nucleus, DRN) regions taken from SERT-overexpressing and wildtype mice (male, CBAxC57BL6J; 20–35 g). Electrical stimuli (200 μsec pulse width) consisted of trains of 20 pulses at a range of frequencies (10, 20, 50 and 100 Hz) delivered by a local bipolar electrode positioned approximately 100 μm from the CFM. Electrical stimulation evoked a frequency-dependent increase in $[5\text{-HT}]_o$ in both the SNr and DRN of wildtype mice. In the SNr of SERT-overexpressing mice, the magnitude of electrically evoked $[5\text{-HT}]_o$ was less than in wildtype mice at all frequencies, with the greatest difference seen at high stimulation frequencies. This reduction in evoked $[5\text{-HT}]_o$ was reversed, but only partially by bath application of the SERT inhibitor citalopram (100 nM). In contrast to the SNr, no difference in evoked $[5\text{-HT}]_o$ was detected in the DRN of SERT over-expressing versus wildtype mice. These findings suggest that SERT-over-expressing mice show a deficiency in evoked $[5\text{-HT}]_o$ at 5-HT terminals, but not somatodendrites, and that this deficiency is particularly apparent at higher stimulation frequencies. The finding that SERT blockade only partially reversed the deficiency in evoked $[5\text{-HT}]_o$ in the SNr of SERT-over-expressing mice suggests that enhanced 5-HT reuptake at the nerve terminal is not the sole cause of the deficit and that 5-HT release mechanisms may also be impaired. Impaired 5-HT release at 5-HT terminals may in turn underlie the low anxiety phenotype linked with SERT over-expression.

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SCP050

Inhibition of TPH2 expression by RNA interference

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Serotonin (5-HT) controls a wide range of biological functions. In the brain, it is involved in the physiology of the food intake, nociception, maternal and sexual behaviours, sleep-wakefulness cycles, motor activity, mood and stress. In the periphery, 5-HT acts as a neuro-hormone and contributes to multitude of other physiological and homeostatic processes. As a growth regulator, it participates in developmental events. In humans, as well as in most other mammalian spieces, serotonin is produced by two distinct enzymes. TPH1, wich is responsible for the synthesis of peripheral serotonin and TPH2, which is restricted to neurons of the raphe nuclei and the enteric nervous system. In the brain, the levels of 5-HT synthesis are essentially dependent on TPH2 activity. Altered 5-HT neurotransmission has been associated with impairements of sleep and emotional behaviours. To inhibit central 5-HT synthesis in the adult mouse (C57BL/6), we selected an siRNA directed against *Tph2*-mRNA. By using a reporter system *in vitro*, we demonstrated that this siRNA inhibits 5-HT synthesis efficiently in 293T HEK cells. Lentiviral vectors which express both the corresponding shRNA and the GFP have been constructed and their transducing and inhibiting efficiency have been validated *in vitro* in primary cultures of serotonergic neurons. Targeting viral vector to the dorsal raphe nucleus in adult mice has been already established in our group. Currently, the lentiviral vectors are delivered *in vivo* by stereotaxic injection into the DRN in adult mouse. We investigate on the consequences of the inhibition of 5-HT synthesis in the neurons of the DRN. Focus will be given on the 5-HT receptors, 5-HTT, VMAT2 and MAOA proteins of the serotonergic system. The impact of a lack of a 5-HT will be investigated not only on the fate of neurotransmitter-lacking neurons and their post-synaptic targets, but also on emotional behaviours and other integrated functions such as sleep-wakefulness patterns. Such studies should contribute to establishing functional maps of serotonergic nuclei and clarify the links between neurotransmission impairment, anatomical and biochemical changes and functional deficits.

SCP051

Bidirectional manipulation of tryptophan hydroxylase-2 expression in dorsal raphe modulates anxiety in ovarectomized rats

