Current Topics in Medicinal Chemistry, 2006, *6 (18)*, 1909-25

Central Serotonin$_{2C}$ Receptor: From Physiology to Pathology

Giuseppe Di Giovanni[1], Vincenzo Di Matteo[2], Massimo Pierucci[2], Arcangelo Benigno[1] and Ennio Esposito[2,*]

[1]*Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana "G. Pagano", Università di Palermo, C.so Tuköry, 129 I-90134 Palermo, Italy,* [2]*Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, 66030 Santa Maria, Imbaro (Chieti), Italy*

Abstract: Since the 1950s, when serotonin (5-HT) was discovered in the mammalian central nervous system (CNS), an enormous amount of experimental evidence has revealed the pivotal role of this biogenic amine in a number of cognitive and behavioural functions. Although 5-HT is synthesized by a small group of neurons within the raphe nuclei of the brain stem, almost all parts of the CNS receive serotonergic projections. Furthermore, the importance of 5-HT modulation and the fine-tuning of its action is underlined by the large number of 5-HT binding sites found in the CNS. Hitherto, up to 15 different 5-HT receptors subtypes have been identified.

This review was undertaken to summarize the work that has explored the pathophysiological role of one of these receptors, the 5-HT$_{2C}$ receptor, that has been emerged as a prominent central serotonin receptor subtype. The physiology, pharmacology and anatomical distribution of the 5-HT$_{2C}$ receptors in the CNS will be firstly reviewed. Finally, their potential involvement in the pathophysiology of depression, schizophrenia, Parkinson's disease and drug abuse will be also discussed.

Keywords: Serotonergic receptors, Depression, Schizophrenia, Drug of abuse, selective 5-HT$_{2C}$ drugs.

BASIC ANATOMY OF 5-HT SYSTEM

More than fifty years have passed since Twarog and Page [1] isolated an indole, identified as serotonin (5-HT), in the mammalian brain. Subsequently, Brodie and colleagues [2] suggested that 5-HT might serve as a neurotransmitter in the central nervous system (CNS). The result was one of the most important discoveries in science, giving birth to a new branch of neuroscience [3]. Serotonin is one of the oldest biologically active compound on earth, found in a variety of plants and animals. In vertebrates, the majority of the neurons containing 5-HT are grouped in 9 nuclei named B1 to B9, located in the medial part of the brainstem, generically called the raphe nuclei [4] (Fig. **1**). These midline clusters can be divided into two major groups. The caudal or inferior group, localized in the medulla, contains the three nuclei projecting essentially to the grey matter of the spinal cord: the nucleus raphe magnus (NRM, cell group B5), nucleus raphe obscurus (NRO, cell groups B1-B2-B3), and nucleus raphe pallidus (NRP, cell group B4). The rostral or superior group, situated in the pons/mesenchephalon, contains the dorsal raphe nucleus (DRN, cell groups B6 and B7) and the median raphe nucleus (MRN, cell group B8). These nuclei supply about 80% of the serotonergic innervation of the forebrain. Even if in many brain areas, the innervation coming from the two nuclei overlaps, in certain regions the innervation comes exclusively or prevalently from one nucleus only. For example, the dorsal hippocampus receives a serotonergic innervation only from MRN, other areas innervated preferentially from this nucleus are: the medial preoptic area, the suprachiasmatic nucleus, the olfactory bulb and the medial septum nucleus. The dorsal raphe nucleus innervates the corpus striatum, the globus pallidus, the lateral septum nucleus and the amygdala, and provides most of the innervation of the prefrontal cortex. Serotonin-containing cell bodies of the raphe send projections to both dopaminergic cells in the ventrategmental area (VTA) and substantia nigra (SN), and to their terminal fields [5-8]. A comparison between the number of 5-HT terminals and the total number of axon terminals in the VTA reveals that the majority of these are serotonergic. Moreover, electron microscopy demonstrates the presence of synaptic contacts of [^{3}H]5-HT labeled terminals with both dopaminergic and non-dopaminergic dendrites in all subnuclei of the VTA and the SN pars compacta and reticulata [5, 8, 9]. These 5-HT axon terminals form many symmetric (inhibitory) contacts with non-dopaminergic and non-accumbens projecting neurons in the VTA, suggesting that 5-HT exerts an inhibitory influence on certain non-DA VTA neurons [7]. The DRN innervates, together with the MRN the ventral part of the hippocampus, the nucleus accumbens and various nuclei of the thalamus and the hippocampus [10-12]. Moreover, extensive serotonergic connections between the DRN and the MRN also exist [13].

5-HT RECEPTORS

The first evidence for the existence of multiple subtypes of 5-HT receptors was provided by Gaddum and Picarelli whuch discovered the so-called M and D receptors [14]. Definitive evidence for two distinct recognition sites was reported by Peroutka and Snyder [15] who classified 5-HT in two subtypes 5-HT$_1$ or 5-HT$_2$ depending on their affinity to [^{3}H]-5-HT and [^{3}H]-spiperone, respectively. A vast amount of research has led to the discovery and characterisation of a plethora of 5-HT receptor subtypes. At present, seven classes

*Address correspondence to this author at the Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri Sud", 66030 Santa Maria Imbaro (Chieti), Italy; Tel: (+39) 872-5701; Fax: (+39) 872-570416; E-mail: esposito@negrisud.it

1568-0266/06 $50.00+.00

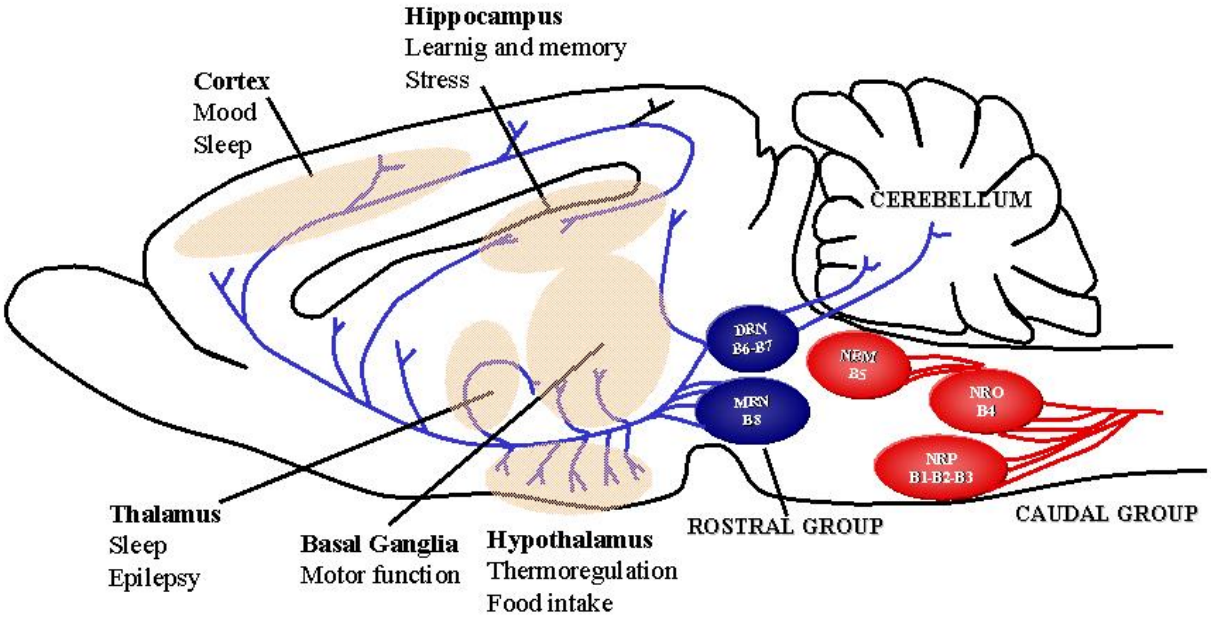

Fig. (1). Midsaggital view of the rat brain with serotonin-immunoreactive cell bodies. The blue and red ovals encompass the two major subdivisions of the brain serotonergic system. The major functions of 5-HT_{2C} receptors, and the brain areas where they occur are also shown. Abbreviations: DRN, dorsal raphe nucleus ; MRN, median raphe nucleus; NRM; nucleus raphe magnus; NRO, nucleus raphe obscurus. Cell groups B1 to B9 according to the terminology of Dahlström and Fuxe (1964).

of 5-HT receptors (5-HT_{1-7} receptors) have been identified which comprise at least a total of 15 subtypes [16] (Table **1**). Not surprisingly, with so many potential targets, distributed throughout all the CNS (Table **2**), 5-HT is a major neurotransmitter involved in a such large number of physiological and pathological processes. 5-HT receptors are cell surface receptors that have been classified on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organisation, and second messenger coupling pathways [16, 17]. Except for the 5-HT_3 receptor, which is a ligand gated channel, all 5-HT receptors belong to the seven transmembrane domain G-protein-coupled receptor (GPCR) superfamily [16, 18, 19]. The 5-HT_3 receptors have three intracellular loops, an extracellular N-terminus, and an itrancellular C-terminus. Of the three intracellular loops, the third (i3) loop is typically the longest. In the brain, high densities of 5-HT_3 receptors are found in the area postrema, cerebral cortex and limbic regions such as the hippocampal formation and the amygdala [20, 21]. The 5-HT_1 receptor class is comprised of five receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}), which, in humans, share 40–63% overall sequence identity and couple preferentially, although not exclusively, to Gi/o to inhibit cAMP formation (see Table **1**). To date, a functional role in a variety of tissues has only been demonstrated for the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors. The 5-HT_{1C} designation is now vacant, since this receptor was reclassified as 5-HT_{2C} due to structural, operational and transductional similarities with the 5-HT_2 receptor subclass [16]. Dense labeling of 5-HT_1 receptor binding sites is typically found in the hippocampus, septum, some amygdaloid nuclei and the cortex including the frontal and cingulate regions. Furthermore, high density of 5-HT_{1A} receptors are localised to the raphe nuclei where they have been shown to act as somatodendritic autoreceptors regulating the firing activity of 5-HT neurons [20]. The 5-HT_2 subfamily will be discussed in the further paragraph. The 5-HT_3 receptor is the oldest of the serotonin receptors, it is a ligand-gated cation channel belonging to the nicotine/GABA receptor superfamily with four putative transmembrane domains. It is a pentamer consisting of five monomers, at least three different monomers (5-$HT_{3A/3B/3C}$) have been found in the brain [22, 23]. The 5-HT_4, 5-HT_6 and 5-HT_7 receptors have all been demonstrated to be preferentially coupled to Gs and promote cAMP formation. However, the sequence dissimilarity justifies classification into different groups. Although the functional significance remains to be revealed, the interest in these receptor subtypes have increased after the demonstration that several antipsychotic drugs including clozapine, chloropramazine, risperidone and olanzapine display a significant affinity for these sites [24]. Two subtypes of the 5-HT_5 receptor (5-HT_{5A} and 5-HT_{5B}), sharing 70% overall sequence identity, have been found in rodents, whereas only the 5-HT_{5A} subtype has been found in humans [25, 26]. Recent studies have shown that the human recombinant 5-HT_{5A} receptor activation produced an inhibition of cAMP production, indicating negative coupling to cAMP via Gi and Go [27]; however, the receptor may also couple positively to cAMP. The human 5-HT_{5B} gene does not encode a functional protein because its coding sequence is interrupted by stop codons. The 5-HT_{5B} receptor is the first example of a brain-specific protein not found in human [28].

5-HT_2 FAMILY

Among the multiple classes of 5-HT receptors described in the central nervous system [16, 17, 29], much attention has been devoted to the 5-HT_2 receptor family since it has been shown by experimental and clinical observation that it

Table 1. Nomenclature and Classification of the Serotonin Receptor Subtypes

Receptor	Agonist	Antaganist
Seven-transmembrane-spanning, G-protein-coupled receptors		
Negatively coupled to adenylate cyclase ($G_{i/o}$)		
5-HT$_{1A}$	8-OH- DPAT	WAY-100635
5-HT$_{1B}$	CP 93129	GR127935
5-HT$_{1D}$	PNU 109291	BRL-15572
5-HT$_{1E}$	-	-
5-HT$_{1F}$	LY-334370	-
Positively coupled to adenylate cyclase (G_s)		
5-HT$_4$	RS67333, SB205149	RS39604, SB-207266
5-HT$_6$	-	RO630563, SB-271046
5-HT$_7$	-	SB-258719, SB-269970
Coupled to phospholipase C (G_q)		
5-HT$_{2A}$	DOI	
5-HT$_{2B}$	BW-723C86	RS-127445, SB-215505
5-HT$_{2C}$	RO-600175, SB-206553 (inverse agonist)	SB-242084, SB-243213
Unknowing coupling		
5-HT$_{5A}$	-	-
5-HT$_{5B}$	-	-
Ligand-gated ion channel/receptor		
5-HT$_{3A}$	SR 57227	Granisetron, Ondansetron
5-HT$_{3B}$	-	-
5-HT$_{3C}$	-	-

may represent a possible therapeutic target for the development of drugs for a range of CNS disorders such as schizophrenia, depression, drug abuse, eating disorders, Parkinson's disease and epilepsy [30-33]. In fact, 5-HT$_2$ receptors are major targets for a wide array of psychoactive drugs, ranging from non-classical antipsychotic drugs, anxiolytics and anti-depressants, which have a 5-HT$_2$ antagonistic action, to hallucinogens, which are agonists of the 5-HT$_2$ receptors [34-37]. Furthermore, recently it has been shown that 5-HT$_2$ receptors have a potential significance in brain development [38], and in experience-dependent plasticity in the visual cortex [39]. The 5-HT$_2$ receptor form a closely related subgroup of G-protein-coupled receptors and show the typical heptahelical structure of an integral membrane protein monomer. They are currently classified as 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ subtypes [16, 17, 21, 29], based on their close structural homology, pharmacology and signal transduction pathways [16, 17, 21, 29] and they are differently localized in the brain (Table **3**). The amino acid sequence of the 5-HT$_2$ receptors shares an high degree (>70%) of identity within the transmembrane segments [16, 17, 21, 29], consequently, it is not surprising that many compounds bind with high affinity to all these three receptor subtypes.

Initial studies on 5-HT$_2$ receptor signalling showed that these receptors activate the heterotrimeric G proteins that contain the α_q subunit, thereby stimulating phospholipase Cβ and leading to phosphatidyl inositol hydrolysis [35]. These receptors also stimulate phospholipase A_2 and the NO/cyclic GMP (cGMP) pathway. However, some differences in the signal transduction characteristics of these receptors have been reported [40, 41]. Studies focusing on the regulation of the 5-HT$_2$ receptor family have also indicated that 5-HT$_2$ receptors are non-classically regulated. Indeed, 5-HT$_2$ receptor responsiveness is not only decreased following chronic agonist exposure but also, paradoxically, after chronic treatment with antagonists such as antipsychotic compounds [37].

