5-HT2 ligands in the treatment of anxiety and depression

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Introduction: One third of depressed patients do not respond adequately to conventional antidepressants including the selective serotonin reuptake inhibitors (SSRIs). Therefore, multi-target drugs or augmentation strategies have been developed for the management of SSRIs-resistant patients. In this context, the 5-HT2 receptor subtypes represent promising targets but their precise roles have yet to be determined.

Areas covered: The aim of this review is to shed some light on the preclinical evidence supporting the use of 5-HT2A and/or 5-HT2C receptor antagonists such as antipsychotics, as potential effective adjuncts in SSRIs-resistant depression. This review synthesizes the current literature about the behavioral, electrophysiological and neurochemical effects of 5-HT2 receptors ligands on the monoaminergic systems but also on adult hippocampal neurogenesis.

Expert opinion: Although studies support the hypothesis that the inactivation of 5-HT2A and/or 5-HT2C receptors might be of interest to reinforce different facets of the therapeutic activity of SSRIs, this pharmacological strategy remains debatable notably because of the lack of chronic data in relevant animal models. Conversely, emerging evidence suggests that the activation of 5-HT2B receptor is required for antidepressant-like activity, opening the way to new therapeutic approaches. However, the potential risks related to the enhancement of monoaminergic neurotransmissions could represent a major concern.

Keywords: 5-HT2 receptors, antidepressant, anxiety, atypical antipsychotics, depression, monoamines, neurogenesis, selective serotonin reuptake inhibitors.

1. Introduction

Anxiety and depression are major burdens on society. They affect 7% of the world’s population, while severe forms of depression impact 2 – 5% of the US population [1]. Furthermore, approximately 32 – 35 million adults in the United States (16%) experience an episode of major depression in their lifetime [2]. While many classes of drugs with antidepressant activity such as selective serotonin reuptake inhibitors (SSRIs) have been developed and approved [3], one third of patients do not respond to these medications [4]. Moreover, for patients who respond, 2 – 4 weeks of treatment are required to achieve a clinically meaningful effect [5]. This delayed action can be somewhat puzzling since SSRIs produce a rapid blockade of the serotonin (5-HT) transporter (SERT) and a concomitant increase in extracellular levels of 5-HT in vivo [6]. This gap in timing between SSRIs near immediate effect on neurotransmitter system and the slow symptomatic recovery has been explained, at least in part, from electrophysiological and microdialysis studies. Indeed, at presynaptic level, SSRIs-induced inhibition of SERT results in the activation of 5-HT1A and 5-HT1B autoreceptors, which produces a rapid suppression of the firing activity of 5-HT neurons and limits 5-HT release; respectively. When the treatment is prolonged, inhibitory 5-HT1A autoreceptor progressively
The pathophysiology of anxiety and depression results, at least in part, from a dysregulation of brain monoaminergic (serotonin, 5-HT; norepinephrine, NE; and dopaminergic, DA) neurotransmission. Monoaminergic neurons have complex functional and reciprocal interactions intimately linked to 5-HT₂ receptors and any action on one system may reverberate in the other systems.

Preclinical studies indicate that the 5-HT₂A and 5-HT₂C receptor subtypes represent promising targets in selective serotonin reuptake inhibitors (SSRIs)-resistant depression. In particular, their pharmacological inactivation with antipsychotics produces antidepressant-like activities per se and potentiates the behavioral effects of SSRIs. Conversely, growing evidence suggests that the stimulation of 5-HT₂B receptor subtype is required for SSRIs-induced antidepressant-like activity.

Because anxiety is a comorbid illness usually observed in depressed patients after acute administration of SSRIs, the beneficial effects of 5-HT₂A and 5-HT₂C receptor antagonists or 5-HT₇B receptor agonists on the antidepressant response could involve anxiolytic activities.

The enhancement of monoaminergic neurotransmission constitutes another property underpinning the putative beneficial effect of 5-HT₂A and 5-HT₂C receptor antagonists in SSRIs-resistant depression.

5-HT₂A and 5-HT₂C receptors are expressed in the dentate gyrus of the hippocampus but their putative involvement in the enhancement of adult neurogenesis has yet to be confirmed.

Lastly, the lack of selective 5-HT₇ receptor ligands prompts fundamental research to combine pharmacological and genetic approaches to highlight the respective contribution of 5-HT₇ receptor subtypes in the modulation of anxiety/depression and related neuronal pathways.

This box summarizes key points contained in the article.

desensitizes thereby producing an enhancement of 5-HT neurotransmission at nerve terminal [6,7]. By using a new strategy to manipulate 5-HT₁A receptor, it has been shown recently that mice with a low expression of 5-HT₁A autoreceptor in the dorsal raphe (DR) display a more rapid increase in extra-cellular levels of 5-HT associated with antidepressant-like phenotype in response to repeated administration of fluoxetine compared to wild-types. These results establish a causal relationship between the delay of antidepressant activity and 5-HT₁A receptor levels [8]. Among the strategies developed for producing faster acting antidepressants, the inactivation of 5-HT₁A autoreceptor with pindolol proved to be effective presumably by preventing the initial decrease in firing activity of 5-HT neurons [5]. However, several studies suggest that stimulation of 5-HT₁A receptor with gepirone or buspirone may also represent a valuable approach to accelerate the therapeutic activity of SSRIs [5]. Although the mechanism of action by which these 5-HT₁A receptor agonists would produce beneficial effects has not been completely solved yet, it is possible that the stimulation of post-synaptic 5-HT₁A heteroreceptor positively affects mood [9,10]. Alternatively, one would expect a more rapid desensitization of 5-HT₁A autoreceptors by combining 5-HT₁A receptor agonist and an SSRI [7,11]. Evidence has also linked the onset of action of SSRIs with the stimulation of adult neurogenesis in the hippocampus, supporting the notion that this event is necessary for antidepressant activity. Increasing serotonergic neurotransmission in response to the chronic administration of SSRIs is thus believed to produce post-synaptic effects including the synthesis of neurotrophic substances such as brain-derived neurotrophic factor (BDNF) and the proliferation/differentiation of stem cells into neurons [12]. The identification of the post-synaptic 5-HT receptors involved in this mechanism is of particular interest.

Certain postsynaptic 5-HT heteroreceptors including 5-HT₃A receptor subtypes have warranted consideration in anxiety, depression and the mechanism of action of related treatments. 5-HT₃A receptors are members of the 7 transmembrane-spanning receptor superfamily frequently referred to as G protein coupled receptors (GPCRs). 5-HT₂A, 5-HT₂B and 5-HT₂C receptors couple to multiple cellular signaling pathways and are involved in the regulation of a variety of physiological brain functions [13] thereby constituting therapeutic targets for obesity, sleep, memory, addiction or psychiatric disorders such as schizophrenia, anxiety and depression [14-16]. The connection between mood disorders, antidepressant responses and 5-HT₃ receptors (mainly 5-HT₂A and 5-HT₂C subtypes) is based on several preclinical and clinical observations. First, pharmacogenetic studies in human have reported that one of the most investigated polymorphism within the 5-HT₂A gene A-1438 (rs6311) is associated with antidepressant response [17]. Regarding the 5-HT₂C receptor, although Cys23ser polymorphism-associated changes in 5-HT₂C receptor function were found to influence the vulnerability to affective disorders [18,19], there are, however, no unequivocal results for such association with antidepressant response [20]. Second, an hypersensitivity/upregulation of 5-HT₂A receptors [21,22] as well as an increased RNA editing and/or functional activity of 5-HT₂C receptor has been reported in depressed patients or in animal models of depression. Conversely, the progressive therapeutic improvement achieved with some SSRIs in rodents is accompanied by a downregulation of 5-HT₂A and 5-HT₂C receptors in rodents [23]. Accordingly, studies performed in 5-HT transporter knock-out (KO) mice, indicated that a lifelong elevation of the synaptic 5-HT concentration resulted in a downregulation of 5-HT₂A and 5-HT₂C receptor-mediated phospholipase A2 (PLA2) signaling [24] and in a reduced expression of these receptors [25]. Although the extrapolation of these findings to humans remains indeterminate, a decrease in platelet functional response mediated by 5-HT₂A receptors following imipramine treatment was reported in depressed patients. Hence, the desensitization or downregulation of
this receptor could be linked to the therapeutic effects of some antidepressants [26]. Imaging studies also supported this hypothesis since a downregulation of 5-HT\textsubscript{2A} receptor was detected in the brain of depressed patients in response to SSRI treatment [27]. It is noteworthy that citalopram, does not produce 5-HT\textsubscript{2A} or 5-HT\textsubscript{2C} receptor downregulation [28] while being as effective as other SSRIs in the treatment of depression and depressive-like states, thus suggesting that such adaptive change is not sufficient to account for the therapeutic activity of this class of antidepressants. Third, the intrinsic antidepressant activity of compounds displaying 5-HT\textsubscript{2A} or 5-HT\textsubscript{2C} receptor antagonistic activity such as atypical antipsychotics in placebo-controlled trials [29] and the reports of the augmenting action of these pharmacological agents in SSRIs-resistant patients [30,31], further support the interest of inactivating both receptors in the treatment of depression. In an attempt to determine the mechanism of action of atypical antipsychotics, particularly on monoaminergic neurotransmission, it has been demonstrated that the sustained inactivation of the SERT induced by SSRIs progressively dampens the firing activity of noradrenergic and dopaminergic neurons via activation of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors in the locus coeruleus (LC) and ventral tegmental area (VTA), respectively [32-34]. The blockade of these electrophysiological responses with aripiprazole, olanzapine, paliperidone or risperidone [35] increases extracellular levels of catecholamines [36-38] which exert excitatory influence upon the 5-HT system. In addition, since the enhancement of NE or DA neurotransmission achieved with NE and/or DA reuptake inhibitors can lead to an antidepressant activity, it is conceivable that these actions account, at least in part, for the beneficial therapeutic effects of antipsychotics in SSRIs-resistant depression. Finally, there is a co-localization between 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors within brain monoaminergic areas such as the DR nucleus, the LC, VTA and their respective nerve terminal regions eg the frontal cortex (FCx), the nucleus accumbens (NAc), the amygdala and the hippocampus that might add to our comprehension of the mechanism of action of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptor agonists and antagonists.