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Previous studies have shown that chronic estrogen treatment increases tryptophan hydroxylase-2 (TpH2) mRNA in the caudal dorsal raphe nucleus (cDRN), and this increase was associated with decreased anxiety. The present study explored the behavioral effects of direct manipulation of TpH2 mRNA in rat brain using antisense knockdown or viral overexpression in cDRN of ovariectomized rats replaced with empty or estrogen capsules (OVX, OVX/E, respectively) on anxiety. Female Sprague–Dawley rats (~300 g) were microinfused with TpH2 or scrambled morpholino antisense oligonucleotides into cDRN and tested in open field test and elevated plus maze. In a separate experiment, animals were microinfused with herpes simplex viral vectors expressing TpH2 and green fluorescent protein (GFP) or GFP alone into cDRN then tested in the open field and shock-probe burying tests. Estrogen decreased anxiety in all behavioral measures. In the OVX/E group, TpH2 knockdown significantly decreased time spent in the center of the open field, but not in OVX group, suggesting that TpH2 knockdown reversed the anxiolytic effects of estrogen. Conversely, TpH2 overexpression in OVX animals mimicked the effects of estrogen, as measured by increased time spent in the center of the open field. Interestingly, TpH2 overexpression in the OVX/E group decreased time spent in the center of the open field, suggesting that increased levels of TpH2 was anxiogenic in these animals. Since estrogen induces TpH2 expression, knockdown of TpH2 reverses and TpH2 overexpression mimics estrogen's anxiolytic effects in OVX rats, we suggest that estrogen's anxiolytic effects may be substantially mediated by TpH2 expression in DRN. However, at very high levels, TpH2 may increase anxiety, suggesting a U-shaped relationship between TpH2 and anxiety. This idea may, in part, explain some gender differences in the effect of serotonin on anxiety and have important clinical implications for women suffering from affective disorders.

SCP052

5-Chloroindole potentiates human 5-HT_{3A} receptor function assessed by $[Ca^{2+}]_i$

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The human 5-HT_{3A} receptor subunit forms a functional homomeric receptor, likely to comprise a pentameric structure around a central ion-channel pore that provides a useful model to study the family of cys-cys loop ligand-gated ion channels. A number of compounds have been shown to act as positive allosteric modulators of 5-HT_3 receptors including alcohols, volatile anaesthetics and indoles. Recent studies have identified the FlexStation as an accurate method of measuring 5-HT_{3A} receptor function using intracellular Ca^{2+} sensitive dyes. During the present study a similar methodology was employed and various 5-HT_3 receptor agonists (mCPBG, 2-methyl-5HT, DDP 733, quipazine and the endogenous agonist, 5-HT) generated pEC_{50} and Hill slope values, consistent with the pharmacology of the 5-HT_3 receptor. In the presence of 5-chloroindole (10–100 μM), 5-HT_3 receptor agonist-induced responses were increased and prolonged (as apposed to the relatively rapid desensitization seen with agonist alone). Emax values (percentage of max 5-HT response in the absence of 5-chloroindole) determined for 5-HT increased to 117 ± 5% in the presence of 5-chloroindole (10 μM). In addition, the partial 5-HT_3 receptor agonists, quipazine, 2-methyl-5-HT and mCPBG, appeared as full agonists in the presence of 5-chloroindole (10 μM), with Emax value increases of 46 ± 9% to 115 ± 14%, 72 ± 8% to 121 ± 18%, and 89 ± 2% to 106 ± 10%, respectively. 5-Chloroindole potentiation also appeared concentration dependent with 10, 30 and 100 μM having an increasingly greater effect on 5-HT_3 receptor function. All 5-HT_3 receptor agonist-induced responses in the absence and presence of 5-chloroindole (either 10 μM or 100 μM) were blocked by pre-incubation with the selective 5-HT_3 receptor antagonist, ondansetron (500 nM). The selective 5-HT_3 receptor antagonists, ramosetron, palonosetron, BRL 46470, ondansetron and alosetron, failed to evoke an increase in $[Ca^{2+}]_i$ in the presence (or absence) of 5-chloroindole (10, 30 or 100 μM). The present study demonstrates the ability of 5-chloroindole to potentiate 5-HT_{3A} receptor function in an agonist-independent manner.

SCP053

Pharmacological comparison of the heteromeric h5-HT_{3A3C} receptor with the homomeric h5HT_{3A} receptor