5-HT$_{2A}$ RECEPTORS

The 5-HT$_{2A}$ receptor (formerly called "5-HT$_2$", "serotonin 2" or "S2" receptors and thought to correspond to the D receptors) is comprised of 471 amino acids in rats,

Table 2. The Anatomical Distribution of Serotonin Receptors in the CNS

Receptor subtype	Receptor Distribution
5-HT_{1A}	Raphe nucli (somatodendritic autoreceptors), limbic system (amygdala, hippocampus, septum).
5-HT_{1B}	Basal ganglia (terminal autoreceptors).
5-HT_{1D}	Raphe nuclei.
5-HT_{1E}	Caudate putamen, entorhinal cortex, parietal cortex, thalamus, hypothalamus.
5-HT_{1F}	Dorsal raphe, hippocampus, cortex, striatum, thalamus, hypothalamus.
5-HT_{2A}	Cortex, basal ganglia, spinal cord.
5-HT_{2B}	Cerebellum, lateral septum, hypothalamus.
5-HT_{2C}	Dorsal raphe, choroid plexus, medulla, pons, striatum, nucleus accumbens, hippocampus, hypothalamus, ventrotegmental area, substantia nigra, spinal cord.
5-$HT_{3A/3B/3C}$	Caudate putamen, hippocampus, entorhinal cortex, frontal cortex, cingulate cortex, and dorsal horn ganglia.
5-HT_{4}	Striatum, brainstem, thalamus, hippocampus, substantia nigra.
5-$HT_{5A/5B}$	Hippocampus, cortex, cerebellum, habenula, spinal cord.
5-HT_{6}	Caudate putamen, olfactory tubercle, nucleus accumbens, cortex, hippocampus.
5-HT_{7}	Hippocampus, hypothalamus, thalamus, raphe nuclei.

Table 3. Comparison of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}

Receptor	Signal transduction	Receptor Distribution	Splice Variants
5-HT_{2A}	PLC, c-fos, ion flux, PKC activation	Cortex > basal ganglia > hippocampus	None identified yet
5-HT_{2B}	Ras activation, Ca2+ flux	Small amounts in various brain areas	None identified yet
5-HT_{2C}	PLC, PLA2, PLD	Choroid plexus > hippocampus > striatum	Yes

mice and humans and it is heterogeneously distributed in the mammalian brain [16, 17, 42]. It is abundantly present in the telencephalon (olfactory system, cerebral cortex, basal forebrain, neostriatum, hippocampus, amygdala). The 5-HT_{2A} receptors occur in the diencephalon (dorsal thalamus, hypothalamus), the mesencephalon (superior collicolus, substantia nigra reticulata), the methencephalon (pedunculopontine nucleus, and the latero dorsal tegmental nucleus) and the myelencephalon (spinal cord neurons and sympathetic and sensory neurons). The match between mRNA and protein distribution suggests a predominant somato-dendritic localisation supported also by the evidence that the 5-HT_{2A} receptor densities are not diminished by specific lesion of the serotonergic system [20]. The 5-HT_{2A} label was detected in the medial prefrontal cortex in some large- and medium-sized GABAergic interneurons [43-45] in almost all pyramidal cells [46] and on dopaminergic nerve terminals [47]. Surprisingly, these studies also revealed a prominent intracellular localisation of the 5-HT_{2A} receptors without any enrichment at the plasma membrane level [48]. The role of the intracellular pool remains to be elucidated. The 5-HT_{2A} receptors within the prefrontal cortex have been implicated in the pathogenesis and treatment of schizophrenia. Indeed, post-mortem studies have revealed a change in 5-HT_{2A} receptor density within the prefrontal cortex of subjects with schizophrenia [49]. Moreover, these receptors are targets for a large number of non-classical anti-psychotic drugs. Changes in 5-HT_{2A} receptor density and activity induced by these compounds likely account for their therapeutic action in schizophrenia and other pathologies. In the ventral tegmental area (VTA), 5-HT_{2A} receptors are present on GABAergic cells, but they are also expressed in rat and human VTA and substantia nigra pars compacta (SNc) dopaminergic neurons [50, 51] providing a potential anatomical basis for the modulation of DA neurons in the VTA either directly, by 5-HT_{2A} receptors localized on DA cells, or indirectly, through localisation on non–DA (GABAergic) neurons [51, 52]. In the basal forebrain, 5-HT_{2A} receptors are presumably localised on cholinergic cells of the lateral septal nucleus and the basal nucleus of Meynert [53]. The reduction of 5-HT_{2A} receptors in the brains of patients suffering from Alzheimer's disease may be a consequence of the degeneration of cholinergic neurons [53]. Dense labelling of 5-HT_{2A} receptor immuno-reactivity was found in the pedunculopontine and latero-dorsal tegmental nuclei. In these nuclei, 5-HT_{2A} receptors seem to occur only on local GABAergic interneurons and not on cholinergic cells [54, 55].

5-HT$_{2B}$ RECEPTORS

In contrast to the other 5-HT$_2$ receptors the 5-HT$_{2B}$ subtype is expressed principally in the periphery and only sparsely in the CNS [56-58]. Loric and colleagues [59] isolated the mouse 5-HT$_{2B}$ (formerly 5-HT$_{2F}$) receptor from a brain cDNA library and detected transcripts in the mouse intestine, heart, kidney and brain, but not in the liver or spleen. The mRNA of the 5-HT$_{2B}$ receptor is present only in very low concentrations in mouse [59-61] and human brain [61]. The human 5-HT$_{2B}$ receptor is made up of 481 amino acids and is relatively homologous with the other 5-HT$_2$ receptors [60]. Using more sensitive techniques, several groups have also shown the presence of both 5-HT$_{2B}$ receptor mRNA and protein [62] in the rat brain, more specifically in the cerebellum, cerebral cortex, hippocampus and amygdala. At present, little is known about the function of the 5-HT$_{2B}$ receptor as result of the lack of selective ligands at these sites. However, it seems that the 5-HT$_{2B}$ receptor plays an important role during embryonic development of various apparatuses [63] including the CNS [64].

5-HT$_{2C}$ RECEPTORS

The 5-HT$_{2C}$ receptor has received less attention in psychopharmacology compared to the 5-HT$_{2A}$ sites. This may relate to the inadequacy of techniques to determine the receptor in tissue samples. However, evidence from a variety of sources has involved this receptor in several important physiological and psychological processes including motor function, anxiety, ingestive behaviour and in brain development [33, 38, 65-67]. 5-HT$_{2C}$ receptor-deficient mice are overweight as a result of abnormal control of feeding behaviour [68, 69], thus this receptor could play a role in the serotonergic control of appetite. Mutant animals are also prone to spontaneous death from seizures, suggesting that receptors of this type mediate tonic inhibition of neuronal network excitability. Direct activation of the 5-HT$_{2C}$ receptors by agonists in normal rats decreases their food intake, and central 5-HT systems probably directly activate pro-opiomelanocortin (POMC) neurons via the 5-HT$_{2C}$ receptors [70-73]. The 5-HT$_{2C}$ receptor knockout mice are also more wakeful, and show several abnormalities in rapid eye movement (REM) sleep expression and enhanced response to sleep deprivation compared with wild-type control mice. These findings suggest that the 5-HT$_{2C}$ receptors may mediate several effects on sleep that have been ascribed to 5-HT [27]. Furthermore, some interesting pharmacogenetic observations have focused on the potential importance of this receptor for the development of novel antipsychotic, antidepressant, and antiepileptic drugs [31, 32, 65].

5-HT$_{2C}$ RECEPTOR STRUCTURE, SIGNAL TRANSDUCTION AND PHARMACOLOGY

The 5-HT$_{2C}$ receptor gene is unique among the members of the 5-HT receptors family by virtue of its genomic organization. The human 5-HT$_{2C}$ receptor gene has been mapped to X-chromosome band q24 [74] and shown to contain six exons and five introns. The coding region of the gene, unlike many other genes for GPCRs, is interrupted by three introns, and the positions of the intron/exon junctions are conserved between the human and the rodent genes [75]. The gene product is a protein of 460 amino acids, heavily glycosylated and migrating as a 60-kDa protein [76]. The 5-HT$_{2C}$ receptor is a member of the GPCR superfamily with 7-transmembrane spanning domains and three intracellular loops connecting them (Fig. **2**). Among the intracellular loops the second one that connects transmembrane helices 3 and 4 has an important role in G protein coupling [77]. However, full G protein activation has been shown to require both the second (i2) and the third intracellular loops (i3), suggesting they act in synergy [78]. The 5-HT$_{2C}$ receptor is the only known GPCRs whose mRNA undergoes post-trascriptional editing to yield different isoforms [79]. A naturally occurring variation in i2 was shown as result of adenine deaminase editing of the receptor mRNA. This leads to substitution of amino acids in the i2 of the receptor at position 156, 158 and 160 (Fig. **2**). Although RNA editing produces at least 14 functional isoforms (and potentially many more) of the receptor, these isoforms are associated with a variation in the efficacy of their interaction with G protein [80-82]. In this regard, it was recently reported that the depletion of 5-HT increases expression of 5-HT$_{2C}$ mRNA isoforms encoding receptors with higher sensitivity to 5-HT. This evidence suggests that this editing acts as a fine-tuning mechanism that adjusts receptor function to changes in synaptic activity, to maintain their normal response properties to agonist stimulation [83]. Interestingly, alteration of the 5-HT$_{2C}$ pre-mRNA editing has been implied in several psychiatric disorders. Indeed, distinct site-specific alteration of this editing has been found in the prefrontal cortex of patients with schizophrenia and major depression. Hitherto, the most complex alteration in 5-HT$_{2C}$ pre-mRNA editing was retrieved in brains of depressed suicide victims. In these brains, 5-HT$_{2C}$ receptor isoforms with reduced function were expressed at significantly increased levels, suggesting that the regulation of editing by synaptic 5-HT is defective [84]. Decreased agonist binding affinity has also been linked to RNA editing, however antagonist binding remained unaltered [85].

A splice variant of the 5-HT$_{2C}$ receptor has been identified, but due to a truncated C terminus, displayed neither G-protein coupling nor ligand binding [81]. Recently, it has been reported that the 5-HT$_{2C}$ receptors, like other GPCRs, exist as constitutive homodimers on the plasma membrane of living cells [86]. The same authors have subsequently shown that one 5-HT$_{2C}$ dimer binds two molecules of ligand and one G-protein [87]. Whilst it has been clearly shown that homodimerization of GPCRs regulates ligand binding, second messanger activation, and receptor trafficking [88], the functional significance of 5-HT$_{2C}$ receptor dimerization, remains unknown. If this phenomenon plays a role in regulating the function of the 5-HT$_{2C}$ receptor, it could have important implications for the development of novel serotonergic therapeutic agents.

The 5-HT$_{2C}$ receptors share extensive sequence similarities with the 5-HT$_{2A}$ receptors. Thus, it is not surprising that they have very similar pharmacological profiles and that they activate the same signalling pathways. However, some differences in signal transduction characteristics of both receptors have been reported. Agonist binding to the 5-HT$_{2C}$

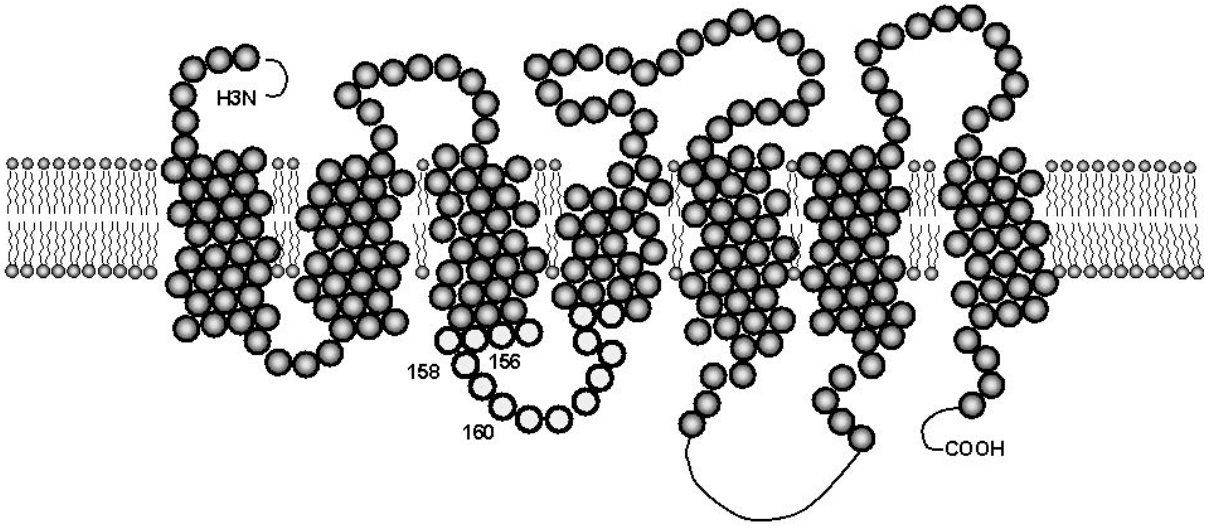

Fig. (2). Helical net representing the amino acid sequence of the unedited 5-HT_{2C} receptor. Residues in the second intracellular loop where the pre-mRNA editing occurs are not coloured and are highlighted by a thick dark circle.

receptor activates phospholipase C (PLC) via G protein ($G_{q/11}$) (Fig. **3**). Thus phospholipase C catalyses the hydrolysis of phosphadytilinositol-4,5-biphosphate to inositol 1,4,5-triphosphate (IP3) and diacylglycerol. IP3, acting as a second messenger, diffuses through the cell cytoplasm and stimulates the release of Ca^{2+} sequestered in the endoplasmatic reticulum which in turn activates numerous cellular processes through the intermediacy of calmodulin and its homologues. The diacylglycerol remains associated with the plasma membrane where it activates protein kinase C to phosphorylate and thereby modulates the activities of a number of cellular proteins [89-92]. However, other different signalling pathways for the 5-HT_{2C} receptor subtype have been shown (Fig. **3**). Indeed, some agonists binding to the 5-HT_{2C} receptor can instead activate phospholipase A_2 (PLA_2) leading to the release of arachidonic acid (AA) [41]. Furthermore, 5-HT_{2C} receptors can also activate phospholipase D (PLD), involving $G_{\alpha 13}$ and free $G_{\beta-\gamma}$ subunits [93], and the mitogen-activated protein kinase, extracellular signal-regulated kinase (ERK) 1 and 2 [94]. Interestingly, it was shown that coupling of 5-HT_{2C} receptors to $G_{\alpha 13}$ and $G_{q/11}$ can be differentially stimulated by certain agonists; ergots preferentially $G_{q/11}$, whereas 5-HT and (S)-2-(chloro-5-flouro-indo-l-yl)-l-methylethylamine fumarate (RO 60-0175), a selective 5-HT_{2C} receptor agonist, are equally efficacious towards both pathways. Differential G-protein coupling appeared to depend on receptor reserve [95] and was regulated by RNA editing [80, 96]. The latter may alter the ability of LSD to stimulate IP3 formation. These observations suggested that it may be possible to develop agonists which preferentially act in specific tissues via a specific signalling pathway. Amongst the 5-HT_2 receptor family, 5-HT_{2C} appears more prone to exhibit intrinsic constitutive activity. In this regard, studies have demonstrated the considerable ability of the native 5-HT_{2C} receptor to spontaneously activate intracellular signalling pathways, including PLC and PLA_2, in the absence of agonist stimulation [97-100]. This quite peculiar 5-HT_{2C} receptor characteristic occurs not only in recombinant cells but also in natural tissues and it seems to play an important role in the tonic control of the central dopaminergic function [101, 102]. The occurrence of constitutive receptor activity allows the detection of compounds that can block this activity, the so-called inverse agonists [97]. Inverse agonists may show differential pharmacological and clinical effects as compared to mere antagonists and bring up important therapeutic perspectives [103]. For instance, several atypical antipsychotic drugs display inverse agonist activity at constitutive 5-HT_{2C} receptors [102, 104]. Furthermore, it has been shown that the typical antipsychotic haloperidol despite its absence of affinity for the 5-HT_{2C} receptors increases the level of their constitutive activity [102], and this, most likely, through an action at the level of common pathways [105]. Recently, it has been shown that the affinity and efficacy of 5-HT for 5-HT_{2C} receptors can be potentiated also via allosteric modulation by amphipathic lipid metabolites [106]. Im and colleagues hypothesized that this modulation could also occur in nature and represent another route of constitutive activation of the 5-HT_{2C} receptors [106].

