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Short title: 5-HT$_2$ receptors, therapeutic

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Keywords: 5-HT$_2$ receptors, selectivity, pathological function, therapeutic track

ABSTRACT
Serotonergic dysfunction is mainly associated with neuropsychiatric and cardiovascular disorders but has also been linked with many other pathological situations. Serotonin (5-hydroxytryptamine, 5-HT) mediates numerous physiological functions in the brain and the periphery by activating a variety of receptors. 5-HT receptors are divided in four classes, three of them belonging to the G-protein coupled receptor family. This review provides an overview of the recent pharmacological developments involving the Gq-coupled 5-HT$_2$ receptors subfamily as well as the pathological implications of this receptor subfamily with regard to fibrosis, the central nervous system, cardiovascular disorders and cancer. The final section highlights new therapeutic opportunities and emerging research revealing unexplored medical opportunities for this class of 5-HT receptors. The development of biased 5-HT$_2$ receptor ligands appears to be an interesting topic in various areas. In the light of recent discoveries, the need for the development of new and safer drugs should take into account the risk of cardiovascular side effects such as pulmonary hypertension and heart valve disease.

Contents

1. Introduction

This review focuses on three serotonin (5-hydroxytryptamine, 5-HT) receptors belonging to the 5-HT$_2$ receptor subfamily: the 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ subtypes. Although the work by D. Julius et al.,(1988) was the first reporting the cloning of a full-length functional serotonin receptor from rat, the 5-HT$_{1c}$ receptor, this publication was shortly followed by considerable efforts from several groups that cloned other unidentified 5-HT receptors. The classical 5-HT$_2$ receptor described by Peroutka et al.,(1981) was cloned in rats slightly later in 1988 (Pritchett, et al., 1988) followed by human analogue (Branchek, et al., 1990; Saltzman, et al., 1991) and was renamed 5-HT$_{2A}$. The 5-HT$_{1c}$ receptor was renamed 5-HT$_{2C}$ because of its structural similarity to the other 5-HT$_2$ receptor, identical second messenger pathways, and similar pharmacological properties. Pharmacological studies attempting to characterize the contractile serotonergic receptor in the rat stomach fundus initially documented its similarity to the 5-HT$_{2C}$ receptor. Despite the absence of detectable 5-HT$_{2C}$ receptor mRNA in the rat stomach fundus, only homology cloning permitted the identification of a new receptor in 1992 in rat and mouse that was named 5-HT$_{2B}$ (Foguet, Hoyer, et al., 1992; Foguet, Nguyen, et al., 1992; Kursar, et al., 1992; Loric, et al., 1992; Wainscott, et al., 1993) and in 1994 in humans (Choi, et al., 1994; Kursar, et al., 1994; Schmuck, et al., 1994; Wainscott, et al., 1996).

The investigation on the contribution of these three 5-HT$_2$ receptors in mammalian physiology leads to a large amount of reports in nearly all functions and organs. Some selective compounds stimulating or blocking these receptors provided an opportunity to explore various areas of human diseases. In this review, we will emphasize some important aspects of the cellular and molecular biology of these receptors and highlight some clinical situations in which these receptors appear as pathophysiological cornerstones.

2. 5-HT$_2$ receptors: structure, coupling, oligomerization, selective ligands, allosteric modulators, biased agonists

The closely related 5-HT$_2$ receptors are members of the rhodopsin family of G protein–coupled receptors (GPCRs) that all activate multiple intracellular signaling networks. The classical signal transduction pathway for this subfamily is Gq/11-coupled activation of phospholipase C (PLC) although these 5-HT$_2$ receptors can also activate phospholipase D and phospholipase A2 by interacting with additional pathways. These 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors are post-transcriptionally modified by alternative RNA splicing, a common mechanism for achieving protein diversity. RNA editing, on the other hand, is a less common process for generating molecular diversity. In fact, the 5-HT$_{2C}$ receptor is one of the few GPCRs known to be edited. RNA editing of the 5-HT$_{2C}$ receptor generates functionally distinct protein variants by altering the genetic code at the mRNA level.

2.1. Structure

5-HT$_2$ receptors are 7 transmembrane domain receptors, with fairly long extracellular N-terminal loops ranging from 55 amino acids for the human 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors to 75 amino acids for the human 5-HT$_{2A}$ receptors and an intracellular C-terminus ranging from 85 amino acids for the human 5-HT$_{2B}$ receptors to 75 amino acids for the human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. A new and unanticipated role of the 5-HT$_{2B}$ receptor N-terminus as a negative modulator, affecting both constitutive and agonist-stimulated activity of the receptor has been shown (Belmer, et al., 2014). The recently published crystal structure of the 5-HT$_{2B}$ receptor bound to ergotamine showed that this receptor exhibits conformational characteristics in both the active and inactive states: an active-like state in the helix VII conformation of the 5-HT$_{2B}$ receptor but only partial changes in helix VI. The differential signaling patterns were also mirrored in the crystal structures, which showed features of a β-arrestin-biased activation state for the 5-HT$_{2B}$ receptor (Wacker, et al., 2013; Wang, et al., 2013). A likely structural explanation for the distinct conformational features and biased pharmacology of ergotamine for 5-HT$_{2B}$ receptors can be found in the region of the extracellular loop 2 (ECL2) junction with helix V (E212-R213-F214), which forms an additional helical turn stabilized by a structured water molecule at the extracellular tip of helix

V. The segment of ECL2 connecting helices III and V *via* the conserved disulfide bond is shortened in the 5-HT$_{2B}$ receptor, and creates a conformational constraint on the position of the extracellular tip of helix V (Martí-Solano, et al., 2014). However, this structured water molecule involved in ECL2 junction with helix V has been challenged since differential interactions of ergotamine with the top of helices V and VI could determine the rotational freedom of helix VI (Liu, et al., 2013). No crystal structures have reported yet for 5-HT$_{2A}$ or 5-HT$_{2C}$ receptor.

More work is needed to precisely understand the structure and function of these receptors and their specific properties.

2.2. Selective agonists

There is virtually no highly selective agonist for a particular 5-HT$_2$ receptor:

- BW723C86: 1-methyl-2- [5-(2-thienylmethoxy)-1H-indole-3-yl] ethylamine hydrochloride, has been reported to have 10-fold selectivity over the human 5-HT$_{2C}$ and 100-fold selectivity over the 5-HT$_{2A}$ receptors (Cussac, et al., 2008; Jerman, et al., 2001; Knight, et al., 2004; Porter, et al., 1999). Lorcaserine [(1R)-8-chloro- 2,3,4,5-tetrahydro-1-methyl-1H-3 benzazepine] has approximately 10-fold higher affinity for 5-HT$_{2C}$ receptor (Thomsen, et al., 2008) over 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors.

- Nor-dexfenfluramine (metabolite of dexfenfluramine), methylergonovine (metabolite of methysergide), and Ro 60-0175: 2(S)-1-(6-chloro-5-fluoro-1H-indol-1-yl)-2-propanamine fumarate are all preferential 5-HT$_{2B}$ agonists with about 10-fold selectivity over 5-HT$_{2C}$ receptor (Cussac, et al., 2002).

- 2,5-dimethoxy-4-iodoamphetamine (DOI) is a non-selective nearly full agonist at 5-HT$_2$ receptors with similar affinity to 5-HT$_{2A}$ 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors (Cussac, et al., 2008; Jerman, et al., 2001; Knight, et al., 2004; Porter, et al., 1999).

- alpha-methyl-5-HT is a non-selective nearly full agonist at 5-HT$_2$ receptors with similar affinity to 5-HT$_{2A}$ 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors (Jerman, et al., 2001; Knight, et al., 2004; Porter, et al., 1999).

2.3. Selective antagonists

A few selective antagonists are available for 5-HT$_2$ receptor subtypes:

- The first highly selective 5-HT$_{2A}$ receptor antagonist reported was MDL100907 [(R)-(+)-α-(2,3- dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]]-4-piperidine- methanol] (Knight, et al., 2004). Sarpogrelate [Succinic acid mono-(1-dimethylaminomethyl-2-(2-[2-(3-methoxyphenyl) ethyl] phenoxy) ethyl) ester hydrochloride], SR46349B [4-((3Z)-3-(2-dimethylaminoethyl)oxyimino-3-(2-fluorophenyl)propen-1-yl)phenol hemifumarate], and ketanserin [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]quinazoline-2,4(1H,3H)-dione] are preferential 5-HT$_{2A}$ receptor antagonists with a 10-fold higher affinity over the 5-HT$_{2C}$ and/or 5-HT$_{2B}$ sites.

- The first highly selective 5-HT$_{2B}$ receptor antagonist reported was LY266097: 1-(2-chloro-3,4- dimethoxybenzyl)- 6-methyl- 1,2,3,4-tetrahydro- 9Hpyrido [3,4-b]indole hydrochloride with a pKi of 9.7 for the human cloned 5-HT$_{2B}$ receptor and a 100-fold greater selectivity over human 5-HT$_{2C}$ and 5-HT$_{2A}$ sites (Audia, et al., 1996). SB204741: N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea has been reported as a selective 5-HT$_{2B}$ receptor antagonist with approximately 100-fold selectivity over the 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors but with a low potency (Ki around 100 nM) (Bonhaus, et al., 1995). The tetrahydro-β-carboline, LY272015 [6-chloro-5-methyl -N-(5-quinolinyl) -2,3-dihydro -1H-indole-1-carboxamide] is also a fairly selective and highly potent antagonist (Cohen, et al., 1996). RS127445 [2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine] was found to have sub-nanomolar affinity for the 5-HT$_{2B}$ receptor (pKi = 9.5) and 1,000 fold selectivity for this receptor as compared to numerous other receptor and ion channel binding sites and appears as the most selective, high affinity 5-HT$_{2B}$ receptor antagonist available now (Bonhaus, et al., 1999). SB215505 [6-chloro-5-methyl-N-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide] behaves as a high affinity and preferential inverse agonist at 5-HT$_{2B}$ receptors (Reavill, et al., 1999).

- SB242084 [6-chloro-5-methyl-1-[6- (2-methylpyridin-3-yloxy)pyridin-3-yl-carbamoyl]indoline] and RS-102221 [N-[5-[5-(2,5-dioxo- spiro[imidazolidine-4,4'-piperidin]-1'-yl)pentanoyl]-2,4-dimethoxy- phenyl]-4-(trifluoromethyl)benzenesulfonamide]are selective 5-HT$_{2C}$ antagonists(Bonhaus, et al., 1997; Knight, et al., 2004).

- SB206553 [5-methyl-N-(3-pyridyl)-1,2,3,5-tetrahydrobenzo[1,2-b:4,5-b']dipyrrole-1-carboxamide] is a mixed 5-HT$_{2C}$/5-HT$_{2B}$ receptor antagonist. It has been reported as a selective 5-HT$_{2C}$/$_{2B}$ receptor inverse agonist with 50- to 100-fold lower affinity for the 5-HT$_{2A}$ and other sites (Kennett, et al., 1996; Knight, et al., 2004).

Non-selective 5-HT$_2$ receptor antagonists such as ritanserin and mesulergine block 5-HT$_2$ receptor-mediated effects. Atypical antipsychotics including clozapine, asenapine, or cariprazine also have fairly high affinity for all 5-HT$_2$ receptors (Kiss, et al., 2010; Millan, et al., 2003; Shahid, et al., 2009; Wainscott, et al., 1996). Aripiprazole (OPC-14597) is a novel atypical antipsychotic drug, which has higher antagonist affinity (EC$_{50}$ = 11 nM) for the human 5-HT$_{2B}$ receptor than at the 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors (Shapiro, et al., 2003).

See table 1. and PDSP database, http://kidbdev.med.unc.edu/databases/pdsp.php

2.4. Coupling

Intracellular signals are inherently challenging targets because they are often ubiquitous; indeed, drug vectorization comes from GPCRs, not transduction pathways. Nonetheless, multi-target drugs acting at two key nodes in a signaling network offer one answer. Another approach to this concept is the manipulation of interactions between 5-HT receptors and their protein partners. One good example is the use of small peptides to decouple 5-HT$_{2C}$ receptors from their PDZ partners, which mimics the desensitization elicited by antidepressants (Gavarini, et al., 2006). Conversely, blocking the interactions between 5-HT$_{2C}$ receptors and the phosphatase PTEN (phosphatase with tensin homology) reproduces the 5-HT$_{2C}$ agonist-induced inhibition of the excitation of mesolimbic dopaminergic neurons by cannabinoids, thereby preventing their rewarding effects (Ji, et al., 2006). Hence, interference with the association between 5-HT$_{2C}$ receptors and PTEN might be an interesting way to counteract drug addiction.

β-Arrestins direct the agonist-induced internalization of 5-HT receptors; however, agonist-independent association with β-arrestins has been reported for non-edited (and partially edited) 5-HT$_{2C}$ receptors (Marion, et al., 2004). This interaction leads to constitutive internalization (Marion, et al., 2004), an effect prevented by inverse agonists (Chanrion, et al., 2008). Calmodulin binds to the proximal region of the 5-HT$_{2C}$ receptor C-terminus upon receptor activation by 5-HT. Mutation of this motif inhibits both β-arrestin recruitment to the 5-HT$_{2C}$ receptor and receptor-operated ERK1,2 signaling in HEK-293 cells, which is independent of G proteins and dependent on β-arrestins. Expression of the calmodulin mutant also prevents receptor-mediated ERK1,2 phosphorylation in cultured cortical neurons and choroid plexus epithelial cells that endogenously express 5-HT$_{2C}$ receptors (Labasque, et al., 2008). Intriguingly, although β-arrestins are implicated in hallucinogenic effects mediated by 5-HT$_{2A}$ receptors, their trafficking is independent (Bhatnagar, et al., 2001). The β-Arrestin-2 directs also agonist-induced internalization of 5-HT$_{2B}$ receptors (Janoshazi, et al., 2007). For instance, β-arrestins contribute to activation of ERK by 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors.

PDZ (postsynaptic-density-95/disc-large/zonulla-occludens-1)-domain-containing proteins profoundly influence internalization of 5-HT$_{2A,2B,2C}$ receptors, and PDZ proteins are essential for targeting 5-HT$_{2A}$ receptors to dendrites in cortical neurons (Xia, Hufeisen, et al., 2003). PDZ partners are both receptor and function specific. Thus, the specific sets of PDZ proteins that interact with 5-HT$_{2A}$, 5-HT$_{2B}$, or 5-HT$_{2C}$ receptors differ. Although PSD-95 (postsynaptic-density-95) prevents 5-HT$_{2A}$ receptor internalization (Xia, Gray, et al., 2003), it favors constitutive and agonist-dependent endocytosis of 5-HT$_{2C}$ receptors (Gavarini, et al., 2006). Conversely, MPP3 (membrane protein palmitoylated 3) stabilizes 5-HT$_{2C}$ receptors at the plasma membrane (Gavarini, et al., 2006). The 5-HT$_{2B}$ receptor shares the C-terminal E-X-V/I-S-X-V sequence with 5-HT$_{2C}$ receptors and also binds MUPP1-PDZ domains *in-vitro* (Becamel, et al., 2001). MUPP1 was shown to interact with the -SSV sequence on the C-

terminus of the 5-HT$_{2C}$ receptor. Moreover, 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors sharing the C-terminal -E-X-V/I-S-X-V sequence with 5-HT$_{2C}$ receptors also bind MUPP1-PDZ domains *in-vitro* (Becamel, et al., 2001). The PDZ motif at the C-terminus of the 5-HT$_{2B}$ receptor was also found necessary for the recruitment of the constitutive NO synthase (cNOS-NOS3) (Manivet, et al., 2000). In addition, stimulation of the 5-HT$_{2B}$ receptor triggered intracellular cGMP production through dual activation of NOS3and inducible NOS (iNOS-NOS2). The group I PDZ motif at the carboxy terminus of the 5-HT$_{2B}$ receptor was shown to be required for recruitment of the NOS3 transduction pathways, and NOS2 stimulation was under control of the Gα13 pathways (Manivet, et al., 2000).

The human polyomavirus JCV causes the fatal demyelinating disease progressive multifocal leukoencephalopathy in immuno-compromised patients. Elphick et al. (2004) found that the serotonergic receptor 5-HT$_{2A}$ could act as the cellular receptor for JCV on human glial cells. 5-HT$_{2A}$ receptor antagonists inhibited JCV infection, and monoclonal antibodies directed at 5-HT$_{2A}$ receptors blocked infection of glial cells by JCV, but not by SV40. Transfection of 5-HT$_{2A}$ receptor-negative HeLa cells with a 5-HT$_{2A}$ receptor rescued virus infection, and this infection was blocked by antibody targeting the 5-HT$_{2A}$ receptor. A tagged 5-HT$_{2A}$ receptor colocalized with labeled JCV in an endosomal compartment following internalization. Later, it was observed that endothelial cells not expressing 5-HT$_{2A}$ receptor could be infected. Following this observation, it was reported that virus entry into HEK293A cells was specifically observed when any 5-HT$_{2A,2B,2C}$ receptors were expressed. Recent data confirmed that virus internalization into HEK293A cells was significantly and specifically allowed by either 5-HT$_{2A,2B,2C}$ serotonin receptors in a way somewhat similar to CCR5 chemokine receptor, which acts as a co-receptor for HIV-1 viral entry. This work shows that 5-HT$_{2A,2B,2C}$ serotonin receptors contribute to JCPyV infection by facilitating viral entry (Assetta, et al., 2013).

A better understanding of virus/5-HT$_2$interactions may lead to new antiviral opportunities.

2.5. Oligomerization

Oligomeric associations of GPCRs often comprise signaling units, and 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors may all form homo- or heterodimers (Brea, et al., 2009; Herrick-Davis, et al., 2004; Jaffre, et al., 2009). In contrast to some classes of GPCRs, agonist binding does not greatly influence formation of 5-HT-receptor dimers, indicating constitutive assembly before membrane insertion. For example, 5-HT$_{2C}$ receptors generate dimers in the endoplasmic reticulum and Golgi of living cells (Herrick-Davis, et al., 2005). 5-HT$_{2C}$ receptor dimers possess an interface between transmembrane helices IV and V, and dimer proximity is increased and decreased by agonists and inverse agonists, respectively (Mancia, et al., 2008). Furthermore, analysis of functionally compensating, co-expressed mutant 5-HT$_{2C}$ receptors linked to Gq (fusion proteins) indicates that the dimer is asymmetric versus Gq, with both subunits binding to 5-HT and having distinct roles during signaling(Herrick-Davis, et al., 2005; Mancia, et al., 2008).

The 5-HT$_{2A}$ and metabotropic glutamate 2 (mGlu2) receptors assemble into heterodimers via a transmembrane-IV and -V linking domain (Gonzalez-Maeso, et al., 2008). Intriguingly, mGlu2 receptor agonists blunt heterodimer coupling to Gi providing one substrate for their anti-hallucinogenic properties (Fribourg, et al., 2011; Gonzalez-Maeso, et al., 2008). Direct evidence has been acquired for mGlu2-5-HT$_{2A}$ heterodimers in the human brain and for a reduced density in schizophrenics (Gonzalez-Maeso, et al., 2008). Nonetheless, this remains one of the few demonstrations of heterodimers in tissues. Of particular interest are ligands specific for 5-HT$_{2A}$-mGlu2 complexes, or other putative heterodimers possessing distinctive binding and coupling profiles (Gonzalez-Maeso, et al., 2008). Evidence for a functional crosstalk between 5-HT$_{2A}$ and D$_2$ receptor were reported in HEK293 cells. D2 receptor activation increases the hallucinogenic agonist affinity for 5-HT$_{2A}$ receptor and decreases the inositol phosphate production. Co-immunoprecipitation studies show that the two receptors can physically interact in HEK293 cells and raise the possibility that a receptor heterocomplex mediates the crosstalk observed. *In vivo*, 5-HT$_{2A}$ receptor

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expression is necessary for the full effects of D_2 antagonist on MK-801-induced locomotor activity (Albizu, et al., 2011). Behavioral studies carried out in mice lacking 5-HT$_{2A}$ receptors revealed also a remarkable 5-HT$_{2A}$ receptor-dependent dissociation in the beneficial antinociceptive effects of cannabinoid CB1 receptors agonist delta9-tetrahydrocannabinol (THC) and its detrimental amnesic properties. Biochemical studies showed that CB1 and 5-HT$_{2A}$ receptors form heteromers that are expressed and functionally active in specific brain regions involved in memory impairment. Remarkably, costimulation of both receptors by agonists reduces cell signaling, antagonist binding to one receptor blocks signaling of the interacting receptor, and heteromer formation leads to a switch in G-protein coupling for 5-HT$_{2A}$ receptor from Gq to Gi proteins (Vinals, et al., 2015).

In cardiac fibroblasts, AT1 angiotensin and 5-HT$_{2B}$ receptors have been reported to share common signaling pathways, which support a possible direct interaction between 5-HT$_{2B}$ and AT1 receptors. Using co-immunolocalization and a pull-down assay, the two receptors were shown to interact together, which suggested that these receptors could exist in heterodimeric complexes (Jaffre, et al., 2009), but *in vivo* experimental confirmation is still lacking. Ghrelin, an orexigenic peptide present in the stomach, has gastroprokinetic properties. *In vivo* studies have shown that the ghrelin receptor (GHS-R1a) antagonist D-Lys(3)-GHRP-6 reduces food intake and delays gastric emptying in rodents but these effects are at variance with the normal phenotype of the ghrelin knockout mice. D-Lys(3)-GHRP-6, induced a pronounced contraction of stomach strips that is blocked by the 5-HT$_2$ receptor antagonists methysergide and yohimbine resulting from smooth muscle contractions and suggesting the possibility of direct interactions with 5-HT$_{2B}$ receptors (Depoortere, et al., 2006).

A possibility for GHS-R1a/5-HT$_{2C}$ dimer-induced attenuation of calcium signaling was also observed. Flow cytometry fluorescence resonance energy transfer (fcFRET) assays confirmed the direct interaction between the GHS-R1a receptor and 5-HT$_{2C}$ receptor. Colocalized expression of the 5-HT$_{2C}$ and GHS-R1a receptor in cultured primary hypothalamic and hippocampal rat neurons further supports the biological relevance of such physiological interaction. When 5-HT$_{2C}$ receptor signaling is blocked, ghrelin's orexigenic effect is potentiated *in vivo*. In contrast, the 5-HT$_{2C}$ receptor preferential agonist lorcaserin attenuates ghrelin-induced food intake (Schellekens, et al., 2015). **Physical associations of the melatonin MT2 and 5-HT$_{2C}$ receptors as functional heteromers were also found by co-immunoprecipitation, bioluminescence resonance energy transfer, and pharmacological techniques both in transfected cells and in cells from human cortex and hippocampus. MT2/5-HT$_{2C}$ heteromers amplify the 5-HT-mediated Gq/phospholipase C response and trigger melatonin-induced unidirectional transactivation of the 5-HT2C protomer of MT2/5-HT$_{2C}$ heteromers. Pharmacological studies reveal distinct functional properties for agomelatine, which shows "biased signaling" (Kamal, et al., 2015)**.

Future studies must focus on the putative heterodimerization of native 5-HT receptors and on their pharmacological profiles in the hope of identifying novel targets for therapeutic intervention.

2.6. Allosteric modulators

Positive allosteric modulators (PAMs) represent alternative approaches to orthosteric agonists (i.e., compounds that interact with the native ligand-binding site). PAMs can increase the affinity and/or efficacy of the orthosteric agonist for its target receptor by acting at a site other than the native ligand-binding site (allosteric). Importantly, so-called pure GPCR PAMs, which lack intrinsic agonist activity within a specific signaling pathway, have been described. These compounds modulate the basal tone of the endogenous ligand in a manner that conserves spatial and temporal elements of native neurotransmission (Christopoulos & Kenakin, 2002). Indeed, multiple PAMs have been identified for GPCRs and may circumvent the challenges of orthosteric agonists. First, PAMs would amplify endogenous signaling through the 5-HT$_2$ receptors, likely resulting in a more physiologically relevant enhancement of function compared to a direct orthosteric agonist. Second, because

of a generally higher sequence divergence in allosteric sites relative to the conserved orthosteric domain, PAMs could potentially achieve higher receptor selectivity than orthosteric agonists. Indeed, some 5-HT$_{2C}$ receptor PAMs have been reported although as yet the pharmacological profiles of these compounds have not been widely reported (Ding, et al., 2012). Ergotamine has been shown to occupy two distinct sites in 5-HT$_{2B}$ receptors, the orthosteric site, where the indole nucleus of ergotamine resides, and the extended binding site, where the tripeptide portion is engaged. The allosteric site in the muscarinic M2 receptor is the same extracellular region as that interacting with the tripeptide portion of ergotamine. These similarities in both the M2 and 5-HT$_{2B}$ receptors suggest that the location of the extracellular allosteric site for Class A GPCRs is quite similar, and in fact, argue that ergotamine likely functions as a bitopic ligand; that is it occupies both the orthosteric and putative extracellular allosteric site in the 5-HT$_{2B}$ receptor. It is now thought that a sodium ion allosterically alters the binding pocket to dampen G-protein signaling, leaving β-arrestin recruitment intact. Recent structural consideration support that this sodium pocket is collapsed in the 5-HT$_{2B}$ receptor structure, (McCorvy & Roth, 2015).

The identification of specific PAMs at 5-HT$_2$ receptors may conceivably lead to improved therapeutics.

2.7. Biased agonists

Another area for 5-HT$_2$ receptors agonist development might emerge from compounds so-called biased agonists sharing a functional selectivity for specific intracellular signaling pathways (Kenakin, et al., 2012). 5-HT$_2$ receptors couple to multiple intracellular pathways including PLC and PLA$_2$ and pharmacological evidences using recombinant cell-based systems suggest that non-selective 5-HT$_2$ agonists such as mCPP and quipazine may differentially activate these signaling pathways downstream from the 5-HT$_{2C}$ receptor (Berg, et al., 1998). LSD and ERG displayed bias for β-arrestin signaling at 5-HT$_{2B}$ receptors, as well as other ergolines such as dihydroergotamine, methylergonovine, pergolide, and cabergoline. ERG and DHE, both of which contain a large tripeptide moiety substitution at the amide scaffold, displayed more extreme signaling bias at the 5-HT$_{2B}$ receptor compared to LSD (Wacker, et al., 2013). A further approach is the identification of compounds with 5-HT$_{2C}$ receptor agonist activity combined with antagonist activity at the 5-HT$_{2A}$ receptor. Experimental evidence supports a potential synergy between these two pharmacological properties, raising the theoretical possibility that a single drug possessing both characteristics may be a superior therapeutic compared to either alone (Booth, et al., 2009; Canal, et al., 2014; Cunningham, et al., 2013; Pockros, et al., 2012).

Currently, it is unknown whether this example of functional selectivity could be translated into any therapeutic gain, although this does open up an interesting opportunity for future drug discovery.

