Neuropsychopharmacology

ARTICLE

Serotonin-2B receptor antagonism increases the activity of dopamine and glutamate neurons in the presence of selective serotonin reuptake inhibition

Rami Hamati[1,2], Mostafa El Mansari[2] and Pierre Blier[1,2]

Previous research has implicated the serotonin-2B (5-HT_{2B}) receptor as a possible contributor to the antidepressant-like response. Aripiprazole has been successfully used in combination with selective serotonin reuptake inhibitors (SSRIs) in treatment-resistant depression and it, among all receptors, exhibits the highest affinity for the 5-HT_{2B} receptor. However, the potential contribution of such an antagonistic action on 5-HT_{2B} receptors in the context of adjunct therapy is not known. In vivo electrophysiological recordings of ventral tegmental area (VTA) dopamine (DA) neurons, dorsal raphe nucleus (DRN) 5-HT neurons and pyramidal neurons in the medial prefrontal cortex (mPFC), and the hippocampus were conducted in anaesthetized Sprague-Dawley rats after the administration of 5-HT_{2B} receptor ligands alone or in combination with the SSRI escitalopram. An escitalopram-induced decrease in DA, but not 5-HT firing activity, was rescued by 2-day co-administration of the selective 5-HT_{2B} receptor antagonist LY266097. In the mPFC, 14-day escitalopram administration alone had no effect on pyramidal neuron firing and burst activity, whereas, aripiprazole administered alone or in combination with escitalopram for 14 days increased pyramidal neuron firing and burst activity. Likewise, the administration of LY266097 alone or its addition on the last 3 days of a 14-day escitalopram regimen increased pyramidal neuron firing and burst activity. These results indicated that 5-HT_{2B} receptors play, at least in part, a role in this enhancement. In the hippocampus, 5-HT_{2B} receptor activation by BW723c86 decreased escitalopram-induced inhibition of 5-HT reuptake, which was reversed by a 5-HT_{2B} receptor antagonist. Altogether, these results put into evidence the possibility that 5-HT_{2B} receptor blockade contributes to the therapeutic effect of aripiprazole addition to SSRIs in depression.

Neuropsychopharmacology (2020) 45:2098–2105; https://doi.org/10.1038/s41386-020-0723-y

INTRODUCTION

Initial first-line pharmacotherapy for major depressive disorder (MDD) is associated with a low remission rate [1]. One strategy of optimizing treatment is by the addition of dopamine (DA)/serotonin (5-HT) antagonists and/or receptor partial agonists (previously known as atypical antipsychotics [2]) to a variety of first-line medications including selective 5-HT reuptake inhibitors (SSRIs) and 5-HT-norepinephrine (NE) reuptake inhibitors (SNRIs) at regimens below their antipsychotic range. Of the adjunctive medications, addition of low-dose aripiprazole has consistently been reported to produce a robust antidepressant effect in treatment-resistant depressed (TRD) patients [3].

As aripiprazole binds to several DA and 5-HT receptors, it could interact through actions on one or several of these receptors. For instance, by blocking 5-HT_{2C} and 5-HT_{2A} receptors, aripiprazole addition to escitalopram normalized escitalopram-induced inhibition of firing activity of, respectively, ventral tegmental area (VTA) DA and locus coeruleus NE neurons [4]. In addition, aripiprazole combination with escitalopram rapidly normalized firing activity of dorsal raphe nucleus (DRN) 5-HT neurons due to desensitization of 5-HT_{1A} autoreceptors and an agonist action on their excitatory D_2 receptors [4–6]. Furthermore, the combination of aripiprazole and escitalopram enhanced tonic activation of 5-HT_{1A} receptors in the rat hippocampus but had no effect by themselves on this parameter [7]. Despite aripiprazole possessing the highest affinity for the 5-HT_{2B} receptor among all receptors, where it exerts an antagonist activity [8, 9], the contribution of this activity to the augmenting effect of aripiprazole when added to 5-HT reuptake inhibition has never been investigated. Interestingly, other adjunct medications (olanzapine, quetiapine, and ziprasidone) also have higher affinity for the 5-HT_{2B} receptor relative to the D_2 receptor [9], which strengthens the notion that the blockade of these receptors may contribute to their antidepressant effect.