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The 5-hydroxytrptamine$_3$ (5-HT_3) receptor is a ligand-gated ion channel that is comprised of multiple subunits. Five human genes have been identified that encode 5-HT_3 receptor subunits (5-HT_{3A-E}) although information is lacking concerning the roles of 5-HT_{3C-E}. A recent study using transient expression has shown that the novel putative 5-HT_3 subunits are similar to the characterised 5-HT_{3B} subunit in that they require the co-expression of the h5-HT_{3A} subunit to generate a pentermeric 5-HT_3 receptor. Due to the potential problem arising from transient co-expression of 5-HT_{3A} and 5-HT_{3C} cDNA resulting in cells only expressing either of the subunits, we have generated a stably expressing 5-HT_{3A3C} cell line. Stable transfection was achieved using a myc-tagged h5-HT_{3C} cDNA subunit within pcDNA 3.1 hygromycin (+) into HEK 293 stable 5-HT_{3A} cells. Cells were grown in selection media and plated into a 96 well plate at a density of 1 cell/well. Single colonies were assessed for myc-immunoreactivity with one cell line chosen for further analysis. Only upon expression with the 5-HT_{3A} subunit did the myc5-HT_{3C} subunit express at the cell surface of HEK 293 cells. Whole cell homogenates were used for saturation binding with [^{3}H]-granisetron (Bmax = 1277 ± 155 fmol/mg, Kd = 0.71 ± 0.06 nM, mean ± SEM n = 03). pKi values determined for nine compounds showed no pharmacological differentiation greater than an order of magnitude between the homomeric 5-HT_{3A} receptor and the heteromeric 5-HT_{3A3C} receptor. Electrophysiological recordings were preformed and in both homomeric 5-HT_{3A} and heteromeric 5-HT_{3A3C} cells, 5-HT (10 μM) evoked an inward current. Current-voltage relationships were determined by applying a voltage ramp (-120 to +40 mV; 400 mV/s) at the peak 5-HT response. Current-voltage relationships indicated that the 5-HT-induced responses mediated by both cell types reversed around 0 mV. The present study indicates that co-expression of the h5-HT_{3C} subunit with the h5-HT_{3A} subunit does not majorly modify the resulting pharmacology of the presumed heteromeric h5-HT_{3A3C} receptor compared to the homomeric h5-HT_{3A} receptor.

SCP054

A comparison of serotonin, dopamine, and norepinephrine in the medial prefrontal cortex of male and female adolescent and adult rats

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We have studied how adolescent brains compare with adult brains when assessing neurotransmitter levels in the medial prefrontal cortex (mPFC) of the rat. Recent evidence has shown that the medial prefrontal cortex is an important brain area in the control of impulsivity, decision-making and executive function. The neurotransmitters serotonin (5-HT), dopamine (DA), and norepinephrine (NE) are integral to the function of the mPFC and are increased by the drugs used to treat attentional deficit disorder. The purpose of our investigation was to determine the extracellular concentrations of DA, 5-HT, and NE in the mPFC of adolescent and adult male and female rats using *in vivo* microdialysis. Because some studies have shown differences between the left and right mPFC in terms of extracellular 5-HT and DA we have used dual probes to examine both left and right mPFC. The overlying hypothesis is the adolescent male brain of the rat has a decreased extracellular DA and NE and increased 5-HT compared to adults. Dual microdialysis probes (2 mm) were placed into the left and right ventral mPFC of adolescent (pnd 40–45) and adult rats (pnd 90) from the same litters. Following collection of baseline samples, 100 μM methamphetamine was perfused through the probes for 1 h. Microdialysate was analyzed for DA, 5-HT and NE via HPLC. Our findings show that basal extracellular levels of 5-HT are significantly increased in the left and right ventral mPFC in adolescent male rats when compared to adolescent female as well as adult male rats. There was also a decrease in DA in the mPFC of male adolescent rats compared with female adolescent rats. In comparison with adult male rats, adolescent male rats have lower levels of DA in the right mPFC and lack the laterality in DA levels seen in the adult. Both male and female adolescents had higher levels of NE in the ventral mPFC than adult males. These data suggest that there are differences between adolescent male and female, and adult male rats in the basal extracellular concentrations of serotonin, dopamine and norepinephrine in the ventral mPFC. This may relate to the increased impulsivity and attentional problems noted in human male adolescents. (Supported by NIH grant MH 074811.)

SCP055

Generation of a *Tph2/EGFP* knockin mouse line for the study of the role of serotonin during the central nervous system development