In contrast to most other receptors 5-HT_{2C} is not classically regulated. Indeed, 5-HT_{2C} receptors appear not only to decrease their responsiveness upon chronic agonist stimulation, but also and paradoxically after chronic treatment with antagonists [107, 108]. This mechanism appears to be related to an internalisation process that removes activated cell surface receptors from the plasma membrane involving a phosphorylation step and possible degradation in lysosomes [37]. As a large number of psychotropic drugs, including atypical antipsychotics, antidepressants, and anxiolitics, can all induce down-regulation of 5-HT_{2C} receptors, it has been suggested that this alteration plays a role in the therapeutic action of these drugs [35, 37].

5-HT_{2C} RECEPTOR DISTRIBUTION AND CIRCUITRY IN CNS

Pazos and colleagues first identified the 5-HT_{2C} receptors by radioligand binding ^{3}H-5-HT and ^{3}H-mesulergine in the

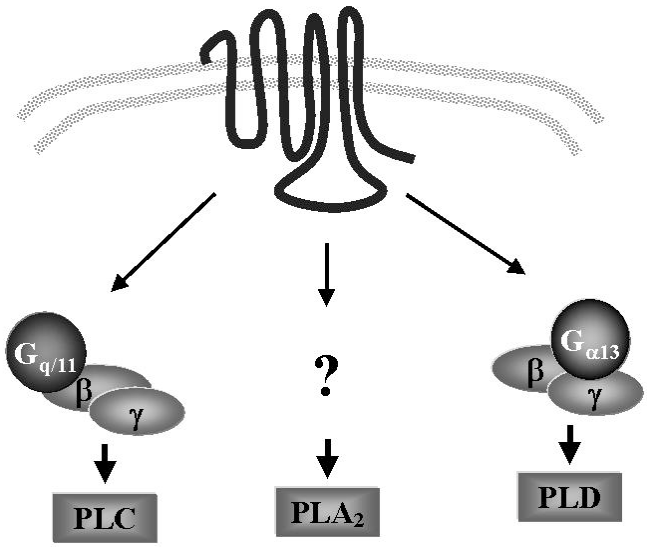

Fig. (3). Schematic drawing showing the different signal transduction pathways of the 5-HT$_{2C}$ receptor. Abbreviations: PLC, phospholipase C; PLA2, phospholipase A2, PLD, phospholipase D; ?, unknown signal transducing molecule.

pig choroid plexus and initially called it 5-HT$_{1C}$ because of its high affinity for tritiated 5-HT [109]. However, once the receptor was cloned and more information about its characteristics became available, a shift to the 5-HT$_2$ receptor family and reclassification as 5-HT$_{2C}$ became accepted. Later studies using autoriadiography with ^{3}H-mesulergine have confirmed a conspicuously high binding site density in the choroid plexus in all mammalian species, including subhuman and human primates. High density is also found in the SNr, globus pallidus and ventro medial thalamus with low amounts detected in many other brain areas [110-112]. A widespread distribution of 5-HT$_{2C}$ receptor mRNA has been demonstrated in rat, monkey and human brains using in situ hybridization techniques [113-116]. 5-HT$_{2C}$ receptors are more abundant and more widely expressed in the brain than 5-HT$_{2A}$ receptors, although there are several areas where both receptors coexist [117]. As was found for 5-HT$_{2C}$ binding sites, the choroid plexus also contained the highest amounts of 5-HT$_{2C}$ receptor mRNA and protein, mainly associated with choroid epithelial cells. Consequently, a role for these receptors in the regulation of the volume and composition of the cerebral fluid has been hypothesized [118]. High levels of both 5-HT$_{2C}$ protein and mRNA have been found in several cortical areas, in the hippocampus, striatum, septal nuclei, thalamic nuclei, midbrain nuclei, brain stem nuclei and the spinal cord [113, 117, 119]. Messenger RNA has been found in the locus coeruleus, SNc and VTA, basal nucleus of Meynert and lateral dorsal tegmental nucleus, suggesting a role for 5-HT$_{2C}$ receptors in the serotonergic regulation of chatecholaminergic and cholinergic systems. A number of studies have concentrated on the localization and potential role of 5-HT$_{2C}$ receptors in the basal ganglia. Studied concomitantly, 5-HT$_{2C}$ and 5-HT$_{2A}$ receptor mRNA was found to be co-localized with substance P and dynorphin in striato-nigral projections and with enkephalin in striato-pallidal projections. However, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor mRNA is differentially localized with regard to the scattered islands of strongly dynorphin mRNA positive cells; 5-HT$_{2C}$ receptor mRNA occurs more in the surroundings areas, as compered to 5-HT$_{2A}$ receptor mRNA [120]. Within the neostriatum, 5-HT$_{2C}$ receptors mRNA labeled neurons are more numerous in the ventral and ventro-lateral than in the dorsal caudate-putamen, where labeled neurons are restricted to isolated clusters. In the striatal target areas, dense labeling is found in the entopeduncular nucleus (internal pallidum) while no mRNA is found in the globus pallidus (external pallidum), an area where 5-HT$_{2C}$ receptor binding sites have been detected. 5-HT$_{2C}$ receptor mRNA also occurs in the subthalamus, where the receptors could be involved in the oral dyskinesia that is induced by drugs with 5-HT$_{2C}$ agonist action [121]. In the substantia nigra and in the VTA, 5-HT$_{2C}$ receptor expression is confined to GABAergic neurons making direct synaptic contact with dopaminergic cell bodies. Other immunoistochemical and electrophysiological studies [7, 122-124] have corroborated this piece of evidence suggesting an indirect control of dopaminergic transmission *via* 5-HT$_{2C}$ receptors. Dense 5-HT$_{2C}$ receptor labeling is found in about half of the neuronal population of the middle and caudal SNc and SNr. 5-HT$_{2C}$ receptors could thus selectively affect discrete neuronal population in the basal ganglia and mesolimbic system [121, 124]. 5-HT$_{2C}$ receptor mRNA is expressed also in the midbrain raphe nuclei and surroundings areas [113, 125]. Similar to other brain areas, experimental findings to date seem to suggest that in the raphe nuclei there is a good concordance between the distribution of 5-HT$_{2C}$ receptors mRNA, 5-HT$_{2C}$ receptors immunoreactivity and 5-HT$_{2C}$ receptors binding sites [110, 113, 117, 125, 126], suggesting that 5-HT$_{2C}$ receptors are located at the level of the cell body and/or dendrites. Furthermore, a recent in situ hybridization study has shown that the 5-HT$_{2C}$ receptors mRNA is not expressed by serotonergic cells but, in contrast, it was found in the majority of GABAergic neurons located within the DRN and MRN [127] showing a similar pattern to the SNc and VTA [121, 124]. In view of these anatomical data and from *in vitro* electrophysiology evidence [128], the blockade of the 5-HT$_{2C}$ receptors on DRN GABAergic interneurons might be used to decrease the GABAergic tone and indirectly increase the firing of 5-HT cells an action belonging to many antidepressant drugs.

In the spinal cord, 5-HT$_{2C}$ receptor mRNA is expressed at high levels in most of the grey matter [129]. The evidence that 5-HT$_{2C}$ receptors activation induces penile erection in rats is consistent with the presence of these receptors on neurons of the lumbo-sacral spinal cord involved in erection and ejaculation [130].

5-HT$_{2C}$ AND CENTRAL DOPAMINERGIC FUNCTION

Among the multiple classes of 5-HT receptors described in the central nervous system [16, 17], much attention has been devoted to the role of the 5-HT$_2$ receptor family in the control of central DA activity, because of the moderate to dense localization of both transcript and protein for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in SN and VTA as well as DA terminal regions of the rat forebrain [51, 52, 117, 131].

The involvement of 5-HT_{2C} receptor subtypes in the control of mesocorticolimbic and nigrostriatal DA neuron activity is now well established [65, 102, 124, 132-140], and evidence has been provided that they exert both tonic and phasic modulation of DA function [101, 135-143]. More than 10 years ago, we suggested that 5-HT could exert an inhibitory action on DA neurons in the VTA by acting through 5-HT_2 receptors [144]. In fact, we showed for the first time that the firing rate of DA neurons in the VTA was reduced by mCPP and trifluoromethylphenylpiperazine (TFMPP), two mixed 5-$HT_{2A/2B/2C}$ receptor agonists [29], whereas these neurons were stimulated by mesulergine [144]. However, these data did not allow us to distinguish the relative contribution of each 5-HT_2 receptor subtype in the control of central DA function. Subsequently, our studies clearly indicated a selective involvement of 5-HT_{2C} receptors on the basis of the evidence that the inhibitory effect of the mixed 5-HT_2 receptor agonists mCPP and 6-chloro-2-(1-piperazinyl)piperazine (MK 212) on the activity of VTA DA-containing neurons and on accumbal DA release was completely prevented by 6-chloro-5-methyl-1-[2-(2-methylpyridiyl-3-oxy)-pyrid-5-yl carbamoyl] indoline (SB 242084), a selective 5-HT_{2C} receptor antagonist [139]. Moreover, SB 242084 blocked the inhibitory action of the selective 5-HT_{2C} receptor agonist RO 60-0175 [138].

Another series of studies has shown that 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) (SB 206553), a selective 5-$HT_{2C/2B}$ receptor inverse agonist [101, 145], increases the basal firing rate and the bursting activity of VTA and SNc DA neurons [136] and enhances DA release in the rat nucleus accumbens, striatum and prefrontal cortex [101, 134, 136, 137, 140]. In addition to a local effect on DA cell bodies, the 5-HT_{2C} receptors located on the nerve terminal regions seem to play a role in the regulation of DA release. Indeed, local administration of SB 206553 increased DA efflux in the striatum and this effect was attenuated by systemic administration of mCPP [140]. Consistent with these findings, 6-chloro-5-methyl-l-[2-(2-methylpyridiyl-3-oxy)-pyrid-5-yl carbomoyl] indoline (SB 242084), a powerful and selective 5-HT_{2C} receptor antagonist [145], selectively enhanced the mesocorticolimbic DA function, while RO 60-0175 and MK 212, two 5-HT_{2C} receptor agonists, reduced it [101, 146, 147]. Recently, it has been demonstrated that the constitutive activity of the 5-HT_{2C} receptors participates in the tonic inhibitory control that they exert on DA release in the rat striatum and nucleus accumbens *in vivo* [101]. Moreover, SB 242084 was found to potentiate phencyclidine-induced increase in accumbal DA release [141], and stress-stimulated DA outflow in the rat prefrontal cortex [142], while stimulation of 5-HT_{2C} receptors by RO 60-0175 in the VTA suppressed it [142], suggesting a role for these receptors on evoked DA release also. Interestingly, some of our data show also that the nigrostriatal DA system is less sensitive to the pharmacological manipulation of the 5-HT_{2C} receptors. Indeed, there is evidence that 5-HT_{2C} receptor agonists such as mCPP, MK 212, and RO 60-0175 do not significantly affect the activity of SNc DA neurons and the *in vivo* DA release in the striatum [139, 146]. On the other hand, the selective 5-$HT_{2B/2C}$ inverse agonist SB 206553 caused only a slight increase in the basal activity of DA neurons in the SNc and in striatal DA release [139]. Consistently, a study carried out in our laboratory has shown that mCPP excites non-DA (presumably GABA-containing) neurons both in the SNr and the VTA by activating 5-HT_{2C} receptors [124]. Despite the evidence that all non-DA neurons in the VTA were being, equally, excited by mCPP, different effects were observed in the SNr. Thus, mCPP caused a marked excitation of presumed GABAergic SNr projection neurons but did not modify the SNr interneurons firing discharge [124]. It is tempting to speculate that this differential response to mCPP might be the basis of the preferential inhibitory effect of 5-HT_{2C} agonists on the mesocorticolimbic versus the nigrostriatal DA function. Recently, it has been hypothesized that 5-HT_{2C} receptor activation may also inhibit DA neurons via a long feedback loop involving glutamatergic pyramidal cells located in the prefrontal cortex [148]. In fact, it has been shown that approximately 60% of VTA mesoprefrontal projections are GABAergic [149] and the activation of this pathway could therefore play an important role in modulating excitatory outputs to sub-cortical structures [148].