5-HT_{2B} receptors are expressed in brain regions that are relevant to depression and may provide an additional target to induce an antidepressant response (see [10] for a review). In the rat DRN, these receptors were found on GABA neurons [11, 12] and in the mouse VTA, about 40% of DA neurons express 5-HT_{2B} receptor mRNA [13]. These receptors are also found in the septum, the amygdala, and on pyramidal neurons in the frontal cortex [14, 15]. 5-HT_{2B} receptors exert a role in the antidepressant-like response in mice [16–19]. Indeed, knocking out the 5-HT_{2B} receptor in mice produces an antidepressant-like phenotype, such that at baseline, these mice exhibit a reduced latency to feed in the novelty-suppressed feeding task, an increased sucrose consumption in the sucrose preference test,

[1]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada and [2]Mood Disorders Research Unit, University of Ottawa Institute of Mental Health Research, Ottawa, ON, Canada
Correspondence: Rami Hamati (rami.hamati@theroyal.ca)

Received: 27 February 2020 Revised: 21 May 2020 Accepted: 22 May 2020
Published online: 30 May 2020

and they express increased BDNF mRNA density and protein levels in the hippocampus [18]. Furthermore, such genetic ablation of the mouse 5-HT_{2B} receptor attenuates the effect of the SSRI fluoxetine in the forced-swim test [18].

The present study investigated whether pharmacological blockade of 5-HT_{2B} receptors have a role in the antidepressant effect when aripiprazole is added to an SSRI. It determined whether blockade of 5-HT_{2B} receptors by aripiprazole or the 5-HT_{2B} antagonist LY266097, in addition to inhibition of the serotonin transporter (5-HTT) by escitalopram in vivo, induces additional change in monoaminergic activity of VTA DA neurons and DRN 5-HT neurons, as well as glutamatergic pyramidal neurons in the medial prefrontal cortex (mPFC). Furthermore, a previous study showed that activation of the 5-HT_{2B} receptor decreases the capacity of SSRIs to bind to the 5-HTT in vitro [20]. Since combination of aripiprazole and escitalopram bind, respectively, to 5-HT_{2B} receptors and 5-HTT, their in vivo interaction was examined in the hippocampus.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, St. Constant, Canada) weighing 250–350 g were housed under standard laboratory conditions (12 h light/dark cycle), with access to food and water ad libitum. Experiments were performed between 10:00 and 18:00. In vivo extracellular recordings were carried out in chloral hydrate anesthetized rats (400 mg/kg, intraperitoneally [i.p.]) preceding fixation of the rodent into the stereotaxic apparatus. A single-barrel glass micropipette (Stoelting, Wood Dale, IL, USA) preloaded with a 2 M sodium chloride solution was used to record in the VTA, DRN, and mPFC and a 5-barrel micropipette (ASI Instruments, Warren, MI, USA) preloaded with the same solution in the hippocampus. Body temperature was maintained at 37 °C throughout the experiment via a water-based heating pad. A catheter was inserted into the lateral tail vein for systemic intravenous (i.v.) injection of pharmacologic agents. All animals were handled according to the Canadian Council on Animal Care guidelines, and all protocols of this study were approved by the local Animal Care Committee (University of Ottawa Institute of Mental Health Research, Ottawa, Canada).

Minipump implantation

Escitalopram was administered subcutaneously (s.c.) using implanted Alzet minipumps. For implantation, after the rat was anesthetized with isoflurane 2–4%, the fur was shaved, and the skin was washed with alcohol and betadine. An incision and a pocket were made and a filled minipump was inserted and the wound was closed with metal clips left in place no longer than 7 days. Minipumps were implanted for 2 and 14 days, and electrophysiological experiments were carried out while minipumps were still implanted. Two different types of minipump were used: model 1003D for 2-day and model 2ML2 for 14-day administration.