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There is growing evidence that serotonin (5-hydroxytryptamine, 5-HT), in addition to its role in synaptic communication, has a major impact on brain development in mammals. 5-HT is synthesized in two steps with tryptophan hydroxylase 2 (*Tph2*) as the rate-limiting enzyme selectively expressed in the serotonergic neurons of the CNS. Neurons producing 5-HT are generated at mid gestation in the ventral region of the brainstem and exhibit wide innervation throughout the CNS at early stages of neurogenesis. The evidence that the timing of appearance of this monoaminergic system in the embryonic telencephalon overlaps with proliferation, migration and neuronal differentiation, has prompted speculation long ago that 5-HT may play a role in neural development. Consistently, several lines of evidence associate an altered serotonergic signalling with neuropsychiatric disorders in humans. Moreover, perinatal perturbation of normal 5-HT levels, using both genetic and pharmacological approaches in rodents, has been shown to result in abnormal development of cortical cytoarchitecture, confirming the involvement of 5-HT in brain development. Despite the importance of this monoamine, the precise role of 5-HT in specific morphogenetic activities during foetal and early postnatal CNS development remains to be clarified. In order to take a step forward in uncovering the role of 5-HT during brain development, we have generated a *Tph2/EGFP* knockin mouse line in which Tph2 was replaced by the reporter gene enhanced Green Fluorescent Protein (*EGFP*). We studied the consequences of *Tph2* inactivation in the newly generated *Tph2/EGFP* mouse line and a dramatic depletion of 5-HT within the CNS of mutant animals was observed. Despite such a drop of 5-HT content in the CNS, mice of a mixed genetic background (C57BL/6 and 129/Ola) lacking *Tph2* survive up to adulthood and are fertile. Growth defects were observed in *Tph2* mutant animals as compared to wild type (wt) mice. Growth rate was monitored throughout the first month of life and statistical analysis using ANOVA showed a striking (e.g. 60% reduction at postnatal day 15, P value < 0.0001) impairment of growth rate in *Tph2* mutants compared to heterozygous and wt littermates. We are currently investigating the origin of such growth impairment

SCP056

Analogues of MDMA/'Ecstasy' as potential anti-neoplastics in non-Hodgkin's lymphoma

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Building on the observation of serotonin entering Burkitt's lymphoma (BL) cells *via* the serotonin transporter, SERT, to engage the cells' apoptotic machinery, we subsequently showed that SERT-binding amphetamine derivatives – including 3,4-methylenodioxymethamphetamine (MDMA/'Ecstasy') – were similarly pro-apoptotic (Meredith *et al.*, 2005). However, while demonstrating efficacy of MDMA in promoting apoptosis in several lymphoma subtypes *in vitro*, we recognised that the high concentrations of drug required could not be translated safely *in vivo*. We therefore proposed the possibility of 'redesigning the designer drug' to isolate desired anti-lymphoma activity from unwanted psychoactivity and neurotoxicity. This process is now underway. From initial analysis of six MDMA analogues with modified alpha-substituent, the incorporation of a phenyl group increased potency 10-fold relative to the parent, MDMA, to drive cell death in biopsy-like BL cell lines. Based on this new lead, related analogues have now been synthesised. The current 'best' compounds – with 1- and 2-naphthyl and *para*-biphenyl substituents – are around 200-fold more potent against lymphoma cells than MDMA. The steric and hydrophobic properties of the substituents were studied and the general trend was that the bigger and more hydrophobic the substituent, the higher the potency for killing BL cells. Also, the type of substituent determines the MDMA analogues' selectivity upon B cells arising from different malignancies, with the highest efficacy of all compounds tested on BL cells, indicating impact upon a common pathway. The mode of cell death has also been studied. BL cells overexpressing a *bcl*-2 transgene die in a caspase-3 independent manner and are only marginally more resistant to the MDMA analogues than those carrying empty vector (control) alone, which die *via* apoptosis. The pathway(s) involved in the MDMA analogue–induced cell death were explored with some evidence consistent with an involvement of oxidative stress. However, the production of extracellular Reactive Oxygen Species (ROS) and associated disruption of cell membranes were excluded. In contrast, preliminary studies implicate intracellular ROS. Ongoing experiments are designed to further unravel the mechanism of action of these novel, potentially therapeutic, compounds for B-cell malignancy.

Reference:

Meredith *et al.* FASEB J. 2005; 19: 1187.

SCP057

Altered analgesic and serotonin syndrome behavioral effects of traditional and non-traditional opioids in serotonin transporter (SERT)-deficient mice