5-HT_{2C} RECPTORS AND DEPRESSION

A large body of evidence suggests that the serotonergic system is impaired in depression. Most neurochemical and neuroendocrine studies of patients with depression are consistent with the existence of a serotonergic deficit. Consistently, selective serotonin re-uptake inhibitors (SSRIs) and monoamino oxidase inhibitors (MAOIs) are clinically effective antidepressants and both increase extraneuronal 5-HT acutely. When mirtazapine (Remeron®), a noradrenergic and specific serotonergic antidepressant (NaSSA) that showed high affinity for the human 5-HT_{2C} receptors, came on to the market attention was drawn to the possible importance of 5-HT_{2C} blocking activity for the treatment of symptoms of depression [33, 150, 151]. This hypothesis is consistent with the evidence that some antidepressant drugs display prominent 5-HT_{2C} receptor binding, e.g. tricyclics (amitriptyline, imipramine), mianserin, nefazodone, opipramole, trazodone [67, 152, 153] and fluoxetine [148, 154]. Based on those findings, Di Matteo and colleagues [155] have carried out experiments showing that acute administration of amitriptyline and mianserin enhances DA release in the rat nucleus accumbens by blocking 5-HT_{2C} receptor subtypes. However, these drugs have multiple receptor and transporter interactions and it is difficult to asses what the real contribution of the 5-HT_{2C} receptor blockade is to the total clinical activity of the drugs.

The possible involvement of the 5-HT_{2C} receptor in the pathogenesis of depressive disorders and in the mode of action of antidepressants is further substantiated by several other observations. The chronic mild stress procedure, which is capable of inducing a depression-like state in animals, was shown to enhance 5-HT_{2C} receptor mediated function, as measured *in vivo* by mCPP induced penile erections. Conversely, two different antidepressant treatments (72-h REM sleep deprivation and 10-day administration of moclobemide, a reversible type A MAOI) resulted in a reduction of this 5-HT_{2C} receptor-mediated function [156], supporting the hypothesis that 5-HT_{2C} receptor may be altered, and presumably may exist in a dysregulated (hypersensitive) state in depressive illness. The adaptive

processes resulting from chronic antidepressant treatment (i.e., desensitization and/or downregulation of 5-HT$_{2C}$ receptors) may normalize this dysregulated state [157]. In this respect, it is interesting to note that chronic treatment with 5-HT$_2$ agonists or antagonists resulted in a paradoxical down-regulation at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors [157-160] and it seems that the down-regulation state obtained after chronic exposure to mianserin in isolated systems as well as in cell cultures, is a direct receptor-mediated mechanism of this drug at these receptors [160]. Therefore, the down-regulating capacity of 5-HT$_{2C}$ agonists and antagonists may play a particularly important role in treating the supersensitivity of the 5-HT$_{2C}$ receptor system resulting from a depressive state [67, 157]. Behavioural effects of antidepressant drugs also support the role of this receptor in the pathogenesis of depression: in the modified rat forced swim test three selective 5-HT$_{2C}$ receptor agonists, WAY 161503, RO 60-0175 and RO 60-0332, were demonstrated to have antidepressant activity, similar to fluoxetine [161]. On the other and, interactions between several doses of RO 60-0175 and antidepressant drugs, such as tricyclics and SSRIs were found effective in the mouse forced swimming test [162], and antagonism at 5-HT$_{2C}$ receptors significantly enhanced the anti-immobility effects of imipramine in the same mouse test [163]. It was also demonstrated that a 5-HT$_{2C}$ receptors mutation may contribute to the pathogenesis of major depression and bipolar disorder [164]. Intriguing evidence also shows an alteration of 5-HT$_{2C}$ pre-mRNA editing in the brains of major depressed and depressed suicide victims especially in the prefrontal cortex, a brain area expressing a large number of differently edited 5-HT$_{2C}$ mRNA isoforms [84]. Since these changes were opposite to those detected in mice chronically treated with fluoxetine [83], the therapeutic effect of SSRIs could be in part explained with the reversing of 5-HT$_{2C}$ receptor mRNA editing abnormalities.

Several lines of evidence indicate that 5-HT$_{2C}$ receptor antagonists maybe used to augment the therapeutic efficacy of SSRIs. [165, 166]. Thus, agomelatine, the first melatonergic antidepressant with 5-HT$_{2C}$ antagonist properties, was observed to produce a robust augmentation of citalopram-, fluoxetine-, and sertraline-induced elevations of hippocampal extracellular 5-HT levels [165, 166]. The potentiation of SSRI-induced increases in hippocampal 5-HT levels was reproduced by the 5-HT$_{2C}$ receptor-selective antagonists SB 242084 and RS 102221, but not by the 5-HT$_{2A}$ receptor-selective antagonist MDL 100,907. Although 5-HT$_{2C}$ receptor antagonists potentiated the actions of SSRIs, they had no effect on extracellular 5-HT levels or tail suspension responses when administered alone. These results corresponded well with independent findings using a line of 5-HT$_{2C}$ receptor null mutant mice [165]. Although this mutation did not affect baseline extracellular 5-HT levels or tail suspension test (TST) behaviour, it enhanced fluoxetine-induced effects on 5-HT levels and immobility in the TST.

Since the 5-HT$_{2C}$ receptors have recently been found to be expressed specifically on GABAergic neurons of the anterior raphe nuclei [127], it is conceivable that blockade of these receptors could disinhibit serotonergic neurons. This hypothesis is strengthened by the evidence that stimulation of the 5-HT$_2$ receptors by serotonin activates local GABA inhibitory inputs to 5-HT-containg neurons in the dorsal raphe nucleus, an effect which is partly mediated by 5-HT$_{2C}$ receptor subtypes [128]. Whether 5-HT/DA interaction is involved in the mechanism of augmentation of the SSRI antidepressant effect exerted by the 5-HT$_{2C}$ receptor antagonists is presently unclear, and it remains to be determined. Nevertheless, it is tempting to speculate that a blockade of 5-HT$_{2C}$ receptors would increase the serotonergic tone to VTA DA neurons, which would be excited by the excess of 5-HT acting on free 5-HT$_{2A}$ receptors. Thus, the pharmacological blockade of 5-HT$_{2C}$ receptors would favour an unbalanced excitatory effect of 5-HT acting through 5-HT$_{2A}$ receptors whose stimulation is known to cause a direct depolarization of DA-containing neurons in the VTA [128]. However, this hypothesis needs experimental demonstration to be confirmed. Another important contribution to the research regarding 5-HT/DA interaction and the effects of antidepressant drugs comes from the data obtained in our laboratory showing that acute administration of fluoxetine causes a dose-dependent inhibition of the firing rate of VTA DA neurons, but it does not affect the activity of DA cells in the SNc [167]. A similar effect, though less pronounced, has been observed with citalopram [167]. Furthermore, mesulergine, an unselective 5-HT$_{2C}$ receptor antagonist [21], as well as the destruction of 5-HT neurons by the neurotoxin 5,7-dihydroxytryptamine (5,7-D-HT), prevented the fluoxetine-induced inhibition of VTA DA cells [167]. These results indicate that fluoxetine inhibits the mesolimbic DA pathway by enhancing the extracellular level of 5-HT, which would act through 5-HT$_{2C}$ receptors [167]. This study also demonstrated that fluoxetine-induced inhibition of DA neurons in the VTA was no longer observed after chronic treatment (21 days) with this drug. Interestingly, mCPP inhibited the firing activity of VTA DA neurons in control animals but not in those chronically treated with fluoxetine [167]. The authors suggested that 5-HT$_{2C}$ receptors might be downregulated after repeated fluoxetine administration. Consistent with this hypothesis is the evidence that chronic treatment with sertraline and other SSRIss, such as sertraline, fluoxetine, fluvoxamine and citalopram, induces tolerance to the hypolocomotor effect of mCPP [168-171]. This hyposensitivity of 5-HT$_{2C}$ receptors might be a key step for the achievement of an antidepressant effect. Indeed, it is possible to argue that the acute inhibitory effect of fluoxetine on the mesolimbic DA system would mask its clinical efficacy in the early stage of treatment. This masking effect would disappear when the hyposensitivity of 5-HT$_{2C}$ receptors occurs. A series of studies carried out in our laboratory have shown that acute administration of SSRIs such as paroxetine, sertraline, and fluvoxamine causes a slight but significant decrease in the basal firing rate of VTA DA neurons [172]. Therefore, it is conceivable that, similar to fluoxetine, these three SSRIs could reduce mesocorticolimbic DA transmission by activating 5-HT$_{2C}$ receptors.

5-HT$_{2C}$ RECPTORS AND SCHIZOPHRENIA

The original observation of the structural similarity between 5-HT and the hallucinogic drug lysergic acid diethylamide (LSD) led to the hypothesis that schizophrenia may be associated with a dysfunction of the 5-HT system

[173, 174]. However, the initial excitement about a 5-HT-related hypothesis of schizophrenia was diminished by the recognition that LSD-induced psychosis differed in several ways from the symptoms seen in schizophrenia [175]. Nevertheless, renewed interest in 5-HT as a player in the pathophysiology of these disorders was brought about when it was shown that the atypical antipsychotic clozapine had high affinity to 5-HT_2 receptors [176] and displayed a superior therapeutic effect as compared to classical antipsychotic in treatment-resistant schizophrenia [177].

Thus, it was hypothesised that the action of atypical antipsychotic drugs (APDs) depends on their interaction with central 5-HT_{2A} or 5-HT_{2C} receptor subtypes, more than with D_2 receptors [34, 178-181]. In fact, by examining *in vitro* receptor binding data, Meltzer and collaborators [34] found that typical and atypical APDs could be distinguished on the basis of their 5-HT_2 to D_2 receptor binding ratios.

Early clinical studies indicated that the selective 5-$HT_{2A/2C}$ receptor antagonist ritanserin [182, 183] can ameliorate negative symptoms as well as attenuate exciting extrapiramidal side effects (EPS) in schizophrenics treated with classical APDs [184, 185]. To date, it is generally accepted that the mechanism of action of atypical APDs is based on their ability to achieve a balanced 5-HT_2 to D_2 receptor antagonistic action and not on their absolute affinity for these receptors *per se*. Clearly, such hypotheses have pushed for the development of novel APDs with combined antiserotonergic and antidopaminergic properties. Canton and colleagues [186], initially showed the high affinity of clozapine and risperidone also for 5-HT_{2C} sites in the rat choroid plexus. These findings were subsequently confirmed [187-191] and extended to brain sections [190-191], and in transiently expressed 5-HT_{2C} receptors [35, 181]. Antagonism at 5-HT_{2C} receptors by several antipsychotics was also observed *in vivo*. Indeed, clozapine produces an increase in extracellular levels of DA in the nucleus accumbens [192, 193], reverses the inhibition of accumbal DA release induced by the 5-HT_{2C} agonist RO 60-0175 [192] and blocks the hypolocomotion induced by the 5-HT_{2C} agonist mCPP [194].

It is worth noting that clozapine, like several atypical APDs, behaves as a 5-HT_{2C} inverse agonist in heterologus expression systems *in vitro* [195, 102, 104] and *in vivo* [102]. Thus, the 5-HT_{2C} receptor inverse agonist might underlie the unique clinical properties of atypical APDs [102, 195]. The modification of 5-HT_{2C} receptors constitutive activity may also participate in the effects of the typical APD haloperidol. Indeed, it has been reported that the increase in striatal DA release induced by haloperidol is dramatically potentiated by the 5-HT_{2C} inverse agonist SB 206553 [102]. Therefore, bearing in mind that haloperidol does not bind to 5-HT_{2C} receptors it was suggested that it could act at the level of the common effector pathway [102]. A preferential increase of DA release in the medial prefrontal cortex seems to be a common mechanism of action of atypical APDs, an effect which might be relevant for their therapeutic action on the negative symptoms of schizophrenia [196]. In this respect, it is important to note that the selective 5-HT_{2C} receptor antagonist SB 242084 [130] markedly increases DA release in the frontal cortex of awake rats [134, 147]. Thus, it is possible to argue that a blockade of 5-HT_{2C} receptors might contribute to the preferential effect of atypical antipsychotics on DA release in the prefrontal cortex and mitigate side effects induced by D_2 antagonism.

For the moment, neither a highly selective 5-HT_{2C} receptor antagonist nor a true mixed 5-$HT_{2A/2C}$ receptor antagonist has been evaluated in schizophrenia. Behavioural studies with SB 228537, a selective 5-$HT_{2B/2C}$ receptor antagonist and using selective 5-HT_{2A} and 5-HT_{2B} receptor antagonists as controls indicate that 5-HT_{2C} receptor antagonism can produce a significant reversal of haloperidol-induced catalepsy [197]. However, not all atypical APDs are 5-HT_{2C} receptor antagonists, for example, risperidone [181].

5-HT_{2C} RECEPTORS AND PARKINSON'S DISEASE

Another interesting application of the data regarding the functional role of 5-HT_{2C} receptors in the basal ganglia is the possible use of 5-HT_{2C} receptor antagonists in the treatment of Parkinson's disease. The neural mechanisms underlying the generation of parkinsonian symptoms are thought to involve reduced activation of the primary motor and premotor cortex and supplementary motor areas, secondary to overactivation of the output regions of the basal ganglia, i.e. SNr and globus pallidus internus (GPi) [198], largely because of an excessive excitatory drive from the subthalamic nucleus (STN). Therapy of Parkinson's disease consists mainly of amelioration of symptoms with classical dopaminomimetics [199]. This treatment, however, is characterized by a declining efficacy and the occurrence of disabling side-effects [200]. Functional inhibition of GPi or STN has provided an alternative to lesioning, by deep brain stimulation associated with modest side-effects [201]. As already mentioned, 5-HT_{2C} receptors are located in the SNr and medial segment of the pallidal complex in the rat and human brain [121, 131], and enhanced 5-HT_{2C} receptor-mediated transmission within the output regions of the basal ganglia in parkinsonism appears to contribute to their overactivity [202]. In addition, 5-HT_{2C} -like receptor binding is increased in a rat model of parkinsonism [203] and in human parkinsonian patients [204]. This suggests a compensatory up-regulation of 5-HT_{2C} receptors as a consequence of a reduced 5-HT level in output regions of the basal ganglia, leading to a decreased activity of these nuclei and an increase in abnormal movements like dyskinesia [205, 206]. Furthermore, systemic administration of SB 206553 was shown to enhance the action of the anti-parkinsonian action of the dopamine D1 and D2 agonists in the 6-hydroxydopamine-lesioned rats [205, 207], suggesting that the use of a 5-HT_{2C} receptor antagonist in combination with a dopamine receptor agonist may reduce the reliance upon dopamine replacement therapies and may thus reduce the problems associated with long term use of currently available antiparkinsonian agents [202].

It would, therefore, appear that 5-HT_{2C} receptors play a significant role in the basal ganglia and that drugs acting on 5-HT_{2C} receptors could have the potential to alleviate the dyskinesia in the parkinsonian patient, and also the comorbid depression that often accompanies Parkinson's disease by elevating accumbal DA levels [101, 136, 140], thereby alleviating the anhedonia associated with depression [208].