Experimental groups and treatments

Acute experiments. In the VTA, the preferential 5-HT_{2B} receptor agonist BW723c86 (pK_i 5-HT_{2A}: 6.6, pK_i 5-HT_{2B}: 7.9, pK_i 5-$HT_{2C:}$ 6.3 [10, 21]) and the selective 5-HT_{2B} receptor antagonist RS127445 (pK_i 5-HT_{2A}: 6.3, pK_i 5-HT_{2B}: 9.5, pK_i 5-$HT_{2C:}$ 6.4 [10, 22] were administered i.v. and s.c., respectively. Doses used were based on previous studies: BW723c86, 1–6 mg/kg, [21] and RS127445, 2 mg/kg, [23, 24]. In the mPFC, a dose of aripiprazole 0.6 mg/kg was used based on a previous study [25].

Short-term (2-day) administration. In this group, the SSRI escitalopram was administered s.c. for 2 days with a minipump. The selective 5-HT_{2B} receptor antagonist LY266097 (pK_i 5-HT_{2A}: 7.7, pK_i 5-HT_{2B}: 9.8, pK_i 5-$HT_{2C:}$ 7.6 [10, 26] was administered i.p. alone or concomitantly with escitalopram for 2 days. Doses used were based on previous studies: escitalopram 2 mg/kg [27] and LY266097 0.6 mg/kg [28].

Long-term (14-day) administration experiments. In this group, the same dose of escitalopram was administered for 14 days with a minipump. LY266097 or aripiprazole were administered alone or concomitantly with escitalopram for 3 days and 14 days, respectively, preceding electrophysiological recordings. Doses of LY266097 and escitalopram were the same to those used for 2 days, and a dose of aripiprazole 2 mg/kg was used based on a previous study [4].

In vivo electrophysiological recordings

Recording of VTA DA neurons. Putative DA neurons were recorded by positioning a single-barrel glass micropipette according to the following coordinates (in millimeters [mm] from lambda): A–P, 3.2–3.7; M–L, 0.6–1.0; D–V, 7.0–9.0 [29]. DA neurons were identified according to the following electrophysiological properties: 1) a firing rate of 2–10 Hz, which may include burst firing, 2) a biphasic or triphasic action potential with a "notch" in the rising phase and a prominent negative inflection, and 3) a spike duration >1.1 ms from spike initiation to the trough of the negative inflection. DA neurons burst activity was analyzed using the following criteria: a series of 2–10 spikes of decreasing amplitude, with a maximal interspike interval (ISI) of 80 ms for initiation of the spike train, and a maximal ISI of 160 ms for the continuation of the spike train [30, 31]. Nine electrode descents were carried out in each rat and neurons were recorded for 2 min after stabilization. The number of spontaneously active neurons per track was identified by recording multiple tracks in a 400–400 µm grid [28].

Recording of DRN 5-HT neurons. Putative 5-HT neurons were recorded by positioning a single-barrel glass micropipette according to the following coordinates (in mm from lambda): A–P, 0.8–1.2; M–L, 0; D–V, 5.0–7.0 [29]. 5-HT neurons were identified according to the following electrophysiological properties: 1) a firing rate of 0.5–3 Hz, 2) a biphasic or triphasic action potential with steady, regular firing and, 3) a spike duration of 1.5–3.0 ms [32, 33]. Four to five electrode descents were carried out in each rat and neurons were recorded for 2 min after stabilization.

Recording of pyramidal neurons in the mPFC. Previous works have reported pyramidal neurons in the mPFC are glutamatergic in nature [34, 35]. Putative mPFC pyramidal neurons were recorded by positioning a single-barrel glass micropipette according to the following coordinates (in mm from bregma): A–P, 3.2–3.4; M–L, 0.6–0.8; D–V, 2.5–5.5 [29]. Pyramidal neurons were identified according to the following electrophysiological properties: 1) a firing rate of 0.01–3 Hz, 2) a biphasic or triphasic action potential with highly irregular firing and 3) a spike with a positive inflection duration >0.36 ms and negative inflection duration >1.08 ms to exclude any fast-spiking interneurons [36]. Burst firing of pyramidal neurons was analyzed using the following criteria: a series of 2 or more spikes, with a maximal ISI of 45 ms for the initiation and continuation of the spike train [37]. For acute experiments in the mPFC, 5–8 neurons were recorded per rat to establish baseline firing and burst activity, then aripiprazole was administered i.v. After the injection, another 5–8 neurons were recorded per rat. For long-term experiments, 4–5 electrode descents were carried out in each rat and neurons were recorded for 5 min after stabilization.