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In addition to their actions on opioid receptors, non-traditional opioids including tramadol and meperidine block serotonin and norepinephrine reuptake. Recently, tramadol and meperidine have been implicated in the serotonin syndrome, a potentially lethal side effect of serotonin-enhancing drugs (Gillman *et al.*, 2005). As serotonin transporter (SERT)-deficient mice show exaggerated serotonin syndrome behavioral responses (Fox *et al.*, 2007), we assessed the serotonin syndrome behavioral effects of tramadol and meperidine, and their analgesic effects in the hot plate test, compared to morphine, in SERT wildtype (+/+), heterozygous (+/−) and knockout (−/−) mice (Bengal *et al.*, 1998). Tramadol and meperidine induced serotonin syndrome behaviors in mice of all genotypes compared to controls administered vehicle or morphine. This response was exaggerated in SERT +/− and −/− mice administered tramadol, and in SERT −/− mice administered meperidine, compared to SERT +/+ mice. In SERT −/− mice, pretreatment with the 5-HT_{1A} antagonist WAY 100 635 decreased tramadol-induced serotonin syndrome behaviors, suggesting that 5-HT_{1A} receptors mediate this response, whereas WAY 100 635 had no effect in SERT +/− mice. Further, the analgesic effects of morphine, tramadol and meperidine were decreased in SERT −/− mice. Thus, SERT-deficient mice show decreased anti-nociceptive, but enhanced serotonin syndrome behavioral responses to tramadol and meperidine. These findings may have implications for individuals with SERT polymorphisms that may reduce SERT expression and function by more than 50% (Lesch *et al.*, 1996), and suggest that these individuals may be at increased risk for developing the serotonin syndrome when treated with non-traditional opioid analgesic medications.

References:

Bengal *et al.* Mol Pharmacol 1998; 53: 649–55.
Fox *et al.* Neuropharmacology 2007; 53: 643–56.
Gillman PK *et al.* Br J Anaesth 2005; 95: 434–41.
Lesch *et al.* Science 1996; 274: 1527–31.

SCP058

Changes in serotonergic activity during sturgeon embryogenesis

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Serotonin behaves as strong morphogene. The aim of this work was to study the time course of serotonergic system activity during embryonic development of fish eggs in normal conditions and under crude oil impact. The Sturgeon eggs *(Acipencer stellatus)* were fertilized and incubated up to hatching in water, containing 500 ppm of the crude oil. Controls were fertilized in the clean water. The serotonin activity was evaluated by measuring the level of serotonin-modulating anticonsolidation protein (SMAP). The level of SMAP was determined by the ELISA before/after fertilization and at the stages of cleavage stage, gastrula, neurula, heart beating and hatching. In embryos exposed to the oil the level of SMAP differed significantly from controls. At the stages of cleavage, gastrula and neurula changes in SMAP content had strong inverse trend relatively to the controls. These changes were accompanied with dramatic disturbances in process of embryonic development. Passage from the gastrula to the neurula stages was accompanied with high rate of mortality (52.9%) in the experimental group, while in the controls this index was 20.7%. In spite of high level of the hatching in the oil-exposed group, there were many larvae with abnormal development. Interestingly, that unhatched larvae had high levels of SMAP in comparison to low levels of this protein in controls. Our results, showing the participation of serotonergic system in embryonic development of Sturgeon eggs, assumed that teratogenic effects of oil chemicals are mediated by alteration of serotonin's morphogenic activity.

SCP059

BDNF+/- mice exhibit region-specific decreases in 5-HT_{1A} receptor function and increased vulnerability to mild handling stress

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Brain-derived neurotrophic factor (BDNF) has profound effects on the plasticity and structural stability of synapses, and promotes monoaminergic neurotransmission. A deficiency in BDNF has been implicated in the pathophysiology of stress-related psychiatric disorders, such as major depression. Increased BDNF signaling is believed to contribute to the therapeutic action of antidepressant treatments. Conversely, exposure to chronic stress may be detrimental in that it serves to decrease BDNF mRNA expression in hippocampus and frontal cortex. BDNF+/- mice exhibit region-specific abnormalities in serotonergic neurotransmission. Serotonergic neurotransmission is increased in hippocampus, a brain region that plays an important role in the negative regulation of the stress response, as well as in the antidepressant-like effect of increased BDNF signaling. In the present study, we examined in male mice the effect of a constitutive deficiency in BDNF on 5-HT_{1A} receptor function in forebrain and serotonergic cell body areas, and on hormonal and behavioural responses to acute or mild stress. 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding was decreased in hippocampus, but not frontal cortex, and in the median but not dorsal raphe nucleus of BDNF+/- mice. The binding of the agonist radioligand [^{3}H]8-OH-DPAT was not altered in any area of brain examined. These data indicate that in BDNF+/- mice the capacity of 5-HT_{1A} receptors to activate G proteins is attenuated in median raphe and hippocampus, which is heavily innervated by serotonergic neurons arising from the median raphe. In response to a single i.p. injection of saline, plasma ACTH and corticosterone levels peaked at 30 min post injection in both wild-type and BDNF+/- mice. Baseline and peak plasma levels of these hormones did not differ between genotypes. However, BDNF+/- mice appeared to be very sensitive to mild handling stress, exhibiting increased immobility in the forced swim test after injection of saline 24 h, 4 h and 1 h before the forced swim test. We are currently investigating whether these neurochemical and behavioural abnormalities in BDNF+/- mice are normalized by treatment with selective serotonin reuptake inhibitors. BDNF+/- mice may be a useful model in which to study how region-specific changes in neuronal populations in response to a constitutive deficiency in BDNF contribute to behaviours relevant to stress-related mood disorders, i.e. anxiety and depression. Supported by US PHS grants MH 71488, MH 52369.