5-HT$_{2C}$ RECEPTORS AND DRUG OF ABUSE

Substantial evidence indicates that the mesolimbic pathway, particularly DA cells innervating accumbal areas, is implicated in the reward value of both natural and drug reinforcers, such as, for example, sexual behaviour or psychostimulants, respectively [209-211]. The fact that drugs of abuse act through different cellular mechanisms leads to the possibility that their effect on DA release could be modulated differentially by each of the 5-HT$_2$ receptor subtypes. Moreover, it has been reported that the increased locomotor activity, as well as the accumbal DA release, elicited by phencyclidine is further enhanced by the blockade of 5-HT$_{2C}$ receptors [141], while antagonism at 5-HT$_{2A}$ receptors had opposite effects [212]. A similar picture emerges when looking at the influence of these receptors on 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy)-induced effects on DA neuron activity. Thus, the selective 5-HT$_{2A}$ antagonist MDL 100,907 significantly reduced the hyperlocomotion and stimulated DA release produced by MDMA while the selective 5-HT$_{2C}$ antagonists SB 242084 and SB206553 potentiated it [213-216].

Recently, it was found that SB 206553 administration potentiates both the enhancement of DA release in the nucleus accumbens and striatum and the increase in VTA and SNc DA neuron firing rate induced by morphine [217]. Consistent with this finding, stimulation of central 5-HT$_{2C}$ receptors has been shown to inhibit morphine-induced increase in DA release in the nucleus accumbens of freely moving rats [143]. Blockade of 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors had opposite effects on cocaine-induced locomotor activity. Thus, 5-HT$_{2A}$ receptor blockade with M100,907 attenuated cocaine-induced locomotion, whereas 5-HT$_{2C}$ blockade with SB 242084 or SB 206553 enhanced cocaine-induced activity [216, 218-220]. It has been found that RO 60-0175 reduces cocaine-reinforced behaviour by stimulating 5-HT$_{2C}$ receptors [221]. Moreover, these authors have also shown that RO 60-0175 reduces ethanol- and nicotine-induced self-administration and hyperactivity [222, 223]. Consistent with these evidences, we recently showed that the selective activation of 5-HT$_{2C}$ receptors, by RO 60-0175 administration, blocks the stimulatory action of nicotine on SNc DA neuronal activity and the nicotine-induced DA release in the corpus striatum [224, 225]. The mesolimbic DA system appeared to be less sensitive to the inhibitory effect of 5-HT$_{2C}$ receptors activation on nicotine-induced stimulation, indeed a higher dose of RO 60-0175 was necessary to prevent the enhancement in VTA DA neuronal firing elicited by acute nicotine. Furthermore, pretreatment with the 5-HT$_{2C}$ agonist did not effect nicotine-induced DA release in the nucleus accumbens [224, 225]. Interestingly, in animals treated repeatedly with nicotine, pretreatment with RO 60-0175 reproduced the same pattern of effects on the enhancement in DA neuronal firing caused by challenge with nicotine, resulting effective only at a higher dose in preventing nicotine excitation in the VTA compared to the SNc. Furthermore, the 5-HT$_{2C}$ receptors agonist counteracted nicotine-induced DA release both in the striatum and in the nucleus accumbens in rats chronically treated with this alkaloid, even if this effect was observed only with the highest dose of RO 60-0175 [224, 225]. Therefore, we hypothesized that after repeated nicotine exposure an up-regulation of 5-HT$_{2C}$ receptors occurs only in the DA mesolimbic system and the blocking of its hyperfunction by 5-HT$_{2C}$ receptor activation might be a useful approach in reducing nicotine reward and eventually helping in smoking cessation.

CONCLUSIONS

Twenty five years of 5-HT$_{2C}$ receptors research have generated detailed information on the molecular biology, regional and cellular localization of these receptors. Furthermore, the 5-HT$_{2C}$ receptor seems to be involved in the regulation of mood, anxiety, cognitive function, stress, nociception, sexual functions, feeding, drug abuse, and other aspects of behaviour. Thus, the 5-HT$_{2C}$ receptors antagonism appears to be an important feature of antipsychotic and antidepressants drugs with broad pharmacological profiles, properties that probably contribute to the treatment of negative and depressive symptoms, respectively, or to the mitigation of side-effects. Although several selective agents for this receptor have been discovered, none have reached the market for the treatment of CNS disorders to date, essentially as a result of their limited efficacy. Several companies are still very active on 5-HT$_{2C}$ receptors research. Organon has several molecules under clinical evaluation. Org-12962 stimulates the 5-HT$_{2C}$ receptors and this compound is now in Phase 2 trials and should at the very least help to clarify the role of these receptors in depression. Furthermore, ADP356 (Arena Pharmaceutical) and BVT933 (Biovitrum/GSK), two members of the new generation of 5-HT$_{2C}$ selective agonists, are currently undergoing clinical trials for the treatment of obesity, hopefully free of their predecessors' side effect.

There are also many avenues that remain unexplored, so there are undoubtedly further advances to be made. In the next few years, we certainly will see compounds selective for 5-HT$_{2C}$ receptors making further significant impacts on the treatment of the major neuropsychiatric disturbances.

ACKNOWLEDGEMENTS

We want to thank Ms. Samantha Austen for the English revision of the manuscript.

REFERENCES

[1] Twarog, B. M.; Page, I. H. Serotonin content of some mammalian tissues and urine and a modelfor its determination. *Am. J. Physiol*. **1953**, *175*, 157-161.

[2] Brodie, B. B.; Pletscher, A.; Shore, P. A. Evidence that serotonin has a role in brain flinctions. *Science* **1955**, *122*, 968.

[3] Whitaker-Azmitia, P. M. The discovery of serotonin and its role in neuroscience. *Neuropsychopharmacology* **1999**, *21*, 2S-8S.

[4] Dahlström, A.; Fuxe, K. Evidence for the existence of monamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.* **1964**, *62*, 1-55.

[5] Moukhles, H.; Bosler, O.; Bolam, J. P.; Vallee, A.; Umbriaco, D.; Geffard, M.; Doucet. G. Quantitative and morphometric data indicate precise cellular interactions between serotonin terminals and postsynaptic targets in rat substantia nigra. *Neuroscience* **1997**, *76*, 1159-1171.

[6] Van Bockstaele, E .J.; Biswas, A.; Pickel, V. M. Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res*. **1993**, *624*, 188-198.

[7] Van Bockstaele, E. J.; Cestari, D. M.; Pickel, V. M. Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: potential sites for modulation of mesolimbic dopamine neurons. *Brain Res*. **1994**, *647*, 307-322.

[8] Herve, D.; Pickel, V. M.; Joh, T. H.; Beaudet, A. Serotonin axon terminals in the ventral tegmental area of the rat: finestructure and synaptic input to dopaminergic neurons. *Brain Res*. **1987**, *435*, 71-83.

[9] Kalivas, P. W. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res. Brain Res. Rev*. **1993**, *18*, 75-113.

[10] Azmitia, E. C.; Segal, M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol*. **1978**, *179*, 641-667.

[11] McQuade, R.; Sharp, T. Release of cerebral 5-hydroxytryptamine evoked by electrical stimulation of the dorsal and median raphe nuclei: effect of a neurotoxic amphetamine. *Neuroscience* **1995**, *68*, 1079-1088.

[12] McQuade, R.; Sharp, T. Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. *J. Neurochem*. **1997**, *69*, 791-796.

[13] Jacobs, B. L.; Azmitia, E. C. Structure and function of the brain serotonin system. *Physiol. Rev.* **1992**, *72*, 165-229.

[14] Gaddum, J. H.; Picarelli, Z. P. Two kinds of tryptamine receptors. *Br. J. Pharmacol.* **1957**, *12*, 323-328.

[15] Peroutka, S. J.; Snyder, S. H. Multiple serotonin receptors: differential binding of [3H)5-hydroxytryptamine, [3H] lysergic acid diethylamide and [3H]spiroperidol. *Mol. Pharmacol*. **1979**, *16*, 687-699.

[16] Hoyer, D.; Hannon, J. P.; Martin, G. R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav*. **2002**, *71*, 533-54.

[17] Barnes, N. M.; Sharp, T. A review of central 5-HT receptors and their function. *Neuropharmacology* **1999**, *38*, 1083-1152.

[18] Leonard, D. A.; Evans, T.; Hart, M.; Cerione, R. A.; Manor, D. Investigation of the GTP-binding/GTPase cycle of Cdc42Hs using fluorescencespectroscopy. *Biochemistry* **1994**, *33*, 12323-12328.

[19] Kroeze, W. K.; Kristiansen, K.; Roth, B. L. Molecular biology of serotonin receptors structure and function at the molecular level. *Curr. Top. Med. Chem*. **2002**, *2*, 507-528.

[20] 20 Zifa, E.; Fillion, G. 5-Hydroxytryptamine receptors. *Pharmacol. Rev*. **1992**, *44*, 401-458.

[21] Boess, F. G.; Martin, I. L. Molecular biology of 5-HT receptors. *Neuropharmacology* **1994**, *33*, 275-317.

[22] Hapfelmeier, G.; Tredt, C.; Haseneder, R.; Zieglgansberger, W.; Eisensamer, B.; Rupprecht, R.; Rammes, G. Co-expression of the 5-HT3B serotonin receptor subunit alters the biophysics of the 5-HT3 receptor. *Biophys. J.* **2003**, *84*, 1720-1733.

[23] Niesler, B.; Frank, B.; Kapeller, J.; Rappold, G. A. Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes -HTR3C, -HTR3D and -HTR3E. *Gene* **2003**, *310*, 101-111.

[24] Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, A.; Monsma, F. J. Jr.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther*. **1994**, *268*, 1403-1410.

[25] Erlander, M. G.; Lovenberg, T. W.; Baron, B. M.; de Lecea L, Danielson, P. E.; Racke, M.; Slone, A. L.; Siegel, B. W.; Foye, P. E.; Cannon, K.; *et al*. Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3452-3456.

[26] Matthes, H.; Boschert, U,; Amlaiky, N., Grailhe, R.; Plassat, J. L.; Muscatelli, F.; Mattei, M. G.; Hen, R. Mouse 5-hydroxytryptamine5A and 5-hydroxytryptamine5B receptors define a new family of serotonin receptors: cloning, functional expression, and chromosomal localization. *Mol. Pharmacol.* **1993**, *43*, 313-9.

[27] Frank, M. G.; Stryker, M. P.; Tecott, L. H. Sleep and sleep homeostasis in mice lacking the 5-HT2c receptor. *Neuropsychopharmacology* **2002**, *27*, 869-873.

[28] Grailhe, R.; Waeber, C.; Dulawa, S. C.; Hornung, J. P., Zhuang, X; Brunner, D.; Geyer, M. A.; Hen, R. Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. *Neuron* **1999**, *22*, 581-91.

[29] Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Harting, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. A. VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev*. **1994**, *46*, 157-203.

[30] Higgins, G. A.; Fletcher, P. J. Serotonin and drug reward: focus on 5-HT2C receptors. *Eur. J. Pharmacol*. **2003**, *480*, 151-162.

[31] Isaac, M. Serotonergic 5-HT2C receptors as a potential therapeutic target for the design antiepileptic drugs. *Curr. Top. Med. Chem*. **2005**, *5*, 59-67.

[32] Reynolds, G. P.; Templeman, L. A.; Zhang, Z. J. The role of 5-HT2C receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. Prog *Neuropsychopharmacol. Biol. Psychiatry* **2005**, *29*, 1021-1028.

[33] Baxter, G. S.; Kennett, G. A.; Blaney, F.; Blackburn, T. 5-HT2 receptor subtypes: a family reunited? *Trends Pharmacol. Sci.* **1995**, *16*, 105-110.

[34] Meltzer, H. Y.; Matsubara, S.; Lee, J. C. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D1, D2 and serotonin2 pKi values. *J. Pharmacol. Exp. Ther*. **1989**, *251*, 238-246.

[35] Roth, B. L.; Meltzer, H. Y.; Khan, N. Binding of typical and atypical antipsychotic drugs to multiple neurotransmitter receptors. *Adv. Pharmacol*. **1998**, *42*, 482-485.

[36] Aghajanian, G. K.; Marek, G. J. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Res. Brain Res. Rev.* **2000**, *31*, 302-312.

[37] Van Oekelen, D.; Luyten, W. H.; Leysen, J. E. 5-HT2A and 5-HT2C receptors and their atypical regulation properties. *Life Sci.* **2003**, *72*, 2429-2449.

[38] Li, Q. H.; Nakadate, K.; Tanaka-Nakadate, S.; Nakatsuka, D.; Cui, Y.; Watanabe, Y. Unique expression patterns of 5-HT2A and 5-HT2C receptors in the rat brain during postnatal development: Western blot and immunohistochemical analyses. *J. Comp. Neurol*. **2004**, *469*, 128-140.

[39] Kojic. L.; Dyck, R.; H.; Gu, Q.; Douglas, R. M.; Matsubara, J.; Cynader, M. S. Columnar distribution of serotonin-dependent plasticity within kitten striate cortex. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1841-1844.

[40] Berg, K. A.; Clarke, W. P.; Sailstad, C.; Saltzman, A.; Maayani, S. Signal transduction differences between 5-hydroxytryptamine type 2A and type 2C receptor systems. *Mol. Pharmacol.* **1994**, *46*, 477-484.

[41] Berg, K. A.; Maayani, S.; Goldfarb, J.; Scaramellini, C.; Leff, P.; Clarke, W. P. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.* **1998**, *54*, 94-104.

[42] Leysen, J. E. 5-HT2 receptors. *Curr. Drug Targets CNS Neurol. Disord*. **2004**, *3*, 11-26.

[43] Morilak, D. A.; Somogyi, P.; Lujan-Miras, R.; Ciaranello, R. D. Neurons expressing 5-HT2 receptors in the rat brain: neurochemical identification of cell types by immunocytochemistry. *Neuropsychopharmacology* **1994**, *11*, 157-66.

[44] Willins, D. L.; Deutch, A. Y.; Roth, B. L. Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse* **1997**, *27*, 79-82.

[45] Jakab, R. L.; Goldman-Rakic, P. S. Segregation of serotonin 5-HT2A and 5-HT3 receptors in inhibitory circuits of the primate cerebral cortex. *J. Comp. Neurol.* **2000**, *417*, 337-348.

[46] Jakab, R. L.; Goldman-Rakic, P. S. 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 735-740.

[47] Miner, L. A.; Backstrom, J. R.; Sanders-Bush, E.; Sesack, S. R. Ultrastructural localization of serotonin2A receptors in the middle layers of the rat prelimbic prefrontal cortex. *Neuroscience* **2003**, *116*, 107-117.

[48] Cornea-Hebert, V.; Riad, M.; Wu, C.; Singh, S. K.; Descarries, L. Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. *J. Comp. Neurol.* **1999**, *409*, 187-209.