Recording and microiontophoresis in CA3 dorsal hippocampus. Glutamatergic dorsal hippocampus pyramidal neurons [38, 39]

were recorded by positioning a five-barrel glass micropipette according to the following coordinates (in mm from lambda): A–P, 4.0–4.2; M–L; 4.0–4.2; D–V, 3.5–4.5 [29]. Since pyramidal neurons in the hippocampus do not discharge spontaneously under chloral hydrate anesthesia, quisqualic acid was used to activate these neurons within their physiological range (10–15 Hz). Pyramidal neurons were identified according to the following electrophysiological properties: 1) large amplitude (0.5–1.2 mV), 2) long duration (0.8–1.2 ms) simple action potentials alternating with 3) complex spike discharges [40]. The following compounds were used to fill the 5-barrel electrode: 10 mM 5-HT in 200 mM NaCl (pH 4), 10 mM BW723c86 in 200 mM NaCl (pH 1.3), 1.5 mM quisqualate in 200 mM NaCl (pH 8), and 2 M NaCl used for automatic current balancing. The central barrel filled with NaCl 2 M is used for recordings. Iontophoretic ejection of 5-HT for 50 sec (s) suppresses the firing activity of CA3 pyramidal neurons. The inhibited pyramidal neurons gradually regain their initial firing activity after the cessation of ejection due to reuptake of 5-HT. To reliably determine the activity of 5-HT transporter (5-HTT) in vivo, RT-50 index was determined. It is defined as the time elapsed from the cessation of iontophoretic application of 5-HT to 50% recovery of the initial firing rate [41, 42].

Drugs

Escitalopram was generously provided by Lundbeck A/S Ltd. (Valby, Denmark) and was dissolved in water. Aripiprazole (LKT Laboratories, St. Paul, USA) was dissolved in 2% lactic acid. BW723c86 and RS127445 (Tocris, Burlington, ON, Canada) were dissolved in 10% lactic acid. LY266097 (Tocris, Burlington, ON, Canada) was dissolved in 20% hydroxypropyl-beta-cyclodextrin. Apomorphine and haloperidol (SigmaAldrich, Oakville, ON, Canada) were dissolved in 0.5% lactic acid. In all solutions, distilled water was the main solvent, and the pH was adjusted.

Data acquisition and statistical analyses

Recordings were acquired using CED Spike2 data acquisition software (Cambridge Electronic Design, Cambridge, UK). Firing and bursts were analyzed using burstiDAtor [43], except for burst of pyramidal neurons in mPFC were analyzed using an additional package ('bursts.s2s') in Spike2. Data in figures were expressed as means ± S.E.M. and were analyzed using SigmaPlot 12.5. The paired *t*-test, the Kruskal–Wallis one-way ANOVA on Ranks, the one-way repeated-measures ANOVA, and the two-way repeated-measures ANOVA were used in this study. Pairwise comparisons of parametric and nonparametric tests were performed using, respectively, Holm–Sidak and Dunn's method. Threshold for statistical significance was set to 0.05.

RESULTS

Acute 5-HT$_{2B}$ receptor activation inhibits VTA DA firing and burst activity

RS127445 by itself did not alter firing and burst activity of DA neurons (paired samples *t*-test; $t\ [7] = 1.5$, $p > 0.05$ and $t\ [7] = -0.4$, $p > 0.05$; data not shown). A two-way repeated measures ANOVA conducted on the percentage of firing activity of DA neurons showed a significant effect of pre-treatment (saline or RS127445; $F[1,39] = 5.1$; $p < 0.05$) and an interaction between the dose of BW723c86 administered and pre-treatment ($F[3,39] = 6.0$; $p < 0.01$). However, Holm–Sidak pairwise comparisons showed that in rats that received RS127445 pre-treatment, acute injection of BW723c86 at 4 and 6 mg/kg did not inhibit DA neuron firing activity ($p > 0.05$; Fig. 1a–c). On the percentage of burst activity of DA neurons, even though there was no significant effect of pre-treatment ($F[1,39] = 2$; $p > 0.05$), an interaction was statistically significant ($F[3,39] = 3.8$; $p < 0.05$; two-way repeated measures ANOVA). Holm–Sidak pairwise comparisons indicated that at a dose of 6 mg/kg, the inhibitory effect of BW723c86 on the number of bursts per minute was blocked by pre-injection of RS127445 ($p > 0.05$; Fig. 1a, b, d).