SCP060

Evidence for topographically organized endogenous 5-HT-1A receptor-dependent feedback inhibition of the ascending serotonin system

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Raphe and extra-raphe 5-HT-1A receptors contribute to feedback inhibition of serotonin (5-HT) neurons; however, the endogenous function of 5-HT-1A receptor dependent feedback inhibition remains poorly understood. Here, the possibility that 5-HT-1A mediated feedback inhibition of the raphe nuclei is topographically organized was examined. This was done by testing the effect of systemic blockade of 5-HT-1A receptors on Fos expression in 5-HT neurons in the dorsal and median raphe (DR and MR). Male Sprague-Dawley rats (225–275 g) were used. The premise was that appearance of Fos after 5-HT-1A receptor blockade would implicate endogenous inhibition via 5-HT-1A-dependent processes. 5-HT-1A-receptor antagonist administration (WAY 100 635) in rats returned to their home cage significantly increased the number of Fos-5-HT cells in the lateral wings and the ventral caudal part of the DR compared to vehicle-injected controls, suggesting that tonic activity of brain 5-HT-1A receptors impacts these regions. In rats receiving vehicle injections, swim, a behavior known to influence 5-HT neurotransmission, increased the number of Fos-5-HT cells only in the caudal third of DR. Administration of WAY 100 635 preceding a swim did not change Fos in the caudal DR, but increased Fos-labeled 5-HT cells in the rostral DR, lateral wings of the DR and the MR. These results confirm using an imaging approach, that 5-HT-1A receptor-dependent feedback inhibition depends on behavioral state (return to home cage vs. swim). Moreover, they reveal that the effect of 5-HT-1A receptor blockade in each case is subregionally organized.

SCP061

Effects of subchronic restraint stress on brain-derived neurotrophic factor in serotonin transporter deficient mice

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Mood and anxiety disorders arise from a complex combination of factors leading to a heterogeneous pathogenesis with stress identified as an important contributor. In humans, heightened risk for depression following stressful events has been associated with a promoter polymorphism, the 5-*HTTLPR*, in the serotonin transporter (SERT) gene. Mice with constitutive reductions in SERT have also been found to be hyperresponsive to stress and to exhibit an anxious phenotype paralleling the effects of the low expressing allele of the 5-*HTTLPR* in humans. Critical post-synaptic molecular modifications, such as a brain-derived neurotrophic factor (BDNF)-dependent mechanism, likely accompany monoaminergic signaling alterations associated with these psychiatric conditions. In support of this, a variety of anti-depressants appear to up-regulate BDNF mRNA. By contrast, chronic stress decreases hippocampal cell proliferation and BDNF expression. The BDNF gene in rodents and humans contains multiple upstream non-coding regions alternately spliced to a single downstream coding region resulting in multiple transcripts. Recent reports indicate promoter-specific regulation in response to anti-depressant treatment and acute and chronic forms of stress. We are investigating the effects of subchronic restraint stress on the expression patterns of all currently identified BDNF splice variants in frontal cortex, hippocampus, and brain stem of SERT-deficient mice. Core body temperatures recorded during restraint revealed reductions in temperature in stressed wildtype and SERT-deficient mice compared to unstressed mice of each genotype. Decreases in body weight were observed only in stressed mice with SERT−/− mice showing a statistically greater degree of weight loss. We measured total BDNF protein levels by ELISA and these did not change significantly with respect to genotype or stress in any of the three brain regions. RT-qPCR is being used to investigate changes in the expression of individual BDNF transcripts. We anticipate identifying additional key BDNF transcripts modulated by stress and SERT deficiency.