[49] Dean, B. The cortical serotonin2A receptor and the pathology of schizophrenia: a likely accomplice. *J. Neurochem.* **2003**, *85*, 1-13.

[50] Ikemoto, K.; Nishimura, A.; Okado, N.; Mikuni, M.; Nishi, K.; Nagatsu, I. Human midbrain dopamine neurons express serotonin 2A receptor: an immunohistochemical demonstration. *Brain Res.* **2000**, *853*, 377-380.

[51] Nocjar, C.; Roth, B. L.; Pehek, E. A. Localization of 5-HT2A receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience* **2002**, *111*, 163-176.

[52] Doherty, M. D.; Pickel, V. Ultrastructural localization of serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res.* **2000**, *864*, 176-185.

[53] Heider, M.; Schliebs, R.; Rossner, S.; Bigl, V. Basal forebrain cholinergic immunolesion by 192IgG-saporin: evidence for apresynaptic location of subpopulations of alpha 2- and beta-adrenergic as well as 5-HT2A receptors on cortical cholinergic terminals. *Neurochem. Res.* **1997**, *22*, 957-966.

[54] Fay, R.; Kubin, L. 5-HT(2A) receptor-like protein is present in small neurons located in rat mesopontine cholinergic nuclei, but absent from cholinergic neurons. *Neurosci. Lett.* **2001**, *314*, 77-81.

[55] Luttgen, M.; Ogren, S. O.; Meister, B. 5-HT1A receptor mRNA and immunoreactivity in the rat medial septum/diagonal band of Broca-relationships to GABAergic and cholinergic neurons. *J. Chem. Neuroanat.* **2005**, *29*, 93-111.

[56] Enguix, M. J.; Sanchez, L.; Villazon, M.; Brea, J.; Tristan, H.; Caruncho, H. J.; Cadavid, M. I.; Loza, M. I. Differential regulation of rat peripheral 5-HT(2A) and 5-HT(2B) receptor systems: influence of drug treatment. *Naunyn Schmiedebergs Arch. Pharmacol.* **2003**, *368*, 79-90.

[57] Eglen, R. M. The pharmacology and distribution of human 5-hydroxytryptamine2B (5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. *Br. J. Pharmacol.* **1995**, *115*, 622-628.

[58] Borman, R. A.; Tilford, N. S.; Harmer, D. W.; Day, N.; Ellis, E. S.; Sheldrick, R. L.; Carey, J.; Coleman, R. A.; Baxter, G. S. 5-HT(2B) receptors play a key role in mediating the excitatory effects of 5-HT in human colon *in vitro*. *Br. J. Pharmacol.* **2002**, *135*, 1144-51.

[59] Loric, S.; Launay, J. M.; Colas, J. F.; Maroteaux, L. New mouse 5-HT2-like receptor. Expression in brain, heart and intestine. *FEBS Lett.* **1992**, *312*, 203-207.

[60] Foguet, M.; Nguyen, H.; Le, H.; Lubbert, H. Structure of the mouse 5-HT1C, 5-HT2 and stomach fundus serotonin receptor genes. *Neuroreport* **1992**, *3*, 345-348.

[61] Schmuck, K.; Ullmer, C.; Engels, P.; Lubbert, H. Cloning and functional characterization of the human 5-HT2B serotonin receptor. *FEBS Lett.* **1994**, *342*, 85-90.

[62] Duxon, M .S.; Flanigan, T. P.; Reavley, A. C.; Baxter, G. S.; Blackburn, T. P.; Fone, K. C. F. Evidence for expression of the 5-hydroxytryptamine2B receptor protein in the rat central nervous system. *Neuroscience* **1997**, *76*, 323-329.

[63] Nebigil, C. G.; Etienne, N.; Schaerlinger, B.; Hickel, P.; Launay, J. M.; Maroteaux, L. Developmentally regulated serotonin 5-HT2B receptors. *Int. J. Dev. Neurosci.* **2001**, *19*, 365-372.

[64] Choi, D. S.; Ward, S. J.; Messaddeq, N.; Launay, J. M.; Maroteaux, L. 5-HT2B receptor-mediated serotonin morphogenetic functions in mouse cranial neural crest and myocardiac cells. *Development* **1997**, *124*, 1745-1755.

[65] Di Giovanni, G.; Di Matteo, V.; Esposito, E. Serotonin/dopamine interaction-focus on 5-HT2C receptor, a new target of psychotropic drugs. *Indian J. Exp. Biol.* **2002**, *40*, 1344-1352.

[66] Kennett, G. A. 5-HT1C receptors and their therapeutic relevance. *Curr. Opin. Invest. Drugs* **1993**, *2*, 317-362.

[67] Jenck, F.; Bös, J.; Wichmann, J.; Stadler, H.; Martin, J. R.; Moreau, J. L. The role of 5-HT2C receptors in affective disorders. *Exp. Opin. Invest. Drugs* **1998,** *7*, 1587-1599.

[68] Tecott, L. H.; Sun, L. M.; Akana, S. F.; Strack, A. M.; Lowenstein, D. H.; Dallman, M. F.; Julius, D. Eating disorder and epilepsy in mice lacking 5-HT2C serotonin receptors. *Nature* **1995**, *374*, 542-546.

[69] Heisler, L. K.; Chu, H. M.; Tecott, L. H. Epilepsy and obesity in serotonin 5-HT2C receptor mutant mice. *Ann. N. Y. Acad. Sci.* **1998**, *861,* 74-78.

[70] Bickerdike, M. J.; Vickers, S. P.; Dourish, C. T. 5-HT2C receptor modulation and the treatment of obesity. *Diabetes Obes. Metab.* **1999**, *1*, 207-214.

[71] Bickerdike, M. J. 5-HT2C receptor agonists as potential drugs for the treatment of obesity. *Curr. Top. Med. Chem.* **2003**, *3*, 885-897.

[72] Applegate, C. D.; Tecott, L. H. Global increases in seizure susceptibility in mice lacking 5-HT2C receptors: a behavioral analysis. *Exp. Neurol.* **1998**, *154*, 522-530.

[73] Raap, D. K.; Van De Kar, L. D. Selective serotonin-reuptake inhibitors and neuroendocrine function. *Life Sci.* **1999**, *65*, 1217-1235.

[74] Milatovich, A.; Hsieh, C. L.; Bonaminio, G.; Tecott, L.; Julius, D.; Francke, U. Serotonin receptor 1c gene assigned to X chromosome in human (band q24) and mouse (bands D-F4). *Hum. Mol. Genet.* **1992**, *1*, 681-684.

[75] Xie, E.; Zhu, L.; Zhao, L.; Chang, L. S. The human serotonin 5-HT2C receptor: complete cDNA, genomic structure, and alternatively spliced variant. *Genomics* **1996**, *35*, 551-561.

[76] Abramowski, D.; Staufenbiel, M. Identification of the 5-hydroxytryptamine2C receptor as a 60-kDa N-glycosylated protein in choroid plexus and hippocampus. *J. Neurochem.* **1995**, *65*, 782-790.

[77] Visiers, I.; Hassan, S. A.; Weinstein, H. Differences in conformational properties of the second intracellular loop (IL2) in 5-HT(2C) receptors modified by RNA editing can account for G protein coupling efficiency. *Protein Eng.* **2001**, *14*, 409-414.

[78] Cypess, A. M.; Unson, C. G.; Wu, C. R.; Sakmar, T. P. Two cytoplasmic loops of the glucagon receptor are required to elevate cAMP or intracellular calcium. *J. Biol. Chem.* **1999**, *274*, 19455-19464.

[79] Sanders-Bush, E.; Fentress, H.; Hazelwood, L. Serotonin 5-ht2 receptors: molecular and genomic diversity. *Mol. Interv.* **2003**, *3*, 319-330.

[80] Burns, C. M.; Chu, H.; Rueter, S. M.; Hutchinson, L. K.; Canton, H.; Sanders-Bush, E.; Emeson, R. B. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature* **1997**, *387*, 303-308.

[81] Wang, Q.; O'Brien, P. J.; Chen, C. X.; Cho, D. S.; Murray, J. M.; Nishikura, K. Altered G protein-coupling functions of RNA editing isoform and splicing variant serotonin2C receptors. *J. Neurochem.* **2000**, *74*, 1290-1300.

[82] Fitzgerald, L. W.; Iyer, G.; Conklin, D. S.; Krause, C. M,; Marshall, A.; Patterson, J. P.; Tran, D. P.; Jonak, G. J.; Hartig, P. R. Messenger RNA editing of the human serotonin 5-HT2C receptor. *Neuropsychopharmacology* **1999**, *21*, 82S-90S.

[83] Gurevich, I.; Englander, M. T.; Adlersberg, M.; Siegal, N. B.; Schmauss, C. Modulation of serotonin 2C receptor editing by sustained changes in serotonergic neurotransmission. *J. Neurosci.* **2002**, *22*, 10529-10532.

[84] Schmauss, C. Serotonin 2C receptors: suicide, serotonin, and runaway RNA editing. *Neuroscientist* **2003**, *9*, 237-242.

[85] Quirk, K.; Lawrence, A.; Jones, J.; Misra, A.; Harvey, V.; Lamb, H.; Revell, D.; Porter, R. H.; Knight, A. R. Characterisation of agonist binding on human 5-HT2C receptor isoforms. *Eur. J. Pharmacol.* **2001**, *419*, 107-112.

[86] Herrick-Davis, K.; Grinde, E.; Mazurkiewicz, J.E. Biochemical and biophysical characterization of serotonin 5-HT2C receptor homodimers on the plasma membrane of living cells. *Biochemistry* **2004**, *43*, 13963-13971.

[87] Herrick-Davis, K.; Grinde, E.; Harrigan, T. J.; Mazurkiewicz, J. E. Inhibition of serotonin 5-HT2C receptor function through heterodimerization: Receptor dimers bind two molecules of ligand and one G-protein. *J. Biol. Chem.* **2005**, *280*, 40144-40151.

[88] Terrillon, S.; Bouvier, M. Roles of G-protein-coupled receptor dimerization. *EMBO Rep.* **2004**, *5*, 30-34.

[89] de Chaffoy de Courcelles, D.; Leysen, J. E.; De Clerck, F.; Van Belle, H.; Janssen, P. A. Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S2 receptor sites. *J. Biol. Chem.* **1985**, *260*, 7603-7608.

[90] de Chaffoy de Courcelles, D.; Roevens, P.; Wynants, J.; Van Belle, H. Serotonin-induced alterations in inositol phospholipid metabolism in human platelets. *Biochim. Biophys Acta* **1987**, *927*, 291-302.

[91] Conn, P. J.; Sanders-Bush, E. Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5-HT-2 binding site. *J. Neurosci.* **1986**, *6*, 3669-3675.

[92] Chang, M.; Zhang, L.; Tam, J. P.; Sanders-Bush, E. Dissecting G protein-coupled receptor signaling pathways with membrane-permeable blocking peptides. Endogenous 5-HT(2C) receptors in choroid plexus epithelial cells. *J. Biol. Chem.* **2000**, *275*, 7021-7029.

[93] McGrew, L.; Chang, M. S.; Sanders-Bush, E. Phospholipase D activation by endogenous 5-hydroxytryptamine 2C receptors is

mediated by Galpha13 and pertussis toxin-insensitive Gbetagamma subunits. *Mol. Pharmacol.* **2002**, 62, 1339-1343.

[94] Werry, T. D.; Gregory, K. J.; Sexton, P. M.; Christopoulos, A. Characterization of serotonin 5-HT2C receptor signaling to extracellular signal-regulated kinases 1 and 2. *J. Neurochem.* **2005**, *93*, 1603-1615.

[95] Cussac, D.; Newman-Tancredi, A.; Duqueyroix, D.; Pasteau, V.; Millan, M. J. Differential activation of Gq/11 and Gi(3) proteins at 5-hydroxytryptamine(2C) receptors revealed by antibody capture assays: influence of receptor reserve and relationship to agonist-directed trafficking. *Mol. Pharmacol.* **2002**, *62*, 578-589.

[96] Berg, K. A.; Cropper, J. D.; Niswender, C. M.; Sanders-Bush, E.; Emeson, R. B.; Clarke, W. P. RNA-editing of the 5-HT(2C) receptor alters agonist-receptor-effector coupling specificity. *Br. J. Pharmacol.* **2001**, *134*, 386-392.

[97] Barker, E. L.; Westphal, R. S.; Schmidt, D.; Sanders-Bush, E. Constitutively active 5-hydroxytryptamine2C receptors reveal novel inverse agonist activity of receptor ligands. *J. Biol. Chem.* **1994**, *269*, 11687-11690.

[98] Berg, K. A.; Stout, B. D.; Cropper, J. D.; Maayani, S.; Clarke, W. P. Novel actions of inverse agonists on 5-HT2C receptor systems. *Mol. Pharmacol.* **1999**, *55*, 863-872.

[99] Niswender, C. M.; Copeland, S. C.; Herrick-Davis, K.; Emeson, R. B., Sanders-Bush, E. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.* **1999**, *274*, 9472-9478.

[100] Grotewiel, M. S.; Sanders-Bush, E. Differences in agonist-independent activity of 5-Ht2A and 5-HT2c receptors revealed by heterologous expression. *Naunyn. Schmiedebergs Arch. Pharmacol.* **1999**, *359*, 21-27.

[101] De Deurwaerdere, P.; Navailles, S.; Berg, K. A.; Clarke, W. P.: Spampinato, U. Constitutive activity of the serotonin2C receptor inhibits *in vivo* dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.* **2004**, *24*, 3235-3241.

[102] Navailles, S.; Deurwaerdere, P. D.; Spampinato, U. Clozapine and Haloperidol Differentially Alter the Constitutive Activity of Central Serotonin(2C) Receptors *In Vivo*. *Biol. Psychiatry*. **2006**, *59*, 568-575.

[103] Milligan, G.; Bond, R. A.; Lee, M. Inverse agonism: pharmacological curiosity or potential therapeutic strategy? *Trends Pharmacol. Sci.* **1995**, *16*, 10-13.

[104] Rauser, L.; Savage, J. E.; Meltzer, H. Y.; Roth, B.,L. Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine2C receptor. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 83-89.

[105] Dwivedi, Y.; Pandey, G. N. Effects of treatment with haloperidol, chlorpromazine, and clozapine on protein kinase C (PKC) and phosphoinositide-specific phospholipase C (PI-PLC) activity and on mRNA and protein expression of PKC and PLC isozymes in rat brain. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 688-704.

[106] Im, W. B.; Chio, C. L.; Alberts, G. L.; Dinh, D. M. Positive allosteric modulator of the human 5-HT2C receptor. *Mol. Pharmacol.* **2003**, *64*, 78-84.