3. Pathologies and 5-HT$_2$ receptors
3.1. Fibrosis and inflammation

Healing is the process of the restoration of health in an unbalanced, diseased or damaged organism. Fibrogenesis is a critical process in wound repair that generates scar tissue. It helps to protect an injured organ until damaged or lost cells are regenerated. Where an injury naturally resolves, the fibrogenic response is usually limited and the temporary scar tissue replaced by healthy functional cells (Mann & Oakley, 2013). States of chronic tissue infection or damage, ageing, tumors, feeding habits and/or exposure to drugs that generate microenvironments in which the inflammatory and fibrogenic phases of wound healing fail to resolve, cause a state of overactive wound healing and loss of the normal regenerative process (Kapetanaki, et al., 2013; Mann & Oakley, 2013). This latter state can lead to the development of progressive fibrotic disease, in which normal tissue is gradually replaced by scar tissue. Such a fibrotic disease progression can occur as a consequence of uncontrolled repair processes in many organs in response to a wide variety of chronic insults. Unless the underlying injury process is effectively managed, the spread of fibrotic matrix ultimately

impairs the architecture and functioning of the organ. In fact, fibrosis is the final common pathological outcome of many chronic and inflammatory diseases that subsequently leads to permanent scarring, organ malfunction and, ultimately, death, as exemplified by end-stage liver disease, chronic kidney disease, idiopathic pulmonary fibrosis and heart failure. Fibrosis is also a major pathological feature of many chronic autoimmune diseases like scleroderma, rheumatoid arthritis, Crohn's disease, ulcerative colitis, glomerulonephritis and myelofibrosis. Pathologic fibrosis can affect a single organ or be systemic when it affects numerous organs and its incidence increases with low exercise or high fat high sugar diet and with age and thus with the ageing of the population. However, the causative agents triggering the development of fibrosis are often unknown. The penetrance varies among gender, the female: male ratio is 3:1 for systemic sclerosis or lung fibrosis and nearly the opposite for kidney, liver, or heart fibrosis.

Over the last decade, several investigations revealed the fact that the 5-HT system is activated during the early phases of wound repair and has a major influence on fibrogenesis. Modulating the activities of specific 5-HT receptors that trigger the activation of fibrogenic signal transduction seems a promising way to control pathological fibrosis. Mechanistic links between fibrosis and 5-HT were first reported in the 1960s for the condition called carcinoid syndrome, caused by tumors of the neuroendocrine enterochromaffin cells synthesizing 5-HT in the gut (carcinoid tumors that secrete vast quantities of 5-HT). While there is still much to be learned about the way by which 5-HT and its different receptors combine in various target cell types to regulate tissue fibrotic repair, initial evidences were produced about the implication of $5-HT_2$ receptor subtypes in different pathological fibrotic tissues, including skin (Dees, et al., 2011), lung (Launay, et al., 2002), heart (Jaffre, et al., 2009; Pavone, et al., 2012), valves (Ayme-Dietrich, et al., 2012), and liver (Ebrahimkhani, et al., 2011). In addition to fibroblasts, these same receptors have been identified in hematopoietic stem cells (Amireault, et al., 2011; Launay, et al., 2012) and immune cells (de Las Casas-Engel, et al., 2013) that also contribute to fibrosis.

However, there is still a complete lack of therapeutic compounds targeting a specific 5-HT receptor and being selective for fibrotic diseases.

3.1.1. Lung fibrosis
In mouse lung homogenates, 5-HT concentrations increase significantly over the time course of bleomycin-induced fibrosis, with a maximum at day seven, together with the expression of 5-HT receptors $5-HT_{2A}$ and $5-HT_{2B}$(Königshoff, et al., 2010). Pharmacological blockade of either $5-HT_{2A}$ or $5-HT_{2B}$ receptors reduces bleomycin-induced lung fibrosis, as demonstrated by reduced lung collagen content and reduced procollagen 1 and procollagen 3 mRNA expressions. Serotonin antagonists promote an antifibrotic environment by decreasing the lung mRNA levels of TGF-β1, connective tissue growth factor and plasminogen activator inhibitor-1 and JunD mRNA. Interestingly, the $5-HT_{2B}$ receptor is strongly overexpressed by fibroblasts in the fibroblastic foci in human idiopathic pulmonary fibrosis samples and in bleomycin-induced pulmonary fibrosis in rodents (Fabre, et al., 2008).

3.1.2. Liver fibrosis
In the liver, fibrogenic hepatic stellate cells (HSC), which are negative regulators of hepatocyte regeneration, are known to express $5-HT_{2A}$ and $5-HT_{2B}$ receptors that regulate TGF-β1 and the downstream signaling Smads (Li, et al., 2006). HSCs are key cellular components of hepatic wound healing and fibrosis. After HSC activation, expression of $5-HT_{2A}$ and $5-HT_{2B}$ receptors is 100- and 50-fold that of quiescent cells, respectively. Treatment of HSCs with $5-HT_2$ receptor antagonists suppresses proliferation and elevates their rate of apoptosis. Serotonin synergizes with platelet-derived growth factor to stimulate increased HSC proliferation (Ruddell, et al., 2006). Distinct from quiescent cells, activated HSCs exhibit [Ca2+]i transients following treatment with 5-HT that are blocked by $5-HT_2$ receptor antagonists (Park, et al., 2011). Stimulation of $5-HT_{2B}$ receptors on HSC by 5-HT was recently shown to activate expression of TGF-β1 (a powerful suppressor of hepatocyte proliferation) via ERK/JunD signaling. Selective antagonism of $5-HT_{2B}$ receptors enhanced

hepatocyte growth in models of acute and chronic liver injury. Similar effects are observed in mice lacking 5-HT$_{2B}$ or JunD, and when HSC are selectively depleted. Antagonism of 5-HT$_{2B}$attenuates CCl4-induced liver fibrogenesis and improves liver function in disease models in which fibrosis is pre-established and progressive (Ebrahimkhani, et al., 2011).

3.1.3. Skin fibrosis

In skin of systemic sclerosis (SSc), expression of 5-HT$_{2B}$ receptors was strongly increased in fibrotic tissue of patients and almost all fibroblasts stained positive for 5-HT$_{2B}$ receptors. Dermal fibrosis is reduced in *Htr$_{2B}$*$^{-/-}$ mice using both inducible and genetic models of fibrosis. Pharmacologic inactivation of 5-HT$_{2B}$ receptor also effectively prevents the onset of experimental fibrosis and ameliorates established fibrosis by decreasing mRNA levels of TGF-β1, connective tissue growth factor, plasminogen activator inhibitor-1 and Smad-3(Dees, et al., 2011). Moreover, inhibition of platelet activation prevents fibrosis in different rodent models (including bleomycin) of skin fibrosis. Consistently, mice deficient for TPH1, the rate-limiting enzyme for 5-HT production outside the central nervous system, show reduced experimental skin fibrosis (Dees, et al., 2011).

3.1.4. Heart fibrosis

In rats, treatment of neonatal cardiac fibroblasts with 5-HT increases the expression of smooth muscle α-actin, a marker of fibroblast differentiation into myofibroblasts, stimulates their migration, and enhances secretion of TGF-β1 and expression of MMPs, which seem to be mediated through 5-HT$_{2A}$ receptors (Yabanoglu, et al., 2009). Independently, 5-HT- or AngII-stimulated cytokine release in adult cardiac fibroblasts is sensitive to 5-HT$_{2B}$ receptor blockade, including secretion of IL-6, IL-1β, TNF-α, and TGF-β1. Treatments with epidermal growth factor receptor (EGFR, ErbB1/4)-selective inhibitors or with selective inhibitors of MMPs also abolish AngII- and 5-HT-induced cytokine release. Finally, the use of HB-EGF-/- cardiac fibroblasts confirms that EGFR transactivation is absolutely required for AngII- and 5-HT-dependent cytokine release. Collectively, these results reveal that convergent actions of AngII and 5-HT via interactions between AT1 and 5-HT$_{2B}$ receptors coexpressed by non-cardiomyocytes are limiting key events in cardiac fibroblast activation (Jaffre, et al., 2009).

Embryonic morphogenesis of cardiac valves and fibrotic events is a critical event linked to endothelial-mesenchymal transformation (EMT). Inducers of EMT during valvulogenesis include VEGF, TGF-β1, and Wnt/β-catenin, which are regulated in a spatiotemporal manner. Serotonin can initiate TGF-βsignaling, which in turn has been strongly implicated in fibrosis. Recent evidence suggests that degenerative valvular disease may be mediated by developmental pathways including bone morphogenic protein (BMP), Wnt and Notch signaling, nitric oxide, and angiotensin II (Orton, et al., 2012). Wnt2 acts as an angiogenic factor for endothelium *in vitro* and *in vivo* whose target genes undergo complex regulation by the tissue microenvironment (Klein, et al., 2009). By gene profiling, 5-HT$_{2B}$ was identified as a down-regulated target gene of Wnt2 signaling in HUVEC. The existence of valve interstitial cells derived at different times and from different origins (i.e., embryonic epicardium and endocardial cushions and the adult bone marrow) raises the interesting possibility that these populations of fibroblasts are functionally different and, thus, differ in their susceptibility to and/or participation in fibrotic pathological processes (Visconti, et al., 2006).

If correct, deciphering the contribution of 5-HT$_2$ receptors in these events should advance our understanding of fibrosis and lead to new antifibrotic compounds.

3.2. Central nervous system (depression, psychosis, addiction, feeding, impulsivity)
3.2.1. Feeding and anorexigens

Maintenance of energy balance requires regulation of the amount and timing of food intake. Eating disorders are an important health problem in developed countries (Leibowitz & Alexander, 1998; Vickers & Dourish, 2004) see (http://www.cdc.gov/obesity/data/facts.html). It is now well established that in the central nervous system, 5-HT is one major neurotransmitter that controls numerous physiological processes affecting food intake. The

most extensive characterization of the serotonergic influences on energy balance pathways relates to the modulation of the arcuate nucleus POMC and NPY/AgRP neuronal populations. Previous studies have established that released 5-HT (i) hyperpolarizes and inhibits AgRP/NPY neurons and decreases an inhibitory drive onto POMC cells by activation of 5-HT$_{1B}$ receptors and (ii) activates POMC/CART neurons via stimulation of 5-HT$_{2C}$ receptors (Heisler, et al., 2006). This leads to reciprocal increases in α-MSH release and decreases in AgRP release at melanocortin 4 receptors in target sites. Subsequent increased 5-HT neurotransmission has also been shown to regulate the hypothalamic-pituitary-adrenal (HPA) axis upstream corticotropin-releasing hormone among others (Heisler, et al., 2007). Many studies have suggested that 5-HT$_{1B}$ receptors inhibit neurons that promote hunger, while 5-HT$_{2C}$ receptors activate neurons that promote satiety in the hypothalamic nuclei (Heisler, et al., 2006; Lam, et al., 2008; Nonogaki, et al., 2007). However, activation of these receptors is not sufficient to fully explain the modulatory effects of 5-HT in feeding behavior. Other 5-HT receptors, such as 5-HT$_4$ or 5-HT$_6$, have also been suggested to participate in the control of energy intake (Conductier, et al., 2005; Jean, et al., 2007; Vickers & Dourish, 2004).

Dexfenfluramine (d-fenfluramine, DF) is an amphetamine congener that has been utilized therapeutically as a highly efficient anorectic molecule for the treatment of obesity (Garfield & Heisler, 2009). Previously, DF was used in the treatment of obesity as well as having potential for the treatment of bulimia. However, clinical use of DF has been associated with several unacceptable side effects, including primary pulmonary hypertension and valvular heart disease (Fitzgerald, et al., 2000; Launay, et al., 2002; Rothman, et al., 2000) and this anorexigen was withdrawn from the market in 1997. Mennini and colleagues, in early 1980s, have performed pioneering work describing initially the effect of DF and derivatives in the release of 5-HT into nerve terminals by targeting the serotonin transporter (SERT) (Garattini, et al., 1986). Administration of DF suppresses food intake in both animals and humans. Animal studies have reported either a complete or partial blockade of DF-induced hypophagia by the 5-HT$_2$ antagonist ritanserin (Goodall, et al., 1993; Neill & Cooper, 1989), the 5-HT$_{2B}$/$_{2C}$ antagonist SB-200646 (Bourson, et al., 1996) or the 5-HT$_{2C}$ antagonist SB242084 (Clifton, et al., 2000). Thus, the anorectic effect of DF has been proposed to be mediated by activation of 5-HT$_{2C}$ receptors (Vickers, et al., 1999; Vickers, et al., 2001), while 5-HT$_{2B}$ receptors have been shown to participate in the DF-induced pulmonary hypertension (Launay, et al., 2002) and valvulopathy (Setola, et al., 2005). Since the hypophagic effect of DF persisted in 5-HT$_{2C}$ receptor knockout ($Htr_{2C}^{-/-}$) mice (Vickers, et al., 1999), other 5-HT receptor subtypes must be involved in DF-induced hypophagia.

The 5-HT$_{2B}$ receptor has been proposed to play also a role in the regulation of food intake (De Vry & Schreiber, 2000), and an early study showed an orexigenic effect of the preferential 5-HT$_{2B}$ receptor agonist BW723C86 (Kennett, et al., 1997). Furthermore, it has been reported that 5-HT regulates appetite possibly via 5-HT$_{2B}$ receptors on hypothalamic neurons (Yadav, et al., 2009). In particular, POMC-specific $Htr_{2B}^{-/-}$ mice show mild hypophagia and a reduction in fat pad mass. Like all 5-HT$_2$ receptors, 5-HT$_{2B}$ receptor is Gq-coupled and therefore excitatory, while POMC neurons have a well-established anorexigenic function. The mechanisms through which 5-HT$_{2B}$ receptors on POMC neurons may produce orexigenic effects have not been established. Reports that 5-HT$_{2B}$ receptors on POMC neurons mediate orexigenic effects are especially puzzling since a series of studies recently reported that expression of 5-HT$_{2C}$ receptor on POMC neurons has a critical anorexigenic function (Xu, et al., 2008).

Whether and how multiple types of 5-HT receptors might be working at cross-purposes in the same or different population of POMC neurons are open questions, which require additional investigation.

Interestingly, the hypophagic response to the anorexigen and 5-HT releaser, DF, observed in wild-type (WT) mice was eliminated in $Htr_{2B}^{-/-}$ mice or in WT mice treated with the highly selective 5-HT$_{2B}$ receptor antagonist, RS127445. Using microdialysis, the DF-induced hypothalamic peak of 5-HT release was found strongly reduced in $Htr_{2B}^{-/-}$ conscious mice

compared with WT. Moreover, the strong 5-HT release observed upon DF stimulation of a synaptosomal preparation from WT was not observed in synaptosomes from $Htr_{2B}^{-/-}$ mice (Banas, et al., 2011). A 5-HT$_{2B}$ receptor-dependent phosphorylation of SERT (Launay, et al., 2006) may explain the requirement for 5-HT$_{2B}$ receptors in the releaser action. These findings strongly support that activation of 5-HT$_{2B}$ receptors is a limiting step in the SERT dependent-releasing effect of DF, whereas other 5-HT receptors may act downstream with respect to feeding behavior. Results using the 5-HT$_{2C}$ receptor agonist WAY-161503 (Rosenzweig-Lipson, et al., 2006) in $Htr_{2B}^{-/-}$ mice confirm the participation of 5-HT$_{2C}$ receptors in feeding behavior(Banas, et al., 2011) as observed in humans during the recent clinical trial for another 5-HT$_{2C}$ receptor preferential agonist lorcaserin (Smith, et al., 2010; Thomsen, et al., 2008). Moreover, it has been reported that 5-HT$_{2C}$ receptor-expressing POMC neurons are required to control energy and glucose homeostasis (Berglund, et al., 2013). Nevertheless, postsynaptic receptors including 5-HT$_{1B}$, 5-HT$_{2C}$ and possibly 5-HT$_{2B}$ receptors seem to be indirectly activated via DF-induced SERT- and 5-HT$_{2B}$ receptor-dependent 5-HT release. Central 5-HT neurons have recently been reported to play a major role in regulating glucose and lipid homeostasis, through recruitment and metabolic activation of brown and beige adipocytes (McGlashon, et al., 2015).

The interplay between central and peripheral 5-HT regulation of feeding behavior and energy homeostasis are not yet solved.

3.2.2. Raphe neurons and depression
In the raphe nuclei, neurotransmission by 5-HT is tightly regulated by autoreceptors that fine-tune serotonergic neurotransmission through negative feedback inhibition at the cell bodies (predominantly 5-HT$_{1A}$) or at the axon terminals (predominantly 5-HT$_{1B}$); however, different roles for 5-HT$_{2B}$ receptors have also been detected (McDevitt & Neumaier, 2011). The therapeutic effects induced by serotonin-selective reuptake inhibitor (SSRI) antidepressants are initially triggered by blocking the SERT and rely on long-term adaptations of pre- and post-synaptic receptors.

The 5-HT$_2$ receptor agonist DOI decreases the firing rate of 5-HT neurons in the dorsal raphe (DR) nucleus of WT anesthetized mice. This inhibitory response persists in $Htr_{2C}^{-/-}$ but is completely blunted in $Htr_{2A}^{-/-}$ mutant mice. Moreover, the reducing effect of DOI on DR 5-HT neuronal activity in WT mice can be attenuated by a loss of norepinephrine (NE) neurons. In WT mice, pharmacological inactivation of 5-HT$_{2A}$ receptors by the selective antagonist MDL100907 reverses escitalopram-induced decrease in DR 5-HT neuronal activity. In microdialysis experiments, a single injection of escitalopram increases cortical extracellular 5-HT, but not NE, levels in awake WT mice. Although the addition of MDL100907 does not potentiate 5-HT neurotransmission, it allows escitalopram to increase cortical NE outflow and consequently to elicit an increase in swim time in the forced swimming test. Blockade of the 5-HT$_{2A}$ receptor may strengthen the antidepressant-like effect of escitalopram by facilitating the enhancement of the brain NE transmission(Quesseveur, et al., 2013).

These results provide support for the use of atypical antipsychotics, which target 5-HT$_2$ receptors, with SSRIs as a relevant antidepressant augmentation strategy.

Both short-term and long-term behavioral and neurogenic SSRI effects were abolished after either genetic or pharmacologic inactivation of 5-HT$_{2B}$ receptors (Diaz, et al., 2012). Conversely, direct agonist stimulation by the preferential 5-HT$_{2B}$ receptor agonist, BW723C86, induced an SSRI-like response in acute behavioral and chronic neurogenic assays. The 5-HT$_{2B}$ receptor is expressed by raphe serotonergic neurons, as shown by single cell PCR. The SSRI-induced increase in hippocampal extracellular 5-HT concentration was strongly reduced in the absence of functional 5-HT$_{2B}$ receptors. These results support a positive regulation of serotonergic neurons by 5-HT$_{2B}$ receptors (Diaz, et al., 2012).

The 5-HT$_{2B}$ receptor appears, therefore, to positively modulate serotonergic activity and to be required for the therapeutic actions of SSRIs, although direct 5-HT$_{2B}$ agonists

cannot be used as therapeutic option, unless biased agonist without harmful cardiopulmonary effect could be developed.

Evidence from various sources indicates alterations in 5-HT$_{2C}$ receptor functions in anxiety, depression, suicide, and other stress-related disorders treated with antidepressant drugs. Although the notion of a 5-HT$_{2C}$ receptor desensitization following antidepressant treatments is rather well anchored in the literature, this concept is mainly based on *in vitro* assays and/or behavioral assays (hypolocomotion, hyperthermia) that have poor relevance to anxio-depressive disorders. Various serotonergic projections to distinct 5-HT$_{2C}$ receptor populations exert complex modulations. Targeting 5-HT$_{2C}$ receptor in specific brain areas rather than activating or blocking them in the whole brain would be the most rational therapeutic strategy (Martin, et al., 2014). Nevertheless, agomelatine, which is a potent melatonin receptor agonist, is an effective antidepressant and a potent 5-HT$_{2B/2C}$ receptor antagonist as well (Millan, et al., 2003). Administration of melatonin twice daily increases the number of spontaneously active dopamine (DA) neurons but leaves the firing of NE neurons unaltered. Long-term administration of melatonin and the 5-HT$_{2C}$ receptor antagonist, SB242084, have, by themselves, no effect on the firing rate and burst parameters of dorsal raphe 5-HT and ventral tegmental area DA neurons. The combination of both, however, enhances only the number of spontaneously active DA neurons, while leaving the firing of 5-HT neurons unchanged. The addition of the selective 5-HT$_{2B}$ receptor antagonist LY266097, which by itself is devoid of effect, to the previous regimen increases for DA neurons the number of bursts per minute and the percentage of spikes occurring in bursts (Chena, et al., 2014). In conclusion, the combination of melatonin receptor activation and 5-HT$_{2C}$ receptor blockade results in a disinhibit ion of DA neurons; when 5-HT$_{2B}$ receptors are also blocked, the firing and the bursting activity of DA neurons are both enhanced, thus reproducing the antidepressant effect of agomelatine, supporting an effect of these receptors on DA neurons.

3.2.3. Drugs of abuse

Dopaminergic projections to the striatum inhibit the medium spiny neurons (MSN) in the striatopallidal (indirect) pathway and excite MSNs in the striatonigral (direct) pathway. There are dense 5-HT projections to the striatum from the dorsal raphe nucleus and it is known that increased 5-HT in the striatum facilitates DA release from terminals. The direct pathway excites various cortical nuclei and some of these nuclei send inhibitory projections to the DRN (dorsal raphe nuclei). Among drugs of abuse, 1) cocaine blocks all 3 monoamine transporters at similar concentrations2) amphetamine and methamphetamine are most potent at norepinephrine transporter (NET), while being 5- to 9-fold less potent at dopamine transporter (DAT), and 200- to 500-fold less potent at SERT; 3) The 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') has higher affinity for SERT than for DAT and NET (Han & Gu, 2006).

3.2.3.1. MDMA and its metabolite MDA

The amphetamine derivative MDMA is a psychostimulant drug, widely used recreationally among young people in Europe and North America. MDMA is metabolized into N-demethylated metabolite 3,4-methylenedioxyamphetamine (MDA). The serotonergic system appears crucial for MDMA reinforcing properties. MDMA binds preferentially to, and reverses, the activity of the SERT, causing also a release of 5-HT stores from nerve terminals. Subsequent activation of postsynaptic 5-HT receptors by released 5-HT had been shown to be critical for the unique psychostimulatory effects of MDMA.

Current evidences indicate that 5-HT$_{2A}$ receptors modulate mesolimbic DA activity and several behavioral responses related to the addictive properties of psychostimulants. A study evaluating the role of 5-HT$_{2A}$ receptors in MDMA-induced reinforcement, hyperlocomotion and the reinstatement of MDMA-seeking behavior investigated basal and MDMA-stimulated extracellular levels of DA in the nucleus accumbens (NAcc) and 5-HT and NE in the prefrontal cortex. Self-administration of MDMA is blunted in *Htr$_{2A}^{-/-}$* mice compared to WT littermates. Horizontal locomotion is increased by MDMA to a higher extent in *Htr$_{2A}^{-/-}$*

than in WT mice. DA outflow in the NAcc is lower in $Htr_{2A}^{-/-}$ compared to WT mice under basal conditions and after MDMA challenge. In WT mice, priming does not reinstate MDMA-seeking behavior, while cue-induced reinstatement is prominent. This cue-induced reinstatement is blocked by administration of the preferential 5-HT$_{2A}$ receptor antagonist, SR46349B (eplivanserin). 5-HT$_{2A}$ receptors are crucial for MDMA-induced reinforcement and cue-induced reinstatement of MDMA-seeking behavior. These effects are probably due to the modulation of mesolimbic dopaminergic activity (Orejarena, et al., 2011). An increase in the functionality of cortical 5-HT$_{2A}$ receptors was observed in mice pretreated with MDMA compared with mice pretreated with saline, but this activation was significantly greater in mice pretreated in the locomotor environment. In contrast, the functional activity of striatal D2 receptors is significantly decreased only in mice pretreated with MDMA. These results reveal neuroadaptations in cortical 5-HT$_{2A}$ and striatal D2 receptors after MDMA-induced behavioral sensitization in mice (Varela, et al., 2011). Using *in vivo* microdialysis and locomotor activity monitoring, repeated injections of MDMA induce a long-term sensitization of noradrenergic and serotonergic neurons, which correlates with behavioral sensitization. The development of this phenomenon, which lasts for at least 1 month after withdrawal, requires repeated stimulation of α$_{1B}$-adrenergic and 5-HT$_{2A}$ receptors. Moreover, behavioral and neuroendocrine assays indicate that hyper-reactivity of noradrenergic and serotonergic networks is associated with a persistent desensitization of somatodendritic α$_{2A}$-adrenergic, 5-HT$_{2A}$, and 5-HT$_{1A}$ receptors. Repeated MDMA exposure causes strong neural and behavioral adaptations and inhibitory feedback mediated by α$_{2A}$-adrenergic and 5-HT$_{1A}$ autoreceptors has an important role in the physiopathology of this addictive behavior (Lanteri, et al., 2014).

The central 5-HT$_{2B}$ receptor has been only recently considered as an interesting pharmacological target to treat drug addiction. Acute pharmacological inhibition or genetic ablation of the 5-HT$_{2B}$ receptor in mice completely abolishes MDMA-induced hyperlocomotion and 5-HT release in NAcc and ventral tegmental area. Furthermore, the 5-HT$_{2B}$ receptor dependence of MDMA-stimulated release of endogenous 5-HT from superfused midbrain synaptosomes suggests that 5-HT$_{2B}$ receptors act, to favor MDMA-stimulated 5-HT release. Thus, the 5-HT$_{2B}$ receptor is a regulatory component in the actions of MDMA (Doly, et al., 2008). $Htr_{2B}^{-/-}$ mice did not exhibit behavioral sensitization or conditioned place preference following MDMA injections. In addition, MDMA-induced reinstatement of conditioned place preference after extinction and locomotor sensitization development are each abolished by RS127445 in WT mice. Accordingly, MDMA-induced dopamine D1 receptor-dependent phosphorylation of extracellular regulated kinase in NAcc is abolished in mice lacking functional 5-HT$_{2B}$ receptors. These results underpin the importance of 5-HT$_{2B}$ receptors in the reinforcing properties of MDMA (Doly, et al., 2009).

The selective 5-HT$_{2C}$ receptor antagonist RS102221 can suppress MDMA-induced hyperlocomotion. These findings provide evidence that the inactivation of 5-HT$_{2C}$ receptors may reduce motor response to MDMA (Conductier, et al., 2005). A role has been ascribed to the inhibitory effects of 5-HT$_{2C}$ receptor activation on physiology and behavior mediated by the mesolimbic dopaminergic pathway, particularly in the terminal region of the NAcc. The influence of this receptor subtype on functions mediated by the nigrostriatal dopaminergic pathway is less clear.

Therefore, the contribution of 5-HT$_2$ receptors to MDMA psychostimulant effects is complex, 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors being responsible for enhanced effects, whereas 5-HT$_{2C}$ receptor lowering MDMA effects.

3.2.3.2. Amphetamine

Although locomotor response to d-Amphetamine is mediated by an increased release of DA and NE, blockade of either α$_{1B}$-adrenergic or 5-HT$_{2A}$ receptors almost completely inhibits d-amphetamine-induced locomotor response in mice. In agreement, mice lacking α$_{1B}$-adrenergic receptors hardly respond to d-amphetamine. Paradoxically, mice lacking 5-HT$_{2A}$ receptors ($Htr_{2A}^{-/-}$) exhibit a twofold higher locomotor response to d-amphetamine than WT littermates. Repeating amphetamine injections still increases $Htr_{2A}^{-/-}$ mice locomotor response to d-amphetamine at a level similar to that of sensitized WT mice. Prazosin a α$_{1B}$-

adrenergicantagonist, at 1 mg/kg completely blocks d-amphetamine-induced locomotor response in $Htr_{2A}^{-/-}$ naïve animals but 3 mg/kg is necessary in sensitized $Htr_{2A}^{-/-}$ mice. Because naïve $Htr_{2A}^{-/-}$ mice exhibit an increased cortical noradrenergic response to d-amphetamine, these data confirmed that repeated d-amphetamine modifies noradrenergic transmission in $Htr_{2A}^{-/-}$ mice. Stimulation of 5-HT$_{2A}$ receptors would inhibit noradrenergic neurons. Dramatic decrease in 5-HT$_{2A}$ receptor antagonist efficiency in sensitized WT mice indicates that a disruption of the regulating role of 5-HT$_{2A}$ receptors on noradrenergic transmission occurs during sensitization and thus represents a putative physiological basis of behavioral sensitization to d-Amphetamine (Salomon, et al., 2007).