SCP062

Improved route learning in aging mice with constitutive reductions in brain-derived neurotrophic factor

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Brain-derived neurotrophic factor (BDNF) plays an important role in the genesis, survival, growth and synaptic plasticity of neurons in the developing and adult brain and is known to have a complex co-regulatory relationship with the serotonin (5-HT) system. Adult BDNF+/− mice show increases in extracellular 5-HT in hippocampus that have been correlated with reduced uptake by the serotonin transporter (SERT) *in vivo*. In aging BDNF+/− mice, there is an accelerated age-related loss of 5-HT innervation to the hippocampus associated with reduced extracellular 5-HT levels. Some reports indicate that BDNF+/− mice show anxiety-related behaviors and impairments in spatial learning. In the present study, we investigated behavior in two separate cohorts of mice having constitutive reductions in BDNF. Mixed sex groups of BDNF+/+ and BDNF+/− mice were 2 months (n = 25) or 25 months (n = 56) of age at the time they were tested in the elevated plus maze, open field, and the Lashley III maze, a low-stress route-learning task. No significant differences were found between BDNF+/+ and BDNF+/− mice in the elevated plus maze and no differences in horizontal or vertical movement were observed in the open field, suggesting that constitutive reductions in BDNF have no effect on anxiety-related behavior or locomotor activity, even in older mice. By contrast, striking differences between the two genotypes were observed in the Lashley III maze in aging animals. Highly significant main effects of genotype occurred in the number of errors ($P < 0.001$), days to criterion ($P < 0.001$), and learning index ($P < 0.001$), with BDNF+/− mice of both sexes performing *better* than age-matched BDNF+/+ littermates on each of these measures of maze learning. These data suggest that older mice with lifelong reductions in BDNF learn this type of low stress task faster than mice with normal BDNF levels and that the nature of the conditions under which learning occurs may result in qualitatively different outcomes in learning behavior. We are currently carrying out studies to determine the role of brain region-specific SERT activity in mediating the effects of stress on learning behaviors in young and aging mice with reduced BDNF expression.

SCP063

Serotonin as a key factor involved in fertility and embryonic development

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Serotonin (5-HT) is a neurohormone with several known functions in the central nervous system and periphery. Recently we showed that 5-HT could also be a crucial factor for normal embryonic development. Using mice knocked-out for the gene expressing the 5-HT-synthesizing enzyme, tryptophan hydroxylase 1 (TPH1), which have low levels of peripheral 5-HT, we observed that offspring experience early embryonic anomalies that are strictly linked to their mother's genotype (TPH1−/−), regardless of their own. This indicates a purely maternal effect and underlines the importance of 5-HT for embryo development. We also showed the presence of a serotonergic network in reproductive tissues that is likely to take part in local regulation of key processes from oocyte growth to embryo development. In light of these results, we addressed the requirements for 5-HT derived from the maternal reproductive tract in fertility by verifying reproductive performances of the TPH1−/− females. First, the study of estrous cycles (using vaginal smears) revealed a prolonged diestrus in TPH1−/− females (3–5 days) as compared to control mice (1–2 days). To establish the fertility of the TPH1−/− mice, wild-type,

heterozygous and null mutant TPH1−/− females were bred with wild-type males. A significant difference was seen in the numbers of pups born to the TPH1−/− mice as compared with control or heterozygous mice. However, the numeration of ovulated oocytes showed no difference between control ant mutant females, suggesting *in vitro* death of embryos. Finally, the expression of serotonergic components and that of 5-HT itself in reproductive tissues was evaluated and shown to vary with the stage of the estrus cycle. Our results will set the first basis establishing to what extent maternal 5-HT may be at play to influence reproduction and can be correlated to prescriptions of serotonergic drugs. While any possible effect of these compounds on early women's fertility is neither known nor even considered, there is increasing concern about their use at later stages of pregnancies. For example, use of specific serotonin reuptake inhibitors (SSRIs) was associated with increased birth defects, particularly cardiac malformations and a higher risk of pulmonary hypertension in newborns among mothers taking SSRIs while other studies indicated a higher risk of spontaneous abortions, congenital malformations or low-birth weight, preterm birth and fetal death.