The present review describes the distribution and the behavioral properties of 5-HT\textsubscript{2} receptors in relation with anxiety and depression in rodents supporting the idea that 5-HT\textsubscript{2A}, 5-HT\textsubscript{2C}, but also 5-HT\textsubscript{2B} receptor agonists/antagonists, might be useful in the treatment of these pathologies given either alone or in combination with conventional antidepressants. The impact of 5-HT\textsubscript{2} receptors and related pharmacological agents in the modulation of monoaminergic neurotransmission is also addressed particularly from electrophysiological and neurochemical studies. Lastly, since growing evidence suggests that part of the antidepressant activity of SSRIs is mediated by the stimulation of adult neurogenesis in response to increased monoaminergic tone in the hippocampus [12], this review raises the possibility that 5-HT\textsubscript{2} receptor ligands might significantly influence cell fate.

Importantly, current research is facing the challenge of the absence of selective 5-HT\textsubscript{2} receptor subtype ligands (Table 1). As an example, after cloning the mouse 5-HT\textsubscript{2C} receptor cDNAs, the respective affinity for 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C} receptors of commercially available compounds was recently determined. The study revealed that, among these compounds, many of the so-called selective 5-HT\textsubscript{2C} compounds have similar (if not higher) affinity for 5-HT\textsubscript{2B} receptors in mice [39]. This point is important since it considerably limits the understanding of the precise role of the 5-HT\textsubscript{2} receptor subtypes in the control of mood and monoaminergic neurotransmission and might have directed researches in wrong directions. Similar remarks can be made with the lack of selective 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C} receptor antibodies that force us to consider immunohistochemistry data with caution. In this prospect, combination of pharmacological and genetic approaches will be extremely helpful and could even contradict some obvious demonstrations.

2. Distribution of 5-HT\textsubscript{2} receptor subtypes in rodent brain regions related to mood disorders

In the rat brain, 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors have a wide-spread distribution particularly in monoaminergic regions (Figure 1 and Table 2). Their first mapping comes from an in situ hybridization study consisting in visualizing specific mRNA probes for 5-HT\textsubscript{2} receptors. In this initial study, high levels of 5-HT\textsubscript{2A} receptor mRNA were detected in cortical areas including the frontal, cingulate and piriform cortices, but also in the amygdala [40]. In the hippocampus, 5-HT\textsubscript{2A} receptor mRNA levels were low in the pyramidal cell layer of the CA2 and CA1 but they were very high in the ventral part of CA3 and the dentate gyrus (DG) [40]. Intermediate levels of 5-HT\textsubscript{2A} receptor were seen in the NAc. Finally, in brainstem nuclei, low levels of 5-HT\textsubscript{2A} receptor mRNA were found in the raphe nuclei [40,41], while no signals were detected in the LC and the VTA [40]. Advances in the field of autoradiography have also yielded considerable information on the regional distribution of 5-HT\textsubscript{2} binding sites. However, autoradiography, as well as in situ hybridization approaches, lacks high resolution prompting research to develop immunohistochemistry at the light and electron microscopic levels. Using this approach, Descaries' group confirmed the localization of 5-HT\textsubscript{2A} receptor obtained from in situ hybridization studies. However, differences in both the number of labeled cells and the intensity of immunolabeling between anatomical regions were reported [42]. For example, in the cingulate, frontal and parietal cortices, a high number of large layer V pyramidal neurons exhibit intense 5-HT\textsubscript{2A} receptor labeling of their soma and dendrites [42,43]. In the amygdala, most nuclei displayed soma-dendritic labeling of weak to strong intensity but relatively low abundance, except in the basolateral nucleus [42]. The hippocampal formation showed some of the most intense and abundant 5-HT\textsubscript{2A} immunostaining in the rat brain,
particularly in the DG and throughout the pyramidal neurons in CA1, CA2 and CA3 [42]. Interestingly, in contrast to in situ hybridization, immunoreactive soma/dendrites and axons were visible at all monoaminergic brainstem levels. Abundant, but less intense, immunoreactivity was observed in the median raphe nucleus. There were no labeled soma/dendrites within the dorsal raphe nucleus. In the pons, only few weakly labeled dendrites, but no labeled soma, were visible in the LC. In contrast, moderate to strong somatodendritic labeling was found in the VTA [42]. Recently, it has been reported that cells within the rat VTA displayed a strong immunoreactivity for 5-HT2A receptor co-localized with tyrosine hydroxylase suggesting the presence of this receptor subtype on DA neurons cell bodies [44,45]. Although more rare, non-DA neurons would also express 5-HT2A receptor immunoreactivity in the VTA [45].

Regardless 5-HT2C receptor, immunohistochemical analysis of the rat brain unveiled the presence of this receptor subtype in the cerebral cortex with highest levels in the frontal, parietal and cingulate cortices [46,47]. Most of the nuclei in the amygdala contained dense 5-HT2C receptor labeling. Abundant immunopositive neurons were found in the pyramidal cell layer of the CA1, CA2 and CA3 regions of the hippocampus [46,47]. In the brainstem, the raphe nucleus is endowed with a rich population of 5-HT2C receptor suggesting the presence of this protein on 5-HT neurons. Using double in situ hybridization to examine the cellular localization of 5-HT2C receptor mRNA in relation to serotonergic and GABAergic neurons, it was reported that in the dorsal and median raphe nuclei, 5-HT2C receptor mRNA was not detected in serotonergic cells identified as those expressing 5-HT transporter mRNA. In contrast, 5-HT2C receptor mRNA was found in most GABAergic cells, identified by the presence of glutamic acid decarboxylase (GAD) mRNA [48]. Although data are lacking regarding the putative localization of 5-HT2C receptor in the LC, using a double double-label immunofluorescence techniques in the VTA, it has been recently reported that the 5-HT2C receptor was present on GABA neurons in rats [49] but also present in cells that contained immunoreactivity for tyrosine hydroxylase, validating the localization of this receptor type directly on DA neurons which project to the NAc [49,50].

Finally, in the rat brain 5-HT2B receptor is present in discrete nuclei and evidence for its presence in the central nervous system (CNS) mainly comes from pharmacological studies. The first characterization of the 5-HT2B receptor in the adult brain was obtained from the mouse brain using a subtype-specific antiserum directed against this receptor. Data showed that the expression of this receptor was mainly found in cerebellar nuclei [51].

### 3. The Role of 5-HT2 receptors in anxiety and depression

Pharmacological evidence supports a role of 5-HT2 receptors in the modulation of anxiety and depression. As an example, the non-selective 5-HT2 receptor agonist mCPP precipitates anxiogenic responses in naive rodents [52-56], whereas numerous examples unveiled an anxiolytic-like effect of non-selective 5-HT2 receptor antagonists including ritanserin, mesulergine and ketanserin [56-61]. Despite these data, little is known about the specific contribution of each 5-HT2 receptor subtype in the modulation of these behavioral responses because of the lack of highly selective receptor ligands. This chapter synthesizes the current knowledge about the effects of the preferential 5-HT2A, 5-HT2B and 5-HT2C receptor agonists/antagonists in animal paradigms used to screen anxiolytic-/antidepressant-like activities (Table 3). It also provides an overview of the electrophysiological and neurochemical effects of these ligands on the monoaminergic systems that might explain, at least in part, their behavioral effects.

#### 3.1 The 5-HT2A receptor

#### 3.1.1 Behavioral studies

In the mouse elevated plus maze (EPM), the preferential 5-HT2A receptor agonist DOI produced anxiolytic-like activity that was attenuated by mianserin, ketanserin or the 5-HT2A receptor antagonist SR46949B [62]. The observation that the 5-HT2C or 5-HT2B receptor antagonists RS10-2221 and SB206553, respectively, failed to block DOI-induced anxiolysis demonstrated the selective involvement of 5-HT2A receptor in this activity [61,63]. This response is apparently mediated by post-synaptic 5-HT2A receptor since

Table 1. Affinity constants for various 5-HT2 receptor agonists and antagonists.