Acute exposure to the selective 5-HT$_{2B}$ receptor antagonist LY266097 significantly diminished the increase in DA outflow induced by d-amphetamine in the ventral striatum/NAcc, but not in the dorsal striatum (DSt) (Auclair, et al., 2010). The locomotor response of $Htr_{2B}^{-/-}$ mice to d-amphetamine was found significantly enhanced as compared with $Htr_{2B}^{+/+}$ mice (Pitychoutis, et al., 2015) supporting the participation of this receptor in the control of DA/NE pathways. The $Htr_{2C}^{-/-}$ mice showed marked alterations in the activity and functional output of DA pathway. $Htr_{2C}^{-/-}$ mice displayed increased activity of substantia nigra pars compacta (SNc) DA neurons, elevated baseline extracellular DA concentrations in the DSt, alterations in grooming behavior, and enhanced sensitivity to the stereotypic behavioral effects of d-amphetamine and DAT blocker GBR 12909. These psychostimulant responses occurred in the absence of phenotypic differences in drug-induced extracellular DA concentration, suggesting a phenotypic alteration in behavioral responses to released DA. This was further suggested by enhanced behavioral responses of $Htr_{2C}^{-/-}$ mice to the D1 receptor agonist SKF 81297, also observed in $Htr_{2B}^{-/-}$ mice (Bevilacqua, et al., 2010). Differences in DSt D1 or D2 receptor expression were not found, nor were differences in medium spiny neuron firing patterns or intrinsic membrane properties following DA stimulation (Abdallah, et al., 2009).

Thus 5-HT$_{2B/2C}$ receptors regulate nigrostriatal dopaminergic activity and function both at SNc dopaminergic neurons and at a locus downstream of the DSt and 5-HT$_{2A}$ receptors act at NE neurons.

3.2.3.3. Cocaine

Several studies indicate that acute treatment with 5-HT$_{2A}$ receptor antagonists attenuates the reinstatement of cocaine-maintained behavior but not cocaine self-administration in rodents. Investigation of the effects of the selective 5-HT$_{2A}$ receptor antagonist MDL100907 on intravenous cocaine self-administration and drug and cue-primed reinstatement was performed in rhesus macaques. The role of 5-HT$_{2A}$ receptors in cocaine-induced DA overflow in the NAcc and the caudate nucleus/DSt was evaluated using *in vivo* microdialysis. MDL100907 significantly attenuates drug- and cue-induced reinstatement but had no significant effects on cocaine self-administration across a range of maintenance doses. Importantly, MDL100907 attenuates cocaine-induced DA overflow in the DSt but not in the NAcc (Murnane, et al., 2013).

This work revealed that 5-HT$_{2A}$ receptors differentially contribute to abuse-related effects of cocaine and cocaine-induced nigrostriatal and mesolimbic DA overflow.

The peripheral administration of selective 5-HT$_{2B}$ receptor antagonists RS127445 or LY266097 significantly reduced basal DA outflow in the NAcc shell, but had no effect on cocaine-induced DA outflow in this brain region. Also, RS 127445 failed to modify both basal and cocaine-induced DA outflow in the NAcc core and the DSt. This interaction may take place downstream to DA neurons and could involve an action at the level of DSt and/or NAcc DA transmission, in keeping with the importance of these brain regions in the behavioral responses of cocaine. A regulatory control may thus be exerted by the 5-HT$_{2B}$ receptor on ascending DA pathways (Devroye, et al., 2015).

Thus 5-HT$_{2B}$ receptors may also exert, in addition to serotonergic neurons, a facilitatory control on mesoaccumbens DA pathway activity.

The $Htr_{2C}^{-/-}$ mice devoid of 5-HT$_{2C}$ receptors display enhanced exploration of a novel environment and increased sensitivity to the locomotor stimulant effects of cocaine. In an

operant intravenous self-administration model under a progressive ratio schedule of reinforcement, $Htr_{2C}^{-/-}$ mice display elevated levels of lever pressing for cocaine injections, indicating that the drug is more reinforcing in these mice. Moreover, $Htr_{2C}^{-/-}$ mice exhibit enhanced cocaine-induced elevations of DA levels in the NAcc, a brain region implicated in the stimulant and rewarding properties of cocaine. In contrast, phenotypic differences in DSt DA levels were not produced by cocaine treatment. These findings strongly implicate 5-HT$_{2C}$ receptors in the serotonergic suppression of DA-mediated behavioral responses to cocaine (Rocha, et al., 2002). Depleting forebrain 5-HT induces compulsive cocaine seeking in rats with a limited cocaine taking history; this can be reversed by systemic treatment with a 5-HT$_{2C}$ receptor agonist and mimicked by systemic treatment with a 5-HT$_{2C}$ receptor antagonist in intact animals (Pelloux, et al., 2012).

These results indicate the causal involvement of reduced serotonergic transmission in the emergence of compulsive drug seeking after a long cocaine-taking history depending on 5-HT$_{2C}$ receptors.

3.2.4. Impulsivity

A feature of multiple neuropsychiatric disorders is impulsivity. Recent studies have implicated 5-HT systems in medial prefrontal cortex (mPFC) in mediating individual differences in motor impulsivity, notably the 5-HT$_2$ receptor. High and low impulsive rats were identified in a 1-choice serial reaction time task (1-CSRTT). In western blots, protein levels of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors predict the intensity of motor impulsivity and their ratio in mPFC positively correlates with levels of premature responses in individual outbred rats. High phenotypic motor impulsivity is associated with diminished mPFC synaptosomal 5-HT$_{2A}$:5-HT$_{2C}$ receptor co-immunoprecipitations. The shRNA knockdown of mPFC 5-HT$_{2C}$ receptor results in increased motor impulsivity and triggers a functional disruption of the local 5-HT$_{2A}$:5-HT$_{2C}$ receptor balance as evidenced by a compensatory upregulation of 5-HT$_{2A}$ receptor protein expression and a leftward shift in the potency of M100907 to suppress impulsive behavior. There seem to be a direct relationship between the mPFC 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, and their imbalance may be a functionally relevant mechanism in motor impulsivity (Anastasio, et al., 2015).

A functional polymorphism in the human HTR_{2B} gene introducing a stop codon after 20 amino-acids (Q20*) was found to enhance impulsive behavior (Bevilacqua, et al., 2010). Especially under conditions where control was impaired, the carriers of the stop codon being more vulnerable to alcohol were more impulsive if they drank. Similarly, $Htr_{2B}^{-/-}$ mice displayed more impulsive choice in delayed discounting tasks, sought novelty, and were more active after receiving a D1 dopamine receptor agonist (Bevilacqua, et al., 2010). The phenotype of $Htr_{2B}^{-/-}$ mice results from a combination of both the direct absence of 5-HT$_{2B}$ receptor signaling and the neural adaptations triggered by the permanent lack during development of this receptor.

In operant-based behavioral paradigms, $Htr_{2C}^{-/-}$ mice display deficits in executive functions. $Htr_{2C}^{-/-}$ mice are impaired in the acquisition of a visiospatial attention task as assessed in the 5-CSRTT. In this task, $Htr_{2C}^{-/-}$ mice exhibit marked impairment of attentional processes, with normal response inhibition. By in vivo microdialysis, elevated extracellular DA concentrations in the NAcc of $Htr_{2C}^{-/-}$ mice, during task performance, indicate that 5-HT$_{2C}$ receptors impact DA homeostasis during a visiospatial attention task. The disinhibition of mesolimbic DA pathways may contribute to impaired attention and perturbed task performance in $Htr_{2C}^{-/-}$ mice. Additionally, in a spatial reversal-learning task, $Htr_{2C}^{-/-}$ mice failed to improve their performance over a series of reversals, indicating that intact 5-HT$_{2C}$ receptor signaling is required to accurately respond to repeated changes in reward contingencies. In contrast to the $Htr_{2C}^{-/-}$ mice phenotype in the 5-CSRTT, WT mice treated with the 5-HT$_{2C}$ receptor antagonist SB242084 exhibited diminished response inhibition, suggesting different effects of acute pharmacological blockade and constitutive loss of 5-HT$_{2C}$ receptor activity (Pennanen, et al., 2013). These findings suggest that impaired 5-HT$_{2C}$ receptor signaling during development may predispose to executive function disorders.

3.2.5. Psychosis

The head-twitch behavioral response (HTR-rapid lateral movements of the head) in rodents is reliably elicited by a variety of psychedelics (e.g. DOI, DOM, DOB, mescaline, LSD and psilocin). It was proposed in 1982 that the mescaline-induced HTR is mediated by the 5-HT$_{2A}$ receptor, based on the fact that the relative potency of 5-HT antagonists to block the behavior is correlated with their 5-HT$_{2A}$ affinities. Furthermore non-hallucinogenic 5-HT$_2$ receptor agonists, such as lisuride and ergotamine, do not induce this behavioral response. Thus, HTR seems to serve as a mouse bioassay that is able to predict the hallucinogenic-specific signaling and effects of 5-HT$_{2A}$ receptor agonists in humans. Numerous studies have shown MDL100907 blocks the HTR induced by hallucinogens. Mice lacking the 5-HT$_{2A}$ receptor gene do not produce HTRs in response to mescaline, DOI, DOM, LSD, DMT, 5-MeO-DMT, psilocin, or 1-methylpsilocin (Halberstadt, 2015), although the response can be rescued by selectively restoring the 5-HT$_{2A}$ receptor gene to cortical regions.

Interestingly, NMDA receptor antagonists including phencyclidine (PCP)-like drugs also induce HTR in rodents enhance the 5-HT$_{2A}$-receptor-mediated HTR in mice (Nabeshima, et al., 1987). In animals, clozapine, ritanserin, amesergide and ketanserin block PCP-dependent locomotion and HTRs, which indicates a potential role of circuits working through the 5-HT$_2$ receptor in mediating the behavioral responses induced by NMDA antagonism. Similar results were reported with the selective 5-HT$_{2A}$ receptor antagonist MDL100907. Atypical antipsychotics reverse the cellular responses produced by PCP-like drugs in cortical pyramidal neurons and block the 5-HT and glutamate release in the prefrontal cortex. The mGlu2 receptor activation abolishes the hallucinogen-specific signaling activated by LSD at the 5-HT$_{2A}$/mGlu2 receptor complex. Microdialysis experiments in the cortex have demonstrated that extracellular glutamate increases more slowly than extracellular 5-HT after intraperitoneal administration of PCP-like drugs. These observations support that 5-HT and glutamate acting through the 5-HT$_{2A}$/mGlu2 receptor complex might each be responsible for different LSD-like and PCP-like symptoms of schizophrenia (Gonzalez-Maeso, et al., 2008). Serotonergic and glutamatergic drugs bind the 5-HT$_{2A}$/mGlu2 receptor heterocomplex, which then balances Gi- and Gq-dependent signaling. The 5-HT$_{2A}$/mGlu2 receptor-mediated changes in Gi and Gq activity predict the psychoactive behavioral effects of a variety of pharmacological compounds (Fribourg, et al., 2011). Prefrontal serotonin 5-HT$_{2A}$ receptor activation also enhances NMDA transmission and gates the induction of temporal-dependent plasticity mediated by NMDA receptors at thalamocortical synapses in acute slices. Expressing 5-HT$_{2A}$ receptors in the mediodorsal thalamus of 5-HT$_{2A}$ receptor-deficient mice, using a viral gene-delivery approach, rescued the otherwise absent potentiation of NMDA transmission, induction of temporal plasticity, and deficit in associative memory (Barre, et al., 2016).

In a Finnish cohort of impulsive patients (Bevilacqua, et al., 2010), early-onset schizophrenia was more prevalent in HTR_{2B} Q*20 carriers. Recently, it was shown that domains related to the positive, negative and cognitive symptom-clusters of schizophrenia are affected in $Htr_{2B}$$^{-/-}$ mice, as shown by deficits in sensorimotor gating, in selective attention, in social interactions and in learning and memory processes. In addition, $Htr_{2B}$$^{-/-}$ mice present with enhanced locomotor response to the NMDA receptor antagonist dizocilpine and psychostimulant amphetamine, and with robust alterations in sleep architecture. Importantly, selected schizophrenic-like phenotypes and endophenotypes are rescued by chronic haloperidol treatment (Pitychoutis, et al., 2015). Owing to its relatively low expression level in the brain, the 5-HT$_{2B}$ receptor has not received much of the limelight as a possible regulator of the DOI-induced HTR. The DOI HTR-inducing effects are increased in 5-HT$_{2B}$ receptor knockout mice (Luc Maroteaux, unpublished). Interestingly, although the ergoline analogue, lisuride, has 5-HT$_{2A}$ receptor agonist activity, it does not produce the HTR, and it is a potent antagonist of 5-HT$_{2B}$ receptors. It is worth considering the 5-HT$_{2B}$ receptor as a potential modulator of the behavioral effects of DOI. For example, 5-HT$_{2B}$ receptor affinity of hallucinogenic phenylisopropalamines is significantly correlated with their human hallucinogenic potency (Nelson, et al., 1999). Studies showed that the 5-HT$_{2B}$ receptor is required for the releasing effects of the 5-HT releasers DF and MDMA, findings

that argue brain 5-HT$_{2B}$ receptors may be involved in cognitive and/or behavioral effects of psychoactive drugs (Canal & Morgan, 2012).

There is a consensus in the literature that the ability of DOI to induce the HTR is not blocked by selective 5-HT$_{2C}$ receptor antagonists. There is some evidence that 5-HT$_{2B}$/$_{2C}$ sites may play a modulatory role. Non-selective 5-HT$_{2B/2C}$ receptor agonists Ro 60-0175, MK-212, and mCPP, do not induce the HTR in rats unless administered in combination with the 5-HT$_{2C}$ antagonist, SB 242084. There is also evidence that the ability of DOI to induce the HTR is significantly attenuated by pretreatment with 5-HT$_{2B/2C}$ agonists, including Ro 60-0175, and mCPP. These findings indicate 5-HT$_{2C}$ receptor activation reduces expression of the HTR (Halberstadt, 2015). Likewise, DOI produces a biphasic dose–response curve, and SB 242084 reportedly shifts the descending arm of the DOI response to the right (Fantegrossi, et al., 2010). Both the 5-HT$_{2B}$/$_{2C}$ receptor selective antagonist/inverse agonist, SB206553, and the 5-HT$_{2C}$ receptor antagonist, SB242084, attenuates the HTR elicited by DOI by 50% in mice, and the DOI-induced HTR was attenuated by ~50% in $Htr_{2C}^{-/-}$ mice, relative to their WT littermates (Canal & Morgan, 2012; Canal, et al., 2010).

In conclusion, both 5-HT$_{2B}$ and 5-HT$_{2C}$ receptor modulate the antipsychotic responses. Atypical antipsychotics behaving as 5-HT$_{2A}$ receptor inverse agonists might have efficacy against negative symptoms without evoking motor perturbation (Meltzer, 2013). Finally, with a view to exploiting ligand-directed signaling, 5-HT$_{2A}$-receptor agonists favoring Gq versus Gi activation and devoid of hallucinogenic properties are attractive possibilities.

3.2.5. Prader-Willi syndrome

Prader-Willi syndrome (PWS) is caused by loss of function of paternally expressed genes in the 15q11-q13 region. This region contains a small nucleolar RNA (snoRNA), HBII-52, that exhibits sequence complementarity to the alternatively spliced exon Vb of the serotonin receptor 5-HT$_{2C}$ receptor. HBII-52 regulates alternative splicing of 5-HT$_{2C}$ receptor by binding to a silencing element in exon Vb. Prader-Willi syndrome patients do not express HBII-52. They have different 5-HT$_{2C}$ receptor mRNA isoforms than healthy individuals (Kishore & Stamm, 2006). Using whole genome microarrays to analyze gene expression, microarray and quantitative RT-PCR analysis, alterations in expression of serotonin receptor genes (e.g., *HTR2B*) and genes involved in eating behavior and obesity (*ADIPOR2, MC2R, HCRT, OXTR*) were noted. Other genes of interest with reduced expression in PWS subjects included STAR (a key regulator of steroid synthesis) and SAG (an arrestin family member which desensitizes G-protein-coupled receptors). Quantitative RT-PCR for *SAG, OXTR, STAR, HCRT*, and *HTR2B* using RNA isolated from their lymphoblastoid cells and available brain tissue (frontal cortex) from separate individuals with PWS and control subjects and normalized to *GAPD* gene expression levels validated microarray gene expression data (Bittel, et al., 2007).

3.3. Cardiovascular system

Expression of the 5-HT$_{2C}$ receptors in cardiovascular system is undetectable.

3.3.1. Heart failure and cardiac hypertrophy

Both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors were implicated in cardiac hypertrophy and failure and are expressed at the cardiomyocyte cell surface. The 5-HT$_{2A}$ receptor activation triggers positive inotropic responses (Brattelid, et al., 2007). Noteworthy, despite a similar coupling to the 5-HT$_{2A}$ receptor subtype, the 5-HT$_{2B}$ receptor stimulation does not elicit any contractile response, although the response to β-adrenergic receptor agonist dobutamine in$Htr_{2B}^{-/-}$ cardiomyocytes was impaired (Nebigil, et al., 2001). Serotonin via the Gq-coupled 5-HT$_{2B}$ receptor protects cardiomyocytes against serum deprivation-induced apoptosis as manifested by DNA fragmentation, nuclear chromatin condensation, and TUNEL labeling. Serotonin prevents cytochrome c release and caspase-9 and -3 activation after serum deprivation via cross-talks between phosphatidylinositol-3 kinase/Akt and extracellular signal-regulated kinase ERK1/2 signaling pathways. Serotonin binding to 5-HT$_{2B}$-receptor activates ERK kinases to inhibit Bax expression induced by serum deprivation. Serotonin via

phosphatidylinositol-3 kinase/Akt can activate NF-kB that is required for the regulation of the mitochondrial adenine nucleotide translocator (ANT-1). Parallel to these observations, ultrastructural analysis in $Htr_{2B}^{-/-}$ mice heart revealed pronounced mitochondrial defects in addition to altered mitochondrial enzyme activities (cytochrome oxidase and succinate dehydrogenase) and ANT-1 and Bax expressions. These findings identify 5-HT as a novel survival factor targeting mitochondria in cardiomyocytes via 5-HT$_{2B}$ receptors (Nebigil, et al., 2003).

A cardioprotective role of 5-HT$_{2A}$ receptor blockade was also recently suggested by Blasco-Fontecilla et al. (Blasco-Fontecilla, et al., 2010). These authors emphasized that atypical antipsychotic drugs could reduce the risk of cardiovascular events in schizophrenia. In fact, the cardiovascular risk is increased in these patients due to cigarette smoking, metabolic disorders and cardiac arrhythmias due to QT prolongation. Independently of lifestyle, some of these troubles can be favored by antipsychotics themselves but, interestingly, Tiihonen et al. (Tiihonen, et al., 2009) suggested that long term exposure to antipsychotics could reduce overall cardiovascular mortality, the best profile being obtained for antipsychotic drugs with the higher 5-HT$_2$ receptor affinity i.e. clozapine, quetiapine, olanzapine and thioridazine. It is quite difficult to identify a mechanism in such epidemiological studies but a reduction of platelet aggregation and thrombus formation combined with a limitation of coronary spasms, both linked with 5-HT-mediated 5-HT$_{2A}$ receptor activation, could contribute to cardiovascular protection in these patients.

Another role of cardiac 5-HT$_2$ receptors is myocardial hypertrophy. Genetic studies failed to show genetic polymorphisms in the 5-HT$_{2A}$ receptor gene in patients with hypertrophic cardiomyopathy of genetic origin or due to hypertension. Conversely, the expression of this receptor is increased in cardiac hypertrophy and its pharmacological blockade can prevent the development of cardiac hypertrophy induced by transverse aortic constriction in mice (Lairez, et al., 2013). Caveolin-3 (Mialet-Perez, et al., 2012) and the calcineurin/NFAT (Vindis, et al., 2010) pathways could be involved in these regulations giving to 5-HT a pathophysiological role in cardiac hypertrophy. In human adults, 5-HT$_{2B}$ receptors were also found to be overexpressed in heart from patients with congestive heart failure, this overexpression being positively correlated with cytokine and norepinephrine plasma levels (Jaffre, et al., 2009). Serotonin plasma levels are also increased in patients with heart failure and in animal studies with cardiac hypertrophy induced by aortic constriction. These findings may indicate that 5-HT induces cardiac hypertrophy or heart failure through the 5-HT$_{2B}$ receptor. The 5-HT$_{2B}$ receptor has been shown to be involved in cardiac hypertrophy by acting directly on cardiac myocytes. After two weeks of aortic banding surgery, mRNA and protein expression of 5-HT$_{2B}$ receptors increased significantly. The antagonist, SB215505, significantly reduced the increase in heart weight, heart wall thickness, left ventricular mass and the expression of the brain natriuretic peptide (BNP) but did not attenuate the up-regulation of 5-HT$_{2B}$ receptor protein expression in rats after aortic banding. Following *in-vitro* mechanical stretch of cardiomyocytes and incubation with 5-HT, the level of 5-HT$_{2B}$ receptors and BNP protein increased time-dependently. When transfected with specific siRNA for 5-HT$_{2B}$ receptors in cardiomyocytes, the increase of NF-κB translocation and BNP protein induced by 5-HT incubation plus mechanical stretch were both reversed (Liang, et al., 2006).

By mimicking sympathetic stimulation *in-vivo*, mice globally lacking serotonin 5-HT$_{2B}$ receptors did not develop isoproterenol-induced left ventricular hypertrophy (Jaffré, et al., 2004). The exact cardiac cell type(s) expressing 5-HT$_{2B}$ receptors (cardiomyocytes versus non-cardiomyocytes) involved in this pathological heart hypertrophy was addressed *in-vivo*: mice expressing the 5-HT$_{2B}$ receptor solely in cardiomyocytes, like global 5-HT$_{2B}$ receptor-null mice, are resistant to isoproterenol-induced cardiac hypertrophy and dysfunction, as well as to isoproterenol-induced increases in plasma cytokine levels (Jaffre, et al., 2009) pointing a fibroblast effect. In primary culture of cardiac fibroblasts, angiotensin II and isoproterenol stimulated NOX activity that was prevented by a selective antagonist (SB215505). The 5-HT$_{2B}$ receptor blockade by SB215505 prevented the increase in cardiac superoxide generation and hypertrophy in two models of cardiac hypertrophy, i.e., angiotensin II and isoproterenol infusions in mice (Monassier, et al., 2008). A functional interaction between

AT1 and 5-HT$_{2B}$ receptors *via* a transinhibition mechanism that may involve heterodimeric receptor complexes was shown to trigger cytokine release in cardiac fibroblasts (Jaffre, et al., 2009) and could be a new therapeutic target.

The involvement of 5-HT$_{2B}$ receptors was reported in the generation of apoptotic events associated with cardiac remodeling during increased adrenergic stimulation (Bai, et al., 2010). Based on these data, the effect of a chronic 5-HT$_{2B}$ receptor blockade by the selective antagonist, RS127445, was investigated in spontaneously hypertensive rats showing a left ventricular hypertrophy combined with diastolic dysfunction and an apparently normal ejection fraction (Ayme-Dietrich, et al., 2015; Marzak, et al., 2014). In this model, the 5-HT$_{2B}$ receptor is overexpressed in the left ventricle but the antagonist did not improve cardiac function and hypertrophy. An increase in subendocardial ventricular fibrosis was observed that was reproduced by 5-HT injections in WT but amplified in Htr$_{2B}$$^{-/-}$ animals. Therefore, in hypertensive cardiomyopathy, 5-HT$_{2B}$ receptors could also be associated to cardioprotection through an endothelial mediated coronary vasodilatation (Ayme-Dietrich, et al., 2015).

These data revealed a dual role of 5-HT$_{2A/2B}$ receptors on both cardiomyocytes and cardiac fibroblasts in regulating cardiac hypertrophy *in-vivo*.

3.3.2. Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a progressive and often fatal disorder in humans that results from an increase in pulmonary blood pressure associated with abnormal vascular proliferation. Serotonin is associated with the pathogenesis of PAH (Chan & Loscalzo, 2008). Therapeutic drugs with PAH as a side effect, like the amphetamine derivative and anorexigen dexfenfluramine, are potent 5-HT releasers acting at SERT and/or agonists at 5-HT$_{2B}$ receptors (Weir, et al., 2008). Blockade of 5-HT$_{2B}$ receptors using independent approaches, either genetic (*Htr$_{2B}$$^{-/-}$*) or pharmacologic inactivation (5-HT$_{2B}$ receptor antagonist RS127445), completely prevented the development of hypoxia-induced pulmonary hypertension in mice including the increase in pulmonary blood pressure and lung remodeling, the increase in vascular proliferation, elastase activity and transforming growth factor-β-1 (TGFβ1) levels (Launay, et al., 2002). Using the monocrotaline-induced pulmonary hypertension model, recent studies confirmed that other 5-HT$_{2B}$ receptor antagonists (terguride, PRX-08066, or C-122) significantly reduced pulmonary pressure, arterial wall thickening and lumen occlusion but maintained cardiac function (Dumitrascu, et al., 2011; Porvasnik, et al., 2010; Zopf, et al., 2011). Pulmonary hypertension is associated with a substantial increase in 5-HT$_{2B}$ receptor expression in pulmonary arteries of rodents and humans (Dumitrascu, et al., 2011; Launay, et al., 2002). Activation of 5-HT$_{2B}$ receptors appears to be, therefore, a limiting step in the development of pulmonary hypertension. Recently, the restricted expression of 5-HT$_{2B}$ receptors to bone-marrow cells was shown as necessary and sufficient for pulmonary hypertension to develop *via* an action at hematopoietic stem cell differentiation in response to either hypoxia or monocrotaline (Launay, et al., 2012).

These findings reveal a limiting role of 5-HT$_{2B}$ receptors in PAH development and shift the contribution of 5-HT to PAH to an extrapulmonary, hematopoietic event and open new possibility for therapeutic interventions.

3.3.3. Vascular tone of systemic and coronary arteries

The pharmacology of 5-HT is complex in systemic arteries. 5-HT can elicit vasoconstriction through its interaction with 5-HT$_{2A}$ receptors on vascular smooth muscle cells but also vasodilatation via a nitric oxide-dependent mechanism involving the 5-HT$_{1B}$/$_{2B}$ receptors. In the coronary circulation, the effects of the preferential 5-HT$_{2A}$ receptor antagonist, sarpogrelate, were investigated in dogs submitted to acute reduction of the coronary blood flow (30% of the baseline flow) in the anterior wall (Fujita, et al., 2004). Sarpogrelate amplified 5-HT release in the myocardium and 5-HT$_{1B}$ receptor-mediated dilatation involving NO synthase. Such a mechanism was also suggested in an ischemic hind limb model in diabetic mice where sarpogrelate restored perfusion through the stimulation of the eNOS/Akt

pathway involving the $5-HT_{1B}$ receptor (Iwabayashi, et al., 2012). Nevertheless, an effect in the absence of 5-HT could also happen due to the inverse agonist action of the drug (Hossain, et al., 2012; Muntasir, et al., 2006). In humans, sarpogrelate given as a single 200 mg oral administration also increased basal and maximal (adenosine triphosphate) average peak velocity of the coronary blood flow in patients with coronary artery disease. Such a vasodilatation was also observed in rabbit cerebral arteries (Kawamura, et al., 2013) and experimental vein grafts (Kodama, et al., 2009). The mechanisms of these sarpogrelate effects are not fully understood. An unmasking vasodilation linked to 5-HT-induced $5-HT_{1B}$ receptor stimulation is one possible explanation but other mechanisms were proposed. In rats chronically treated with sarpogrelate, serotonergic stimulation involving $5-HT_7$ and $5-HT_{1D}$ receptors counteracts the pressure response elicited by sympathetic nervous system stimulation (Garcia-Pedraza, et al., 2014). This phenomenon involves smooth muscle cell hyperpolarization and the COX-2 pathway. Interestingly, 5-HT can induce a $5-HT_{2A}$ receptor-mediated COX2-dependant prostacyclin synthesis by smooth muscle cells involving the PKC/Src/MAPK pathway. This biological effect could be involved in vasodilatation and implicates the $5-HT_{2A}$ receptor because it is antagonized by sarpogrelate (Machida, et al., 2011).