SCP064

5-HT4-elicited positive inotropic response is mediated by cAMP and regulated by PDE3 in failing rat and human cardiac ventricle

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The ventricle of the failing rat heart becomes sensitive to serotonin (5-HT) paralleled by appearance of functional Gs-coupled 5-HT4 receptors. The objective of this study was to explore regulatory functions of phosphodiesterases in the ventricular 5-HT4-mediated functional effects induced in failing rat and human heart. Post-infarction heart failure was induced by coronary artery ligation in male Wistar rats. Contractility was measured in rat left ventricular papillary muscles 6 weeks after surgery and in ventricular trabeculae from explanted human hearts. cAMP was quantified by RIA. In papillary muscles from post-infarction rat hearts 5-HT4 stimulation exerted positive inotropic and lusitropic effects accompanied by an increase of cAMP. The inotropic effect was increased by the non-selective PDE inhibitor IBMX (10 μM) and by the PDE3 inhibitor cilostamide (1μM). The PDE2 inhibitor EHNA (10 μM) and the PDE4 inhibitor rolipram (10 μM) did not increase the inotropic response. Combined PDE3/4 inhibition enhanced the inotropic response beyond the effect of PDE3 inhibition alone and increased the sensitivity to serotonin. The lusitropic effect was increased only during combined PDE inhibition. In failing human ventricles, the 5-HT4-mediated positive inotropic response was regulated by PDEs in a similar manner as in post-infarction rat hearts. The 5-HT4-mediated positive inotropic response in the failing rat ventricle is cAMP-dependent. PDE3 is the main regulator of this response and the involvement of PDE4 is demasked by inhibition of PDE3 in both post-infarction rat and failing human hearts.

SCP065

Antagonist-mediated down-regulation of 5-HT7 receptors by the atypical anti-psychotics clozapine and olanzapine: Evidence for functional selectivity

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Classically, ligands of G protein-coupled receptors have been classified primarily upon their affinity and efficacy to activate a signal transduction pathway. More recent reports indicate that the efficacy of a particular ligand can vary depending on the receptor mediated response measured (e.g. activating G protein(s), other downstream responses, inducing internalization). At the Gs-coupled 5-HT7 serotonin receptor, we have previously demonstrated that one out of three inverse agonists (SB269 970) induced both homo- and heterologous desensitization, similar to agonist stimulation (Krobert *et al.*, 2006). The primary objective of this study was to determine whether different antagonists/inverse agonists at the 5-HT7 receptor also induced receptor internalization and/or degradation of 5-HT7 receptors. The agonist 5-HT and three out of four inverse agonists tested induced internalization, but only the atypical anti-psychotics clozapine and olanzapine (inverse agonists) induced degradation of 5-HT7 receptors (~60% reduction within 24 h). Incubation with only clozapine or olanzapine targeted 5-HT7 receptors to lysosomes and inhibiting lysosomal degradation with chloroquine blocked the down regulation of 5-HT7 receptor density. Incubation with SB269 970 decreased both 5-HT7(b) internalization and receptor density but increased 5-HT7(d) receptor density, indicating differential regulation among the 5-HT7 splice variants. Taken together, the results show that various ligands differentially activate regulatory processes governing receptor internalization and degradation in addition to signal transduction. Thus, these data provide support for functional selectivity at the 5-HT7 receptor.

Reference:

Krobert KA *et al.* Eur J Pharmacol. 2006; 532: 1–10.

SCP066

Interaction of oestrogen with central serotonergic mechanisms in sensory gating: A study using the loudness dependence of the auditory evoked potential in healthy subjects

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The central serotonergic system is involved in the pathophysiology of schizophrenia and other psychiatric disorders. Women have a later onset of schizophrenia and a better treatment outcome compared to men, suggesting that estrogen may be protective. The loudness dependence of the auditory evoked potential (LDAEP) has been suggested as a non-invasive electrophysiological marker of central serotonin function, with reduced serotonergic activity thought to increase the LDAEP slope. The present study aimed to explore the effect of estrogen on serotonergic mechanism using LDAEPs using dipole source localization. Because 5-HT1A receptors have been suggested to play a role in schizophrenia, we also examined the interaction of estrogen pretreatment with the effect of the 5-HT1A receptor partial agonist, buspirone. In a double-blind, repeated measures design, 13 female healthy volunteers were treated with placebo/placebo, estrogen (2 mg)/placebo, placebo/buspirone (5 mg) and estrogen/buspirone. Data were analyzed with repeated-measures ANOVA. Oestrogen treatment resulted in a marked and significant increase of LDAEPs slope on its own. Buspirone treatment significantly enhanced LDAEP slope in the placebo condition but was without any effect in the estrogen condition. In conclusion, these results suggest that buspirone treatment increased LDAEP by inhibiting 5HT function and that this inhibition was prevented by estrogen pretreatment. In addition, estrogen was found to increase LDAEP, possibly by a direct action on cortical pyramidal cells or by an interaction with 5-HT1A receptors. These results could be important for our understanding of the mechanism by which estrogen protects against schizophrenia.