Blockade of 5-HT$_{2B}$ receptors rescues SSRI-induced inhibition of DA but not 5-HT neurons

In the VTA, a 2-day regimen of escitalopram (10 mg/kg/day; s.c.) significantly decreased the firing activity of DA neurons ($H[3] = 25.6$, $p < 0.001$; Kruskal–Wallis One-Way ANOVA on Ranks followed by Dunn's method; Fig. 2a). However, there was no change in the number of spontaneously active DA neurons encountered per electrode descent (population activity; $H[3] = 1.8$, $p > 0.05$; Kruskal–Wallis One-Way ANOVA on Ranks; Fig. 2b). Also, there was no alteration in the percentage of spikes occurring in burst ($H[3] = 3.3$, $p > 0.05$; Kruskal–Wallis One-Way ANOVA on Ranks; Fig. 2c) and the number of bursts per minute ($H[3] = 2.8$, $p > 0.05$; Kruskal–Wallis One-Way ANOVA on Ranks; Fig. 2d). Whereas the administration of the selective 5-HT$_{2B}$ receptor antagonist LY266097 (0.6 mg/kg/day for 2 days; i.p.) alone had no effect on these parameters, its co-administration counteracted the inhibitory effect of escitalopram on the firing activity of DA neurons, resulting in a recovery to control level ($p > 0.05$; Fig. 2a).

In the DRN, a 2-day regimen of escitalopram significantly decreased the firing activity of 5-HT neurons ($H[3] = 102$, $p < 0.001$; Kruskal–Wallis One-Way ANOVA on Ranks followed by Dunn's method, $p < 0.05$; Fig. 3). Despite an increase of 40%, the administration of LY266097 alone for two days had no significant effect on the firing activity of 5-HT neurons. The co-administration of LY266097 did not rescue escitalopram-induced inhibition of 5-HT neuron firing activity ($p < 0.05$; Fig. 3).

The increase of mPFC pyramidal neuron activity by aripiprazole alone and in combination with escitalopram may be mediated by 5-HT$_{2B}$ receptors

Acute aripiprazole administration (0.6 mg/kg; i.v.) increased the firing activity of mPFC pyramidal neurons by 30%, but it was not statistically significant ($t\ [7] = -1.8$, $p > 0.05$; paired samples *t*-test; data not shown). However, it significantly increased their burst activity by 50% ($t\ [7] = -3.0$, $p < 0.05$; paired samples *t*-test; data not shown).

Long-term administration of escitalopram had no effect on the firing and burst activity of mPFC pyramidal neurons. After a 14-day regimen, aripiprazole alone and in combination with escitalopram significantly increased the firing and burst activity of pyramidal neurons by 105 and 150%, respectively (For firing: $H[3] = 15.8$, $p < 0.01$ and for bursts: $H[3] = 12.1$, $p < 0.01$; Kruskal–Wallis One-Way ANOVA on Ranks followed by Dunn's method; Fig. 4a, b).

To assess whether 5-HT$_{2B}$ receptors were involved in the increase of mPFC pyramidal neurons firing and burst activity induced by aripiprazole, the 5-HT$_{2B}$ receptor antagonist LY266097 was administered alone or concomitantly with escitalopram. The long-term administration of LY266097 both alone and when combined with escitalopram, significantly enhanced the firing and burst activity of pyramidal neurons by 165 and 125%, respectively (For firing: $H[3] = 27.3$, $p < 0.001$ and for burst: $H[3] = 15.2$, $p < 0.01$; Kruskal–Wallis One-Way ANOVA on Ranks followed by Dunn's method; Fig. 4c, d), similar to the combination of aripiprazole and escitalopram.