Therefore, in some conditions, a $5-HT_{2A}$ receptor stimulation could sensitize smooth muscle cells towards vasodilation triggered by other serotonergic receptors.

Beside its contribution to vasomotion, $5-HT_{2A}$ receptors also appear as key contributors to atherosclerosis and arterial wall remodeling. In rabbits fed a high cholesterol diet, sarpogrelate reduced the extent of atherosclerotic deposits in the aorta in parallel to an induction of endothelial nitric oxide synthase (Hayashi, et al., 2003). These chronic effects combined with a reduction of 5-HT and angiotensin II-induced vascular smooth muscle cell proliferation (Sharma, et al., 2001; Watanabe, et al., 2001) could limit vascular remodeling. This is now clear for peripheral arterial disease in which sarpogrelate improves outcome of symptomatic patients (Ren, et al., 2013) but it remains to be demonstrated after endovascular therapy. Retrospective studies showed a reduction of major clinical endpoints such as amputation or death from any cause after endovascular therapy for critical hindlimb ischemia (Takahara, et al., 2014) and the first preliminary results of a recent prospective study suggest that sarpogrelate associated to aspirin is as efficacious as sarpogrelate associated to clopidogrel for the prevention of restenosis following femoropopliteal arterial stenting (Chen, et al., 2015).

The reduction of neointimal hyperplasia in combination with the improvement of endothelial function by sarpogrelate (Miyazaki, et al., 2007) highly encourages its use in peripheral arterial disease and emphasizes the contribution of the $5-HT_{2A}$receptor in chronic artery disease.

Diabetes mellitus is a pathological situation at high risk for cardiovascular events. In experimental type 2 diabetes, 5-HT increases plasma epinephrine and glucose making rats insulin resistant. This resistance involves $5-HT_{2A}$ receptors because is blocked by a sarpogrelate pretreatment (Takishita, et al., 2004). The insulin-sensitizing effect of sarpogrelate has been confirmed in diabetic patients (Kokubu, et al., 2006) and in mice when combined with the PPAR-γ agonist, pioglitazone (Iizuka, et al., 2009). High glucose promotes endothelial dysfunction and 5-HT has been suggested as a key pathophysiological player in vascular complications of diabetes in the context of the metabolic syndrome. When rat aortic rings are incubated in the presence of a high glucose concentration, the endothelium-dependent vasodilatation triggered by acetylcholine is reduced, whereas the endothelium-independent dilatation induced by an NO-donor is unaffected. Interestingly, sarpogrelate can restore the endothelial NO production and, as a consequence, NO-mediated dilatation (Sun, et al., 2011). Nevertheless, these effects were obtained at high sarpogrelate concentrations (10 μM) in a culture medium in which the concentration of 5-HT was not measured. This work emphasizes that blocking $5-HT_{2A}$ receptors could protect endothelial function in diabetic patients. In a rat model of type 1 diabetes, sarpogrelate

reduced blood glucose and the endothelial PECAM-1 overexpression, therefore limiting 5-HT-induced thrombosis (Yamada, et al., 2012). Interestingly, this receptor also affects neointimal proliferation and restenosis following arterial stenting, although not expressed by endothelial cells. A paracrine mechanism involving cytokines released by smooth muscle cells or the regulation of the homing of circulating cells can be suggested to explain some of the vascular beneficial effects following 5-HT$_{2A}$ receptor blockade.

Taken together, all these data argue in favor of a major contribution of the 5-HT$_{2A}$ receptor in the regulation of vasomotor tone and in the interaction between endothelial and smooth muscle cells trough a subtle equilibrium involving other receptors.

5-HT$_{2B}$ receptors are also involved in vascular responsiveness in hypertension. Mesenteric arteries from deoxycorticosterone- (DOCA) salt hypertensive rats predominantly contract *via* 5-HT$_{2B}$ receptors. PCR analyses indicate an increase in mRNA for the 5-HT$_{2B}$ receptor in mesenteric arteries of DOCA-salt hypertensive arteries, supporting an increase in receptor number (Watts, et al., 1996). The endothelium-denuded isolated superior mesenteric artery of DOCA-salt rats displays a marked increase in maximum contraction to the 5-HT$_{2B}$ receptor agonist BW723C86 compared with that of arteries from sham rats, confirming that the 5-HT$_{2B}$ receptor plays a greater role in 5-HT-induced contraction in arteries from DOCA-salt rats. In chronically instrumented rats, the 5-HT$_{2B}$receptor antagonist LY272015 significantly reduced the mean blood pressure (Watts & Fink, 1999). LY272015 produced a 4-fold rightward shift to 5-HT in aorta from hypertensive rats made by exposure to the nitric-oxide synthase inhibitor N(omega)-nitro-L-arginine, and blockade by ketanserin did not occur at low concentrations of 5-HT(Russell, et al., 2002).

These findings reveal that the 5-HT$_{2B}$ receptor plays an important role in 5-HT-induced contraction in arteries of hypertensive individuals that could be targeted for therapeutic purpose.

3.3.4. Valvular heart disease

Carcinoid heart disease occurs in over 65% of patients with the carcinoid syndrome and is characterized by fibrous thickening of cardiac valves, leading to heart failure for review (Roth, 2007). Correlation of high plasma 5-HT levels with valvular abnormalities detected by cardiac catheterization and echocardiography has been reported. Thus, 5-HT overproduction has been proposed to be responsible for cardiac valvular disease in patients with carcinoid tumors(Robiolio, et al., 1995). The similarity to lesions in carcinoid heart disease and in methysergide-associated valvular disease suggested direct stimulation of myofibroblast growth by 5-HT agonism (Hendrikx, et al., 1996). The occurrence of fenfluramine-associated valvular heart disease has raised concerns that other serotonergic medications might also increase the risk of developing valvular heart disease (Connolly, et al., 1997). Dexfenfluramine was approved in the United States for long-term use as an appetite suppressant until it was reported to be associated with valvular heart disease. The valvular changes (myofibroblast proliferation) are histopathologically indistinguishable from those observed in carcinoid disease or after long-term exposure to 5-hydroxytryptamine 5-HT$_2$-preferring ergot drugs (ergotamine, methysergide). The amphetamine derivative MDMA and its metabolite MDA each preferentially bind to and activate human recombinant 5-HT$_{2B}$ receptors and like fenfluramine and its N-methylated metabolite norfenfluramine, elicit myogenic responses in human valvular interstitial cells *via* activation of 5-HT$_{2B}$ receptors (Stole, et al., 2003).

Based on strikingly similar echocardiographic and histopathological features, it is now considered that ergot-derived dopamine agonists may cause a valvular heart disease nearly identical to that seen in patients with carcinoid syndrome (Horvath, et al., 2004). Population studies of patients with Parkinson's disease compared with non-parkinsonian controls have reported that pergolide and cabergoline have a similar risk of inducing fibrotic changes in cardiac valve leaflets. Pergolide and cabergoline have high affinity for the 5-HT$_{2B}$receptor. The frequency of moderate-to-severe regurgitation in at least one heart valve was higher in patients receiving cabergoline or pergolide than in patients taking non-ergot agonists or

controls, and the incidence of new-onset valvulopathy was high in patients taking the ergot-derived drugs (Antonini & Poewe, 2007; Roth, 2007). A simultaneous mitral bioprostheses hypertrophic scaring and native aortic valve fibrosis was recently reported during benfluorex therapy in a 40-year-old woman. The bioprostheses and aortic valves exhibited similar histopathological lesions. Thickening and plaque deposits made by smooth muscle α-actin- and vimentin-positive cells in a glycosaminoglycan matrix were observed supporting that activated by 5-HT$_{2B}$ receptor norfenfluramine (also metabolite of benfluorex) triggers the development of drug-induced heart disease (Ayme-Dietrich, et al., 2012). 5-HT$_{2B}$ and 5-HT$_{2A}$ receptor transcripts are easily detected in heart valves, while no 5-HT$_{2C}$ receptor transcript is detectable. Preferential stimulation of valvular 5-HT$_{2B}$ receptors (with or without accompanying 5-HT$_{2A}$ receptor activation) may contribute to valvular fibroplasia in humans (Fitzgerald, et al., 2000). Mitral valve regurgitation has been associated with increased mRNA expression of valvular 5-HT$_{2B}$ receptors and SERT in pigs (Cremer, Zois, et al., 2015). Canine myxomatous mitral valve disease was associated with higher expression of 5-HT$_{2B}$ receptors in mitral valve (Cremer, Moesgaard, et al., 2015).

These findings suggest that 5-HT$_{2B}$signaling links vascular damage and platelet activation to tissue remodeling and identify 5-HT$_{2B}$ as a novel therapeutic target to treat valvular heart diseases.

3.3.5. Migraine
A role for 5-HT in migraine has been supported by changes in circulating levels of 5-HT and its metabolites during the phases of a migraine attack. A migraine headache is thought to be transmitted by the trigeminal nerve from the meninges and their associated blood vessels. Correlation of the receptor affinities with the potencies used in migraine prophylaxis showed significant correlations for the 5-HT$_{2B}$ receptor. Various human meningeal tissues express 5-HT$_{2B}$ mRNAs (Schmuck, et al., 1996). The 5-HT$_{2B}$ receptor can activate the release of NO, and induce relaxation of the cerebral arteries and the jugular vein. 5-HT$_{2B}$ receptors located in endothelial cells of meningeal blood vessels may trigger migraine headache through the formation of NO, which results in the dilation of cerebral blood vessels and the concomitant activation of sensory trigeminovascular afferents, thus initiating the manifestation of head pain (Johnson, et al., 2003; Schmuck, et al., 1996). In addition, a recent genetic study identified 5-HT$_{2B}$ receptors as a susceptibility gene to migraine (Corominas, et al., 2010).

Endothelial 5-HT$_{2B}$ receptors may thus trigger dilation of meningeal blood vessels, which by activating sensory trigeminovascular afferents induces head pain.

3.4. Cancer
In addition to its numerous physiological functions, 5-HT has been shown to be a mitogenic factor in a wide range of normal and tumor cells. The following will emphasize the role of 5-HT$_2$ receptors in cancers.

3.4.1. Carcinoid tumors
The 5-HT$_{2B}$ receptor expression was observed in spontaneous human carcinoid tumors, along with coupling to p21^{ras} activation, ERK1/2 activation and proliferation (Launay, et al., 1996; Nebigil, et al., 2000). The tumor proliferative activity of small intestinal neuroendocrine tumors (including cell growth and the development of desmoplasia) is associated with the particular microenvironment in the peritoneum and tumor cells support this necessary milieu through the secretion of profibrotic/ angiogenetic factors (Svejda, et al., 2010), but an autocrine action of 5-HT/5-HT$_{2B}$ receptor is likely.

3.4.2. Breast tumors
Increased 5-HT biosynthetic capacity accompanied by multiple changes in 5-HT receptor expression and signaling favor malignant progression of human breast cancer cells (for example, stimulated proliferation, inappropriate cell survival). Expression levels for 5-HT$_{1F}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_{5A}$ and 5-HT$_7$ receptors show an overall increase in breast cancers. Among these receptors, 5-HT$_{2B}$ receptor expression was found increased in breast cancers

and the HTR_{2B} mRNA is also expressed in untransformed human mammary epithelium (Pai, et al., 2009). HTR_{2B} mRNA expression was found lower in basal tumors estrogen receptor (ER) negative, compared with luminal tumors, which are most commonly ER positive. HTR_{2B} mRNA was elevated in carcinomas, increased with tumor stage, and concomitantly was higher in lymph node-positive tumors as compared to node-negative tumors. This observation was supported by a study, showing that c-Myc transformation induced an increase in HTR_{2B} expression (Pai, et al., 2009). In human breast cancer, correlation analysis revealed a significant correlation of HTR_{2B} with ER-α (Kopparapu, et al., 2013). Although 5-HT_{2A} receptor expression was detected in human breast cancer cell line (MCF-7) and evoked as responsible for the mitogenic effect of 5-HT, preliminary data have to be confirmed (Sonier, et al., 2006).

3.4.3. Melanomas
Uveal (ocular) melanoma is an aggressive cancer that often forms undetectable micro metastases before diagnosis of the primary tumor. High increases in HTR_{2B} mRNA transcript levels were found in all uveal melanomas with monosomy 3 compared with low expression in all tumors with disomy 3. As monosomy 3 is associated with metastatic disease, HTR_{2B} expression has been proposed as a marker to identify patients with poor prognosis (Tschentscher, et al., 2003). The 5-HT_{2B} receptor signals through the heterotrimeric GTPase, $GNAQ$, which is mutated in half of uveal melanomas (Onken, et al., 2010). The 5-HT_{2B} receptor is among the genes, which show the highest overexpression in class 2 uveal melanoma (van Gils, et al., 2008). A PCR-based 15-gene assay comprising 12 discriminating genes including HTR_{2B} are now part of a prognostic assay, which provides an important addition to the armamentarium for managing patients with uveal melanoma (Onken, et al., 2010). These genes, including HTR_{2B}, provide candidates for distinguishing whether uveal melanomas contain liver metastases and thus aid in the diagnosis and prevention of uveal melanoma liver metastases, based on their different features (Zhang, et al., 2014).

3.4.4. Prostate cancer
Prostate cancer is the most commonly diagnosed non-cutaneous cancer in men. Despite this fact, many of the genetic changes that coincide with prostate cancer progression remain enigmatic. The 5-HT_{2B} receptor was found to be upregulated in tumors relative to benign glands (Magee, et al., 2001). Overexpression of receptors to neuroendocrine cell products has been suggested to contribute to development of hormone-refractory prostate cancer. Immunostaining for the 5-HT_{2B} receptor was observed in low-grade and high-grade tumors, prostatic intra-epithelial neoplastic and benign prostatic hyperplasia cells, and in vascular endothelial cells. Antagonists for the 5-HT_{2B} receptor have been reported to inhibit proliferation of prostate cancer cells in a dose-dependent manner (Dizeyi, et al., 2005).

3.4.5. Adrenocortical carcinoma
Interestingly, gene expression profiles of adrenocortical tumors identified underexpression of HTR_{2B} mRNA as a marker of malignant adrenocortical carcinoma (Fernandez-Ranvier, et al., 2008). Analysis of biomarkers of malignancy of adrenocortical cancers in the meta-analysis has revealed that the combination of overexpressed anillin ($ANLN$) and underexpressed HTR_{2B} mRNA appeared to be the best predictor of malignancy (Zsippai, et al., 2011). However, in adrenocorticotropin-dependent adrenal hyperplasias, the mechanisms responsible for the ectopic adrenal expression of glucose-dependent insulinotropic peptide (GIP) receptor ($GIPR$) in GIP-dependent Cushing's syndrome are unknown. Chronic adrenal stimulation by GIP in GIP-dependent ACTH-independent macronodular adrenal hyperplasia leads to the significant induction of the $GPR54$, HTR_{2B}, $GPR4$, and endothelial differentiation sphingolipid receptor $EDG8$ (Lampron, et al., 2006).

3.4.6. Hepatocellular carcinoma
Among 64 genes for which mRNA expression levels differed between non-hepatitis B, non-hepatitis C compared to hepatitis C-type hepatocellular carcinoma (HCC), the most affected

was found HTR_{2B} (Iizuka, et al., 2004). The function of 5-HT as a survival factor of HCC cells was recently demonstrated: activation of the 5-HT$_{2B}$ receptor leads to sustained phosphorylation of two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy. Inhibiting the 5-HT$_{2B}$ receptor reduced cancer cell growth *in-vitro* and *in-vivo*. The presence of 5-HT$_{2B}$ receptors in HCC and the activation of autophagy-related mechanisms demonstrate novel insights of 5-HT in cancer biology and propose 5-HT-mediated signaling as a therapeutic target (Soll, et al., 2010). The 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors were found expressed, respectively, in 32% and 35% of the patients with hepatocellular cancer. Both receptors were associated with an increased proliferation index (Soll, et al., 2012). The 5-HT$_{2B}$ receptor mediates 5-HT-induced proliferation in the serum-deprived HCC Huh7 cells. Additionally, inhibition of 5-HT$_{2B}$ receptor in Huh7 cells using SB204741, a selective 5-HT$_{2B}$ receptor antagonist, significantly decreased the expression of FOXO3a, demonstrating that FOXO3a was a target of 5-HT in Huh7 cells (Liang, et al., 2013).

3.4.7. Choriocarcinoma

This gestational trophoblastic disease is due to abnormal growth of trophoblast cells. The 5-HT$_{2A}$ receptor expression was found in human choriocarcinoma cell lines like JEG-3 and BeWo and in normal human placental tissue.5-HT stimulation leads to a concentration-dependent increase in proliferation of choriocarcinoma cell lines (maximal increase in JEG-3 cell proliferation of 25.6±6.8% with 40µM of 5-HT and 11.1±4% with 20µM of 5-HT for BeWo cells, compared to untreated control cells) (Sonier, et al., 2006). The same team confirmed the role of 5-HT$_{2A}$ receptor in cell viability and cell-cycle progression, by treating JEG-3 and BeWo cells with the 5-HT$_2$ receptor agonist DOI and reversing the concentration-dependent increase in cell viability with ketanserin, a preferential5-HT$_{2A}$ receptor antagonist. Moreover, stimulation of 5-HT$_2$ by DOI in these two choriocarcinoma cell lines activated both MEK-ERK1/2 and JAK2-STAT3 signaling pathways (Oufkir, et al., 2010).

3.4.8. Glioma

Human malignant gliomas are particularly invasive making a complete surgical resection very difficult. Therefore, pharmacological approaches are explored. Merzak et al.(1996) identified, 5-HT$_2$ receptor expression by RT-qPCR in eight glioma cell lines and a weak expression in normal fetal astrocytes coming from the cerebellum and the left hemisphere. Moreover, these authors have shown that 5-HT stimulates proliferation, migration and invasion of glioma cells. Nevertheless, they did not clearly establish the receptor type involved. Other *in vitro* experiments made on rat astrocytes and 9L glioma cells detected 5-HT$_{2A}$ receptor expression by RT-qPCR among twelve 5-HT receptor subtypes explored (Sarrouilhe, et al., 2015). In this work, 5-HT stimulation of C6 glioma cells increased glial fibrillary acidic protein (GFAP) expression (mRNA and protein levels), suggesting that 5-HT could induce the differentiation of these cells(Morita, et al., 2006). Finally, stimulation with 5-HT of C6 glioma cells increased the glial cell-line derived neurotrophic factor (GDNF) release, an effect, which was blocked by ketanserin and cyproheptadine treatments. These results suggest that 5-HT$_{2A}$ receptors could be involved in survival, proliferation or migration of glioma cell lines through GDNF release (Hisaoka, et al., 2004; Tsuchioka, et al., 2008).

3.4.9. T-cell Leukemia

A proteasome inhibitor, bortezomib, could be a potential therapeutic agent in treating adult T-cell leukemia (ATL) patients. A network including HTR_{2B} was identified that converges to secreted protein acidic and rich in cysteine (*SPARC*) gene, a tumor-invasiveness related gene, which may act as a possible modulator of bortezomib-induced cell death in ATL cells (Ohyashiki, et al., 2008), although the putative role of 5-HT was not investigated.

3.4.10. Osteosarcoma

Htr$_{2A}$ mRNA is found in anaplastic osteoblasts as well as in differentiated and matured osteoblasts, whereas*Htr$_{2B}$* mRNA is only expressed in differentiated and matured osteoblasts

(Hirai, et al., 2009; Westbroek, et al., 2001). The 5-HT$_{2A}$ receptor has been shown to regulate MC3T3-E1 osteoblast cell proliferation, by activating the ERK pathway. This effect is reversed by the preferential 5-HT$_{2A}$ receptor antagonist ketanserin (Hirai, et al., 2010; Hirai, et al., 2009). Bracha S et al.(Bracha, et al., 2013) reported the serotonergic signaling pathway triggered by 5-HT$_{2A}$ receptor in non-neoplastic canine osteoblasts and in osteosarcoma cell line. They demonstrated that intracellular second messenger signal is different between normal and malignant osteoblast cells: ritanserin, a 5-HT$_2$ receptor antagonist decreased ERK phosphorylation and cell viability in non-neoplastic osteoblasts cell lines, whereas it decreased cell viability by CREB phosphorylation in osteosarcoma cell lines. The lack of selectivity of 5-HT$_2$ antagonists used precludes any conclusion about the exact receptor involved.

3.4.11. Myosarcoma
In the pathogenesis of uterine leiomyosarcoma, a cDNA microarray analysis also identified a four fold overexpression of *HTR$_{2B}$* among the most overexpressed genes (Arslan, et al., 2005; Matsumura, et al., 2006).

3.4.12. Tumor angiogenesis
In tumor-infiltrating macrophages, 5-HT does not enhance colon cancer tumor cell proliferation but may act as a regulator of angiogenesis by reducing the expression of MMP-12, entailing lower levels of angiostatin—an endogenous inhibitor of angiogenesis (Nocito, et al., 2008). Serotonin can stimulate the phosphorylation of ERK1/2 in bovine endothelial cells, and the 5-HT$_{2B}$ receptor was reported to play a role in the activation of eNOS in human endothelial cells. In SB204741-treated mice, the selective blockade of the 5-HT$_{2B}$ receptor resulted in the reduction of tumor angiogenesis and growth through the inhibition effect of ERK1/2 and eNOS (Asada, et al., 2009). Therefore the possibility that 5-HT$_{2B}$ receptors participate in tumor angiogenesis is a likely possibility that remains to be evaluated in other tumors subtypes.

In conclusion, the contribution of 5-HT$_2$ receptors in tumors remains to be determined but is depending on the type of tumors.

3.5. Skeleton
3.5.1. Bone development and osteoporosis
The *Htr$_{2B}$* mRNA, undetected in anaplastic osteoblasts, appears in differentiated and matured osteoblasts (Bliziotes, et al., 2001; Westbroek, et al., 2001). The differentiation and maturation of osteoblasts might thus be regulated by the activation of the 5-HT$_{2B}$ receptor (Hirai, et al., 2009). Of interest, *Htr$_{2B}$$^{-/-}$* female mice displayed reduced bone density that was significant from the age of 4 months and intensifies by 12 and 18 months. This histomorphometrically confirmed that osteopenia was due to reduced bone formation since i) the alkaline phosphatase-positive colony-forming unit capacity of bone marrow precursors was markedly reduced in *Htr$_{2B}$$^{-/-}$* mice from 4 to 12 months of age, ii) *ex-vivo* primary osteoblasts from *Htr$_{2B}$$^{-/-}$* mice exhibited reduced proliferation and delayed differentiation, and iii) calcium incorporation was markedly reduced in osteoblasts after 5-HT$_{2B}$ receptor depletion (produced genetically or by pharmacological inactivation) (Collet, et al., 2008). Using the osteoprogenitor cell line C1, blockade of 5-HT$_{2B}$ receptor intrinsic activity was reported to affect the efficiency of mineralization by decreasing calcium incorporation. Optimal bone matrix mineralization involves both NO and PLA2 signaling pathways and the 5-HT$_{2B}$ receptor promotes prostaglandin E2 production through cyclooxygenase (COX) activation. When C1 osteoblasts undergo conversion into osteocyte-like cells, COX activity was quenched. The 5-HT$_{2B}$ receptor contributed in an autocrine manner to osteogenic differentiation (Locker, et al., 2006). A functional link between the 5-HT$_{2B}$ receptor and the activity of the tissue-non specific alkaline phosphatase (TNAP) was established. Agonist stimulation of the receptor increased TNAP activity during the initial mineralization phase. Indeed, inhibition of 5-HT$_{2B}$ receptor intrinsic activity prevented TNAP activation. In contrast, agonist stimulation of the receptor further increased TNAP activity during the initial

mineralization phase. Previous observations indicated that the 5-HT$_{2B}$ receptor coupled to PLA2 pathway and prostaglandin production at the beginning of mineral deposition. The 5-HT$_{2B}$ receptor also controlled leukotriene synthesis *via* PLA2 at the terminal stages of differentiation. These two 5-HT$_{2B}$ receptor-dependent eicosanoid productions delineate distinct time-windows of TNAP regulation during the osteogenic program. Finally, prostaglandins or leukotrienes were shown to relay the post-translational activation of TNAP *via* stimulation of the phosphatidylinositol-specific phospholipase C. In agreement with the above findings, primary calvarial osteoblasts from *Htr$_{2B}$$^{-/-}$* mice were shown to exhibit defects in TNAP activity (Baudry, et al., 2010). Brain 5-HT was proposed to favor indirectly bone mass accrual following activation of 5-HT$_{2C}$ receptors on ventromedial hypothalamic neurons and 5-HT$_{2B}$ receptors on arcuate neurons (Yadav, et al., 2009). Compared to control osteoblasts, the lack of 5-HT$_{2B}$ receptors was associated with a 10-fold over-production of prostacyclin (PGI2). Also, a specific prostacyclin synthase inhibitor (U51605) rescued totally osteoblast aggregation and matrix mineralization in *Htr$_{2B}$$^{-/-}$* osteoblasts without having any effect on WT osteoblasts. Prostacyclin is the endogenous ligand of PPAR-β/δ, and its inhibition in *Htr$_{2B}$$^{-/-}$* cells rescued totally the alkaline phosphatase and osteopontin mRNA levels, cell-cell adhesion, and matrix mineralization. The absence of 5-HT$_{2B}$ receptors leads to the overproduction of prostacyclin, inducing reduced osteoblast differentiation due to peroxisome proliferator-activated receptor (PPAR)-β/δ-dependent target regulation and defective cell-cell adhesion and matrix mineralization (Chabbi-Achengli, et al., 2013). The *HTR$_{2A}$* was also found expressed only in osteoblasts, whereas *HTR$_{2B}$* expression increased from precursor to mature osteoclasts (Hodge, et al., 2013). A recent study highlights the contribution of the 5-HT$_{2A}$ receptor in bone metabolism. This receptor appears involved in osteoblast differentiation and bone mineral density. The 5-HT$_{2A}$ receptor blockade by MDL11939 decreased bone mass and led to skeletal fragility in mice. Moreover, the pharmacological blockade of 5-HT$_{2A}$ receptors significantly decreased cellular differentiation in MC3T3-E1 cells and osteoblasts primary culture from the right femur in mice(Tanaka, et al., 2015).

All these results drive to the hypothesis according which5-HT and the 5-HT$_{2A/2B}$ receptor signaling pathways may contribute to bone formation and cellular differentiation.