<table>
<thead>
<tr>
<th></th>
<th>5-HT2A</th>
<th>5-HT2B</th>
<th>5-HT2C</th>
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<tbody>
<tr>
<td>Agonists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>6.50 ± 0.13</td>
<td>6.73 ± 0.09</td>
<td>6.69 ± 0.12</td>
</tr>
<tr>
<td>mCPP</td>
<td>7.26 ± 0.02</td>
<td>7.39 ± 0.02</td>
<td>7.85 ± 0.07</td>
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<tr>
<td>DOI</td>
<td>9.03 ± 0.11</td>
<td>7.55 ± 0.13</td>
<td>8.08 ± 0.11</td>
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<tr>
<td>BW723C6</td>
<td>ND</td>
<td>7.89 ± 0.01</td>
<td>6.90 ± 0.01</td>
</tr>
<tr>
<td>Ro600175</td>
<td>7.44 ± 0.04</td>
<td>8.27 ± 0.06</td>
<td>8.22 ± 0.28</td>
</tr>
<tr>
<td>MK212</td>
<td>5.99 ± 0.06</td>
<td>6.21 ± 0.09</td>
<td>7.01 ± 0.09</td>
</tr>
<tr>
<td>WAY161503</td>
<td>ND</td>
<td>7.28 ± 0.19</td>
<td>7.46 ± 0.05</td>
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</table>

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>5-HT2A</th>
<th>5-HT2B</th>
<th>5-HT2C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ritanserin</td>
<td>8.34 ± 0.09</td>
<td>8.67 ± 0.09</td>
<td>8.18 ± 0.15</td>
</tr>
<tr>
<td>MDL100907</td>
<td>8.73 ± 0.20</td>
<td>5.99 ± 0.06</td>
<td>7.52 ± 0.13</td>
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<tr>
<td>RS127455</td>
<td>6.03 ± 0.13</td>
<td>8.97 ± 0.09</td>
<td>6.33 ± 0.10</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>8.09 ± 0.08</td>
<td>6.13 ± 0.07</td>
<td>7.21 ± 0.12</td>
</tr>
<tr>
<td>Mianserin</td>
<td>7.74 ± 0.13</td>
<td>7.92 ± 0.03</td>
<td>8.26 ± 0.07</td>
</tr>
<tr>
<td>S20066</td>
<td>6.00 ± 0.07</td>
<td>8.03 ± 0.05</td>
<td>8.43 ± 0.06</td>
</tr>
<tr>
<td>SB206553</td>
<td>5.64 ± 0.09</td>
<td>7.65 ± 0.07</td>
<td>7.79 ± 0.07</td>
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<tr>
<td>SB242084</td>
<td>6.07 ± 0.13</td>
<td>6.84 ± 0.28</td>
<td>8.15 ± 0.10</td>
</tr>
<tr>
<td>SDZ SER082</td>
<td>6.29 ± 0.03</td>
<td>6.69 ± 0.05</td>
<td>8.12 ± 0.07</td>
</tr>
</tbody>
</table>

Higher the pKi value higher is the affinity for the corresponding human recombinant 5-HT2 receptor subtype. These values have been obtained from binding studies into transfected HEK-293 or CHO cells.

Adapted from [39,245].
the lesion of the serotonergic or the noradrenergic system did not affect the anxiolytic-like effect of DOI evaluated, for example, in the four plate test (FPT) [64]. In order to determine the brain region involved in this behavior, the possibility that the anxiolytic-like effects of DOI resulted from the activation of 5-HT2A receptors in the hippocampus [65] or the periaqueductal grey (PAG) [66] has been proposed notably after its local microinjection in these brain regions. Interestingly, studies indicate that the 5-HT2A receptor antagonist MDL100907 alone lacked consistent activity in selected rodent models of anxiety [60,67], thus suggesting that 5-HT2A receptor is not tonically activated to regulate this behavior or that distinct neuro-anatomical pathways are involved with major regional differences. To illustrate this latter hypothesis, a reduced level of anxiety has been reported in constitutive 5-HT2A receptor KO mice evaluated in the EPM, the open field (OF) and the light-dark [68]. The selective re-introduction of the 5-HT2A receptor in the cortex normalized anxiety-like behavior in these 5-HT2A KO mice. Consequently, although data emphasized that 5-HT2A receptor activation in the hippocampus or PAG produced anxiolysis, a specific anxiogenic role of this receptor in the cortex is also possible [68].

Numerous studies have associated 5-HT2A receptor activation with a depressive-like behavior. In behavioral paradigms relevant to depression, DOI significantly increased immobility time in the mouse forced swim test (FST) [73], and this effect was abolished by a pretreatment with MDL100907 [69]. This result strongly suggested that 5-HT2A receptor antagonists might produce antidepressant-like activity. Accordingly, in the rat FST, the 5-HT2A receptors antagonists EMD281014 or MDL100907 decreased the immobility time [70,71]. Similarly, the novel 5-HT2A receptor antagonist BIP-1 has been shown to produce antidepressant-like activities in the mouse FST and TST [72]. Although, these findings are consistent with the observation that antisense-mediated downregulation of the 5-HT2A receptor decreased immobility in the mouse FST [73], the role and mechanism of action of 5-HT2A receptor in other animal paradigms relevant to depression or after chronic administration should inspire us for future investigations. In this prospect, it has been demonstrated recently that the sustained administration of BIP-1 attenuated behavioral anomalies detected in bulbectomized rats [72]. Finally, the blockade of 5-HT2A receptor by MDL100907 augmented the antidepressant-like effects of fluoxetine in rats [74] underlying the possible synergic effects between these pharmacological agents.

**3.1.2 Electrophysiological studies**

*In vitro* recordings in the DR showed that the local application of 5-HT produced hyperpolarization of tryptophane hydroxylase (Tph) positive neurons [75]. Similarly, in rat brain slices, DOI induced a concentration-dependent increase in the frequency of inhibitory postsynaptic currents (IPSCs). Together with the observation that these effects were blocked by bicuculline or MDL100907, a local interaction between 5-HT2A receptor and GABAergic neurons in the DR has been proposed [76]. Indeed, although low levels of 5-HT2A receptor mRNA have been detected in the DR [40,41], endogenous 5-HT would act on excitatory 5-HT2A receptor located on GABA neurons that would ultimately suppress the firing of DR 5-HT neurons. This mechanism is consistent with the hypothesis that the 5-HT2A receptor antagonists could prevent the inhibitory effects of SSRIs on 5-HT neurons by preventing the local release of GABA within the DR. In *in vivo* studies performed in rodents confirmed these *in vitro* data since the systemic or local administration of DOI in the DR reduced the discharge of 5-HT neurons [77-82].

![Figure 1. Distribution of the 5-HT2A, 5-HT2B and 5-HT2C receptors in the brain monoaminergic regions involved in anxiety and depression.](Image)
and these effects were reversed by ritanserin or MDL100907 [81]. Importantly, the systemic administration of DOI increased c-Fos immunoreactivity in the DR specifically in GABAergic interneurons [83]. The role of 5-HT$_{2A}$ receptor in the regulation of DR 5-HT neuronal activity might also involve indirect mechanisms. As an example, the LC that sends noradrenergic projections to the DR [84,85], expresses 5-HT$_{2A}$ receptor. It is well accepted that sustained SSRIs administration enhanced 5-HT transmission to NE neurons in the LC [32,86,87] thereby producing a marked suppression of the firing activity of NE neurons through activation of excitatory 5-HT$_{2A}$ receptors located on GABAergic interneurons [32,86,87]. Given the excitatory influence of noradrenergic terminals on 5-HT neurons in the DR (Figure 2) [88,89], activation of 5-HT$_{2A}$ receptor in the LC seemingly favors the inhibition of the serotonergic system and might account for the depressive-like behavior induced by acute administration of DOI.

Another modality of interaction between 5-HT$_{2A}$ heteroreceptor and DR 5-HT neurons concerns the recruitment of the medial prefrontal cortex (mPFCx) [42]. Several studies from Dr Arigas’ group demonstrated that the local application of DOI in this cortical area increased the firing rate of DR 5-HT neurons [80,82]. This might result from activation of excitatory cortical glutamatergic pyramidal neurons projecting to the DR in response to DOI administration (Figure 2) and might explain, at least in part, that the systemic administration of 5-HT$_{2A}$ receptor antagonists such as risperidone or clozapine, dose-dependently decreased DR 5-HT neuronal activity [90,91]. In addition, evidence also demonstrated that 5-HT$_{2A}$ receptor located in the mPFCx modulated the neuronal activity of VTA DA neurons [92] which have strong anatomical and functional interactions with the DR [85]. Electrophysiological studies showed that the systemic or local injection of DOI in the mPFCx increased VTA DA firing rate [93], an effect that might contribute to facilitate the activation of 5-HT neurons since several studies demonstrated the excitatory impact of DA in the DR [85,89,94]. Therefore, it appears that 5-HT$_{2A}$ receptor activation elicits inhibitory or excitatory influences on DR 5-HT neuronal activity depending on the brain region where this receptor type is recruited.