5-HT$_{2B}$ receptor agonism impairs the effect of escitalopram on the 5-HTT in vivo

CA3 pyramidal neuronal firing activity was suppressed by microiontophoretic application of 5-HT and displayed a recovery time to 50% of baseline firing (RT-50) with an average of 70 ± 12 s. Because 5-HTTs were inhibited following i.v. injection of escitalopram (0.2 mg/kg; i.v.), the suppressant effect of 5-HT was significantly prolonged to a RT-50 of 140 ± 14 s ($F[3,8] = 7.3$, $p < 0.05$; One-Way Repeated Measures ANOVA followed by Holm–Sidak method; Fig. 5a, b). This escitalopram-induced

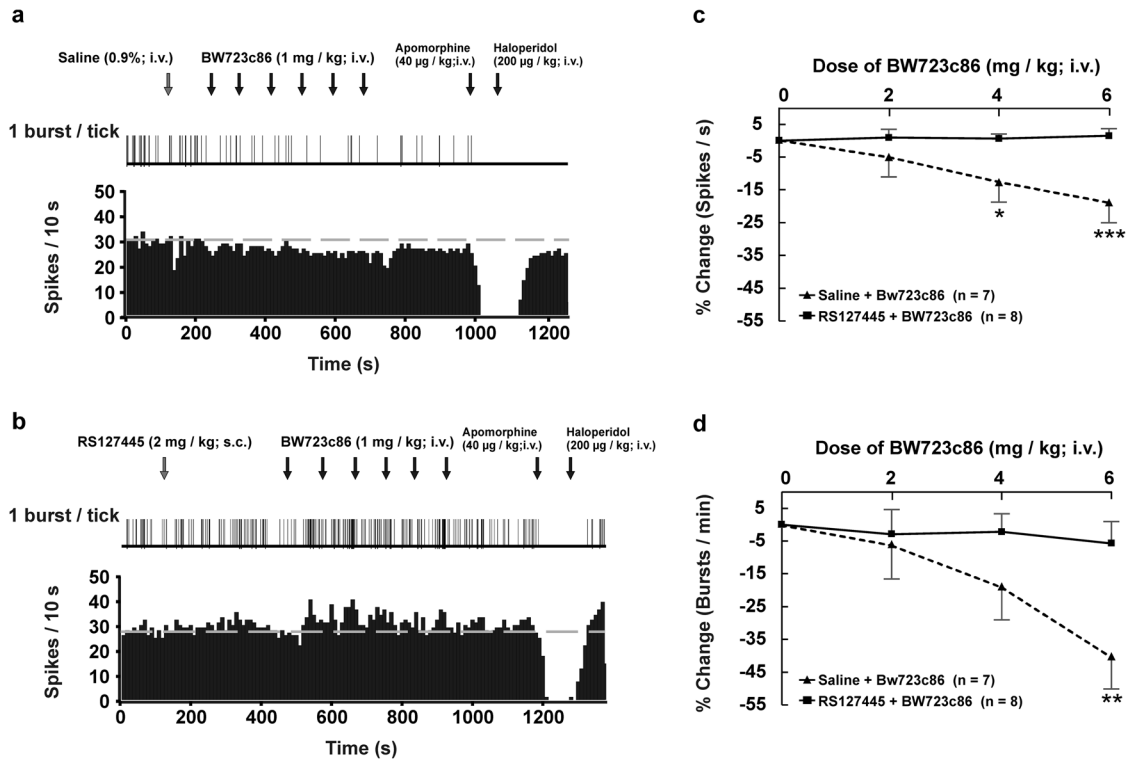

Fig. 1 5-HT2B receptors modulate firing and burst activity of DA neurons. a Integrated firing rate histogram illustrating the inhibitory effects of BW723c86 on a DA neuron. The upper trace in these panels corresponds to burst activity of the neuron. Apomorphine and haloperidol were injected to ascertain the DA nature of recorded neuron. **b** Integrated firing rate histogram illustrating RS127445 pre-administration blocking the inhibitory effects of BW723c86 on a DA neuron. The upper trace in these panels corresponds to burst activity of the neuron. Apomorphine and haloperidol were injected to ascertain the DA nature of recorded neuron. **c** Percentage change in the firing rate of VTA DA neurons in rats that received an acute cumulative dose of the 5-HT_{2B} agonist BW723c86 ($N = 7$; dotted lines) following a pre-treatment with saline or the selective 5-HT_{2B} antagonist RS127445 ($N = 8$; solid lines). **d** Percentage change in the burst rate of DA neurons in rats administered an acute cumulative dose of the 5-HT_{2B} agonist BW723c86 ($N = 7$; dotted lines) after pre-administration of saline or the selective 5-HT_{2B} antagonist RS127445 ($N = 8$; solid lines). Note that the acute inhibitory effects of BW723c86 on DA neurons firing and burst activity were prevented by RS127445. Data are presented as mean ± S.E.M. *$p < 0.05$, **$p < 0.01$,*** $p < 0.001$ between pretreatments for a given dose.

prolongation was blocked by the subsequent microiontophoretic application of the 5-HT_{2B} receptor agonist BW723c86 (RT-50 = 69 ± 12 s; Holm–Sidak method; $p > 0.05$, Fig. 5a, b). Following blockade of 5-HT_{2B} receptors by RS127445 (2 mg/kg; s.c.), the RT-50 recovered back to 104 ± 10 s, a duration that was not significantly different from that induced by escitalopram (Holm–Sidak method; $p > 0.05$, Fig. 5a, b).

DISCUSSION