3.5.2. Teeth development

Periodontal diseases occur in patients treated with antidepressants such as SSRIs (e.g., Prozac), which target the SERT. In the molar of *Htr$_{2B}$$^{-/-}$* mice, rod curvatures and twisting were altered compared to WT mice, suggesting involvement of *Htr$_{2B}$* at early stages of enamel formation. The volume of the *Htr$_{2B}$$^{-/-}$* enamel layer was also reduced, with smaller crystallite thickness. The outer aprismatic enamel border was 1.5- to 2-fold larger in *Htr$_{2B}$$^{-/-}$* compared to WT mice. Finally, although no noticeable difference was observed in dentin, the three-dimensional pulp reconstruction evidenced a decrease in both length and width of dentin formation in the root canals of the *Htr$_{2B}$$^{-/-}$* versus WT mice (Dimitrova-Nakov, et al., 2014).

Therefore, 5-HT$_{2B}$ receptors may mediate some harmful effects of long term use of SSRIs on bone and teeth regeneration.

4. Emerging research and new therapeutic opportunities

4.1. Hematopoiesis

Besides its role as a neurotransmitter, 5-HT was shown to regulate inflammation and tissue repair *via* a set of receptors whose pattern of expression varies among cell lineages. Previous studies have shown that 5-HT is a growth factor for hematopoietic stem/progenitor cells. A 5-HT$_{2B}$ receptor expression was identified in megakaryocytic cell lineage. Serotonin promoted the megakaryocytes (MKs) proliferation and reduced the cell apoptosis *via* the activation of 5-HT$_{2B}$ receptor and Akt pathway (Liu & Fanburg, 2006). Serotonin increased proplatelet bearing MKs and polymerized actin level *via* ERK1/2 (Ye, et al., 2014). Mice

deficient in peripheral 5-HT ($Tph1^{-/-}$) displayed morphological and cellular features of ineffective erythropoiesis. The central event occurred in the bone-marrow where the absence of 5-HT hampered progression of erythroid precursors expressing 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors toward terminal differentiation. In addition, red blood cells from 5-HT-deficient mice were more sensitive to macrophage phagocytosis and have a shortened *in-vivo* half-life (Amireault, et al., 2011). In addition, a 5-HT$_{2B}$ receptor expression was detected in c-kit+ bone-marrow cells (Launay, et al., 2012). The 5-HT$_{2B}$ receptor antagonist RS127445 decreased colony-forming capacity, with inhibition of both CFU-GEMM and BFU-E formation attributed to a reduction of cell proliferation and/or an apoptotic effect. By contrast, 5-HT significantly enhanced the expansion of CD34+ cells to early stem/progenitors (CFU-GEMM) and committed progenitors (BFU/CFU-E) (Yang, et al., 2007). Treatment with aggregated (1-40 or 1-42) and oligomeric (1-42) amyloid β (Aβ-found in Alzheimer's disease) promoted differentiation of bone marrow-derived mesenchymal stem cells without toxic effects. The effect of Aβ was shown to be mediated by GPCRs, neuropeptide Y1 and the 5-HT$_{2B}$ receptor, *via* PI3K-dependent activation of the MAPK/ERK1/2 pathway (Kim, et al., 2009).

In human macrophages, 5-HT was reported to inhibit the lipopolysaccharide-induced release of proinflammatory cytokines, to upregulate the expression of M2 polarization-associated genes, and to reduce the expression of M1-associated genes. The 5-HT$_{2B}$ receptor mediates the pro-M2 skewing effect of 5-HT. In fact, blockade of this receptor during *in-vitro* monocyte-to-macrophage differentiation preferentially modulates the acquisition of M2 polarization markers. *Htr$_{2B}$* mRNA was found to be preferentially expressed by anti-inflammatory M2 (M-CSF) macrophages and was detected *in-vivo* in liver Kupffer cells and in tumor-associated macrophages (de Las Casas-Engel, et al., 2013). Recently, expression of the 5-HT$_{2B}$ receptor was reported on postnatal microglia, supporting that 5-HT participates in temporal and spatial synchronization of microglial functions (Kolodziejczak, et al., 2015).

Htr$_{2B}$ mRNA expression was found in spleen, thymus, and peripheral blood lymphocytes (Stefulj, et al., 2000). Study of the expression of serotonergic receptors on human dendritic cells showed that immature dendritic cells expressed *Htr$_{2B}$* mRNA. Moreover, 5-HT$_{2B}$ receptor stimulation induced intracellular Ca^{2+} mobilization in immature, but not mature, dendritic cells. Serotonin stimulated, in a maturation-dependent manner, different signaling pathways in dendritic cells (Idzko, et al., 2004). A proper balance between different T helper (Th) cell subsets is necessary for normal functioning of the adaptive immune system. In human umbilical cord blood, T helper cells cultured in absence and presence of cytokines promoting Th1 or Th2 differentiation were found to specifically express *HTR$_{2B}$* among 50 th2 differentially expressed genes (Aijö, et al., 2012). In gene expression profiles during human CD41 T cell differentiation, *HTR$_{2B}$*, was found to be SP4-specific (~10-fold) among the sixteen transcripts that were expressed in SP4 thymocytes at levels 3-fold or higher than in any other isolated T cell subpopulation (Lee, et al., 2004).

Rheumatoid arthritis is a chronic disease that results in a disabling and painful condition as it progresses to destruction of the articular cartilage and ankylosis of the joints. Although the cause of the disease is still unknown, evidence argues that autoimmunity plays an important part. There are increasing views regarding 5-HT as being associated with activation of immunoinflammatory pathways and the onset of autoimmune reactions. In *Tph1$^{-/-}$* mice with arthritis, a significant increase in osteoclast differentiation and bone resorption was observed with an increase in IL-17 levels in the paws and in Th17 lymphocytes in the draining lymph nodes, whereas T-regulatory cells were dampened. Ex vivo 5-HT and agonists of the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors restored IL-17 secretion from splenocytes and Th17 cell differentiation in *Tph1$^{-/-}$* mice. These findings indicate that serotonin plays a fundamental role in arthritis through the regulation of the Th17/T-regulatory cell balance and osteoclastogenesis (Chabbi-Achengli, et al., 2016).

Therefore, the 5-HT via 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors is mediating the balance among various hematopoietic lineages.

4.2. Amyotrophic lateral sclerosis
Amyotrophic lateral sclerosis (ALS) is the major adult onset motor neuron disease, and

represents the third most frequent neurodegenerative disease after Alzheimer's and Parkinson's diseases. ALS is characterized by the selective degeneration of upper motor neurons in the cerebral cortex and lower motor neurons in spinal cord and brainstem, and leads to progressive paralysis and death within 3–5 years after onset. Spinal cord injury leads to an initial phase of hyporeflexia followed by hyperreflexia, often referred to as spasticity. Spasticity is a common and disabling symptom also observed in patients with ALS. A rat tail-spasticity model with a caudal spinal transection identified that the expression of 5-HT$_{2B}$ receptors was down regulated at 21 days post-injury (Wienecke, et al., 2010). Motoneurons, which recover from denervation, function autonomously exhibiting large persistent calcium currents that help with functional recovery and contribute to uncontrolled muscle spasms. Application of agonists to 5-HT$_{2B}$ receptors (including BW723C86) significantly increased persistent calcium currents. 5-HT$_{2B}$ receptors on motoneurons ultimately contribute to recovery of motoneuron function and emergence of spasms (Murray, et al., 2011). In ALS, spasticity is traditionally thought to be the result of degeneration of the upper motor neurons in the cerebral cortex, although degeneration of other neuronal types, in particular serotonergic neurons, might also represent a cause of spasticity. In SOD1 (G86R) mice, a transgenic model of ALS, 5-HT levels were decreased in brainstem and spinal cord before onset of motor symptoms. Furthermore, there was a noticeable atrophy of 5-HT neuronal cell bodies along with neuritic degeneration at disease onset. In SOD1 (G86R) mice, tail muscle spastic-like contractions were observed at end-stage. Importantly, they were abolished by 5-HT$_{2B/2C}$ receptors inverse agonist SB206553. In line with this result, 5-HT$_{2B}$ receptor expression was strongly increased at disease onset (Dentel, et al., 2013).

In summary, 5-HT$_{2B}$ receptors on motoneurons may ultimately contribute to recovery of motoneuron function and emergence of spasms.

Microglia are the resident mononuclear phagocytes of the central nervous system and have been implicated in the pathogenesis of neurodegenerative diseases such as ALS. During neurodegeneration, microglial activation is accompanied by infiltration of circulating monocytes, leading to production of multiple inflammatory mediators in the spinal cord. Degenerative alterations in mononuclear phagocytes are commonly observed during neurodegenerative diseases. In mutant SOD1 mice, a 5-HT$_{2B}$ receptor upregulation was observed that was restricted to cells positive for CD11b, a marker of mononuclear phagocytes including microglia. Ablation of 5-HT$_{2B}$ receptor in transgenic ALS mice expressing mutant SOD1 resulted in increased degeneration of mononuclear phagocytes, as evidenced by fragmentation of Iba1-positive cellular processes. This was accompanied by decreased expression of key neuroinflammatory genes but also loss of expression of homeostatic microglial genes. Importantly, the dramatic effect of 5-HT$_{2B}$ receptor ablation on mononuclear phagocytes was associated with acceleration of disease progression (El Oussini, et al., 2016). A study on polymorphisms in the human *HTR$_{2B}$* gene, which encodes the 5-HT$_{2B}$ receptor, in a large cohort of ALS patients, showed that the C allele of SNP rs10199752 in *HTR$_{2B}$*gene was associated with longer survival. Moreover, patients carrying one copy of the C allele of SNP rs10199752 showed increased *HTR$_{2B}$* mRNA expression in spinal cord and displayed less pronounced degeneration of Iba1 positive cells than patients carrying two copies of the more common A allele (El Oussini, et al., 2016).

Thus, the 5-HT$_{2B}$ receptor limits degeneration of spinal cord mononuclear phagocytes, most likely microglia, and slows disease progression in ALS.

4.3. Obesity/Metabolic syndrome
The serotonergic system affects feeding behavior not only at the central nervous system level but also at the peripheral level by regulating glucose and lipid metabolism (McGlashon, et al., 2015). A strong lactogen-dependent upregulation of 5-HT biosynthesis has been shown to occur in a subpopulation of mouse islet β-cells during pregnancy (Schraenen, et al., 2010). This subpopulation of pancreatic islet cells expresses the genes encoding all of the products necessary for synthesizing, packaging, and secreting 5-HT, including tryptophan hydroxylases. Transcriptome analysis of islets isolated from pregnant mice identified very

strong upregulation of the two paralogous genes encoding tryptophan hydroxylase, Tph1 and Tph2 (Goyvaerts, et al., 2016) that is dependent on activation of prolactin receptors. In β-cells, Pet1 can bind to the serotonergic genes but also to a conserved insulin gene regulatory element. In $Tph1^{-/-}$ mice, the absence of 5-HT leads to an impaired insulin secretion and, as a consequence, a reduced glucose tolerance (Ohta, et al., 2011). The mechanism could be an impaired serotonylation of the small GTPases Rab3a and Rab27a, which are two key players for insulin exocytosis (Paulmann, et al., 2009). Nevertheless, it remains debatable whether or not blocking 5-HT receptor signaling in pregnant mice also blocks β-cell expansion and caused glucose intolerance (Goyvaerts, et al., 2016; Kim, et al., 2010). The differential expression of 5-HT receptor genes in human islets coming from non-diabetic and type 2 diabetic patients highlighted the overexpression of HTR_{1D} and HTR_{2A} mRNA in diabetic subjects. In fact 5-HT effects are complex in the pancreas and could vary depending on the pathophysiological status. 5-HT was found to inhibit insulin and glucagon secretion in non-diabetic islets whereas 5-HT increased insulin release in response to glucose from type 2 diabetic islets (Bennet, et al., 2015).

Taken together, these results suggest that 5-HT regulates insulin secretion in non-diabetic islets but, at the opposite, the stimulation of overexpressed 5-HT_{1D} and 5-HT_{2A} receptors contribute to islet dysfunction in type 2 diabetic patients.

On the other hand, gut-derived 5-HT favors lipolysis, liver neoglucogenesis and inhibits glucose uptake in liver and adipose tissue by a signaling involving the 5-HT_{2B} receptor. Inhibition of peripheral 5-HT synthesis and blockade of 5-HT_{2B} receptors improves hyperglycemia in a mouse model of type 2 diabetes (Sumara, et al., 2012). In a high throughput RNA screen with human primary (pre)adipocytes 110 genes were found regulated on the gene expression level during adipogenesis. Among them, HTR_{2B} mRNA was one of our top hits identified. Adipocytes cultured in the presence of the selective 5-HT_{2B} receptor antagonist RS127445 exhibited a significant increase in neutral lipid levels compared to control cells (Söhle, et al., 2012). A recent study highlighted the role of peripheral 5-HT in the brown adipose tissue thermogenesis. Tph1 deficient mice are protected from obesity and insulin resistance by an elevation of brown adipose tissue activity. The mechanism implies an inhibition of the β–adrenergic signaling by 5-HT(Crane, et al., 2015). These studies suggest that peripheral 5-HT regulates glucose and lipid metabolism, fat accumulation and obesity. The last major organ involved in glucose metabolism is the muscle. Recently, in mice fed a high-fat diet, Watanabe et al.(2016) observed an reduction of weight gain, hyperglycemia and insulin resistance after intraperitoneal injections of high doses of 5-HT (0.1 mg, 0.5 mg or 1 mg twice a week). They simultaneously observed a shift in metabolic profile in the muscle fiber of the soleus muscle. 5-HT injections induced an increase mRNA expression of the PPAR coactivator 1α in soleus muscle, that was inhibited by 5-HT_{2A} or 5-HT_7 serotonin receptors antagonists.

These data suggest important roles for 5-HT_{2A} and 5-HT_{2B} receptors in controlling energy homeostasis.

5. Conclusions and prospects

After many years of investigations in various fields such as fibrosis, central nervous system and cardiovascular system regulations, new paradigms of 5-HT_2 receptors pathophysiological contributions were discovered and understood. In parallel, pharmacochemistry identified new selective ligands and highlighted 5-HT_2 receptor intracellular signaling pathways. At that step translational research will be required to point clinical applications. Nevertheless, cardiovascular side effects lit the interest for agonists. In this field the search for allosteric modulators and biased ligands will be of great importance. On the other side, the use of highly selective 5-HT_2 receptors antagonists seems to be safe and could prevent and/or treat many diseases (Cf Figure 1).

Acknowledgments

References

Abdallah, L., Bonasera, S. J., Hopf, F. W., O'Dell, L., Giorgetti, M., Jongsma, M., Carra, S., Pierucci, M., Di Giovanni, G., Esposito, E., Parsons, L. H., Bonci, A., & Tecott, L. H. (2009). Impact of serotonin $_{2C}$ receptor null mutation on physiology and behavior associated with nigrostriatal dopamine pathway function. *J Neurosci, 29*, 8156-8165.

Aijö, T., Edelman, S. M., Lönnberg, T., Larjo, A., Kallionpää, H., Tuomela, S., Engström, E., Lahesmaa, R., & Lähdesmäki, H. (2012). An integrative computational systems biology approach identifies differentially regulated dynamic transcriptome signatures which drive the initiation of human T helper cell differentiation. *BMC Genomics, 13*, 572.

Albizu, L., Holloway, T., Gonzalez-Maeso, J., & Sealfon, S. C. (2011). Functional crosstalk and heteromerization of serotonin 5-HT2A and dopamine D2 receptors. *Neuropharmacology, 61*, 770-777.

Amireault, P., Hatia, S., Bayard, E., Bernex, F., Collet, C., Callebert, J., Launay, J.-M., Hermine, O., Schneider, E., Mallet, J., Dy, M., & Côté, F. (2011). Ineffective erythropoiesis with reduced red blood cell survival in serotonin-deficient mice. *Proc Natl Acad Sci USA, 108*, 13141-13146.

Anastasio, N. C., Stutz, S. J., Fink, L. H. L., Swinford-Jackson, S. E., Sears, R. M., DiLeone, R. J., Rice, K. C., Moeller, F. G., & Cunningham, K. A. (2015). Serotonin (5-HT) 5-HT$_{2A}$ Receptor (5-HT$_{2A}$R):5-HT$_{2C}$R Imbalance in Medial Prefrontal Cortex Associates with Motor Impulsivity. *ACS chemical neuroscience, 6*, 1248-1258.

Antonini, A., & Poewe, W. (2007). Fibrotic heart-valve reactions to dopamine-agonist treatment in Parkinson's disease. *Lancet Neurol, 6*, 826-829.

Arslan, A. A., Gold, L. I., Mittal, K., Suen, T. C., Belitskaya-Levy, I., Tang, M. S., & Toniolo, P. (2005). Gene expression studies provide clues to the pathogenesis of uterine leiomyoma: new evidence and a systematic review. *Hum Reprod, 20*, 852-863.

Asada, M., Ebihara, S., Yamanda, S., Niu, K., Okazaki, T., Sora, I., & Arai, H. (2009). Depletion of serotonin and selective inhibition of $_{2B}$ receptor suppressed tumor angiogenesis by inhibiting endothelial nitric oxide synthase and extracellular signal-regulated kinase 1/2 phosphorylation. *Neoplasia, 11*, 408-417.

Assetta, B., Maginnis, M. S., Gracia Ahufinger, I., Haley, S. A., Gee, G. V., Nelson, C. D. S., O'Hara, B. A., Allen Ramdial, S.-a. A., & Atwood, W. J. (2013). 5-HT2 receptors facilitate JC polyomavirus entry. *J Virol, 87*, 13490-13498.

Auclair, A. L., Cathala, A., Sarrazin, F., Depoortère, R., Piazza, P. V., Newman-Tancredi, A., & Spampinato, U. (2010). The central serotonin $_{2B}$ receptor: a new pharmacological target to modulate the mesoaccumbens dopaminergic pathway activity. *J Neurochemistry, 114*, 1323-1332.

Audia, J. E., Evrard, D. A., Murdoch, G. R., Droste, J. J., Nissen, J. S., Schenck, K. W., Fludzinski, P., Lucaites, V. L., Nelson, D. L., & Cohen, M. L. (1996). Potent, selective tetrahydro-beta-carboline antagonists of the serotonin $_{2B}$ (5-HT$_{2B}$) contractile receptor in the rat stomach fundus. *J. Med. Chem., 39*, 2773-2780.

Ayme-Dietrich, E., Lawson, R., Gasser, B., Dallemand, R., Bischoff, N., & Monassier, L. (2012). Mitral bioprosthesis hypertrophic scaring and native aortic valve fibrosis during benfluorex therapy. *Fundam Clin Pharmacol, 26*, 215-218.

Ayme-Dietrich, E., Marzak, H., Lawson, R., Mokni, W., Wendling, O., Combe, R., Becker, J., El Fertak, L., Champy, M., Matz, R. L., Andriantsitohaina, R., Doly, S., Boutourlinsky, K., Maroteaux, L., & Monassier, L. (2015). Contribution of serotonin to cardiac remodeling associated with hypertensive diastolic ventricular dysfunction in rats. *J Hypertension 33*, 2310-2321.

Bai, C.-F., Liu, J.-C., Zhao, R., Cao, W., Liu, S.-B., Zhang, X.-N., Guo, H.-J., Yang, Q., Yi, D.-H., & Zhao, M.-G. (2010). Role of 5-HT$_{2B}$ receptor in cardiomyocyte apoptosis of norepinephrine-induced cardiomyopathy model in rats. *Clin Exp Pharmacol Physiol, 37*, e145-e151.

Banas, S., Doly, S., Boutourlinsky, K., Diaz, S., Belmer, A., Callebert, J., Collet, C., Launay, J.-M., & Maroteaux, L. (2011). Deconstructing antiobesity compound action: requirement of serotonin 5-HT$_{2B}$ receptors for dexfenfluramine anorectic effects. *Neuropsychopharmacology, 36*, 423–433.

Barre, A., Berthoux, C., De Bundel, D., Valjent, E., Bockaert, J., Marin, P., & Becamel, C. (2016). Presynaptic serotonin 2A receptors modulate thalamocortical plasticity and associative learning. *Proc Natl Acad Sci U S A, 113*, E1382-1391.

Baudry, A., Bitard, J., Mouillet-Richard, S., Locker, M., Poliard, A., Launay, J.-M., & Kellermann, O. (2010). The serotonergic 5-HT$_{2B}$ receptor controls tissue non-specific alkaline phosphatase activity in osteoblasts via eicosanoids and phosphatidylinositol-specific phospholipase C. *J Biol Chem, 285*, 26066-26073.

Becamel, C., Figge, A., Poliak, S., Dumuis, A., Peles, E., Bockaert, J., Lubbert, H., & Ullmer, C. (2001). Interaction of Serotonin 5-HT$_{2C}$ receptors with PDZ10 of the multi PDZ protein MUPP1. *J Biol Chem, 276*, 12974-12982.

Belmer, A., Doly, S., Setola, V., Banas, S. M., Moutkine, I., Boutourlinsky, K., Kenakin, T., & Maroteaux, L. (2014). Role of the N-terminal Region in G-Protein Coupled Receptor Functions: Negative Modulation Revealed by 5-HT$_{2B}$ Receptor Polymorphisms. *Mol Pharmacol, 85*, 127-138.

Bennet, H., Balhuizen, A., Medina, A., Dekker Nitert, M., Ottosson Laakso, E., Essen, S., Spegel, P., Storm, P., Krus, U., Wierup, N., & Fex, M. (2015). Altered serotonin (5-HT) 1D and $_{2A}$ receptor expression may contribute to defective insulin and glucagon secretion in human type 2 diabetes. *Peptides, 71*, 113-120.

Berg, K. A., Maayani, S., Goldfarb, J., Scaramellini, C., Leff, P., & Clarke, W. P. (1998). Effector pathway-dependent relative efficacy at serotonin type $_{2A}$ and $_{2C}$ receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol Pharmacol, 54*, 94-104.

Berglund, E. D., Liu, C., Sohn, J.-W., Liu, T., Kim, M. H., Lee, C. E., Vianna, C. R., Williams, K. W., Xu, Y., & Elmquist, J. K. (2013). Serotonin $_{2C}$ receptors in pro-opiomelanocortin neurons regulate energy and glucose homeostasis. *The Journal of clinical investigation, 123*, 5061-5070.

Bevilacqua, L., Doly, S., Kaprio, J., Yuan, Q., Tikkanen, R., Paunio, T., Zhou, Z., Wedenoja, J., Maroteaux, L., Diaz, S., Belmer, A., Hodgkinson, C., Dell'Osso, L., Suvisaari, J., Coccaro, E., Rose, R., Peltonen, L., Virkkunen, M., & Goldman, D. (2010). A population-specific HTR$_{2B}$ stop codon predisposes to severe impulsivity. *Nature, 468*, 1061-1066.

Bhatnagar, A., Willins, D. L., Gray, J. A., Woods, J., Benovic, J. L., & Roth, B. L. (2001). The dynamin-dependent, arrestin-independent internalization of 5-hydroxytryptamine $_{2A}$ (5-HT$_{2A}$) serotonin receptors reveals differential sorting of arrestins and 5-HT$_{2A}$ receptors during endocytosis. *J Biol Chem, 276*, 8269-8277.

Bittel, D. C., Kibiryeva, N., Sell, S. M., Strong, T. V., & Butler, M. G. (2007). Whole genome microarray analysis of gene expression in Prader-Willi syndrome. *Am J Med Genet A, 143*, 430-442.

Blasco-Fontecilla, H., Baca-Garcia, E., & de Leon, J. (2010). Do atypical antipsychotic drugs reduce the risk of ischemic heart disease and mortality? Possible role of 5-HT$_{2A}$ receptor blockade. *Schizophr Res, 119*, 160-163.

Bliziotes, M. M., Eshleman, A. J., Zhang, X. W., & Wiren, K. M. (2001). Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. *Bone, 29*, 477-486.

Bonhaus, D. W., Bach, C., DeSouza, A., Salazar, F. H. R., Matsuoka, B. D., Zuppan, P., Chan, H. W., & Eglen, R. M. (1995). The pharmacology and distribution of human 5-hydroxytryptamine $_{2B}$ (5-HT$_{2B}$) receptor gene products: comparison with 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. *Br. J. Pharmacol., 115*, 622-628.

Bonhaus, D. W., Flippin, L. A., Greenhouse, R. J., Jaime, S., Rocha, C., Dawson, M., Van Natta, K., Chang, L. K., Pulido-Rios, T., Webber, A., Leung, E., Eglen, R. M., & Martin, G. R. (1999). RS-127445: a selective, high affinity, orally bioavailable 5-HT$_{2B}$ receptor antagonist. *Br J Pharmacol, 127*, 1075-1082.

Bonhaus, D. W., Weinhardt, K. K., Taylor, M., DeSouza, A., McNeeley, P. M., Szczepanski, K., Fontana, D. J., Trinh, J., Rocha, C. L., Dawson, M. W., Flippin, L. A., & Eglen, R. M. (1997). RS-102221: a novel high affinity and selective, 5-HT$_{2C}$ receptor antagonist. *Neuropharmacology, 36*, 621-629.

Booth, R. G., Fang, L., Huang, Y., Wilczynski, A., & Sivendran, S. (2009). (1R, 3S)-(-)-trans-PAT: a novel full-efficacy serotonin 5-HT$_{2C}$ receptor agonist with 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor inverse agonist/antagonist activity. *Eur J Pharmacol, 615*, 1-9.

Bourson, A., Wanner, D., Wyler, R., Petit, N., Zwingelstein, C., Rudler, A., & Sleight, A. J. (1996). Pharmacologic evaluation of the discriminative stimulus of metachlorophenylpiperazine. *Pharmacol Biochem Behav, 53*, 107-114.

Bracha, S., Viall, A., Goodall, C., Stang, B., Ruaux, C., Seguin, B., & Chappell, P. E. (2013). The expression and role of serotonin receptor 5HTR$_{2A}$ in canine osteoblasts and an osteosarcoma cell line. *BMC Vet Res, 9*, 251.

Branchek, T., Adham, N., Macchi, M., Kao, H. T., & Hartig, P. R. (1990). [3H]-DOB(4-bromo-2,5-dimethoxyphenylisopropylamine) and [3H] ketanserin label two affinity states of the cloned human 5-hydroxytryptamine2 receptor. *Mol Pharmacol, 38*, 604-609.

Brattelid, T., Tveit, K., Birkeland, J. A., Sjaastad, I., Qvigstad, E., Krobert, K. A., Hussain, R. I., Skomedal, T., Osnes, J. B., & Levy, F. O. (2007). Expression of mRNA encoding G protein-coupled receptors involved in congestive heart failure--a quantitative RT-PCR study and the question of normalisation. *Basic Res Cardiol, 102*, 198-208.

Brea, J., Castro, M., Giraldo, J., López-Giménez, J. F., Padín, J. F., Quintián, F., Cadavid, M. I., Vilaró, M. T., Mengod, G., Berg, K. A., Clarke, W. P., Vilardaga, J.-P., Milligan, G., & Loza, M. I. (2009). Evidence for distinct antagonist-revealed functional states of 5-hydroxytryptamine$_{(2A)}$ receptor homodimers. *Mol Pharmacol, 75*, 1380-1391.

Canal, C. E., & Morgan, D. (2012). Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. *Drug testing and analysis, 4*, 556-576.

Canal, C. E., Morgan, D., Felsing, D., Kondabolu, K., Rowland, N. E., Robertson, K. L., Sakhuja, R., & Booth, R. G. (2014). A novel aminotetralin-type serotonin (5-HT) $_{2C}$ receptor-specific agonist and 5-HT$_{2A}$ competitive antagonist/5-HT$_{2B}$ inverse agonist with preclinical efficacy for psychoses. *The Journal of pharmacology and experimental therapeutics, 349*, 310-318.