3.1.3 Neurochemical studies

In agreement with the fact that activation of 5-HT$_{2A}$ receptor can reduce the firing activity of DR 5-HT neurons, it has been demonstrated that the systemic administration of DOI to chloral hydrate-anesthetized rats reduced the extracellular 5-HT concentration in the mPFCx, an effect antagonized by MDL100907 [80]. Despite this result, the local application of DOI, through reverse dialysis, in the medial rat or mouse PFCx dose-dependently increased 5-HT local outflow which is blocked by the application of MDL100907, but not of SB242084 [80,82]. Subsequent studies demonstrated that the neurochemical effects of DOI in the mPFCx involved the activation of 5-HT$_{2A}$ receptor expressed on glutamatergic neurons. This activation would enhance the activity of excitatory pyramidal neurons projecting to the DR, thus increasing serotonergic activity and 5-HT release in projection areas. Additionally, glutamate might also activate terminal glutamatergic receptors located on 5-HT terminals and increase 5-HT transmission in the NAc [106]. Interestingly, the electrical release locally in the mPFCx increased VTA DA firing rate [92] which have strong anatomical and functional interactions with the DR [85]. Therefore, it appears that 5-HT$_{2A}$ receptor activation elicits inhibitory or excitatory influences on DR 5-HT neuronal activity depending on the brain region where this receptor type is recruited.

Interactions between 5-HT$_{2A}$ and DA neurons in the PFCx may also be important in the modulation of DA release. In rat, the systemic or local injection of DOI in the mPFCx increased cortical DA release [97-102] probably through the recruitment of a specific population of excitatory cortical pyramidal neurons projecting to the VTA [93,103]. Alone, the 5-HT$_{2A}$ receptor antagonists MDL100907, MDL11939 and SR46549B injected systemically or directly in the rat mPFCx through reverse dialysis had little or no effect on basal DA efflux [98,100]. In the NAc, the systemic administration of DOI also resulted in a robust increase in DA outflow [104,105]. These results agreed with the observation that stimulated DA release was blocked by MDL100907, thus strengthening the hypothesis that 5-HT$_{2A}$ receptor activation favors DA neurotransmission in the NAc [106].

### Table 2. Relative distribution of 5-HT2 receptors in monoaminergic region in rat.

<table>
<thead>
<tr>
<th>Monoaminergic terminal regions</th>
<th>Density of 5-HT2 receptors</th>
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<tbody>
<tr>
<td></td>
<td>5-HT$_{2A}$</td>
</tr>
<tr>
<td>Cortex</td>
<td>+++, ++</td>
</tr>
<tr>
<td>Frontal</td>
<td>++</td>
</tr>
<tr>
<td>Parietal</td>
<td>++</td>
</tr>
<tr>
<td>Cingulate</td>
<td>++</td>
</tr>
<tr>
<td>Amygdala</td>
<td>+</td>
</tr>
<tr>
<td>Basolateral nucleus</td>
<td>+</td>
</tr>
<tr>
<td>Medial nucleus</td>
<td>+</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>CA1/CA2</td>
</tr>
<tr>
<td>CA3</td>
<td>+++</td>
</tr>
<tr>
<td>DG</td>
<td>+++</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>+</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Monoaminergic somatodendritic region</th>
<th>Density of 5-HT2 receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal raphe nucleus</td>
<td>+</td>
</tr>
<tr>
<td>Ventral tegmental area</td>
<td>+</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>ND</td>
</tr>
</tbody>
</table>

The intensity of immunostaining is graded as weak (+), moderate (++) or strong (+++).
Adapted from [42,47].
ND: Not Determined.

Figure 2 Interactions between 5-HT$_{2A}$ and DA neurons in the PFCx...
stimulation of the DR eliciting the release of endogenous 5-HT has been reported to increase the DA neurotransmission in the NAc, and this effect was attenuated by the 5-HT2A receptor antagonist SR46349B, but not the 5-HT2B/2C receptor antagonist SB206553 (Figure 3) [107].

Table 3. Behavioral effects of 5-HT2 receptor ligands given alone in animal paradigms relevant to anxiety/depression.

<table>
<thead>
<tr>
<th>5-HT2 receptor ligand</th>
<th>Route</th>
<th>Test</th>
<th>Specie</th>
<th>Behavioral property</th>
<th>Ref.</th>
</tr>
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<td>Syst</td>
<td>SIT</td>
<td>Mouse</td>
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<td>Mouse</td>
<td>[119]</td>
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<td>Rat</td>
<td>[138]</td>
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</tr>
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<td>Rat</td>
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<td>Mouse</td>
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<tr>
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<td>Rat</td>
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<tr>
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<td>Syst</td>
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<tr>
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<tr>
<td>DOI</td>
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<td>FST</td>
<td>Mouse</td>
<td>[69]</td>
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<td>Mouse</td>
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<td>Rat</td>
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<td>Rat</td>
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<tr>
<td>S32006</td>
<td>Syst</td>
<td>FST</td>
<td>Rat</td>
<td>[131]</td>
<td></td>
</tr>
</tbody>
</table>

Signs † indicate an anxiogenic- or depressive-like activity. Signs ‡ indicate an anxiolytic or antidepressant-like activity. Syst: systemic administration.

Regarding the modulation of NE neurotransmission by 5-HT2A receptor, DOI has been reported to exert an excitatory effect on the release of NE in the FCx of freely-moving rats [62].
moving rats [98] but further studies are required to precise the
nature of interactions between 5-HT 2A receptor and NE out-
flow at nerve terminals.

3.2 The 5-HT2B receptor

3.2.1 Behavioral studies

The involvement of 5-HT 2B receptor in mood disorders has
been poorly studied notably due to the absence of highly
selective compounds. Despite such a deficiency, the recent
demonstration that the 5-HT 2B receptor is located in brain
regions relevant to anxiety and depression, strongly suggests
their role in psychiatric disorders. For example, it has been
proposed, that the stimulation of 5-HT 2B receptor produces
anxiolysis [108]. As observed with benzodiazepines, the
5-HT 2B receptor agonist BW723C86 increased the number
of punishments accepted in a rat Vogel drinking conflict test
indicative of an anxiolytic-like effect whereas this response
was prevented by a 5-HT 2B, but not a 5-HT 2C, receptor
antagonist [109]. The anxiolytic-like effect of BW723C86
would involve the amygdala since its bilateral microinjection
in this brain region increased the total interaction in the
rat social interaction test, whereas this effect was prevented
by the 5-HT 2B/2C receptor antagonist SB200646A [110].

Consistent with these results, BW723C86 also provoked an
anxiolytic-like response in the mouse FPT and the EPM [61,63].

In the mouse FST, recent findings have shown that
BW723C86 significantly reduced immobility time in wild-
types but not in 5-HT 2B KO mice [69]. Interestingly, although
the genetic inactivation of 5-HT 2B receptor failed to affect
the spontaneous behavior of mice in the FST, 5-HT 2B KO

Figure 2. Neuro-anatomical substrates underlying the putative role of 5-HT 2A, 5-HT 2B and 5-HT 2C receptor in the modulation
doctor of dorsal raphe 5-HT neuronal activity. In the dorsal raphe (DR), 5-HT 2A/2C receptors stimulate GABAergic interneurons thus
triggering the inhibition of firing of neighboring 5-HT neurons. The presence of 5-HT 2A receptor in the DR mainly comes from
functional studies. A recent hypothesis assumes that 5-HT 2B receptor would be directed express on DR 5-HT neurons and their
stimulation would exert excitatory influence on this neuronal population. 5-HT 2A/2C receptor are also present in the ventral
tegmental area (VTA) and the locus coeruleus (LC). 5-HT 2A and 5-HT 2C receptors are believed to play a prominent inhibitory
effect on noradrenergic and dopaminergic neurons respectively through the stimulation of local GABA release. Remarkably,
the VTA 5-HT 2C receptor is also express on DA neurons where it could exert excitatory effect. Because VTA DA and LC NE
neurons have strong anatomical and functional (excitatory) interaction with DR 5-HT neurons, the inhibition of
catecholaminergic neurons in response to 5-HT 2A and/or 5-HT 2C receptor would favor the inhibition of serotonergic neurons
through an attenuation of excitatory tone. Finally, there is a high density of 5-HT 2A receptor in the prefrontal cortex (PFCx),
particularly on pyramidal neurons. The activation of these cells in response to 5-HT 2A receptor would enhance the activity of
DR 5-HT by a direct or indirect pathway involving VTA DA neurons. Size of the symbols represent the relative density of 5-HT 2
receptor subtypes.
mutants did not respond to the doses of SSRIs that were efficacious in reducing immobility time in wild-type littermates [69]. These results indicate that 5-HT 2B receptor is required for acute SSRIs effects. Accordingly, it has been recently reported that chronic administration of paroxetine or fluoxetine failed to elicit antidepressant-like effects in 5-HT2B KO mice or in 5-HT2B wild-types treated with the 5-HT2B receptor RS127445 in the novelty suppressed feeding paradigm [111] confirming that this receptor is required for SSRIs-mediated antidepressant-like activity [111].

3.2.2 Electrophysiological studies
The contribution of central 5-HT2B receptors in the modulation of monoaminergic neuronal activities has not been documented, likely due to its modest expression in the rodent brain [51]. Nevertheless, the presence of 5-HT2B receptor has been reported in a large majority Tph2 expressing cells suggesting for the first time a possible existence of an excitatory autoreceptor regulating the 5-HT system [111]. Such a property would explain the fact that the 5-HT2B receptor is required for the antidepressant-like activity of SSRIs. In a near future, electrophysiological studies will likely address this possibility, notably by studying the putative excitatory effect of 5-HT2B receptor agonists on DR 5-HT neuronal activity. In particular such approach would be relevant under conditions that produce a marked attenuation of 5-HT synthesis and/or release.