[107] Hietala, J.; Koulu, M.; Kuoppamaki, M.; Lappalainen, J.; Syvalahti, E. Chronic clozapine treatment down-regulates serotonin 5-HT-1c receptors in rat brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry*. **1992**, *16*, 727-732.

[108] Briddon, S. J.; Leslie, R. A.; Elliott, J. M. Comparative desensitization of the human 5-HT2A and 5-HT2C receptors expressed in the human neuroblastoma cell line SH-SY5Y. *Br. J. Pharmacol.* **1998**, *125*, 727-734.

[109] Pazos, A.; Hoyer, D.; Palacios, J. M. The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.* **1984**, *106*, 539-546.

[110] Pazos, A.; Cortes, R.; Palacios, J. M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.* **1985**, *346*, 231-249.

[111] Pazos, A.; Probst, A.; Palacios, J. M. Serotonin receptors in the human brain-IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience* **1987**, *21*, 123-139.

[112] Marazziti, D.; Rossi, A.; Giannaccini, G.; Zavaglia, K. M.; Dell'Osso, L.; Lucacchini, A.; Cassano, G. B. Distribution and characterization of [3H]mesulergine binding in human brain postmortem. *Eur. Neuropsychopharmacol.* **1999**, *10*, 21-26.

[113] Molineaux, S. M.; Jessell, T. M.; Axel, R.; Julius, D. 5-HT1C receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6793-6797.

[114] Mengod, G.; Pompeiano, M.; Martinez-Mir, M. I.; Palacios, J. M. Localization of the mRNA for the 5-HT2 receptor by in situ hybridization histochemistry. Correlation with the distribution of receptor sites. *Brain Res.* **1990**, *524*,139-143.

[115] Pasqualetti, M.; Ori, M.; Castagna, M.; Marazziti, D.; Cassano, G. B.; Nardi, I. Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. *Neuroscience* **1999**, *92*, 601-611.

[116] Lopez-Gimenez, J. F.; Mengod, G.; Palacios, J. M.; Vilaro, M. T. Regional distribution and cellular localization of 5-HT2C receptor mRNA in monkey brain: comparison with [3H]mesulergine binding sites and choline acetyltransferase mRNA. *Synapse* **2001**, *42*, 12-26.

[117] Pompeiano, M.; Palacios, J. M.; Mengod, G. Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Mol. Brain Res.* **1994**, *23*, 163-178.

[118] Esterle, T. M.: Sanders-Bush, E. Serotonin agonists increase transferrin levels via activation of 5-HT1C receptors in choroid plexus epithelium. *J. Neurosci.* **1992**, *12*, 4775-4782.

[119] Sharma, A.; Punhani, T.;Fone, K .C. F. Distribution of the 5-hydroxytryptamine2C receptor protein in adult rat brain and spinal cord determined using a receptor-directed antibody: effect of 5,7,-dihydroxytryptamine. *Synapse* **1997**, *27*, 45-56.

[120] Ward, R. P.; Dorsa, D. M. Colocalization of serotonin receptor subtypes 5-HT2A, 5-HT2C and 5-HT6 with neuropeptides in rat striatum. *J. Comp. Neurol.* **1996**, *370*, 405-414.

[121] Eberle-Wang, K.; Mikeladze, Z.; Uryu, K.; Chesselet, M-F. Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.* **1997**, *384*, 233-247.

[122] Van Bockstaele, E. J.; Pickel, V. M. GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res.* **1995**, *682*, 215-221.

[123] Steffensen, S. C.; Svingos, A. L.; Pickel, V. M.; Henriksen, S. J. Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J. Neurosci.* **1998**, *18*, 8003-8015.

[124] Di Giovanni, G.; Di Matteo, V.; La Grutta, V.; Esposito, E. m-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. *Neuroscience* **2001**, *103*, 111-116.

[125] Hoffman, B. J.; Mezey, E. Distribution of serotonin 5-HT1C receptor mRNA in adult rat brain. *FEBS Lett.* **1989**, *247*, 453-62.

[126] Wright, D. E.; Seroogy, K. B.; Lundgren, K. H.; Davis, B. M.; Jennes, L. Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J. Comp. Neurol.* **1995**, *351*, 357-373.

[127] Serrats, J.; Mengod, G.; Cortes, R. Expression of serotonin 5-HT2C receptors in GABAergic cells of the anterior raphe nuclei. *J. Chem. Neuroanat.* **2005**, *29*, 83-91.

[128] Liu, R.; Jolas, T.; Aghajanian, G. Serotonin 5-HT(2) receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Res.* **2000**, *873*, 34-45.

[129] Fonseca, M. I.; Ni, Y. G.; Dunning, D. D.; Miledi, R. Distribution of serotonin 2A, 2C and 3 receptor mRNA in spinal cord and medulla oblongata. *Brain Res. Mol. Brain Res.* **2001**, *89*, 11-19.

[130] Kennett, G. A.; Wood, M. D.; Bright, F.; Trail, B.; Riley, G.; Holland, V.; Avenel, K. J.; Stean, T.; Upton, N.; Bromidge, S.; Forbes, I. T.; Brown, A. M.; Middlemiss, D. N.; Blackburn, T. P. SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. *Neuropharmacology* **1997,** *36*, 609-620.

[131] Abramowski, D.; Rigo, M.; Due, D.; Hoyer, D.; Staufenbiel, M. Localization of 5-hydroxytryptamine2C receptor protein in human and rat brain using specific antisera. *Neuropharmacology* **1995,** *35*, 1635-1645.

[132] De Deurwaerdère, P.; Spampinato, U. Role of serotonin2A and serotonin2B/2C receptor subtypes in the control of accumbal and striatal dopamine release elicited *in vivo* by dorsal raphe nucleus electrical stimulation. *J. Neurochem.* **1999**, *73*, 1033-1042.

[133] Gobert, A.; Millan, M. J. Serotonin 5-HT2A receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT,in the frontal cortex of freely-moving rats. *Neuropharmacology* **1999**, *38*, 315-317.

[134] Gobert, A.; Rivet, J-M.; Lejeune, F.; Newman-Tancredi, A.; Adhumeau-Auclair, A.; Nicolas, J-P.; Cistarelli, L.; Melon, C.;

Millan, M. J. Serotonin2C receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: A combined dialysis and electrophysiological analysis in the rat. *Synapse* **2000**, *36*, 205-221.

[135] Lucas, G.; De Deurwaerdère, P.; Caccia, S.; Spampinato, U. The effect of serotonergic agents on haloperidol-induced striatal dopamine release *in vivo*: Opposite role of 5-HT2A and 5-HT2C receptor subtypes and signif-icance of the haloperidol dose used. *Neuropharmacology* **2000**, *39*, 1053-1063.

[136] 136 Di Giovanni, G.; De Deurwaerdère, P.; Di Mascio, M.; Di Matteo, V.; Esposito, E.; Spampinato, U. Selective blockade of serotonin2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined *in vivo* electrophysiological and microdialysis study. *Neuroscience* **1999**, *91*, 587-597.

[137] Di Matteo, V.; Di Giovanni, G.; Di Mascio, M.; Esposito, E. Selective blockade of serotonin 2C/2B receptors enhances dopaminereleasein the rat nucleus accumbens. *Neuropharmacology* **1998**, *37*, 265-272.

[138] Di Matteo, V.; Di Giovanni, G.; Di Mascio, M.; Esposito, E. Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin2C receptors. *Brain Res*. **2000**, *865*, 85-90.

[139] Di Giovanni, G.; Di Matteo, V.; Di Mascio, M.; Esposito, E. Preferential mod-ulation of mesolimbic versus nigrostriatal dopaminergic function by serotonin2C/2B receptor agonists: a combined *in vivo* electrophysiological and microdialysis study. *Synapse* **2000**, *35*, 53-61.

[140] Alex, K. D.; Yavanian, G. J.; McFarlane, H. G.; Pluto, C. P.; Pehek, E. A. Modulation of dopamine release by striatal 5-HT2C receptors. *Synapse* **2005**, *55*, 242-251.

[141] Hutson, P. H.; Barton, C. L.; Jay, M.; Blurton, P.; Burkamp, F.; Clarkson, R.; Bristow, L. J. Activation of mesolimbic dopamine function by phencyclidine is enhanced by 5-HT2C/2B receptor antagonists: Neurochemical and behavioural studies. *Neuropharmacology* **2000**, *39*, 2318-2328.

[142] Pozzi, L.; Acconcia, S.; Ceglia, I.; Invernizzi R. W.; Samanin, R. Stimulation of 5-hydroxytryptamine (5-HT2C) receptors in the ventrotegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. *J. Neurochem*. **2002**, *82*, 93-100.

[143] Willins, D. L.; Meltzer, H. Y. Serotonin 5-HT2C agonists selectively inhibit morphine-induced dopamine efflux in the nucleus accumbens. *Brain Res*. **1998**, *781*, 291-299.

[144] Prisco, S.; Pagannone, S.; Esposito, E. Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study *in vivo*. *J. Pharmacol. Exp. Ther*. **1994**, *271*, 83-90.

[145] Kennett, G. A.; Wood, M. D.; Bright, F.; Cilia, J.; Piper, D. C.; Gager, T.; Thomas, D. R.; Baxter, G. S.; Forbes, I. T.; Ham, P.; Blackburn, T. P. *In vitro* and *in vivo* profile of SB 206553, a potent 5-HT2C/5-HT2B receptor antagonist with anxiolytic-like properties. *Br. J. Pharmacol.* **1996**, *117*, 427-434.

[146] Di Matteo, V.; Di Giovanni, G.; Di Mascio, M.; Esposito, E. SB 242084, a selective serotonin2C receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology* **1999**, *38*, 1195-1205.

[147] Millan, M. J.; Dekene, A.; Gobert, A. Serotonin (5-HT)2C receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT release in the frontal cortex *in vivo*. *Neuropharmacology* **1998**, *37*, 953-955.

[148] Giorgetti, M.; Tecott, L. H. Contributions of 5-HT(2C) receptors to multiple actions of central serotonin systems. *Eur. J. Pharmacol.* **2004**, *488*, 1-9.

[149] Carr, D. B.; Sesack, S. R. GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. *Synapse* **2000**, *38*, 114-123.

[150] Anttila, S. A.; Leinonen., E. V.; A review of the pharmacological and clinical profile of mirtazapine. *CNS Drug Rev*. **2001**, *7*, 249-264.

[151] Millan, M. J.; Gobert, A.; Rivet, J. M.; Adhumeau-Auclair, A.; Cussac, D.; Newman-Tancredi, A.; Dekeyne, A.; Nicolas, J. P.; Lejeune, F. Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of α2-adrenergic and serotonin2C receptors: a comparison with citalopram. *Eur. J. Neurosci*. **2000**, *12*, 1079-1095.

[152] Jenck, F.; Moreau, J. L.; Mutel, V.; Martin, J. R.; Haefely, W. E. Evidence for a role of 5-HT1C receptors in the antiserotonergic properties of some antidepressant drugs. *Eur. J. Pharmacol.* **1993**, *231*, 223-229.

[153] Jenck, F.; Moreau, J. L.; Mutel, V.; Martin, J. R. Brain 5-HT1C receptors and antidepressants. *Progr. Neuropsychopharmacol. Biol. Psychiatr.* **1994**, *18*, 563-574.

[154] Ni, Y. G.; Miledi, R. Blockage of 5-HT2C serotonin receptors by fluoxetine (Prozac). *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2036-2040.

[155] Di Matteo, V.; Di Mascio, M.; Di Giovanni, G.; Esposito, E. Acute administration of amitriptyline and mianserin increases dopamine release in the rat nucleus accumbens: possible involvement of serotonin2C receptors. *Psychopharmacology* **2000**, *150*, 45–51.

[156] Moreau, J-L.; Jenck, F.; Martin, J. R.; Perrin, S.; Haefely, W. E. Effect of repeated mild stress and two antidepressant treatments on the behavioral response to 5-HT1C receptor activation in rats. *Psychopharmacology* **1993**, *110*, 140-144.

[157] Moreau, J-L.; Bös, M.; Jenck, F.; Martin, J. R.; Mortas, P.; Wichmann, J. 5-HT2C receptor agonists exhibit antidepressant -like properties in the anhedonia model of depression in rats. *Eur. Neuropsychopharmacol.* **1996**, *6*, 169-175.

[158] Barker, E. L.; Sanders-Bush, E. 5-Hydroxytryptamine1C receptor density and mRNA levels in choroid plexus epithelial cells after treatment with mianserin and (-)-1-(4-bromo-2,5-dimethoxyphenyl) -2-aminopropane. *Mol. Pharmacol.* **1993**, *44*, 725-730.

[159] Pranzatelli, M. R.; Murthy, J. N.; Tailor, P. T. Novel regulation of 5-HT1C receptors: down-regulation induced both by 5-HT1C/2 receptor agonists and antagonists. *Eur. J. Pharmacol.* **1993**, *244*, 1-5.

[160] Newton, R. A.; Elliott, J. M. Mianserin-induced down-regulation of human 5-hydroxytryptamine2A and 5-Hydroxytryptamine2C receptors stably expressed in the human neuroblastoma cell line SH-SY5Y. *J. Neurochem.* **1997**, *69*, 1031-1038.

[161] Cryan, J. F.; Lucki, I. Antidepressant-like behavioral effects mediated by 5-hydroxytryptamine2C receptors. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1120-1126.

[162] Clenet, F.; De Vos, A.; Bourin, M. Involvement of 5-HT2C receptors in the anti-immobility effects of antidepressants in the forced swimming test in mice. *Eur. Neuropsychopharmacology* **2001**, *11*, 145-152.

[163] Yamada, J.; Sugimoto, Y. Effects of 5-HT2 receptor antagonists on the anti-immobility effects of imipramine in the forced swimming test in mice *Eur. J. Pharmacol.* **2001**, *427*, 221-225.

[164] Lerer, B.; Macciardi, F.; Segman, R. H.; Adolfsson, R.; Blackwood, D.; Blairy, S.; Del Favero, J.; Dikeos, D. G.; Kaneva, R.; Lilli, R.; Massat, I.; Milanova, V.; Muir, W.; Noethen, M.; Oruc, L.; Petrova, T.; Papadimitriou, G. N.; Rietschel, M.; Serretti, A.; Souery, D.; Van Gestel, S.; Van Broeckhoven, C.; Mendlewicz, J. Variability of 5-HT2C receptor cys23ser polymorphism among european populations and vulnerability to affective disorder. *Mol. Psychiatry* **2001**, *6*, 579-585.