Canal, C. E., Olaghere da Silva, U. B., Gresch, P. J., Watt, E. E., Sanders-Bush, E., & Airey, D. C. (2010). The serotonin $_{2C}$ receptor potently modulates the head-twitch response in mice induced by a phenethylamine hallucinogen. *Psychopharmacology, 209*, 163-174.

Chabbi-Achengli, Y., Coman, T., Collet, C., Callebert, J., Corcelli, M., Lin, H., Rignault, R., Dy, M., de Vernejoul, M.-C., & Côté, F. (2016). Serotonin Is Involved in Autoimmune Arthritis through Th17 Immunity and Bone Resorption. *The American journal of pathology, 186*, 927-937.

Chabbi-Achengli, Y., Launay, J.-M., Maroteaux, L., De Vernejoul, M. C., & Collet, C. (2013). Serotonin $_{2B}$ Receptor (5-HT$_{2B}$ R) Signals through Prostacyclin and PPAR-ß/δ in Osteoblasts. *PLoS ONE, 8*, e75783.

Chan, S. Y., & Loscalzo, J. (2008). Pathogenic mechanisms of pulmonary arterial hypertension. *J Mol Cell Cardiol, 44*, 14-30.

Chanrion, B., Mannoury la Cour, C., Gavarini, S., Seimandi, M., Vincent, L., Pujol, J. F., Bockaert, J., Marin, P., & Millan, M. J. (2008). Inverse agonist and neutral antagonist actions of antidepressants at recombinant and native 5-hydroxytryptamine$_{2C}$ receptors: differential modulation of cell surface expression and signal transduction. *Mol Pharmacol, 73*, 748-757.

Chen, Y. X., Wang, W. D., Song, X. J., Gu, Y. Q., Tian, H. Y., Hu, H. J., Zhao, J. C., Li, X. Q., & Liu, C. W. (2015). Prospective Randomized Study of Sarpogrelate Versus Clopidogrel-based Dual Antiplatelet Therapies in Patients Undergoing Femoropopliteal Arterial Endovascular Interventions: Preliminary Results. *Chin Med J (Engl), 128*, 1563-1566.

Chenu, F., Shim, S., El Mansari, M., & Blier, P. (2014). Role of melatonin, serotonin $_{2B}$, and serotonin $_{2C}$ receptors in modulating the firing activity of rat dopamine neurons. *J Psychopharmacol (Oxford), 28*, 162-167.

Choi, D.-S., Birraux, G., Launay, J.-M., & Maroteaux, L. (1994). The human serotonin 5-HT$_{2B}$ receptor: pharmacological link between 5-HT2 and 5-HT1D receptors. *FEBS L, 352*, 393-399.

Christopoulos, A., & Kenakin, T. (2002). G protein-coupled receptor allosterism and complexing. *Pharmacol Rev, 54*, 323-374.

Clifton, P. G., Lee, M. D., & Dourish, C. T. (2000). Similarities in the action of Ro 60-0175, a 5-HT$_{2C}$ receptor agonist and d-fenfluramine on feeding patterns in the rat. *Psychopharmacology (Berl), 152*, 256-267.

Cohen, M. L., Schenck, K. W., Mabry, T. E., Nelson, D. L., & Audia., J. E. (1996). LY272015, a potent, selective and orally active 5-HT$_{2B}$ receptor antagonist. *J Ser Res, 3*, 131-144.

Collet, C., Schiltz, C., Geoffroy, V., Maroteaux, L., Launay, J.-M., & de Vernejoul, M.-C. (2008). The serotonin 5-HT$_{2B}$ receptor controls bone mass via osteoblast recruitment and proliferation. . *FASEB J. , 22*, 418-427.

Conductier, G., Crosson, C., Hen, R., Bockaert, J., & Compan, V. (2005). 3,4-N-methlenedioxymethamphetamine-induced hypophagia is maintained in 5-HT1B receptor knockout

mice, but suppressed by the 5-HT$_{2C}$ receptor antagonist RS102221. *Neuropsychopharmacology, 30*, 1056-1063.

Connolly, H. M., Crary, J. L., McGoon, M. D., Hensrud, D. D., Edwards, B. S., Edwards, W. D., & Schaff, H. V. (1997). Valvular heart disease associated with fenfluramine-phentermine. *N Engl J Med, 337*, 581-588.

Corominas, R., Sobrido, M. J., Ribasés, M., Cuenca-León, E., Blanco-Arias, P., Narberhaus, B., Roig, M., Leira, R., López-González, J., Macaya, A., & Cormand, B. (2010). Association study of the serotoninergic system in migraine in the Spanish population. *Am J Med Genet B Neuropsychiatr Genet, 153B*, 177-184.

Crane, J. D., Palanivel, R., Mottillo, E. P., Bujak, A. L., Wang, H., Ford, R. J., Collins, A., Blumer, R. M., Fullerton, M. D., Yabut, J. M., Kim, J. J., Ghia, J. E., Hamza, S. M., Morrison, K. M., Schertzer, J. D., Dyck, J. R., Khan, W. I., & Steinberg, G. R. (2015). Inhibiting peripheral serotonin synthesis reduces obesity and metabolic dysfunction by promoting brown adipose tissue thermogenesis. *Nat Med, 21*, 166-172.

Cremer, S. E., Moesgaard, S. G., Rasmussen, C. E., Zois, N. E., Falk, T., Reimann, M. J., Cirera, S., Aupperle, H., Oyama, M. A., & Olsen, L. H. (2015). Alpha-smooth muscle actin and serotonin receptors $_{2A}$ and $_{2B}$ in dogs with myxomatous mitral valve disease. *Research in Veterinary Science, 100*, 197-206.

Cremer, S. E., Zois, N. E., Moesgaard, S. G., Ravn, N., Cirera, S., Honge, J. L., Smerup, M. H., Hasenkam, J. M., Sloth, E., Leifsson, P. S., Falk, T., Oyama, M. A., Orton, C., Martinussen, T., & Olsen, L. H. (2015). Serotonin markers show altered transcription levels in an experimental pig model of mitral regurgitation. *Vet J, 203*, 192-198.

Cunningham, K. A., Anastasio, N. C., Fox, R. G., Stutz, S. J., Bubar, M. J., Swinford, S. E., Watson, C. S., Gilbertson, S. R., Rice, K. C., Rosenzweig-Lipson, S., & Moeller, F. G. (2013). Synergism between a serotonin 5-HT$_{2A}$ receptor (5-HT$_{2A}$R) antagonist and 5-HT$_{2C}$R agonist suggests new pharmacotherapeutics for cocaine addiction. *ACS Chem Neurosci, 4,* 110-121.

Cussac, D., Boutet-Robinet, E., Ailhaud, M. C., Newman-Tancredi, A., Martel, J. C., Danty, N., & Rauly-Lestienne, I. (2008). Agonist-directed trafficking of signalling at serotonin 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells. *Eur J Pharmacol, 594*, 32-38.

Cussac, D., Newman-Tancredi, A., Quentric, Y., Carpentier, N., Poissonnet, G., Parmentier, J. G., Goldstein, S., & Millan, M. J. (2002). Characterization of phospholipase C activity at h5-HT$_{2C}$ compared with h5-HT$_{2B}$ receptors: influence of novel ligands upon membrane-bound levels of [3H]phosphatidylinositols. *Naunyn Schmiedebergs Arch Pharmacol, 365*, 242-252.

de Las Casas-Engel, M., Domínguez-Soto, A., Sierra-Filardi, E., Bragado, R., Nieto, C., Puig-Kroger, A., Samaniego, R., Loza, M., Corcuera, M. T., Gómez-Aguado, F., Bustos, M., Sánchez-Mateos, P., & Corbí, A. L. (2013). Serotonin skews human macrophage polarization through HTR$_{2B}$ and HTR7. *J Immunol, 190*, 2301-2310.

De Vry, J., & Schreiber, R. (2000). Effects of selected serotonin 5-HT(1) and 5-HT(2) receptor agonists on feeding behavior: possible mechanisms of action. *Neurosci Biobehav Rev, 24*, 341-353.

Dees, C., Akhmetshina, A., Reich, N., Jüngel, A., Beyer, C., Krönke, G., Zwerina, J., Reiter, R., Alenina, N., Maroteaux, L., Gay, S., Schett, G., Distler, O., & Distler, J. H. W. (2011). Platelet derived serotonin links vascular disease and tissue fibrosis. *J Exp Med, 208*, 961-972.

Dentel, C., Palamiuc, L., Henriques, A., Lannes, B., Spreux-Varoquaux, O., Gutknecht, L., René, F., Echaniz-Laguna, A., Gonzalez de Aguilar, J.-L., Lesch, K. P., Meininger, V., Loeffler, J.-P., & Dupuis, L. (2013). Degeneration of serotonergic neurons in amyotrophic lateral sclerosis: a link to spasticity. *Brain, 136*, 483-493.

Depoortere, I., Thijs, T., & Peeters, T. (2006). The contractile effect of the ghrelin receptor antagonist, D-Lys3-GHRP-6, in rat fundic strips is mediated through 5-HT receptors. *Eur J Pharmacol, 537*, 160-165.

Devroye, C., Cathala, A., Di Marco, B., Caraci, F., Drago, F., Piazza, P. V., & Spampinato, U. (2015). Central serotonin$_{2B}$ receptor blockade inhibits cocaine-induced hyperlocomotion independently of changes of subcortical dopamine outflow. *Neuropharmacology, 97*, 329-337.

Diaz, S. L., Doly, S., Narboux-Nême, N., Fernandez, S., Mazot, P., Banas, S., Boutourlinsky, K., Moutkine, I., Belmer, A., Roumier, A., & Maroteaux, L. (2012). 5-HT$_{2B}$ receptors are required for serotonin-selective antidepressant actions. *Mol Psychiatry, 17*, 154-163.

Dimitrova-Nakov, S., Baudry, A., Harichane, Y., Collet, C., Marchadier, A., Kellermann, O., & Goldberg, M. (2014). Deletion of serotonin $_{2B}$ receptor provokes structural alterations of mouse dental tissues. *Calcif Tissue Int, 94*, 293-300.

Ding, C., Bremer, N. M., Smith, T. D., Seitz, P. K., Anastasio, N. C., Cunningham, K. A., & Zhou, J. (2012). Exploration of synthetic approaches and pharmacological evaluation of PNU-69176E and its stereoisomer as 5-HT$_{2C}$ receptor allosteric modulators. *ACS chemical neuroscience, 3*, 538-545.

Dizeyi, N., Bjartell, A., Hedlund, P., Tasken, K. A., Gadaleanu, V., & Abrahamsson, P. A. (2005). Expression of serotonin receptors $_{2B}$ and 4 in human prostate cancer tissue and effects of their antagonists on prostate cancer cell lines. *Eur Urol, 47*, 895-900.

Doly, S., Bertran-Gonzalez, J., Callebert, J., Bruneau, A., Banas, S. M., Belmer, A., Boutourlinsky, K., Herve, D., Launay, J. M., & Maroteaux, L. (2009). Role of serotonin via 5-HT$_{2B}$ receptors in the reinforcing effects of MDMA in mice*PLoS ONE, 4*, e7952.

Doly, S., Valjent, E., Setola, V., Callebert, J., Herve, D., Launay, J. M., & Maroteaux, L. (2008). Serotonin 5-HT$_{2B}$ receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. *J Neurosci, 28*, 2933-2940.

Dumitrascu, R., Kulcke, C., Königshoff, M., Kouri, F., Yang, X., Morrell, N., Ghofrani, H. A., Weissmann, N., Reiter, R., Seeger, W., Grimminger, F., Eickelberg, O., Schermuly, R. T., & Pullamsetti, S. S. (2011). Terguride ameliorates monocrotaline-induced pulmonary hypertension in rats. *Eur Respir J, 37*, 1104-1118.

Ebrahimkhani, M., Oakley, F., Murphy, L., Mann, J., Moles, A., Perugorria, M., Ellis, E., Burt, A., Douglass, A., Wright, M., White, S., Jaffré, F., Maroteaux, L., & Mann, D. (2011). Stimulating healthy tissue regeneration by targeting the 5-HT$_{2B}$ receptor in chronic liver disease. *Nature Medicine, 17*, 1668-1673.

El Oussini, H., Bayer, H., Scekic-Zahirovic, J., Vercruysse, P., Sinniger, J., Dirrig-Grosch, S., Dieterlé, S., Echaniz-Laguna, A., Larmet, Y., Müller, K., Weishaupt, J. H., Thal, D. R., van Rheenen, W., van Eijk, K., Lawson, R., Monassier, L., Maroteaux, L., Roumier, A., Wong, P. C., van den Berg, L. H., Ludolph, A. C., Veldink, J. H., Witting, A., & Dupuis, L. (2016). Serotonin $_{2B}$ receptor slows disease progression and prevents degeneration of spinal cord mononuclear phagocytes in amyotrophic lateral sclerosis. *Acta Neuropathologica*.

Elphick, G. F., Querbes, W., Jordan, J. A., Gee, G. V., Eash, S., Manley, K., Dugan, A., Stanifer, M., Bhatnagar, A., Kroeze, W. K., Roth, B. L., & Atwood, W. J. (2004). The human polyomavirus, JCV, uses serotonin receptors to infect cells. *Science (New York, NY), 306*, 1380-1383.

Fabre, A., Marchal-Sommé, J., Marchand-Adam, S., Quesnel, C., Borie, R., Dehoux, M., Ruffié, C., Callebert, J., Launay, J.-M., Hénin, D., Soler, P., & Crestani, B. (2008). Modulation of bleomycin-induced lung fibrosis by serotonin receptor antagonists in mice. *Eur Respir J, 32*, 426-436.

Fantegrossi, W. E., Simoneau, J., Cohen, M. S., Zimmerman, S. M., Henson, C. M., Rice, K. C., & Woods, J. H. (2010). Interaction of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in R(-)-2,5-dimethoxy-4-iodoamphetamine-elicited head twitch behavior in mice. *J Pharm Exp Ther, 335*, 728-734.

Fernandez-Ranvier, G. G., Weng, J., Yeh, R. F., Khanafshar, E., Suh, I., Barker, C., Duh, Q. Y., Clark, O. H., & Kebebew, E. (2008). Identification of biomarkers of adrenocortical carcinoma using genomewide gene expression profiling. *Arch Surg, 143*, 841-846.

Fitzgerald, L. W., Burn, T. C., Brown, B. S., Patterson, J. P., Corjay, M. H., Valentine, P. A., Sun, J.-H., Link, J. R., Abbaszade, I., Hollis, J. M., Largent, B. L., Hartig, P. R., Hollis, G. F., Meunier, P. C., Robichaud, A. J., & Robertson, D. W. (2000). Possible role of valvular serotonin 5-HT$_{2B}$ receptors in the cardiopathy associated with fenfluramine. *Mol Pharmacol, 57*, 75-81.

Foguet, M., Hoyer, D., Pardo, L. A., Parekh, A., Kluxen, F. W., Kalkman, H. O., Stühmer, W., & Lübbert, H. (1992). Cloning and functional characterization of the rat stomach fundus serotonin receptor. *EMBO J., 11*, 3481-3487.

Foguet, M., Nguyen, H., Le, H., & Lübbert, H. (1992). Structure of the mouse 5-HT1C, 5-HT2 and stomach fundus serotonin receptor genes. *Neuroreport, 3*, 345-348.

Fribourg, M., Moreno, J. L., Holloway, T., Provasi, D., Baki, L., Mahajan, R., Park, G., Adney, S. K., Hatcher, C., Eltit, J. M., Ruta, J. D., Albizu, L., Li, Z., Umali, A., Shim, J., Fabiato, A., MacKerell, A. D., Brezina, V., Sealfon, S. C., Filizola, M., González-Maeso, J., & Logothetis, D. E. (2011). Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. *Cell, 147*, 1011-1023.

Fujita, M., Minamino, T., Sanada, S., Asanuma, H., Hirata, A., Ogita, H., Okada, K., Tsukamoto, O., Takashima, S., Tomoike, H., Node, K., Hori, M., & Kitakaze, M. (2004). Selective blockade of serotonin 5-HT$_{2A}$ receptor increases coronary blood flow via augmented cardiac nitric oxide release through 5-HT1B receptor in hypoperfused canine hearts. *J Mol Cell Cardiol, 37*, 1219-1223.

Garattini, S., Mennini, T., Bendotti, C., Invernizzi, R., & Samanin, R. (1986). Neurochemical mechanism of action of drugs which modify feeding via the serotoninergic system. *Appetite, 7 Suppl*, 15-38.

Garcia-Pedraza, J. A., Garcia, M., Martin, M. L., San Roman, L., & Moran, A. (2014). The role of endothelium-derived hyperpolarizing factor and cyclooxygenase pathways in the inhibitory serotonergic response to the pressor effect elicited by sympathetic stimulation in chronic sarpogrelate treated rats. *Eur J Pharmacol, 731*, 80-87.

Garfield, A. S., & Heisler, L. K. (2009). Pharmacological targeting of the serotonergic system for the treatment of obesity. *J Physiol, 587*, 49-60.

Gavarini, S., Becamel, C., Altier, C., Lory, P., Poncet, J., Wijnholds, J., Bockaert, J., & Marin, P. (2006). Opposite effects of PSD-95 and MPP3 PDZ proteins on serotonin 5-hydroxytryptamine$_{2C}$ receptor desensitization and membrane stability. *Mol Biol Cell, 17*, 4619-4631.

Gonzalez-Maeso, J., Ang, R. L., Yuen, T., Chan, P., Weisstaub, N. V., Lopez-Gimenez, J. F., Zhou, M., Okawa, Y., Callado, L. F., Milligan, G., Gingrich, J. A., Filizola, M., Meana, J. J., & Sealfon, S. C. (2008). Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature, 452*, 93-97.

Goodall, E. M., Cowen, P. J., Franklin, M., & Silverstone, T. (1993). Ritanserin attenuates anorectic, endocrine and thermic responses to d-fenfluramine in human volunteers. *Psychopharmacology (Berl), 112*, 461-466.

Goyvaerts, L., Schraenen, A., & Schuit, F. (2016). Serotonin competence of mouse beta cells during pregnancy. *Diabetologia*.

Halberstadt, A. L. (2015). Recent advances in the neuropsychopharmacology of serotonergic hallucinogens. *Behavioural brain research, 277*, 99-120.

Han, D. D., & Gu, H. H. (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol, 6*, 6.

Hayashi, T., Sumi, D., Matsui-Hirai, H., Fukatsu, A., Arockia Rani, P. J., Kano, H., Tsunekawa, T., & Iguchi, A. (2003). Sarpogrelate HCl, a selective 5-HT$_{2A}$ antagonist, retards the progression of atherosclerosis through a novel mechanism. *Atherosclerosis, 168*, 23-31.

Heisler, L. K., Jobst, E. E., Sutton, G. M., Zhou, L., Borok, E., Thornton-Jones, Z., Liu, H. Y., Zigman, J. M., Balthasar, N., Kishi, T., Lee, C. E., Aschkenasi, C. J., Zhang, C. Y., Yu, J., Boss, O., Mountjoy, K. G., Clifton, P. G., Lowell, B. B., Friedman, J. M., Horvath, T., Butler, A. A., Elmquist, J. K., & Cowley, M. A. (2006). Serotonin reciprocally regulates melanocortin neurons to modulate food intake. *Neuron, 51*, 239-249.

Heisler, L. K., Pronchuk, N., Nonogaki, K., Zhou, L., Raber, J., Tung, L., Yeo, G. S., O'Rahilly, S., Colmers, W. F., Elmquist, J. K., & Tecott, L. H. (2007). Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin $_{2C}$ receptor stimulation. *J Neurosci, 27*, 6956-6964.

Hendrikx, M., Van Dorpe, J., Flameng, W., & Daenen, W. (1996). Aortic and mitral valve disease induced by ergotamine therapy for migraine: a case report and review of the literature. *J Heart Valve Dis, 5*, 235-237.

Herrick-Davis, K., Grinde, E., Harrigan, T. J., & Mazurkiewicz, J. E. (2005). Inhibition of serotonin 5-hydroxytryptamine$_{2C}$ receptor function through heterodimerization: receptor dimers bind two molecules of ligand and one G-protein. *J Biol Chem, 280*, 40144-40151.

Herrick-Davis, K., Grinde, E., & Mazurkiewicz, J. E. (2004). Biochemical and biophysical characterization of serotonin 5-HT$_{2C}$ receptor homodimers on the plasma membrane of living cells. *Biochemistry, 43*, 13963-13971.

Hirai, T., Kaneshige, K., Kurosaki, T., & Nishio, H. (2010). Functional expression of 5-HT$_{2A}$ receptor in osteoblastic MC3T3-E1 cells. *Biochem Biophys Res Commun, 396*, 278-282.

Hirai, T., Tokumo, K., Tsuchiya, D., & Nishio, H. (2009). Expression of mRNA for 5-HT2 receptors and proteins related to inactivation of 5-HT in mouse osteoblasts. *J Pharmacol Sci, 109*, 319-323.

Hisaoka, K., Nishida, A., Takebayashi, M., Koda, T., Yamawaki, S., & Nakata, Y. (2004). Serotonin increases glial cell line-derived neurotrophic factor release in rat C6 glioblastoma cells. *Brain Res, 1002*, 167-170.

Hodge, J. M., Wang, Y., Berk, M., Collier, F. M., Fernandes, T. J., Constable, M. J., Pasco, J. A., Dodd, S., Nicholson, G. C., Kennedy, R. L., & Williams, L. J. (2013). Selective Serotonin Reuptake Inhibitors Inhibit Human Osteoclast and Osteoblast Formation and Function. *Biol Psychiatry, 74*, 32-39.

Horvath, J., Fross, R. D., Kleiner-Fisman, G., Lerch, R., Stalder, H., Liaudat, S., Raskoff, W. J., Flachsbart, K. D., Rakowski, H., Pache, J. C., Burkhard, P. R., & Lang, A. E. (2004). Severe multivalvular heart disease: a new complication of the ergot derivative dopamine agonists. *Mov Disord, 19*, 656-662.

Hossain, M., Muntasir, H. A., Ishiguro, M., Bhuiyan, M. A., Rashid, M., Sugihara, T., & Nagatomo, T. (2012). Mechanism of inverse agonist action of sarpogrelate at the constitutively active mutant of human 5-HT$_{2A}$ receptor revealed by molecular modeling. *Biol Pharm Bull, 35*, 1553-1559.

Idzko, M., Panther, E., Stratz, C., Müller, T., Bayer, H., Zissel, G., Dürk, T., Sorichter, S., Di Virgilio, F., Geissler, M., Fiebich, B., Herouy, Y., Elsner, P., Norgauer, J., & Ferrari, D. (2004). The serotoninergic receptors of human dendritic cells: identification and coupling to cytokine release. *J Immunol, 172*, 6011-6019.

Iizuka, K., Hamaue, N., Machida, T., Hirafuji, M., & Tsuji, M. (2009). Beneficial effects of sarpogrelate hydrochloride, a 5-HT$_{2A}$ receptor antagonist, supplemented with pioglitazone on diabetic model mice. *Endocr Res, 34*, 18-30.

Iizuka, N., Oka, M., Yamada-Okabe, H., Hamada, K., Nakayama, H., Mori, N., Tamesa, T., Okada, T., Takemoto, N., Matoba, K., Takashima, M., Sakamoto, K., Tangoku, A., Miyamoto, T., Uchimura, S., & Hamamoto, Y. (2004). Molecular signature in three types of hepatocellular carcinoma with different viral origin by oligonucleotide microarray. *Int J Oncol, 24*, 565-574.

Iwabayashi, M., Taniyama, Y., Sanada, F., Azuma, J., Iekushi, K., Kusunoki, H., Chatterjee, A., Okayama, K., Rakugi, H., & Morishita, R. (2012). Role of serotonin in angiogenesis: induction of angiogenesis by sarpogrelate via endothelial 5-HT1B/Akt/eNOS pathway in diabetic mice. *Atherosclerosis, 220*, 337-342.

Jaffre, F., Bonnin, P., Callebert, J., Debbabi, H., Setola, V., Doly, S., Monassier, L., Mettauer, B., Blaxall, B. C., Launay, J. M., & Maroteaux, L. (2009). Serotonin and angiotensin receptors in cardiac fibroblasts coregulate adrenergic-dependent cardiac hypertrophy. *Circ Res, 104*, 113-123.

Jaffré, F., Callebert, J., Sarre, A., Etienne, N., Nebigil, C. G., Launay, J. M., Maroteaux, L., & Monassier, L. (2004). Involvement of the serotonin 5-HT$_{2B}$ receptor in cardiac hypertrophy linked to sympathetic stimulation: control of interleukin-6, interleukin-1 beta, and tumor necrosis factor-alpha cytokine production by ventricular fibroblasts. *Circulation, 110*, 969-974.

Janoshazi, A., Deraet, M., Callebert, J., Setola, V., Guenther, S., Saubamea, B., Manivet, P., Launay, J. M., & Maroteaux, L. (2007). Modified Receptor Internalization upon Co-expression of 5-HT1B Receptor and 5-HT$_{2B}$ Receptors. *Mol Pharmacol, 71*, 1463-1474.

Jean, A., Conductier, G., Manrique, C., Bouras, C., Berta, P., Hen, R., Charnay, Y., Bockaert, J., & Compan, V. (2007). Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. *Proc Natl Acad Sci U S A, 104*, 16335-16340.

Jerman, J. C., Brough, S. J., Gager, T., Wood, M., Coldwell, M. C., Smart, D., & Middlemiss, D. N. (2001). Pharmacological characterisation of human 5-HT2 receptor subtypes. *Eur J Pharmacol, 414*, 23-30.

Ji, S.-P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J. R., & Zhang, X. (2006). Disruption of PTEN coupling with 5-HT$_{2C}$ receptors suppresses behavioral responses induced by drugs of abuse. *Nature medicine, 12*, 324-329.

Johnson, K. W., Nelson, D. L., Dieckman, D. K., Wainscott, D. B., Lucaites, V. L., Audia, J. E., Owton, W. M., & Phebus, L. A. (2003). Neurogenic dural protein extravasation induced by meta-chlorophenylpiperazine (mCPP) involves nitric oxide and 5-HT$_{2B}$ receptor activation. *Cephalalgia, 23*, 117-123.

Julius, D., MacDermott, A. B., Axel, R., & Jessell, T. M. (1988). Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science, 241*, 558-564.

Kamal, M., Gbahou, F., Guillaume, J. L., Daulat, A. M., Benleulmi-Chaachoua, A., Luka, M., Chen, P., Kalbasi Anaraki, D., Baroncini, M., Mannoury la Cour, C., Millan, M. J., Prevot, V., Delagrange, P., & Jockers, R. (2015). Convergence of melatonin and serotonin (5-HT) signaling at MT2/5-HT2C receptor heteromers. *J Biol Chem, 290*, 11537-11546.

Kapetanaki, M. G., Mora, A. L., & Rojas, M. (2013). Influence of age on wound healing and fibrosis. *J. Pathol., 229*, 310-322.

Kawamura, M., Ishiguro, M., Nagamine, T., & Houkin, K. (2013). Sarpogrelate dilates cerebral arteries in the absence of exogenous serotonin. *Neurol Med Chir (Tokyo), 53*, 291-298.

Kenakin, T., Watson, C., Muniz-Medina, V., Christopoulos, A., & Novick, S. (2012). A simple method for quantifying functional selectivity and agonist bias. *ACS Chem Neurosci, 3*, 193-203.