In the VTA, it has been shown that the non-selective 5-HT2B/2C receptor antagonist SB206553 caused a dose-dependent increase in the basal firing rate of DA neurons associated with an increase in bursting activity [112,113]. These results suggest that 5-HT2B receptor exerts an inhibitory influence on DA neurons, but once again, the lack of selectivity of SB206553 do not allow identifying the respective contribution of 5-HT2B and/or 5-HT2C receptors. In the LC, excitatory effects of SB206553 were reported on the firing activity of NE neurons but the observation that these compound reversed the 5-HT2C receptor agonist Ro 600175-induced complete inhibition of LC NE neuronal activity strongly suggested the involvement of 5-HT2C receptor in these electrophysiological response [113].

3.2.3 Neurochemical studies
A recent microdialysis study in mice reported that the local injection of the 5-HT2B Receptor agonist BW723C86 in the DR increased the local extracellular levels of 5-HT whereas pretreatment with the selective 5-HT2B receptor antagonist RS127445 completely blocked this effect [114]. This is compatible with the existence of a positive feedback. However, the 5-HT2B receptor antagonist RS127445 or the genetic inactivation of this receptor type in 5-HT2B KO mice had

Figure 3. Neuro-anatomical substrates underlying the putative role of 5-HT2A, 5-HT2B and 5-HT2C receptor in the modulation of 5-HT release at terminal level. The dorsal raphe (DR) sends serotonergic projection in the nucleus accumbens (NAc) and the frontal cortex (FCx). The release in 5-HT in the NAc and the FCx would be positively regulated by 5-HT2B autoreceptors located presynaptically on 5-HT terminals. Increase in 5-HT release stimulates 5-HT2A and 5-HT2B heteroreceptors located postsynaptically on GABAergic interneurons thus inhibiting the local release of dopamine (DA) and/or norepinephrine (NE). In addition 5-HT2A receptors have been identified on cortical pyramidal neurons and its activation on pyramidal neurons produces release of glutamate which in turn activate the meso- or cortico-limbic pathway at distinct levels i.e., the VTA, the FCx or the NAc.
no effect on basal 5-HT extracellular concentrations in the DR, the hippocampus or the NAc [111,114] suggesting that this receptor is recruited under specific conditions to stimulate the release of 5-HT. Accordingly, it has been shown that the increase in extracellular concentrations of 5-HT in the hippocampus induced by the SSRI paroxetine in WT mice was attenuated in mice pre-treated with the 5-HT<sub>2B</sub> receptor antagonist RS127445 antagonist or in 5-HT<sub>2B</sub> KO mice [111]. Although the mechanism by which activation of 5-HT<sub>2B</sub> receptor may stimulate the release of 5-HT is still not known, the observation that MDMA induced 5-HT release from superfused midbrain synaptosome preparation is blunted in 5-HT<sub>1B</sub> KO mice, reinforced the hypothesis that 5-HT<sub>2B</sub> receptor would act presynaptically in DR 5-HT neurons to enhance 5-HT release [114].

Regarding the other monoaminergic systems, recent studies suggest that 5-HT<sub>2B</sub> receptor agonists or antagonists had no influence on basal DA outflow in the rat NAc [115]. However, in the NAc, it has been shown that the selective 5-HT<sub>2B</sub> receptor antagonist LY266097 significantly reduced basal or stimulated DA release by haloperidol supporting a facilitatory control on mesoaccumbens DA pathway activity [115,116]. Finally, RS127445 completely blocked MDMA-induced increase in DA release in the VTA [114] and this effect could be attributed to a decreased serotonergic tone in the VTA and/or NAc, two brain regions where activation of postsynaptic 5-HT receptors such as 5-HT<sub>2A</sub> receptor are known to exert stimulatory effects [117]. In the mPFCx, RS127445 has been shown to increase basal DA levels or potentiate haloperidol-induced increase in cortical DA levels [116]. These results demonstrate that the 5-HT<sub>2B</sub> receptor exerts a region-dependent modulation of DA ascending pathways by producing opposite facilitatory and inhibitory control of NAc and mPFCx DA release.

### 3.3 The 5-HT<sub>2C</sub> receptor

#### 3.3.1 Behavioral studies

Evidence also suggests the involvement of 5-HT<sub>2C</sub> receptor in anxiety. The observation that the anxiolytic-like effects of the non selective 5-HT<sub>2</sub> receptor agonist mCPP in rats [118-120], were blocked by various 5-HT<sub>2C</sub> receptor antagonists given either systemically or directly injected in the amygdala, supported this hypothesis [121]. The role of amygdala has been strengthened by the observation that the microinjection of the 5-HT<sub>2C</sub> receptor agonists mCPP or CP809101 into the amygdala induced anxiogenic-like effects [121] or mimicked the effect of stress in rats [122]. These results also agree with findings reporting that the systemic injection of the preferential 5-HT<sub>2C</sub> receptor agonists MK-212 or Ro600175 produced anxiogenic-like effects in rodents [55,123-125] whereas such responses were abolished by the microinjection of ritalserin into the basolateral nucleus of the amygdala (BLA) [123]. Given the lack of high selectivity of these agonists, part of their anxiogenic-like activity might have resulted from the activation of 5-HT<sub>2B</sub> receptor. This is, however, unlikely since we have detailed above the anxiolytic effect of 5-HT<sub>2B</sub> receptor activation. A recent study aimed at increasing 5-HT<sub>2C</sub> receptor expression in the mouse amygdala using recombinant viral approach reported a robust correlation between anxiogenic-like effects in the EPM and OF and the density of the receptor [126]. In addition, the observation that the microinjection of MK-212 in the rat ventral, but not dorsal, hippocampus also resulted in anxiogenic-like effects in the EPM [127] indicated that other brain regions of the limbic system are involved in the anxiogenic effect of 5-HT<sub>2C</sub> receptor activation. Conversely, the genetic or pharmacological inactivation of 5-HT<sub>2C</sub> receptor with SB200646A, SB242084 or S32006 reduced anxiety levels [56,125,128,129] but evidence suggests that stressful or anxiogenic conditions are a prerequisite for such behavioral activity [23,130,131].

With respect to depression, initial studies reported that the 5-HT<sub>2C</sub> receptor agonist WAY-163909 decreased immobility time in the rat FST with a significant effect on swimming behavior, but not on climbing. The reduction in immobility produced by WAY163909 were fully reversed by the 5-HT<sub>2C/2B</sub> receptor antagonist SB206553 underlying the role of 5-HT<sub>2C</sub> and/or 5-HT<sub>2B</sub> receptors in mediating these behavioral effects [71,132,133]. Accordingly, antidepressant-like effects of the 5-HT<sub>2C</sub> receptor agonists were confirmed with WAY161503 and Ro600175. It is noteworthy that in these latter studies 5-HT<sub>2C</sub> receptor agonists were injected three times before the test session [71,134], a procedure that might have induced a desensitization of 5-HT<sub>2C</sub> receptor. However, this possibility is unlikely because co-administration of a 5-HT<sub>2C</sub> receptor antagonist prevented the antidepressant-like effect of 5-HT<sub>2C</sub> agonists in the FST. Remarkably, 5-HT<sub>2C</sub> receptor agonists were also shown to produce ant depres sive effects in animal models of depression including in Wistar-Kyoto or bulbectomized rats [133,135] or in the anhedonia model of depression in rat [136]. Despite these clear evidence for a beneficial effect of 5-HT<sub>2C</sub> receptor activation in mediating antidepressant properties, other studies showed that the 5-HT<sub>2C</sub> receptor agonists WAY161503 or CP809101 significantly increased immobility time in the mouse FST [69] or produced learned helplessness-behaviors [137] whereas the novel 5-HT<sub>2C</sub> receptor antagonist S32006 displayed a broad spectrum of antidepressant-like effects notably in the rat FST [71,131] and suppressed anhedonia in a chronic mild stress paradigm after two weeks of treatment [131]. These latter data with S32006 are somewhat puzzling since genetic (i.e., in constitutive 5-HT<sub>2C</sub> KO mice) or acute pharmacological inactivation of this receptor did not affect behavior in the FST or in the tail suspension test (TST) [69,134,138-142], thus reflecting a lack of tonic control of this specific behavior by 5-HT<sub>2C</sub> receptor. However, they concur with the potentiating activity of 5-HT<sub>2C</sub> receptor antagonist on various SSRI-induced antidepressant-like effects in the FST or TST [141-145]. In light of the conflicting data reporting similar antidepressant-like effects of 5-HT<sub>2C</sub> receptor agonists or
antagonists, further analyses are needed to define their precise role in the regulation of depressive-like symptoms. One reason of such discrepancies might come from the weak selectivity of the ligands tested [39]. For example, S32006 display a non-negligible 5-HT_2B antagonistic activity as demonstrated from in vitro data [131]. However, as long as the relative in vivo occupancy at both 5-HT_2C and 5-HT_2B receptors will remain indeterminate, it will be difficult to anticipate the real target of S32006. Alternatively, the experimental conditions and by analogy with anxiety, the levels of stress before the tests, might affect the behavioral response of 5-HT_2C ligands. Strain differences might be considered as well since this parameter has a significant impact on stress sensitivity [146]. It has also been proposed that such contradictions could be related to the defense reaction of the animals submitted to stressful situations particularly in the FST and TST [147]. This implies that a battery of behavioral assays known to recapitulate diverse anomalies observed in depression will help probably refining the role of 5-HT_2C receptor in this disorder. Finally, psychostimulant effects or signs of serotonergic syndrome resulting from 5-HT_2C receptor agonists [69,148] might have represented confounding parameters towards the resignation evaluated in these paradigms. Despite all these considerations, further investigations evaluating the impact of long-term administration of 5-HT_2C Receptor agonists are required. These receptors can be submitted to adaptive changes such as a functional desensitization and this process could provide a cellular explanation for the antidepressant effect of 5-HT_2C receptor agonists and antagonists.