[165] Cremers, T. I.; Giorgetti, M.; Bosker, F. J.; Hogg, S.; Arnt, J.; Mork, A,; Honig. G.; Bogeso, K. P.; Westerink, B. H.; den Boer, H.; Wikstrom, H. V. and Tecott L. H. Inactivation of 5-HT$_{2C}$ receptor potentiates consequences of serotonin reuptake blockade. *Neuropsychopharmacology* **2004**, *29*, 1782-1789.

[166] Gao, K.; Calabrese, J. R. Newer treatment studies for bipolar depression. *Bipolar Disord*. **2005**, *7*, 13-23.

[167] Prisco, S.; Esposito, E. Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopaminergic neurones in the ventral tegmental area. *Br. J. Pharmac.* **1995**, *116*, 1923-1931.

[168] Maj, J.; Moryl, E. Effects of sertraline and citalopram given repeatedly on the responsiveness of 5-HT receptor subpopulations. *J. Neural. Transm. Gen. Sec.* **1992**, *88*, 143-156.

[169] Kennedy, A. J; Gibson, E. L.; O'Connel, M. T.; Curzon G. Effects of housing, restraint and chronic treatments with mCPP and sertraline on behavioural responce to mCPP. *Psychopharmcology* **1993**; *113,* 262-268.

[170] Kennett, G. A.; Lightowler, S.; de Biasi, V.; Stevens, N. C.; Wood, M. D.; Tulloch, I. F.; Blackburn, T. P. Effect of chronic administration of selective 5-hydroxytryptamine and noradrenaline uptake inhibitors on a putative index of 5-HT2C/2B receptor function. *Neuropharmacology* **1994**, *33*, 1581-1588.

[171] Yamauchi, M.; Tatebayashi, T.; Nagase, K.; Kojima, M.; Imanishi, T. Chronic treatment with fluvoxamine desensitizes 5-HT2C receptor-mediated hypolocomotion in rats. *Pharmacol. Biochem. Behav.* **2004**, *78*, 683-689.

[172] Di Mascio, M.; Di Giovanni, G.; Di Matteo, V.; Prisco, S.; Esposito, E. Selective serotonin reuptake inhibitors reduce the spontaneous activity of dopaminergic neurons in the ventral tegmental area. *Brain Res. Bull.* **1998**, *46*, 547-554.

[173] Gaddum, J. H. Drugs antagonistic to 5-hydroxytryptamine. In Ciba foundation symposium on hypertension, Wolstenholme GH Eds.; Little Brown and Company: Boston, **1954**, pp 75-77.

[174] Wooley, D. W.; Shaw. E. A biochemical and pharmacological suggestion about certain mental disorders. *Proc. Natl. Acad. Sci. USA* **1954**, *40*, 228-231

[175] Snyder, S. H. Amphetamine psychosis: a "model" schizophrenia mediated by catecholamines. *Am. J. Psychiat.* **1973**, *130,* 61-67.

[176] Altar, C. A.; Wasley, A, M.; Neale, R. F.; Stone, G. A. Typical and atypical antipsychotic occupancy of D2, and S2 receptors: an autoradiographic analysis in rat brain. *Brain Res. Bull.* **1986**, *16*, 517-525.

[177] Kane, J.; Honigfeld, G.; Singer, J.; Meltzer, H. Clozapine for the treatment-resistent schizophrenic. A double-blind camperison with chlorpromazine. *Arch. Gen. Psychiatry* **1988**, *45*, 789-796.

[178] Schmidt, C. J.; Sorensen, S. M.; Kehne, J. H.; Carr, A. A.; Palfreymann, M. G. The role of 5-HT2A receptors in antipsychotic activity. *Life Sci.* **1995***, 25*, 2209-2222.

[179] Ashby, C. R.; Wang, R. Y.; Pharmacological actions of the atypical antipsychotic drug clozapine. A review. *Synapse* **1996**, *24*, 349-394.

[180] Meltzer, H. Y.; Nash, J. F. VII. Effects of antipsychotic drugs on serotonin receptors. *Pharmacol. Rev.* **1991**, 43, 587-604.

[181] Roth, B.L.; Roland, D.; Ciaranello, D.; Meltzer, H.Y. Binding of typical and atypical antipyschotic agents to transiently expressed 5-HT1C receptors. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 1361-1365.

[182] Leysen, J. E.; Gommeren, W.; Van Gompel, P.; Wynants, J.; Janssen, P. F.; Laduron, P. M. Receptor-binding properties *in vitro* and *in vivo* of ritanserin: a very potent and long acting serotonin-S2 antagonist. *Mol. Pharmacol.* **1985**, *27*, 600-611.

[183] Schotte, A.; de Bruyckere, K.; Janssen, P. F.; Leysen, J. E. Receptor occupancy by ritanserin and risperidone measured using ex vivo autoradiography. *Brain Res.* **1989**, *500*, 295-301.

[184] Bersani, G.; Grispini, A.; Marini, S.; Pasini, A.; Valducci, M.; Ciani, N. 5-HT2 antagonist ritanserin in neuroleptic-induced parkinsonism: a double-blind comparison with orphenadrine and placebo. *Clin. Neuropharmacol.* **1990**, *13*, 500-506.

[185] Miller, C. H.; Fleischhacker, W. W.; Ehrmann, H.; Kane, J. M. Treatment of neuroleptic induced akathisia with the 5-HT2 antagonist ritanserin. *Psychoparm. Bull.* **1990**, *26*, 373-376.

[186] Canton, H.; Verrièle, L.; Colpaert, F.C. Binding of typical and atypical antipsychotics to 5-HT1C and 5-HT2 sites: clozapine potently interacts with 5-HT1C sites. *Eur. J. Pharmacol.* **1990**, *191*, 93-96.

[187] Canton, H.; Verrièle, L.; Millan, M. J. Competitive antagonism of serotonin (5-HT)2C and 5-HT2A receptor-mediated phosphoinositide (PI) turnover by clozapine in the rat: a comparison to other antipsychotics. *Neurosci. Lett.* **1994**, *181*, 65-68.

[188] Kuoppamäki, M.; Syvälahti, E.; Jetala, J. Clozapine and N-desmethylclozapine are potent 5-HT1C receptor antagonist. *Eur. J. Pharmacol.* **1993**, *245*, 179-182.

[189] Kuoppamäki, M.; Pälvimäki, E. P.; Jetala, J.; Syvälahti, E. Differential regulation of rat 5-HT2A and 5-HT2C receptors after chronic treatment with clozapine, chlorpromazine and three putative atypical antipsychotic drugs. *Neuropsychopharmacology* **1995**, *13*, 139-150.

[190] Schotte, A.; Janssen, P. F. M.; Megens, A. A. H.,P.; Leysen, J. E. Occupancy of central neurotransmitter receptors by risperidone, clozapine and haloperidol, measured ex vivo by quantitative autoradiography. *Brain Res.* **1993**, *631*, 191-202.

[191] Schotte, A.; Janssen, P. F. M.; Gommeren, W.; Luyten, W. H. M. L.; Van Gompel, P.; Lesage, A. S.; De Loore, K.; Leysen, J. E. Risperidone compared with new and reference antipsychotic drugs: *in vitro* and *in vivo* receptor binding. *Psychopharmacology* **1996**, *124*, 57-73.

[192] Di Matteo, V.; Cacchio, M.; Di Giulio, C.; Di Giovanni, G.; Esposito, E. Biochemical evidence that the atypical antipsychotic drugs clozapine and risperidone block 5-HT2C receptors *in vivo*. *Pharmacol. Biochem. Behav.* **2002**, *71*, 607–613.

[193] Shilliam, C. S.; Dawson, L. A. The effect of clozapine on extracellular dopamine levels in the shell subregion of the rat nucleus accumbens is reversed following chronic administration: comparison with a selective 5-HT(2C) receptor antagonist. *Neuropsychopharmacology* **2005**, *30*, 372-380.

[194] Prinssen, E. P. M.; Koek, W.; Kleven, M. S. The effects of antipsychotics with 5-HT2C receptor affinity in behavioral assays selective for 5-HT2C receptor antagonist properties of compounds. *Eur. J. Pharmacol.* **2000**, *388*, 57–67.

[195] Herrick-Davis, K.; Grinde, E.; Teitler, M. Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine2C receptors. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 226-232.

[196] Kuroki, T.; Meltzer, H. Y.; Ichikawa, J. Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 774-781.

[197] Reavill, C.; Kettle, A.; Holland, V.; Riley, G.; Blackburn, T. P. Attenuation of haloperidol-induced catalepsy by a 5-HT2C receptor antagonist. *Br. J. Pharmacol.* **1999**, 126, 572-574.

[198] Albin, R.; Young, A. B.; Penney, J. B. The functional anatomy of basal ganglia disorders. *Trends Neurosci.* **1989**, *12*, 366-375.

[199] Hagan, J. J.; Middlemiss, D. N.; Sharp, P. C.; Poste, G. H. Parkinson's disease: prospects for improved drug therapy. *Trends Pharmacol. Sci.* **1997**, *18*, 156-63.

[200] Agid, Y. Levodopa: is toxicity a myth? *Neurology* **1998**, *50*, 858-863.

[201] Rodriguez, M. C.; Obeso, J. A.; Olanow, C. W. Subthalamic nucleus-mediated excitoxicity in Parkinson's disease: a target for neuroprotection. *Ann. Neurol.* **1998**, 44, S175-S188.

[202] Fox, S. H.; Brotchie, J. M. A role for 5-HT2C receptor antagonists in the treatment of Parkinson's disease? *Drugs News Perspect.* **1999**, *12*, 477-483.

[203] Radja, F.; Descarrier, L.; Dewar, K. M.; Reader, T. A. Serotonin 5-HT1 and 5-HT2 receptors in adult rat brain after destruction of nigrostriatal dopamine neurons: a quantitative autoradiographic study. *Brain Res.* **1993**, *606*, 273-285.

[204] Fox, S. H.; Brotchie, J. M. 5-HT2C receptor binding is increased in the substantia nigra pars reticulata in Parkinson's disease. *Mov. Disord.* **2000**, *15*, 1064-1069.

[205] Fox, S. H.; Brotchie, J. M. 5-HT2C receptor antagonists enhance the behavioural response to dopamine D1 receptor agonists in the 6-hydroxydopamine-lesioned rat. *Eur. J. Pharmacol.* **2000**, *398*, 59-64.

[206] Wolf, W.A.; Bieganski, G. J.; Guillen, V.; Mignon, L. Enhanced 5-HT2C receptor signaling is associated with haloperidol-induced "early onset" vacuous chewing in rats: implications for antipsychotic drug therapy. *Psychopharmacology* **2005**, *182*, 84-94.

[207] Fox, S. H.; Moser, B.; Brotchie, J. M. Behavioural effects of 5-HT2C receptor antagonism in the substantia nigra zona reticulata of the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Exp. Neurol.* **1998**, *151*, 35-49.

[208] Blackburn, T. P. Serotonergic agents and Parnkinson's disease. *Drug Disc. Today* **2004**, *1*, 35-41.

[209] Koob, G. F. Drugs of abuse: Anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.* **1992**, *13*, 177-184.

[210] Di Chiara, G.; Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc*. Natl. Acad. Sci. USA* **1988**, *85*, 5274-5278.

[211] Spanagel, R.; Weiss, F. The dopamine hypothesis of reward: past and current status. *Trends Neurosci.* **1999**, *22*, 521-527.

[212] Maurel-Remy, S.; Bervoets, K.; Millan, M. J. Blockade of phencyclidine-induced hyperlocomotion by clozapine and MDL 100,907 in rats reflects antagonism of 5-HT2A receptors. *Eur. J. Pharmacol.* **1995**, *280*, R9-R11.

[213] Kehne, J. H.; Ketteler, H. J.; McCloskey, T. C.; Sullivan, C. K.; Dudley, M. W.; Schmidt, C. J. Effects of the selective 5-HT2A receptor antagonist MDL 100,907 on MDMA-induced locomotor stimulation in rats. *Neuropsychopharmacology* **1996**, *15*, 116-124.

[214] Schmidt, C. J.; Fadayel, G. M.; Sullivan, C. K.; Taylor, V. L. 5-HT2 receptors exert a state-dependent regulation of dopaminergic function: Studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine. *Eur. J. Pharmacol.* **1992**, *223*, 65-74.

[215] Bankson, G. M.; Cunningham, K. A. Pharmacological studies of the acute effects of (+)-3,4-Methylenedioxymethamphetamine on locomotor activity: role of 5-HT1B/1D and 5-HT2 receptors. *Neuropsychopharmacology* **2002**, *26*, 40-52.

[216] Fletcher, P. J.; Phil, D.; Grottick, A. J.; Higgins, G. A. Differential effects of the 5-HT2A receptor antagonist M100,907 and the 5-HT2C receptor antagonist SB242,084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology* **2002**, 27, 576-586.

[217] Porras, G.; Di Matteo, V.; Fracasso, C.; Lucas, G.; De Deurwaerdère, P.; Caccia, S.; Esposito, E.; Spampinato, U. 5-HT2A and 5-HT2C/2B receptor subtypes modulate dopamine release induced *in vivo* by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology* **2002**, *26*, 311-324.

[218] O'Neill, M. F.; Heron-Maxwell, C. L.; Shaw, G. 5-HT2 receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB. *Pharmacol. Biochem. Behav*. **1999**, *63*, 237–243.

[219] McMahon, L. R.; Cunningham, K. A. Antagonism of 5-Hydroxytryptamine2A receptors attenuates the behavioral effects of cocaine in rats. *J. Pharmacol. Exp. Ther*. **2001**, *297*, 357-363.

[220] McCreary, A. C.; Cunningham, K. A. Effects of the 5-HT2C/2B antagonist SB 206553 on hyperactivity induced by cocaine. *Neuropsychopharmacology* **1999**, *20*, 556-564.

[221] Grottick, A. J.; Fletcher, P. J.; Higgins, G. A. Studies to investigate the role of 5-HT2C receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1183-1191.

[222] Tomkins, D. M.; Joharchi, N.; Tampakeras, M.; Martin, J. R.; Wichmann, J.; Higgins, G. A. An investigation of the role of 5-HT2C receptors in modifying ethanol self-administration behaviour. *Pharmacol. Biochem. Behav.* **2002**, *71*, 735-744.

[223] Grottick, A. J.; Corrigall, W. A.; Higgins, G. A. Activation of 5-HT2C receptors reduces the locomotor and rewarding effects of nicotine. *Psychopharmacology* **2001**, *157*, 292–298.

[224] Pierucci, M.: Di Matteo, V.; Esposito, E. Stimulation of serotonin2C receptors blocks the hyperactivation of midbraindopamine neurons induced by nicotine administration. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 109-118.

[225] Di Matteo, V.; Pierucci, M.; Esposito, E. Selective stimulation of serotonin2c receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine administration. *J. Neurochem*. **2004**, *89*, 418-429.