Kennett, G. A., Ainsworth, K., Trail, B., & Blackburn, T. P. (1997). BW 723C86, a 5-HT$_{2B}$ receptor agonist, causes hyperphagia and reduced grooming in rats. *Neuropharmacology, 36*, 233-239.

Kennett, G. A., Wood, M. D., Bright, F., Cilia, J., Piper, D. C., Gager, T., Thomas, D., Baxter, G. S., Forbes, I. T., Ham, P., & Blackburn, T. P. (1996). In vitro and in vivo profile of SB 206553, a potent 5-HT$_{2C}$/5-HT$_{2B}$ receptor antagonist with anxiolytic-like properties. *Br J Pharmacol, 117*, 427-434.

Kim, H., Toyofuku, Y., Lynn, F. C., Chak, E., Uchida, T., Mizukami, H., Fujitani, Y., Kawamori, R., Miyatsuka, T., Kosaka, Y., Yang, K., Honig, G., Van Der Hart, M., Kishimoto, N., Wang, J., Yagihashi, S., Tecott, L. H., Watada, H., & German, M. S. (2010). Serotonin regulates pancreatic beta cell mass during pregnancy. *Nat Med, 16*, 804-808.

Kim, S. W., Shin, I. S., Kim, J. M., Youn, T., Yang, S. J., Hwang, M. Y., & Yoon, J. S. (2009). The 5-HT2 receptor profiles of antipsychotics in the pathogenesis of obsessive-compulsive symptoms in schizophrenia. *Clin Neuropharmacol, 32*, 224-226.

Kishore, S., & Stamm, S. (2006). The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. *Science, 311*, 230-232.

Kiss, B., Horváth, A., Némethy, Z., Schmidt, E., Laszlovszky, I., Bugovics, G., Fazekas, K., Hornok, K., Orosz, S., Gyertyán, I., Agai-Csongor, E., Domány, G., Tihanyi, K., Adham, N., & Szombathelyi, Z. (2010). Cariprazine (RGH-188), a dopamine D(3) receptor-preferring, D(3)/D(2) dopamine receptor antagonist-partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J Pharmacol Exp Ther, 333*, 328-340.

Klein, D., Demory, A., Peyre, F., Kroll, J., Géraud, C., Ohnesorge, N., Schledzewski, K., Arnold, B., & Goerdt, S. (2009). Wnt2 acts as an angiogenic growth factor for non-sinusoidal endothelial cells and inhibits expression of stanniocalcin-1. *Angiogenesis, 12*, 251-265.

Knight, A. R., Misra, A., Quirk, K., Benwell, K., Revell, D., Kennett, G., & Bickerdike, M. (2004). Pharmacological characterisation of the agonist radioligand binding site of $5-HT_{2A}$, $5-HT_{2B}$ and $5-HT_{2C}$ receptors. *Naunyn Schmiedebergs Arch Pharmacol, 370*, 114-123.

Kodama, A., Komori, K., Hattori, K., Yamanouchi, D., Kajikuri, J., & Itoh, T. (2009). Sarpogrelate hydrochloride reduced intimal hyperplasia in experimental rabbit vein graft. *J Vasc Surg, 49*, 1272-1281.

Kokubu, N., Tsuchihashi, K., Yuda, S., Hase, M., Eguchi, M., Wakabayashi, T., Hashimoto, A., Nakata, T., Miura, T., Ura, N., Nagao, K., Tsuzuki, M., Wakabayashi, C., & Shimamoto, K. (2006). Persistent insulin-sensitizing effects of sarpogrelate hydrochloride, a serotonin $_{2A}$ receptor antagonist, in patients with peripheral arterial disease. *Circ J, 70*, 1451-1456.

Kolodziejczak, M., Bechade, C., Gervasi, N., Irinopoulou, T., Banas, S. M., Cordier, C., Rebsam, A., Roumier, A., & Maroteaux, L. (2015). Serotonin Modulates Developmental Microglia via $5-HT_{2B}$ Receptors: Potential Implication during Synaptic Refinement of Retinogeniculate Projections. *ACS Chem Neurosci, 6*, 1219-1230.

Königshoff, M., Dumitrascu, R., Udalov, S., Amarie, O. V., Reiter, R., Grimminger, F., Seeger, W., Schermuly, R. T., & Eickelberg, O. (2010). Increased expression of 5-hydroxytryptamine$_{2A}$/B receptors in idiopathic pulmonary fibrosis: a rationale for therapeutic intervention. *Thorax, 65*, 949-955.

Kopparapu, P. K., Tinzl, M., Anagnostaki, L., Persson, J. L., & Dizeyi, N. (2013). Expression and localization of serotonin receptors in human breast cancer. *Anticancer Res, 33*, 363-370.

Kursar, J. D., Nelson, D. L., Wainscott, D., & Baez, M. (1994). Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine$_{2B}$ receptor. *Mol. Pharmacol., 46*, 227-234.

Kursar, J. D., Nelson, D. L., Wainscott, D. B., Cohen, M. L., & Baez, M. (1992). Molecular cloning, functional expression, and pharmacological characterisation of a novel serotonin (5-Hydroxytryptamine2F) from rat stomach fundus. *Mol. Pharmacol., 42*, 549-557.

Labasque, M., Reiter, E., Becamel, C., Bockaert, J., & Marin, P. (2008). Physical interaction of calmodulin with the 5-hydroxytryptamine2C receptor C-terminus is essential for G protein-independent, arrestin-dependent receptor signaling. *Mol Biol Cell, 19*, 4640-4650.

Lairez, O., Cognet, T., Schaak, S., Calise, D., Guilbeau-Frugier, C., Parini, A., & Mialet-Perez, J. (2013). Role of serotonin $5-HT_{2A}$ receptors in the development of cardiac hypertrophy in response to aortic constriction in mice. *J Neural Transm (Vienna), 120*, 927-935.

Lam, D. D., Przydzial, M. J., Ridley, S. H., Yeo, G. S., Rochford, J. J., O'Rahilly, S., & Heisler, L. K. (2008). Serotonin $5-HT_{2C}$ receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. *Endocrinology, 149*, 1323-1328.

Lampron, A., Bourdeau, I., Hamet, P., Tremblay, J., & Lacroix, A. (2006). Whole genome expression profiling of glucose-dependent insulinotropic peptide (GIP)- and adrenocorticotropin-dependent adrenal hyperplasias reveals novel targets for the study of GIP-dependent Cushing's syndrome. *J Clin Endocrinol Metab, 91*, 3611-3618.

Lanteri, C., Doucet, E. L., Hernández Vallejo, S. J., Godeheu, G., Bobadilla, A.-C., Salomon, L., Lanfumey, L., & Tassin, J.-P. (2014). Repeated exposure to MDMA triggers long-term plasticity of noradrenergic and serotonergic neurons. *Molecular psychiatry, 19*, 823-833.

Launay, J.-M., Birraux, G., Bondoux, D., Callebert, J., Choi, D.-S., Loric, S., & Maroteaux, L. (1996). Ras involvement in signal transduction by the serotonin $5-HT_{2B}$ receptor. *J Biol Chem, 271*, 3141-3147.

Launay, J.-M., Hervé, P., Callebert, J., Mallat, Z., Collet, C., Doly, S., Belmer, A., Diaz, S. L., Hatia, S., Côté, F., Humbert, M., & Maroteaux, L. (2012). Serotonin 5-HT$_{2B}$ receptors are required for bone-marrow contribution to pulmonary arterial hypertension. *Blood, 119*, 1772-1780.

Launay, J. M., Hervé, P., Peoc'h, K., Tournois, C., Callebert, J., Nebigil, C., Etienne, N., Drouet, L., Humbert, M., Simonneau, G., & Maroteaux, L. (2002). Function of the serotonin 5-hydroxytryptamine $_{2B}$ receptor in pulmonary hypertension. *Nat Med, 8*, 1129-1135.

Launay, J. M., Schneider, B., Loric, S., Da Prada, M., & Kellermann, O. (2006). Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT$_{2B}$ receptor signaling in serotonergic neuronal cells. *FASEB J, 20*, 1843-1854.

Lee, M. S., Hanspers, K., Barker, C. S., Korn, A. P., & McCune, J. M. (2004). Gene expression profiles during human CD4+ T cell differentiation. *Int Immunol, 16*, 1109-1124.

Leibowitz, S. F., & Alexander, J. T. (1998). Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biol Psychiatry, 44*, 851-864.

Li, T., Weng, S.-G., Leng, X.-S., Peng, J.-R., Wei, Y.-H., Mou, D.-C., & Wang, W.-X. (2006). Effects of 5-hydroxytamine and its antagonists on hepatic stellate cells. *HBPD INT, 5*, 96-100.

Liang, C., Chen, W., Zhi, X., Ma, T., Xia, X., Liu, H., Zhang, Q., Hu, Q., Zhang, Y., Bai, X., & Liang, T. (2013). Serotonin promotes the proliferation of serum-deprived hepatocellular carcinoma cells via upregulation of FOXO3a. *Mol Cancer, 12*, 14.

Liang, Y. J., Lai, L. P., Wang, B. W., Juang, S. J., Chang, C. M., Leu, J. G., & Shyu, K. G. (2006). Mechanical stress enhances serotonin $_{2B}$ receptor modulating brain natriuretic peptide through nuclear factor-kappaB in cardiomyocytes. *Cardiovasc Res, 72*, 303-312.

Liu, W., Wacker, D., Gati, C., Han, G. W., James, D., Wang, D., Nelson, G., Weierstall, U., Katritch, V., Barty, A., Zatsepin, N. A., Li, D., Messerschmidt, M., Boutet, S., Williams, G. J., Koglin, J. E., Seibert, M. M., Wang, C., Shah, S. T. A., Basu, S., Fromme, R., Kupitz, C., Rendek, K. N., Grotjohann, I., Fromme, P., Kirian, R. A., Beyerlein, K. R., White, T. A., Chapman, H. N., Caffrey, M., Spence, J. C. H., Stevens, R. C., & Cherezov, V. (2013). Serial femtosecond crystallography of G protein-coupled receptors. *Science, 342*, 1521-1524.

Liu, Y., & Fanburg, B. L. (2006). Serotonin-induced Growth of Pulmonary Artery Smooth Muscle Requires Activation of PI3K/Akt/S6K1. *Am J Respir Cell Mol Biol, 34*, 182-191.

Locker, M., Bitard, J., Collet, C., Poliard, A., Mutel, V., Launay, J. M., & Kellermann, O. (2006). Stepwise control of osteogenic differentiation by 5-HT$_{2B}$ receptor signaling: nitric oxide production and phospholipase A2 activation. *Cell Signal, 18*, 628-639.

Loric, S., Launay, J.-M., Colas, J.-F., & Maroteaux, L. (1992). New mouse 5-HT2-like receptor: Expression in brain, heart, and intestine. *FEBS L, 312*, 203-207.

Machida, T., Ohta, M., Onoguchi, A., Iizuka, K., Sakai, M., Minami, M., & Hirafuji, M. (2011). 5-Hydroxytryptamine induces cyclooxygenase-2 in rat vascular smooth muscle cells: Mechanisms involving Src, PKC and MAPK activation [corrected]. *Eur J Pharmacol, 656*, 19-26.

Magee, J. A., Araki, T., Patil, S., Ehrig, T., True, L., Humphrey, P. A., Catalona, W. J., Watson, M. A., & Milbrandt, J. (2001). Expression profiling reveals hepsin overexpression in prostate cancer. *Cancer Res, 61*, 5692-5696.

Mancia, F., Assur, Z., Herman, A. G., Siegel, R., & Hendrickson, W. A. (2008). Ligand sensitivity in dimeric associations of the serotonin 5HT$_{2C}$ receptor. *EMBO Rep, 9*, 363-369.

Manivet, P., Mouillet-Richard, S., Callebert , J., Nebigil, C. G., Maroteaux, L., Hosoda, S., Kellermann, O., & Launay, J.-M. (2000). PDZ-dependent activation of nitric-oxide synthases by the serotonin $_{2B}$ receptor. *J Biol Chem, 275*, 9324-9331.

Mann, D. A., & Oakley, F. (2013). Serotonin paracrine signaling in tissue fibrosis. *Biochim Biophys Acta, 1832*, 905-910.

Marion, S., Weiner, D. M., & Caron, M. G. (2004). RNA editing induces variation in desensitization and trafficking of 5-hydroxytryptamine $_{2C}$ receptor isoforms. *J Biol Chem, 279*, 2945-2954.

Martí-Solano, M., Sanz, F., Pastor, M., & Selent, J. (2014). A dynamic view of molecular switch behavior at serotonin receptors: implications for functional selectivity. *PLoS ONE, 9*, e109312.

Martin, C. B. P., Hamon, M., Lanfumey, L., & Mongeau, R. (2014). Controversies on the role of 5-HT($_{2C}$) receptors in the mechanisms of action of antidepressant drugs. *Neuroscience and biobehavioral reviews, 42*, 208-223.

Marzak, H., Ayme-Dietrich, E., Lawson, R., Mokni, W., Combe, R., Becker, J., Fertak, L. E., Champy, M. F., & Monassier, L. (2014). Old spontaneously hypertensive rats gather together typical features of human chronic left-ventricular dysfunction with preserved ejection fraction. *J Hypertens, 32*, 1307-1316.

Matsumura, N., Mandai, M., Miyanishi, M., Fukuhara, K., Baba, T., Higuchi, T., Kariya, M., Takakura, K., & Fujii, S. (2006). Oncogenic property of acrogranin in human uterine leiomyosarcoma: direct evidence of genetic contribution in in vivo tumorigenesis. *Clin Cancer Res, 12*, 1402-1411.

McCorvy, J. D., & Roth, B. L. (2015). Structure and function of serotonin G protein-coupled receptors. *Pharmacol Ther, 150*, 129-142.

McDevitt, R. A., & Neumaier, J. F. (2011). Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J Chem Neuroanat, 41*, 234-246.

McGlashon, J. M., Gorecki, M. C., Kozlowski, A. E., Thirnbeck, C. K., Markan, K. R., Leslie, K. L., Kotas, M. E., Potthoff, M. J., Richerson, G. B., & Gillum, M. P. (2015). Central serotonergic neurons activate and recruit thermogenic brown and beige fat and regulate glucose and lipid homeostasis. *Cell Metabolism, 21*, 692-705.

Meltzer, H. Y. (2013). Update on typical and atypical antipsychotic drugs. *Annu Rev Med, 64*, 393-406.

Merzak, A., Koochekpour, S., Fillion, M. P., Fillion, G., & Pilkington, G. F. (1996). *Mol. Brain Res., 41*, 1-7.

Mialet-Perez, J., D'Angelo, R., Villeneuve, C., Ordener, C., Negre-Salvayre, A., Parini, A., & Vindis, C. (2012). Serotonin 5-HT$_{2A}$ receptor-mediated hypertrophy is negatively regulated by caveolin-3 in cardiomyoblasts and neonatal cardiomyocytes. *J Mol Cell Cardiol, 52*, 502-510.

Millan, M. J., Gobert, A., Lejeune, F., Dekeyne, A., Newman-Tancredi, A., Pasteau, V., Rivet, J.-M., & Cussac, D. (2003). The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine$_{2C}$ receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther, 306*, 954-964.

Miyazaki, M., Higashi, Y., Goto, C., Chayama, K., Yoshizumi, M., Sanada, H., Orihashi, K., & Sueda, T. (2007). Sarpogrelate hydrochloride, a selective 5-HT$_{2A}$ antagonist, improves vascular function in patients with peripheral arterial disease. *J Cardiovasc Pharmacol, 49*, 221-227.

Monassier, L., Laplante, M. A., Jaffre, F., Bousquet, P., Maroteaux, L., & de Champlain, J. (2008). Serotonin 5-HT$_{2B}$ Receptor Blockade Prevents Reactive Oxygen Species-Induced Cardiac Hypertrophy in Mice. *Hypertension, 52*, 301-307.

Morita, K., Arimochi, H., Itoh, H., & Her, S. (2006). Possible involvement of 5alpha-reduced neurosteroids in adrenergic and serotonergic stimulation of GFAP gene expression in rat C6 glioma cells. *Brain Res, 1085*, 49-56.

Muntasir, H. A., Rashid, M., Komiyama, T., Kawakami, J., & Nagatomo, T. (2006). Identification of amino acid residues important for sarpogrelate binding to the human 5-hydroxytryptamine$_{2A}$ serotonin receptor. *J Pharmacol Sci, 102*, 55-63.

Murnane, K. S., Winschel, J., Schmidt, K. T., Stewart, L. M., Rose, S. J., Cheng, K., Rice, K. C., & Howell, L. L. (2013). Serotonin $_{2A}$ Receptors Differentially Contribute to Abuse-Related Effects of Cocaine and Cocaine-Induced Nigrostriatal and Mesolimbic Dopamine Overflow in Nonhuman Primates. *J Neurosci, 33*, 13367-13374.

Murray, K. C., Stephens, M. J., Ballou, E. W., Heckman, C. J., & Bennett, D. J. (2011). Motoneuron Excitability and Muscle Spasms Are Regulated by 5-HT$_{2B}$ and 5-HT$_{2C}$ Receptor Activity. *J Neurophysiology, 105*, 731-748.

Nabeshima, T., Ishikawa, K., Yamaguchi, K., Furukawa, H., & Kameyama, T. (1987). Phencyclidine-induced head-twitch responses as 5-HT2 receptor-mediated behavior in rats. *Neurosci Lett, 76*, 335-338.

Nebigil, C. G., Etienne, N., Messaddeq, N., & Maroteaux, L. (2003). Serotonin is a novel survival factor of cardiomyocytes: mitochondria as a target of 5-HT$_{2B}$-receptor signaling. *FASEB J, 17*, 1373-1375.

Nebigil, C. G., Hickel, P., Messaddeq, N., Vonesch, J.-L., Douchet, M.-P., Monassier, L., György, K., Martz, R., Andriantsitohaina, R., Manivet, P., Launay, J.-M., & Maroteaux, L. (2001). Ablation of serotonin 5-HT$_{2B}$ receptors in mice leads to abnormal cardiac structure and function. *Circulation, 103*, 2973-2979.

Nebigil, C. G., Launay, J.-M., Hickel, P., Tournois, C., & Maroteaux, L. (2000). 5-Hydroxytryptamine $_{2B}$ receptor regulates cell-cycle progression: Cross talk with tyrosine kinase pathways. *Proc Natl Acad Sci U S A, 97*, 2591-2596.

Neill, J. C., & Cooper, S. J. (1989). Evidence that d-fenfluramine anorexia is mediated by 5-HT1 receptors. *Psychopharmacology (Berl), 97*, 213-218.

Nelson, D. L., Lucaites, V. L., Wainscott, D. B., & Glennon, R. A. (1999). Comparisons of hallucinogenic phenylisopropylamine binding affinities at cloned human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. *Naunyn Schmiedebergs Arch Pharmacol, 359*, 1-6.

Nocito, A., Dahm, F., Jochum, W., Jang, J. H., Georgiev, P., Bader, M., Graf, R., & Clavien, P. A. (2008). Serotonin regulates macrophage-mediated angiogenesis in a mouse model of colon cancer allografts. *Cancer Res, 68*, 5152-5158.

Nonogaki, K., Nozue, K., Takahashi, Y., Yamashita, N., Hiraoka, S., Kumano, H., Kuboki, T., & Oka, Y. (2007). Fluvoxamine, a selective serotonin reuptake inhibitor, and 5-HT$_{2C}$ receptor inactivation induce appetite-suppressing effects in mice via 5-HT1B receptors. *Int J Neuropsychopharmacol, 10*, 675-681.

Ohta, Y., Kosaka, Y., Kishimoto, N., Wang, J., Smith, S. B., Honig, G., Kim, H., Gasa, R. M., Neubauer, N., Liou, A., Tecott, L. H., Deneris, E. S., & German, M. S. (2011). Convergence of the Insulin and Serotonin Programs in the Pancreatic β-Cell. *Diabetes, 60*, 3208-3216.

Ohyashiki, J. H., Hamamura, R., Kobayashi, C., Zhang, Y., & Ohyashiki, K. (2008). A network biology approach evaluating the anticancer effects of bortezomib identifies SPARC as a therapeutic target in adult T-cell leukemia cells. *Adv Appl Bioinform Chem, 1*, 85-98.

Onken, M. D., Worley, L. A., Tuscan, M. D., & Harbour, J. W. (2010). An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. *J Mol Diagn, 12*, 461-468.

Orejarena, M. J., Lanfumey, L., Maldonado, R., & Robledo, P. (2011). Involvement of 5-HT$_{2A}$ receptors in MDMA reinforcement and cue-induced reinstatement of MDMA-seeking behaviour. *Int J Neuropsychopharmacol, 14*, 927-940.

Orton, E. C., Lacerda, C. M. R., & MacLea, H. B. (2012). Signaling pathways in mitral valve degeneration. *J Vet Cardiol, 14*, 7-17.

Oufkir, T., Arseneault, M., Sanderson, J. T., & Vaillancourt, C. (2010). The 5-HT $_{2A}$ serotonin receptor enhances cell viability, affects cell cycle progression and activates MEK-ERK1/2 and JAK2-STAT3 signalling pathways in human choriocarcinoma cell lines. *Placenta, 31*, 439-447.

Pai, V., Marshall, A., Hernandez, L., Buckley, A., & Horseman, N. (2009). Altered serotonin physiology in human breast cancers favors paradoxical growth and cell survival. *Breast Cancer Res, 11*, R81.

Park, K.-S., Sin, P.-J., Lee, D. H., Cha, S.-K., Kim, M.-J., Kim, N.-H., Baik, S.-K., Jeong, S.-W., & Kong, I. D. (2011). Switching-on of serotonergic calcium signaling in activated hepatic stellate cells. *WJG, 17*, 164-173.

Paulmann, N., Grohmann, M., Voigt, J.-P., Bert, B., Vowinckel, J., Bader, M., Skelin, M., Jevsek, M., Fink, H., Rupnik, M., & Walther, D. J. (2009). Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol, 7*, e1000229.

Pavone, L. M., Rea, S., Trapani, F., De Pasquale, V., Tafuri, S., Papparella, S., & Paciello, O. (2012). Role of serotonergic system in the pathogenesis of fibrosis in canine idiopathic inflammatory myopathies. *Neuromuscul Disord, 22*, 549-557.

Pelloux, Y., Dilleen, R., Economidou, D., Theobald, D., & Everitt, B. J. (2012). Reduced forebrain serotonin transmission is causally involved in the development of compulsive cocaine seeking in rats. *Neuropsychopharmacology 37*, 2505-2514.

Pennanen, L., Van Der Hart, M., Yu, L., & Tecott, L. H. (2013). Impact of serotonin (5-HT)$_{2C}$ receptors on executive control processes. *Neuropsychopharmacology, 38*, 957-967.

Peroutka, S. J., Lebovitz, R. M., & Snyder, S. H. (1981). Two distinct central serotonin receptors with different physiological functions. *Science, 212*, 827-829.

Pitychoutis, P., Belmer, A., Moutkine, I., Adrien, J., & Maroteaux, L. (2015). Mice lacking the serotonin Htr$_{2B}$ receptor gene present an antipsychotic-sensitive schizophrenic-like phenotype. *Neuropsychopharmacology, 40*, 2764-2773.

Pockros, L. A., Pentkowski, N. S., Conway, S. M., Ullman, T. E., Zwick, K. R., & Neisewander, J. L. (2012). 5-HT$_{2A}$ receptor blockade and 5-HT$_{2C}$ receptor activation interact to reduce cocaine hyperlocomotion and Fos protein expression in the caudate-putamen. *Synapse, 66*, 989-1001.

Porter, R. H., Benwell, K. R., Lamb, H., Malcolm, C. S., Allen, N. H., Revell, D. F., Adams, D. R., & Sheardown, M. J. (1999). Functional characterization of agonists at recombinant human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors in CHO-K1 cells. *Br J Pharmacol, 128*, 13-20.

Porvasnik, S. L., Germain, S., Embury, J., Gannon, K. S., Jacques, V., Murray, J., Byrne, B. J., Shacham, S., & Al-Mousily, F. (2010). PRX-08066, a novel 5-hydroxytryptamine receptor $_{2B}$ antagonist, reduces monocrotaline-induced pulmonary arterial hypertension and right ventricular hypertrophy in rats. *J Pharmacol Exp Ther, 334*, 364-372.

Pritchett, D. B., Bach, A. W., Wozny, M., Taleb, O., Dal Toso, R., Shih, J. C., & Seeburg, P. H. (1988). *EMBO J., 7*, 4135-4140.

Quesseveur, G., Repérant, C., David, D. J., Gardier, A. M., Sanchez, C., & Guiard, B. P. (2013). 5-HT A receptor inactivation potentiates the acute antidepressant-like activity of escitalopram: involvement of the noradrenergic system. *Experimental Brain Research, 226*, 285-295.

Rashid, M., Manivet, P., Nishio, H., Pratuangdejkul, J., Rajab, M., Ishiguro, M., Launay, J. M., & Nagatomo, T. (2003). Identification of the binding sites and selectivity of sarpogrelate, a novel 5-

HT2 antagonist, to human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptor subtypes by molecular modeling. *Life Sci, 73*, 193-207.

Reavill, C., Kettle, A., Holland, V., Riley, G., & Blackburn, T. P. (1999). Attenuation of haloperidol-induced catalepsy by a 5-HT$_{2C}$ receptor antagonist. *Br J Pharmacol, 126*, 572-574.

Ren, S., Qian, S., Wang, W., Liu, J., & Liu, P. (2013). Prospective study of sarpogrelate hydrochloride on patients with arteriosclerosis obliterans. *Ann Thorac Cardiovasc Surg, 19*, 30-34.

Robiolio, P. A., Rigolin, V. H., Wilson, J. S., Harrison, J. K., Sanders, L. L., Bashore, T. M., & Feldman, J. M. (1995). Carcinoid heart disease. Correlation of high serotonin levels with valvular abnormalities detected by cardiac catheterization and echocardiography. *Circulation, 92*, 790-795.

Rocha, B. A., Goulding, E. H., O'Dell, L. E., Mead, A. N., Coufal, N. G., Parsons, L. H., & Tecott, L. H. (2002). Enhanced locomotor, reinforcing, and neurochemical effects of cocaine in serotonin 5-hydroxytryptamine $_{2C}$ receptor mutant mice. *J Neurosci, 22*, 10039-10045.

Rosenzweig-Lipson, S., Zhang, J., Mazandarani, H., Harrison, B. L., Sabb, A., Sabalski, J., Stack, G., Welmaker, G., Barrett, J. E., & Dunlop, J. (2006). Antiobesity-like effects of the 5-HT$_{2C}$ receptor agonist WAY-161503. *Brain Res, 1073-1074*, 240-251.

Roth, B. L. (2007). Drugs and valvular heart disease. *N Engl J Med, 356*, 6-9.

Rothman, R. B., Baumann, M. H., Savage, J. E., Rauser, L., McBride, A., Hufeisen, S. J., & Roth, B. L. (2000). Evidence for possible involvement of 5-HT$_{2B}$ receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation, 102*, 2836-2841.

Ruddell, R. G., Oakley, F., Hussain, Z., Yeung, I., Bryan-Lluka, L. J., Ramm, G. A., & Mann, D. A. (2006). A role for serotonin (5-HT) in hepatic stellate cell function and liver fibrosis. *Am J Pathol, 169*, 861-876.

Russell, A., Banes, A., Berlin, H., Fink, G. D., & Watts, S. W. (2002). 5-Hydroxytryptamine$_{2B}$ Receptor Function Is Enhanced in the N(omega)-Nitro-L-arginine Hypertensive Rat. *J Pharmacol Exp Ther, 303*, 179-187.