### 3.3.2 Electrophysiological studies

In the DR, in vitro recordings of 5-HT neurons showed that 5-HT application evoked inhibitory GABA-mediated postsynaptic currents which were partially attenuated by the 5-HT_2C receptor antagonist SB242084 [76]. More recently, in vivo electrophysiological studies reported that the systemic administration of the preferential 5-HT_2C receptor agonists WAY161503 and Ro 600175 inhibited DR 5-HT neuronal activity in a SB24204-reversible manner [81,148,149]. Immunohistochemical experiments demonstrated that WAY161503 and Ro 600175 increased Fox expression specifically in GAD-positive neurons located in the DR, whereas SB 242082 antagonized this effect [81,83,148]. These data seemingly confirm that 5-HT_2C receptor activation in the DR stimulated neighboring GABA neurons. Substantial evidence demonstrated that the 5-HT_2C receptor also modulates the neuronal activities of LC NE and VTA DA neurons. Indeed, the 5-HT_2C receptor agonists Ro 600175, MK-212 and WAY163909 decreased the firing activity and/or the number of spontaneously active LC NE [113] and VTA DA neurons [34,150-154] whereas these effects were blocked by SB 242084. Interestingly, in the VTA SB242084 caused a dose-dependent increase in the basal firing rate of DA neurons [154] suggesting that the 5-HT_2C receptor exerts a tonic inhibitory control of mesolimbic dopaminergic pathway (Figure 2) [112,150]. All these inhibitory actions of 5-HT_2C receptor agonists after acute administration on monoaminergic neuronal activities seem in accordance with a depressive-like effect but the it is noteworthy that chronic treatments might produce opposite effects.

### 3.3.3 Neurochemical studies

The role of 5-HT_2C receptor in the regulation of 5-HT extracellular levels at nerve terminals has been poorly documented. A recent study reported however that an acute administration of the 5-HT_2C receptor agonist Ro 600175 reduced extracellular 5-HT levels while increasing GABA levels in the DR of DBA/2 mice [155]. Similar inhibitory properties of Ro 600175 were reported on cortical 5-HT outflow in response to increased serotonergic tone in mice [23]. These results concur with electrophysiological studies reporting an indirect inhibitory effect of 5-HT_2C Receptor activation on 5-HT neuronal activity. Remarkably, the acute systemic and/or intrad DR infusion of 5-HT_2C receptor antagonist had no effect alone on extracellular 5-HT level in the DR and mPFCx in rats [113,156] or mice [143,155] but potentiated escitalopram induced increase in 5-HT outflow in these brain regions in relation with a decrease in GABA concentrations [155]. These results suggest the existence of a low level of constitutive activation of 5-HT_2C receptor involved in the regulation of the serotonergic system. In contrast, in response to an elevation of endogenous 5-HT induced by SSRIs, 5-HT_2C receptor is likely recruited to exert a negative feedback. In this specific condition of elevated 5-HT tone, 5-HT_2C receptor antagonists represent a classical augmentation strategy for antidepressants (see expert opinion section).

The role of this receptor type on DA neurotransmission attracted more attention, particularly in the PFCx and the NAc. In the PFCx, the systemic or intra-VTA administration of Ro 600175 blocked stress-induced increase in DA extracellular levels without altering basal levels [157]. Alone, SB242084 increased basal extracellular DA and completely prevented the effects of Ro 600175 in anaesthetized rats [157] confirming that endogenous 5-HT acting on 5-HT_2C receptor tonically inhibits basal or stimulated DA release in the PFCx. A particularly intriguing hypothesis is that fluoxetine may modulate DA outflow in cortical areas by interaction with 5-HT_2C receptor. Fluoxetine has moderate antagonistic activity towards 5-HT_2C receptors in rats brain [158] and this pharmacological property may favor the release of DA in the PFCx [156], possibly by blocking tonic and phasic 5-HT inhibitory control of VTA DA neurons (Figure 3) [150]. However, this hypothesis should be tempered by the fact that the affinity of fluoxetine for the SERT is 20 fold higher than for the 5-HT_2C receptor and consequently a marginal effect at this receptor could be expected. In the NAc, systemic administration of 5-HT_2C receptor agonists significantly decreased basal or stimulated DA release in rats [150,159-161] and these effects were blocked by SB 242084 [151,161-163]. These effects were mediated by 5-HT_2C receptor located in both VTA and
NAc because intra-VTA or NAc microinjection of SB242084 and/or SB243213 prevented the decrease in accumbal DA outflow induced by Ro 600175 [164]. Conversely, basal DA release was significantly enhanced in the NAc following the systemic administration of selective or non-selective 5-HT$_{2C}$ receptor antagonists such as SB242084 and SB206553, respectively [161,165], indicating that 5-HT$_{2C}$ receptor also exerts a tonic and phasic inhibitory control on mesolimbic DA neuron activity [112]. The observation that SB206553-stimulated DA release was insensitive to reduction of 5-HT neuronal function induced by the 5-HT$_{1A}$ agonist 8-OHDPAT or intra-raphe injections of the neurotoxin 5,7-dihydroxytryptamine provided the first in vivo evidence that constitutive activity of the 5-HT$_{2C}$ receptor tonically inhibits mesencephalic DA neurons [161]. Finally, several studies have shown that NE release is increased following systemic administration of 5-HT$_{2C}$ receptor antagonists [15,113,156].

4. Role of 5-HT2 receptors in adult hippocampal neurogenesis

Preclinical studies emphasized the role of neurogenesis in the pathophysiology of depression and the mechanism of action of SSRIs. Although careful examination of the literature suggests that the complex biological and psychological changes associated with depression cannot be attributed to disturbance in hippocampal neurogenesis alone [166], fundamental research remains of interest to anticipate the putative therapeutic activity of new drugs on this process. 5-HT is a potent regulator of adult hippocampal neurogenesis [12] and an increase in 5-HT tone resulting from prolonged SSRI treatment favors this process [167]. In contrast, decrease in 5-HT is believed to attenuate neurogenesis as suggested by the observation that 5-HT depletion in rats significantly reduced the number of newborn cells in the subgranular zone (SGZ) of the hippocampus [168]. Interestingly, ablation of neurogenesis by X-rays attenuated the antidepressant-like effects of SSRI in mice [169]. All these data suggested that part of the antidepressant-like activity of SSRIs would be mediated through neurogenesis-dependent mechanism in rodents [170,171]. Despite the extensive literature concerning the impact of 5-HT in adult neurogenesis, its precise mechanism of action is not fully understood and various post-synaptic 5-HT receptors might have diverse, possibly opposing effects on different stages of neuronal development in the adult dentate gyrus (DG) of the hippocampus. The first step in improving the knowledge on the effect of 5-HT on the various stages of the neurogenesis phenomenon (proliferation, differentiation, maturation and survival) is to identify the cell types in the course of adult neurogenesis on which 5-HT receptors are expressed.

4.1 Direct stimulation of neurogenesis by 5-HT2 ligands

A dense staining of 5-HT$_{2A}$ receptors in the hilus and of 5-HT$_{2C}$ receptors in the granule cell layer of the DG has been yielded (Table 2) [172] suggesting a role of these receptors in the modulation of adult neurogenesis in the hippocampus. An ex vivo study in rats, pointed out that acute treatment with either the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor agonists DOI or Ro 600175 respectively, had no effect on cell proliferation in the SGZ of the dorsal hippocampus in rats [173,174]. However, these results were challenged by a more recent study showing that an acute administration of the non-selective 5-HT$_2$ receptor agonist alpha-methyl-5-HT and the selective 5-HT$_{2C}$ receptor agonist WAY161503 decreased cell proliferation in this brain region [172]. In this study, the fact that the acute administration of the 5-HT2 receptor antagonist cinanserin increased cell proliferation while reducing the percentage of BrdU+/DCX+ (immature neurons) strongly suggest that the inactivation of 5-HT2 receptor stimulate the proliferation of cells displaying an undermined phenotype [172]. The hypothesis that some modifications could occur following chronic administration as observed with SSRIs has been also examined. It has been observed that the sustained administration of the non-selective 5-HT$_2$ receptor antagonist ketanserin resulted in a robust increase in progenitor proliferation [173] without commensurate change in doublecortin (DCX)-positive immature neurons within this duration and dendritic maturation of DCX-positive newborn neurons [173,174]. Similarly repeated administration of the 5-HT$_{2C}$ receptor antagonists such as cinanserin, SB243213 or S32006 increased cell proliferation [131,172,175] thus suggesting that the specific blockade of 5-HT$_{2C}$ receptor is involved in hippocampal plasticity. Despite these data, the mechanism by which 5HT$_{2A}$ and/or 5HT$_{2C}$ receptor(s) might interfere on neurogenesis remains poorly documented. It was, however, reported that the systemic administration of DOI led to a significantly decrease in brain-derived neurotrophic factor (BDNF) mRNA levels within the DG of the hippocampus [176]. These data suggest that the inactivation of 5-HT$_{2A}$ and/or 5-HT$_{2C}$ receptor might stimulate the production of neurotrophins. To our knowledge, no evidence has been reported that 5-HT$_{2C}$ receptor antagonists stimulate the production of neurotrophins in the hippocampus. Although these receptors may regulate neurogenesis directly, there are some indicators that they might exert their neurotrophic activity through the regulation of NE and/or DA release in the hippocampus. These data are of particular interest since it was reported that inactivation of D2-like receptor decreased neurogenesis [177] and the production of CNTF [178]. With respect to the role of 5-HT$_{2B}$ receptor in the modulation of hippocampal neurogenesis, there is only once recent study showing that sustained administration of the 5-HT$_{2B}$ receptor agonist BW723C86 stimulates cell proliferation whereas SSRI-induced neurogenesis (i.e., cell proliferation and survival) is impaired in 5-HT$_{2B}$ receptor KO [111]. These latter results may be explained by the fact that the enhancement of hippocampal serotonergic transmission induced by SSRI is attenuated in mutant mice.
4.2 Indirect stimulation of neurogenesis by 5-HT2 ligands through modulation of astrocytes function

The role of astrocytes in depression and the therapeutic effects of SSRIs is gaining growing interest [179]. For example, it has been reported that the density of astrocytes was dramatically reduced in the brain of depressed patients [180] and in animal models of depression [181] while lesioning astrocytes, notably in the cortex by the local injection of the gliotoxin L-alpha-aminodipate, produced behavioral changes indicative of depressed-like symptoms [182]. Conversely, the administration of antidepressant would normalize this cellular alteration [179]. 5-HT2A, 5-HT2B and 5-HT2C receptor subtypes were identified in primary cultures of astroglial cells and their activation by SSRIs enhance the metabolic activity of astrocytes [183-185]. In particular, it is well accepted that SSRIs activate 5-HT2A receptor and stimulate signaling intracellular cascades leading to the phosphorylation/activation of extracellular signal-regulated kinases (ERK1/2) [186-188]. Hence, antidepressants would exert their therapeutic activity, at least in part, by stimulating this pathway (Figure 4). In the hippocampus, ERK1/2 have been implicated in mood [189] as suggested by their blunted activation and/or expression in both depressed patient [190] and animal models of depression [191]. Moreover, a downregulation of ERK1/2 has been associated with an impairment of adult hippocampal neurogenesis [192]. All together, these results suggest that astrocytes may constitute a microenvironment permissive for neurogenesis [193]. Recent arguments also suggest that astrocytes could promote the synthesis and release of growth and neurotrophic factors [194,195], a mechanism required for the neurogenesis-dependant activity of SSRIs. Accordingly, in vitro studies reported that SSRIs stimulate the expression of BDNF, Glial-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF and VGF) in primary culture of astrocytes and C6 glioma cells [195-198]. In vivo preliminary data showed that the specific over-expression of BDNF in hippocampal astrocytes produced antidepressant-like effect in relation with an increase in cell proliferation, maturation and survival of new generated cells in the DG of the hippocampus [199].

5. Expert opinion and conclusion

Most antidepressants currently approved act by enhancing 5-HT neurotransmission. SSRIs increase the synaptic availability of 5-HT by inhibiting the 5-HT transporter SERT. This class of antidepressant is well tolerated and safer than the first generation agents. Despite their therapeutic action, residual symptoms remain and may explain the fact that approximately 50% of depressive individuals do not respond adequately to these agents [200]. Moreover, for patients who respond, 2-4 weeks of treatment are required to achieve a clinically meaningful effect [5]. It has been proposed that antidepressants with more than one mechanism of action would be more effective than agents with a single pharmacological target [201] [202]. A network meta-analysis reveals that the dual serotonin/norepinephrine reuptake inhibitors (SNRI) venlafaxine is more efficacious than the SSRIs fluoxetine, fluvoxamine and paroxetine [203] but less than citalopram, escitalopram [204-206] and sertraline [203]. Extending this observation to the fact the SNRI duloxetine failed to display a clinically superiority over SSRIs [203], there is no clear evidence that the simultaneous blockade of the 5-HT and NE transporters offers a beneficial therapeutic alternative in non-responders to SSRIs. Importantly, the superiority of a pharmacological compound lies on both its ability to reverse depressive symptoms (therapeutic efficacy) and its acceptability. Hence, regarding SNRIs, it clearly appears that their «acceptability» is lower than that of SSRIs [203] thereby challenging their superiority. In addition, the current evaluations of antidepressants are mainly focusing on mood, whereas other types of symptoms regulated by 5-HT and NE such as pain [207] or cognition [208] should be considered as well. For example, growing evidence suggests that changes in emotional memory are particularly relevant to antidepressant response [209]. In addition, because both 5-HT and NE are involved in mood, emotion, cognition while symptoms such as vigilance, arousal, interest and energy are most closely associated with NE neurotransmission, SNRIs could be used to trigger residual symptoms that may impede full remission or favor relapse within brief delay [210]. Converging lines of evidence also indicates that drugs enhancing dopaminergic neurotransmission such as bupropion can diminish anhedonia and produce antidepressant activities by itself or in combination with SSRIs in treatment-resistant patients [211]. These clinical considerations, have given rise to a new class of antidepressants, named the Triple Reuptake Inhibitors (TRIs), that simultaneously inhibit 5-HT, NE and DA reuptake, with the hope to offer a clinically relevant advantage over single- or dual-acting agents [84]. However, most of them have been discontinued in part due to the risk of addiction resulting from DA increases in the NAc pointing out the necessity to develop compounds that would regionally elevate DA in brain areas outside the NAc. The 5-HT2B receptor antagonists decreasing DA release in this brain region [114-116] can be interesting in this perspective. An other reason that might explain the disappointment based on TRIs concerns the functional interactions between 5-HT, NE and DA neurons [84] occurring at both somatodendritic and nerve terminals levels. These interactions are strongly regulated by 5-HT1 receptors and an increase in 5-HT levels in the brain induced by SSRIs may produce counter-productive effects on NE or DA neuronal activities but also on the 5-HT systems itself. The first example is provided by the activation of 5-HT2A known to decrease the activity of 5-HT and NE systems. These effects might explain the depressive-like effects produced by the acute systemic administration of selective 5-HT2A receptor agonists. Hence, pharmacological compounds blocking these heteroreceptors might be of interest for preventing SSRI-induced inhibition of NE neurons.
and consequently for improving antidepressant activity. In agreement with this assumption, 5-HT$_{2A}$ receptor antagonists were recently reported to potentiate the neurochemical effects of SSRIs, particularly their ability to increase the extracellular levels of NE (Table 4) [36-38,74,149,212-214]. It is conceivable that the advantages of this combination therapy may bear a relationship with the extracellular concentration of NE rather than 5-HT. In line with this argument, it has been shown that the blockade of 5-HT$_{2A}$ receptor augmented the antidepressant-like effects of fluoxetine in rats without a concurrent increase in extracellular levels of 5-HT [74]. Further preclinical investigations are required to confirm the putative relevance of inactivating 5-HT$_{2A}$ receptor [72] and determine the precise mechanism by which 5-HT$_{2A}$ receptor antagonists (i.e., antipsychotic drugs) such as aripiprazole, clozapine, olanzapine, risperidone, quetiapine, and ziprasidone have been reported to be effective in SSRI-resistant depression [35,215-219] either alone or as adjuncts to SSRIs.

Several reports, also pointed out a clear inhibitory action of 5-HT$_{2C}$ receptor agonists on 5-HT and DA systems [117] and these effects may hamper the antidepressant activity of SSRIs. It can thus be anticipated that the pharmacological inactivation of 5-HT$_{2C}$ receptor might also facilitate 5-HT and DA neurotransmissions. Accordingly, the selective 5-HT$_{2C}$ receptor antagonist SB242084 or the non selective 5-HT$_{2C/2A}$ receptor antagonist ketanserine prevented the inhibitory effect of citalopram on 5-HT cell firing in the DR [220] and potentiated the neurochemical effect of this SSRI on 5-HT extracellular levels in both the hippocampus and frontal cortex (Table 4) [139,220,221]. Additionally, SB-242084 was found to restore the antidepressant-like effect of citalopram in DBA/2N mice that do not respond to SSRIs alone [143] or more generally to enhance the behavioral responses of serotonergic antidepressants in the FST and TST [141,142,144,145]. Regarding the dopaminergic system, as detailed before, it is well known that SSRIs induced decrease in the activity of the dopaminergic system is blocked by 5-HT$_{2C}$ receptor

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**Figure 4. Putative role of 5-HT2A, 5-HT2B and 5-HT2C receptor located on astrocytes in the modulation of adult neurogenesis in the dentate gyrus of the hippocampus.** 5-HT$_2$ receptors are Gq/11 protein-coupled receptors and their activation stimulates phospholipase C (PLC), which generate diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3), by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2). The ultimate step of this intracellular cascade lead to an increase in cytosolic calcium concentration ([Ca$^{2+}$]). Increase in [Ca$^{2+}$], was detected in primary cultures of cortical astrocytes after the stimulation of the 5-HT$_2$ receptor induced by the SSRIs fluoxetine and citalopram. Then, in this signaling cascade, increase in [Ca$^{2+}$], and DAG results in the activation of protein kinase C which in turn, stimulates phosphorylation of extracellular signal-regulated kinases (ERK1/2). On one hand, ERK1/2 may favor the production of neurotrophic factors such as BDNF and GDNF and consequently may play a critical role in the neurogenesis. On the other hand, ERK1/2 may activate PLA2, which is involved in glucose metabolism. Importantly, correlation has been observed between severity of depression and reduction of glucose metabolism in depressed patients whereas chronic treatment with SSRIs normalizes this alteration by activating PLA2. This effect of SSRIs has been attributed to the activation of 5-HT$_{2B}$ located on astrocytes.