Salomon, L., Lanteri, C., Godeheu, G., Blanc, G., Gingrich, J., & Tassin, J. P. (2007). Paradoxical constitutive behavioral sensitization to amphetamine in mice lacking 5-HT$_{2A}$ receptors. *Psychopharmacology (Berl), 194*, 11-20.

Saltzman, A. G., Morse, B., Whitman, M. M., Ivanshchenko, Y., Jaye, M., & Felder, S. (1991). Cloning of the Human Serotonin 5-HT2 and 5-HT1C Receptor Subtypes. *Biochem Biophys Res Commun, 181*, 1469-1478.

Sarrouilhe, D., Clarhaut, J., Defamie, N., & Mesnil, M. (2015). Serotonin and cancer: what is the link? *Curr Mol Med, 15*, 62-77.

Schellekens, H., De Francesco, P. N., Kandil, D., Theeuwes, W. F., Mccarthy, T., van Oeffelen, W. E. P. A., Perelló, M., Giblin, L., Dinan, T. G., & Cryan, J. F. (2015). Ghrelin's Orexigenic Effect Is Modulated via a Serotonin $_{2C}$ Receptor Interaction. *ACS chemical neuroscience*.

Schmuck, K., Ullmer, C., Engels, P., & Lübbert, H. (1994). Cloning and functionnal characterisation of the human 5-HT$_{2B}$ serotonin receptor. *FEBS L, 342*, 85-90.

Schmuck, K., Ullmer, C., Kalkman, H., Probst, A., & Lübbert, H. (1996). Activation of meningeal 5-HT$_{2B}$ receptors: an early step in the generation of migraine headache? *European Journal of Neuroscience, 8*, 959-967.

Schraenen, A., Lemaire, K., de Faudeur, G., Hendrickx, N., Granvik, M., Van Lommel, L., Mallet, J., Vodjdani, G., Gilon, P., Binart, N., in't Veld, P., & Schuit, F. (2010). Placental lactogens induce serotonin biosynthesis in a subset of mouse beta cells during pregnancy. *Diabetologia, 53*, 2589-2599.

Setola, V., Dukat, M., Glennon, R. A., & Roth, B. L. (2005). Molecular determinants for the interaction of the valvulopathic anorexigen norfenfluramine with the 5-HT$_{2B}$ receptor. *Mol Pharmacol, 68*, 20-33.

Setola, V., Hufeisen, S. J., Grande-Allen, K. J., Vesely, I., Glennon, R. A., Blough, B., Rothman, R. B., & Roth, B. L. (2003). 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol Pharmacol, 63*, 1223-1229.

Shahid, M., Walker, G. B., Zorn, S. H., & Wong, E. H. F. (2009). Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. *J Psychopharm, 23*, 65-73.

Shapiro, D. A., Renock, S., Arrington, E., Chiodo, L. A., Liu, L. X., Sibley, D. R., Roth, B. L., & Mailman, R. (2003). Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology, 28*, 1400-1411.

Sharma, S. K., Del Rizzo, D. F., Zahradka, P., Bhangu, S. K., Werner, J. P., Kumamoto, H., Takeda, N., & Dhalla, N. S. (2001). Sarpogrelate inhibits serotonin-induced proliferation of porcine coronary

artery smooth muscle cells: implications for long-term graft patency. *Ann Thorac Surg, 71*, 1856-1864; discussion 1865.

Siuciak, J. A., Chapin, D. S., McCarthy, S. A., Guanowsky, V., Brown, J., Chiang, P., Marala, R., Patterson, T., Seymour, P. A., Swick, A., & Iredale, P. A. (2007). CP-809,101, a selective 5-HT$_{2C}$ agonist, shows activity in animal models of antipsychotic activity. *Neuropharmacology, 52*, 279-290.

Smith, S. R., Weissman, N. J., Anderson, C. M., Sanchez, M., Chuang, E., Stubbe, S., Bays, H., Shanahan, W. R., & Group, B. M. a. L. f. O. a. O. M. B. S. (2010). Multicenter, placebo-controlled trial of lorcaserin for weight management. *N Engl J Med, 363*, 245-256.

Söhle, J., Machuy, N., Smailbegovic, E., Holtzmann, U., Grönniger, E., Wenck, H., Stäb, F., & Winnefeld, M. (2012). Identification of new genes involved in human adipogenesis and fat storage. *PLoS ONE, 7*, e31193.

Soll, C., Jang, J. H., Riener, M.-O., Moritz, W., Wild, P. J., Graf, R., & Clavien, P.-A. (2010). Serotonin promotes tumor growth in human hepatocellular cancer. *Hepatology, 51*, 1244-1254.

Soll, C., Riener, M.-O., Oberkofler, C. E., Hellerbrand, C., Wild, P. J., Deoliveira, M. L., & Clavien, P.-A. (2012). Expression of serotonin receptors in human hepatocellular cancer. *Clin Cancer Res, 18*, 5902-5910.

Sonier, B., Arseneault, M., Lavigne, C., Ouellette, R. J., & Vaillancourt, C. (2006). The 5-HT$_{2A}$ serotoninergic receptor is expressed in the MCF-7 human breast cancer cell line and reveals a mitogenic effect of serotonin. *Biochem Biophys Res Commun, 343*, 1053-1059.

Stefulj, J., Jernej, B., Cicin-Sain, L., Rinner, I., & Schauenstein, K. (2000). mRNA expression of serotonin receptors in cells of the immune tissues of the rat. *Brain Behav Immun, 14*, 219-224.

Sumara, G., Sumara, O., Kim, J. K., & Karsenty, G. (2012). Gut-derived serotonin is a multifunctional determinant to fasting adaptation. *Cell Metab, 16*, 588-600.

Sun, Y. M., Su, Y., Jin, H. B., Li, J., & Bi, S. (2011). Sarpogrelate protects against high glucose-induced endothelial dysfunction and oxidative stress. *Int J Cardiol, 147*, 383-387.

Svejda, B., Kidd, M., Giovinazzo, F., Eltawil, K., Gustafsson, B. I., Pfragner, R., & Modlin, I. M. (2010). The 5-HT$_{2B}$ receptor plays a key regulatory role in both neuroendocrine tumor cell proliferation and the modulation of the fibroblast component of the neoplastic microenvironment. *Cancer, 116*, 2902-2912.

Takahara, M., Kaneto, H., Katakami, N., Iida, O., Matsuoka, T. A., & Shimomura, I. (2014). Effect of sarpogrelate treatment on the prognosis after endovascular therapy for critical limb ischemia. *Heart Vessels, 29*, 563-567.

Takishita, E., Takahashi, A., Harada, N., Yamato, M., Yoshizumi, M., & Nakaya, Y. (2004). Effect of sarpogrelate hydrochloride, a 5-HT2 blocker, on insulin resistance in Otsuka Long-Evans Tokushima fatty rats (OLETF rats), a type 2 diabetic rat model. *J Cardiovasc Pharmacol, 43*, 266-270.

Tanaka, K., Hirai, T., Ishibashi, Y., Izumo, N., & Togari, A. (2015). Modulation of osteoblast differentiation and bone mass by 5-HT$_{2A}$ receptor signaling in mice. *Eur J Pharmacol, 762*, 150-157.

Thomsen, W. J., Grottick, A. J., Menzaghi, F., Reyes-Saldana, H., Espitia, S., Yuskin, D., Whelan, K., Martin, M., Morgan, M., Chen, W., Al-Shamma, H., Smith, B., Chalmers, D., & Behan, D. (2008). Lorcaserin, a novel selective human 5-hydroxytryptamine$_{2C}$ agonist: in vitro and in vivo pharmacological characterization. *J Pharmacol Exp Ther, 325*, 577-587.

Tiihonen, J., Lonnqvist, J., Wahlbeck, K., Klaukka, T., Niskanen, L., Tanskanen, A., & Haukka, J. (2009). 11-year follow-up of mortality in patients with schizophrenia: a population-based cohort study (FIN11 study). *Lancet, 374*, 620-627.

Tschentscher, F., Husing, J., Holter, T., Kruse, E., Dresen, I. G., Jockel, K. H., Anastassiou, G., Schilling, H., Bornfeld, N., Horsthemke, B., Lohmann, D. R., & Zeschnigk, M. (2003). Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res, 63*, 2578-2584.

Tsuchioka, M., Takebayashi, M., Hisaoka, K., Maeda, N., & Nakata, Y. (2008). Serotonin (5-HT) induces glial cell line-derived neurotrophic factor (GDNF) mRNA expression via the transactivation of fibroblast growth factor receptor 2 (FGFR2) in rat C6 glioma cells. *J Neurochem, 106*, 244-257.

van Gils, W., Lodder, E. M., Mensink, H. W., Kiliç, E., Naus, N. C., Brüggenwirth, H. T., van Ijcken, W., Paridaens, D., Luyten, G. P., & de Klein, A. (2008). Gene expression profiling in uveal melanoma: two regions on 3p related to prognosis. *Investigative Ophthalmology & Visual Science, 49*, 4254-4262.

Varela, M. J., Brea, J., Loza, M. I., Maldonado, R., & Robledo, P. (2011). Sensitization to MDMA locomotor effects and changes in the functionality of 5-HT$_{(2A)}$ and D receptors in mice. *Behavioural pharmacology, 22*, 362-369.

Vickers, S. P., Clifton, P. G., Dourish, C. T., & Tecott, L. H. (1999). Reduced satiating effect of d-fenfluramine in serotonin 5-HT$_{2C}$ receptor mutant mice. *Psychopharmacology (Berl), 143*, 309-314.

Vickers, S. P., & Dourish, C. T. (2004). Serotonin receptor ligands and the treatment of obesity. *Curr Opin Investig Drugs, 5*, 377-388.

Vickers, S. P., Dourish, C. T., & Kennett, G. A. (2001). Evidence that hypophagia induced by d-fenfluramine and d-norfenfluramine in the rat is mediated by 5-HT$_{2C}$ receptors. *Neuropharmacology, 41*, 200-209.

Vinals, X., Moreno, E., Lanfumey, L., Cordomi, A., Pastor, A., de La Torre, R., Gasperini, P., Navarro, G., Howell, L. A., Pardo, L., Lluis, C., Canela, E. I., McCormick, P. J., Maldonado, R., & Robledo, P. (2015). Cognitive Impairment Induced by Delta9-tetrahydrocannabinol Occurs through Heteromers between Cannabinoid CB1 and Serotonin 5-HT2A Receptors. *PLoS Biol, 13*, e1002194.

Vindis, C., D'Angelo, R., Mucher, E., Negre-Salvayre, A., Parini, A., & Mialet-Perez, J. (2010). Essential role of TRPC1 channels in cardiomyoblasts hypertrophy mediated by 5-HT$_{2A}$ serotonin receptors. *Biochem Biophys Res Commun, 391*, 979-983.

Visconti, R. P., Ebihara, Y., LaRue, A. C., Fleming, P. A., McQuinn, T. C., Masuya, M., Minamiguchi, H., Markwald, R. R., Ogawa, M., & Drake, C. J. (2006). An in vivo analysis of hematopoietic stem cell potential: hematopoietic origin of cardiac valve interstitial cells. *Circ Res, 98*, 690-696.

Wacker, D., Wang, C., Katritch, V., Han, G. W., Huang, X. P., Vardy, E., McCorvy, J. D., Jiang, Y., Chu, M., Siu, F. Y., Liu, W., Xu, H. E., Cherezov, V., Roth, B. L., & Stevens, R. C. (2013). Structural features for functional selectivity at serotonin receptors. *Science, 340*, 615-619.

Wainscott, D. B., Cohen, M. L., Schenck, K. W., Audia, J. E., Nissen, J. S., Baez, M., Kursar, J. D., Lucaites, V. L., & Nelson, D. L. (1993). Pharmacological characteristics of the newly cloned rat 5-Hydroxytryptamine 2F receptor. *Mol. Pharmacol., 43*, 419-426.

Wainscott, D. B., Lucaites, V. L., Kursar, J. D., Baez, M., & Nelson, D. L. (1996). Pharmacologic characterization of the human 5-hydroxytryptamine$_{2B}$ receptor: evidence for species differences. *J Pharmacol Exp Ther, 276*, 720-727.

Wang, C., Jiang, Y., Ma, J., Wu, H., Wacker, D., Katritch, V., Han, G. W., Liu, W., Huang, X. P., Vardy, E., McCorvy, J. D., Gao, X., Zhou, X. E., Melcher, K., Zhang, C., Bai, F., Yang, H., Yang, L., Jiang, H., Roth, B. L., Cherezov, V., Stevens, R. C., & Xu, H. E. (2013). Structural basis for molecular recognition at serotonin receptors. *Science, 340*, 610-614.

Watanabe, H., Nakano, T., Saito, R., Akasaka, D., Saito, K., Ogasawara, H., Minashima, T., Miyazawa, K., Kanaya, T., Takakura, I., Inoue, N., Ikeda, I., Chen, X., Miyake, M., Kitazawa, H., Shirakawa, H., Sato, K., Tahara, K., Nagasawa, Y., Rose, M. T., Ohwada, S., Watanabe, K., & Aso, H. (2016). Serotonin Improves High Fat Diet Induced Obesity in Mice. *PLoS One, 11*, e0147143.

Watanabe, T., Pakala, R., Katagiri, T., & Benedict, C. R. (2001). Serotonin potentiates angiotensin II--induced vascular smooth muscle cell proliferation. *Atherosclerosis, 159*, 269-279.

Watts, S. W., Baez, M., & Webb, R. C. (1996). The 5-hydroxytryptamine$_{2B}$ receptor and 5-HT receptor signal transduction in mesenteric arteries from deoxycorticosterone acetate- salt hypertensive rats. *J Pharmacol Exp Ther, 277*, 1103-1113.

Watts, S. W., & Fink, G. D. (1999). 5-HT$_{2B}$-receptor antagonist LY-272015 is antihypertensive in DOCA-salt- hypertensive rats. *Am J Physiol, 276*, H944-952.

Weir, E. K., Obreztchikova, M., & Hong, Z. (2008). Fenfluramine: riddle or Rosetta stone? *Eur Respir J, 31*, 232-235.

Westbroek, I., van Der Plas, A., de Rooij, K. E., Klein-Nulend, J., & Nijweide, P. J. (2001). Expression of serotonin receptors in bone. *J Biol Chem, 276*, 28961-28968.

Wienecke, J., Westerdahl, A.-C., Hultborn, H., Kiehn, O., & Ryge, J. (2010). Global gene expression analysis of rodent motor neurons following spinal cord injury associates molecular mechanisms with development of postinjury spasticity. *Journal of Neurophysiology, 103*, 761-778.

Xia, Z., Gray, J. A., Compton-Toth, B. A., & Roth, B. L. (2003). A direct interaction of PSD-95 with 5-HT$_{2A}$ serotonin receptors regulates signal transduction and receptor trafficking. *J Biol Chem, 278*, 21901-21908.

Xia, Z., Hufeisen, S. J., Gray, J. A., & Roth, B. L. (2003). The PDZ-binding domain is essential for the dendritic targeting of 5-HT$_{2A}$ serotonin receptors in cortical pyramidal neurons in vitro. *Neuroscience, 122*, 907-920.

Xu, Y., Jones, J. E., Kohno, D., Williams, K. W., Lee, C. E., Choi, M. J., Anderson, J. G., Heisler, L. K., Zigman, J. M., Lowell, B. B., & Elmquist, J. K. (2008). 5-HT$_{2C}$Rs expressed by pro-opiomelanocortin neurons regulate energy homeostasis. *Neuron, 60*, 582-589.

Yabanoglu, S., Akkiki, M., Seguelas, M. H., Mialet-Perez, J., Parini, A., & Pizzinat, N. (2009). Platelet derived serotonin drives the activation of rat cardiac fibroblasts by 5-HT$_{2A}$ receptors. *J Mol Cell Cardiol, 46*, 518-525.

Yadav, V. K., Oury, F., Suda, N., Liu, Z.-W., Gao, X.-B., Confavreux, C., Klemenhagen, K. C., Tanaka, K. F., Gingrich, J. A., Guo, X. E., Tecott, L. H., Mann, J. J., Hen, R., Horvath, T. L., & Karsenty, G. (2009). A Serotonin-Dependent Mechanism Explains the Leptin Regulation of Bone Mass, Appetite, and Energy Expenditure. *Cell, 138*, 976-989.

Yamada, K., Niki, H., Nagai, H., Nishikawa, M., & Nakagawa, H. (2012). Serotonin potentiates high-glucose-induced endothelial injury: the role of serotonin and 5-HT$_{2A}$ receptors in promoting thrombosis in diabetes. *J Pharmacol Sci, 119*, 243-250.

Yang, M., Li, K., Ng, P. C., Chuen, C. K., Lau, T. K., Cheng, Y. S., Liu, Y. S., Li, C. K., Yuen, P. M., James, A. E., Lee, S. M., & Fok, T. F. (2007). Promoting effects of serotonin on hematopoiesis: ex vivo expansion of cord blood CD34+ stem/progenitor cells, proliferation of bone marrow stromal cells, and antiapoptosis. *Stem Cells, 25*, 1800-1806.

Ye, J. Y., Liang, E. Y., Cheng, Y. S., Chan, G. C. F., Ding, Y., Meng, F., Ng, M. H. L., Chong, B. H., Lian, Q., & Yang, M. (2014). Serotonin Enhances Megakaryopoiesis and Proplatelet Formation via p-Erk1/2 and F-Actin Reorganization. *Stem Cells, 32*, 2973-2982.

Zhang, Y., Yang, Y., Chen, L., & Zhang, J. (2014). Expression analysis of genes and pathways associated with liver metastases of the uveal melanoma. *BMC Med Genet, 15*, 29.

Zopf, D. A., Neves, L. A. A. d., Nikula, K. J., Huang, J., Senese, P. B., & Gralinski, M. R. (2011). C-122, a novel antagonist of serotonin receptor 5-HT$_{2B}$), prevents monocrotaline-induced pulmonary arterial hypertension in rats. *Eur J Pharmacol, 670*, 195-203.

Zsippai, A., Szabó, D. R., Szabó, P. M., Tömböl, Z., Bendes, M. R., Nagy, Z., Rácz, K., & Igaz, P. (2011). mRNA and microRNA expression patterns in adrenocortical cancer. *Am J Cancer Res, 1*, 618-628.

Figure 1: Serotonin 5-HT$_2$ receptors as therapeutic targets for central nervous system and cardiovascular diseases.

Blocking or stimulating 5-HT$_2$ receptors could have many clinical impacts. In this figure, main areas are emphasized. We also show which kind of receptor should be blocked or stimulated.
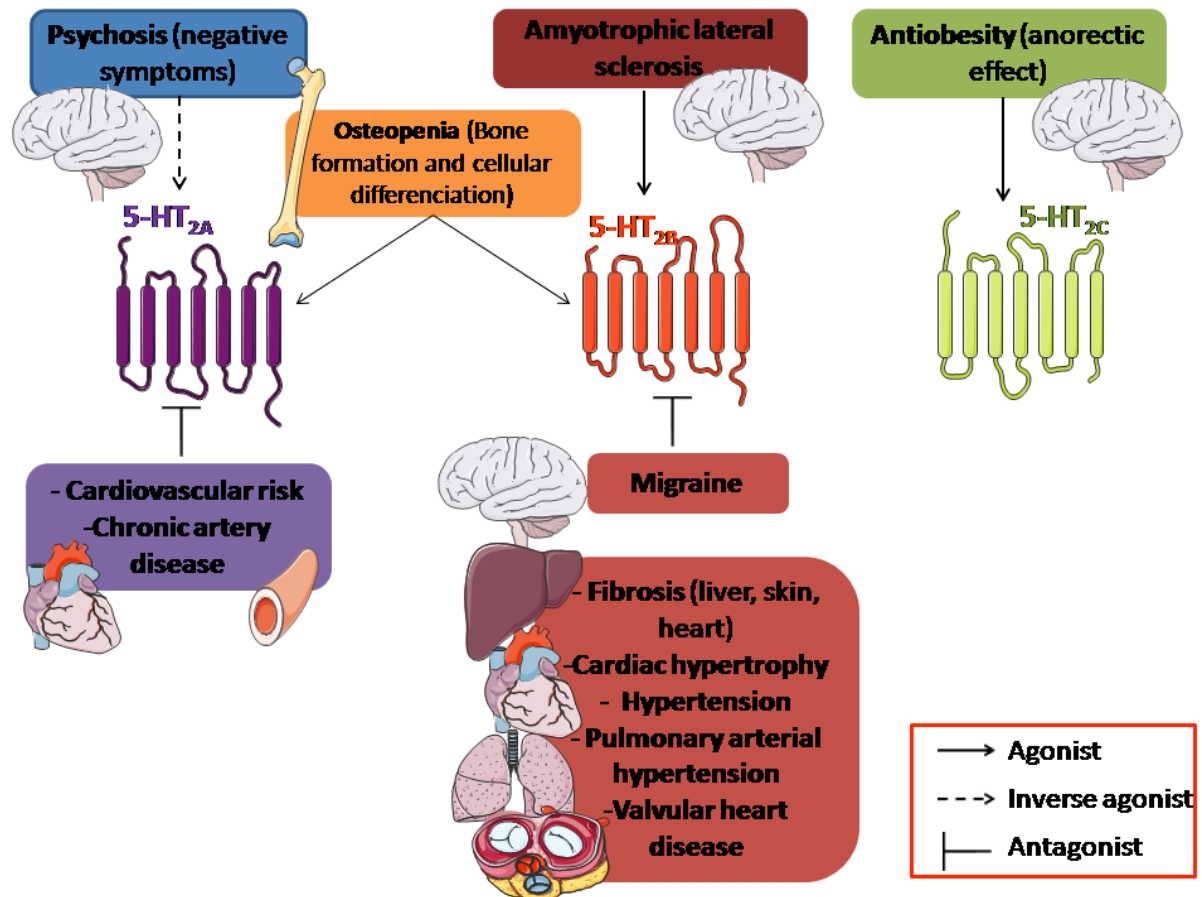
Table I : (Banas, et al., 2011; Cussac, et al., 2008; Cussac, et al., 2002; Knight, et al., 2004; Rashid, et al., 2003; Rosenzweig-Lipson, et al., 2006; Shahid, et al., 2009; Shapiro, et al., 2003; Siuciak, et al., 2007; Thomsen, et al., 2008)

| | | h5-HT$_{2A}$ | r5-HT$_{2A}$ | h5-HT$_{2B}$ | r5-HT$_{2B}$ | m5-HT$_{2B}$ | h5-HT$_{2C}$ | r5-HT$_{2C}$ | m5-HT$_{2C}$ |
		pKi	pKi	pKi	pKi	pKi	pKi	pki	pKi
BW723C86	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			7.89±0.01		8.04±0.15	6.90±0.01		6.78±0.05
	Knight 2004 h$_{2c}$INI	7.2±0.08		7.33±0.03			7.11±0.21		
	Cussac 2008 h$_{2C}$VSV	6.63±0.06		7.85±0.11			7.11±0.01		
RO600175	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			9.01±0.13		8.64±0.14	7.72±0.22		7.35±0.29
	Knight 2004 h$_{2c}$INI	7.44±0.04		8.27±0.06			8.22±0.29		
	Cussac 2008 h$_{2C}$VSV	6.80±0.08		8.66±0.13			7.67±0.07		
WAY161503	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			7.28±0.19		7.84±0.12	7.46±0.05		6.92±0.11
	Rosenzweig 2006 h$_{2c}$?	7.74±0.11		7.22±0.03			8.48±0.14		
CP809101	siuciak $_{2C}$?	8.22±0.15		7.19±0.25			8.80±0.11		
	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			7.86±0.18		8.41±0.18	8.35±0.02		7.72±0.15
DOI	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			8.29±0.18		7.87±0.06	7.60±0.02		7.41±0.24
	Knight 2004 h$_{2c}$INI	9.02±0.11		7.55±0.05			8.08±011		
	Cussac 2008 h$_{2C}$VSV	8.04±0.05		7.78±0.09			7.73±0.04		
Norfenfluramine	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			8.02±0.19		6.76±0.23	7.09±0.62		6.21±0.07
	Knight 2004 h$_{2c}$INI	6.82±0.29		7.00±0.06			7.29±0.04		
D-LSD	Knight 2004 h$_{2c}$INI	9.12±0.06		9.01±0.09			8.96±0.06		
	Cussac 2008 h$_{2C}$VSV	9.49±0.03		9.22±0.02			8.52±0.06		
Lorcaserine	Thomsen $_{2c}$?	6.95±0.03	6.80±0.08	6.76±0.09	6.72±0.01		7.82±0.03	7.54±0.12	
clozapine	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi					7.97±0.09			
	Knight 2004 h$_{2c}$INI	7.60±0.08		7.99±0.09			7.87±0.05		
	Shahid 2009 $_{2c}$?	8.39±0.03		8.79±0.09			8.56±0.06		
aripiprazole	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi					7.21±0.09			
	Schapiro 2003 h$_{2c}$INI	8.06±0.10	7.66±0.08	9.44±0.16			7.12±0.09	7.12±0.04	
	Shahid 2009 $_{2c}$?	8.02±0.16		9.59±0.17				7.55±0.14	
RS1022221	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			6.47±0.02		6.52±0.08	8.01±0.30		7.72±0.22
	Knight 2004 h$_{2c}$INI	5.54±0.03		5.95±0.06			8.30±0.05		
	Cussac 2002 h$_{2C}$VSV			6.63±0.05			8.83±0.04		
SB215505	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			8.12±0.01		7.61±0.21	7.40±0.02		7.24±0.26
	Knight 2004 h$_{2c}$INI								
	Cussac 2002 h$_{2C}$VSV			8.83±0.09			7.95±0.06		
SB206553	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			8.29±0.04		7.06±0.41	8.24±0.01		8.21±0.24
	Knight 2004 h$_{2c}$INI	5.64±0.09		7.65±0.07			7.79±0.07		
	Cussac 2002 h$_{2C}$VSV			8.26±0.17			8.50±0.13		
SB242084	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			6.36±0.02		6.07±0.01	8.19±0.22		5.93±0.27
	Knight 2004 h$_{2c}$INI	6.07±0.18		6.84±0.28			8.15±0.10		
	Cussac 2002 h$_{2C}$VSV			7.34±0.07			9.32±0.06		
Mesulergine	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			8.39±0.2		7.81±0.15	9.01±0.01		8.53±0.21
	Knight 2004 h$_{2c}$INI	7.34±0.03		8.46±0.05			8.74±0.03		
	Cussac 2002 h$_{2C}$VSV			8.71±0.02			8.95±0.06		
RS127445	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			8.51±0.07		8.22±0.24	5.63±0.05		5.33±0.45
	Knight 2004 h$_{2c}$INI	6.03±0.13		8.97±0.09			6.33±0.10		
MDL100907	Banas, 2011, h$_{2c}$INI, m$_{2c}$VNi			5.79±0.60		5.03±0.23	6.79±0.51		6.64±0.17
	Knight 2004 h$_{2c}$INI	8.73±0.20		5.99±0.06			7.52±0.13		
SB204741	Knight 2004 h$_{2c}$INI	<5.00		6.90±0.27			5.56±0.07		
	Cussac 2002 h$_{2C}$VSV			7.29±0.04			5.67±0.11		
Sarpogrelate	Rashid 2003, $_{2c}$?	8.52±0.12		6.57±0.12			7.43±0.03		
Ketanserin	Rashid 2003, $_{2c}$?	9.67±0.12		6.55±0.09			7.39±0.11		