[29,33,91]
antagonists. Moreover, after a few weeks, SSRIs treatments lead to a downregulation of the 5-HT\textsubscript{2} receptors that allows for increased dopaminergic firing, which is proposed to be decisive for the antidepressant effect\cite{222}. Despite these encouraging results, whether 5-HT\textsubscript{2C} activation or inactivation is required to produce antidepressant-like response in preclinical studies remains controversial as emphasized in this review. Future investigations using other paradigms than FST and TST will undoubtedly help determine the real impact of 5-HT\textsubscript{2C} ligands on depressive-like symptoms.

Indeed, the multiplication of behavioral tests known to recapitulate diverse anomalies and symptoms encountered in psychiatric disorders as well as the evaluation of anxiolytic-/antidepressant-like activities of pharmacological compounds in relevant animal models\cite{171,223} is a prerequisite to anticipate their putative activity in human. Despite these considerations the use of complimentary tests may lead to discrepancies. Hence, to overcome these problems the determination of an integrated “emotionality score” in rodents has been created to facilitate the comparison between animal and human studies\cite{224}. It thus appears important to put emphasis on modeling symptoms rather than disorder per se\cite{225}. Taking into consideration all these aspects, it is not clear yet which strategy would be the most appropriate. It is however interesting to note that 5-HT\textsubscript{2C} receptor antagonists may be useful tools to prevent comorbidities, most notably anxiety that can be enhanced in the first day of treatments with SSRIs\cite{130,226,227}.

Using the clinicaltrials.gov website, the Boolean research for the following keywords “antipsychotic” and “major depression” provide 151 results. The detail of the completed studies in patients suffering from unipolar depression without psychotic features and treated with antipsychotics and/or antidepressants/SSRIs (Table 5) shows that 70% of the clinical trials focus on combination studies mainly using aripiprazole, olanzapine, quetiapine and risperidone. None of these studies included a translational approach coupling genetic, serum dosages, imagery techniques and behavioral/clinical

<table>
<thead>
<tr>
<th>SSRI</th>
<th>5-HT\textsubscript{2A} antagonist</th>
<th>Potentiation of SSRI-induced increase in extracellular levels of monoamines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Region</td>
</tr>
<tr>
<td>Citalopram</td>
<td>Risperidone</td>
<td>mPFCx</td>
</tr>
<tr>
<td></td>
<td>MDL100907</td>
<td>HP</td>
</tr>
<tr>
<td></td>
<td>MDL100907</td>
<td>HP</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Olanzapine</td>
<td>PFCx</td>
</tr>
<tr>
<td></td>
<td>Clozapine</td>
<td>PFCx</td>
</tr>
<tr>
<td></td>
<td>Risperidone</td>
<td>PFCx</td>
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<tr>
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<td>MDL100907</td>
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<tr>
<td></td>
<td>Olanzapine</td>
<td>PFCx</td>
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<tr>
<td></td>
<td>Perospirone</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Olanzapine</td>
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</tr>
<tr>
<td></td>
<td>MDL100907</td>
<td>PFCx</td>
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<tr>
<td></td>
<td>Fluvoxamine</td>
<td>Quetiapine</td>
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</tbody>
</table>

Signs + indicate a potentiating effect of the combination over SSRI alone. 0 indicate a lack of potentiating effect.
characterization. Interestingly, some compounds with more than one mode of action, notably with the ability to block simultaneously the 5-HT transporter SERT and 5-HT2A and/or 5-HT2C receptors including the atypical antipsychotics trazodone, nefazodone, arylpiperazine [229-231] or YM992 [232] have been developed. Preclinical studies are now required to determine whether these compounds produced greater antidepressant-like, electrophysiological and neurochemical effects than SSRIs. In particular, since adaptive changes occur after sustained administration of pharmacological compounds, it appears inevitable to test the effect of chronic administration of 5-HT2 ligands in relevant animal paradigms making the distinction between anxiety and/or depression. In this section, we willingly omitted to mention the putative therapeutic interest of central 5-HT2B receptor since its role in the field of neuropsychopharmacology remains poorly documented.

Lastly, it is also important to emphasize the potential central and peripheral risks related to these pharmacological strategies which can be mild, moderate or even life-threatening [233]. Risks of excessive weight loss [234] have been observed in response to the stimulation of 5-HT2A or 5-HT2C receptors. Consequently, it is important to watch over putative weight gain in response to strategies inactivating these receptors. Drug-drug interactions should also be considered when combining antidepressants and antipsychotics since toxicity might be more severe due to the high levels of monoamines [235].

The serotonin syndrome is a serious disorder reported in humans and that most commonly appears after antidepressant overdose or after combining several psychotropic medications [236]. Acute signs of 5-HT syndrome are possibly related to an excess of extracellular 5-HT activating 5-HT2A and 5-HT2C receptors [69,237]. Then, it can be anticipated that the pharmacological blockade of these receptors could prevent this syndrome. However, various atypical antipsychotic drugs, such as olanzapine, have been reported as causing the serotonin syndrome [238]. Finally, suicide is one of the most often risk encountered after overdose [239] and the fact that associations of polymorphisms in HTR2A and HTR2C gene with suicide attempts or suicidal ideation in depressive patients have been described, should draw our attention [240,241]. Regarding 5-HT2B receptor, benfluorex (Mediator), a 5-HT releasing agent and a potent 5-HT2B receptor agonist, has been recently suspected to cause deaths due to valvular insufficiency [242]. The activation of this receptor in the lung and cerebral vasculature has also been incriminated in pulmonary hypertension and migraine attacks, respectively [243,244]. In the CNS, 5-HT2B sites are poorly expressed but the possibility that it acts as an excitatory autoreceptor on the serotonergic system [111] prompt us to be vigilant. Indeed, although preliminary data suggest that activation of this receptor would produce antidepressant-like effects or potentiate the therapeutic activity of SSRIs, the implication of 5-HT2B receptor in the serotonin syndrome is strongly suspected [69].

Table 5. Clinical trials evaluating the antidepressant effect of antipsychotics given either alone or in combination with antidepressants in major depressive disorder (MDD).

<table>
<thead>
<tr>
<th>Antipsychotic</th>
<th>Antidepressant</th>
<th>Comparator(s)</th>
<th>Phase</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quetiapine</td>
<td>Placebo</td>
<td>3</td>
<td>MDD (x6)</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Placebo or duloxetine</td>
<td>3</td>
<td>MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>Placebo</td>
<td>3</td>
<td>MDD (x3)</td>
<td></td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>Sertraline</td>
<td>3</td>
<td>MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>Escitalopram</td>
<td>3</td>
<td>MDD (x2)</td>
<td></td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>Placebo/placebo</td>
<td>3</td>
<td>MDD (x4)</td>
<td></td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>ADs</td>
<td>2/3/4</td>
<td>Augmentation agent in treatment-resistant MDD (x4)</td>
<td></td>
</tr>
<tr>
<td>Iloperidone</td>
<td>SSRIs</td>
<td>4</td>
<td>Augmentation agent in MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>-</td>
<td>1/2</td>
<td>Augmentation agent in treatment-resistant MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Fluoxetine</td>
<td>3</td>
<td>Augmentation agent in treatment-resistant MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Pipamperone</td>
<td>Citalopram</td>
<td>2</td>
<td>MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>ADs</td>
<td>3</td>
<td>MDD (x1)</td>
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<tr>
<td>Quetiapine</td>
<td>SSRIs</td>
<td>2/3/4</td>
<td>Augmentation agent in treatment-resistant MDD (x3)</td>
<td></td>
</tr>
<tr>
<td>Risperidone</td>
<td>ADs</td>
<td>3</td>
<td>Augmentation agent in treatment-resistant MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Risperidone</td>
<td>Citalopram</td>
<td>3</td>
<td>Augmentation agent in treatment-resistant MDD (x1)</td>
<td></td>
</tr>
<tr>
<td>Risperidone</td>
<td>SSRIs</td>
<td>3</td>
<td>Augmentation agent in treatment-resistant MDD (x1)</td>
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<tr>
<td>Risperidone</td>
<td>Bupropion/SSRIs</td>
<td>3</td>
<td>Augmentation agent in treatment-resistant MDD (x1)</td>
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</table>
5-HT2 ligands in the treatment of anxiety and depression

Declarations of interest

G Quesseveur and HT Nguyen declare that they have no conflict of interest. AM Gardier and BP Guiard have received research grants from Lundbeck, Servier and Pierre Fabre laboratories. However these authors have no conflict of interest that might have influenced their opinions expressed in this review. None of the other authors have received any funding and have no competing interests to declare.

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