In the present experiments, the 5-HT_{2B} receptor agonist BW723c86 significantly decreased firing and burst activity of DA neurons. Previous studies, however, did not report a significant effect of BW723c86 on DA neuron firing or DA dialysate levels [44, 45], probably because these investigators used a lower dose than herein. Furthermore, several of the acute anxiolytic effects of BW723c86 were only observed after the administration of a relatively higher dose [21, 46]. Di Matteo et al. [44] showed that another agonist with equivalent affinity for 5-HT_{2B} and 5-HT_{2C} receptors, Ro 60-0175, decreased the firing activity of DA neurons. Considering that BW723c86 has similar affinity for 5-HT_{2B} and 5-HT_{2C} receptors [10], it is possible that the dose used in the present experiments (6 mg/kg, i.v.), acted on both receptor subtypes. While RS127445 administration on its own has been previously reported to decrease the firing of DA neurons [23], it had no effect in the present study. Although the basis for this discrepancy is unclear, it is possible that the electrophysiological effects of RS127445 on DA neurons stemmed from the fact that heterogeneous populations of DA neurons were recorded, as previously reported [47]. Nonetheless, RS127445 prevented the inhibition of DA neuronal firing and burst activity induced by BW723c86. This indicated that the 5-HT_{2B} receptor mediated the inhibition of DA neurons firing and burst activity.

Escitalopram induced a decrease in the firing activity of DA neurons after a 2-day regimen as shown here and in line with previous results [4, 48]. However, 5-HT_{2B} receptor blockade did not change firing and burst activity of DA neurons after 2-day administration of LY266097. Since 5-HT_{2B} receptor mRNA is present in the VTA [13], it is possible that blockade of these receptors underlies the rescue of inhibition exerted by escitalopram on DA neurons firing activity herein. Indeed, the addition of LY266097 to escitalopram for 2 days resulted in recovery of firing of DA neurons, indicating that 5-HT_{2B} receptors are implicated, at least in part, in this normalization of firing activity. Interestingly, a similar rescue of firing activity of DA neurons was also demonstrated when escitalopram was co-administered with aripiprazole, which exerts an antagonist activity at 5-HT_{2B} receptors [4, 8]. A potential role for 5-HT_{2C} receptors in this rescue had been previously established since escitalopram-induced inhibition of DA neurons was restored to the control level by the administration of the 5-HT_{2C} receptor antagonist SB242084 or aripiprazole, which also has a high affinity for 5-HT_{2C} receptors [4, 8, 48]. Altogether, these results indicate that 5-HT_{2B} receptor blockade is probably involved in the rescue of

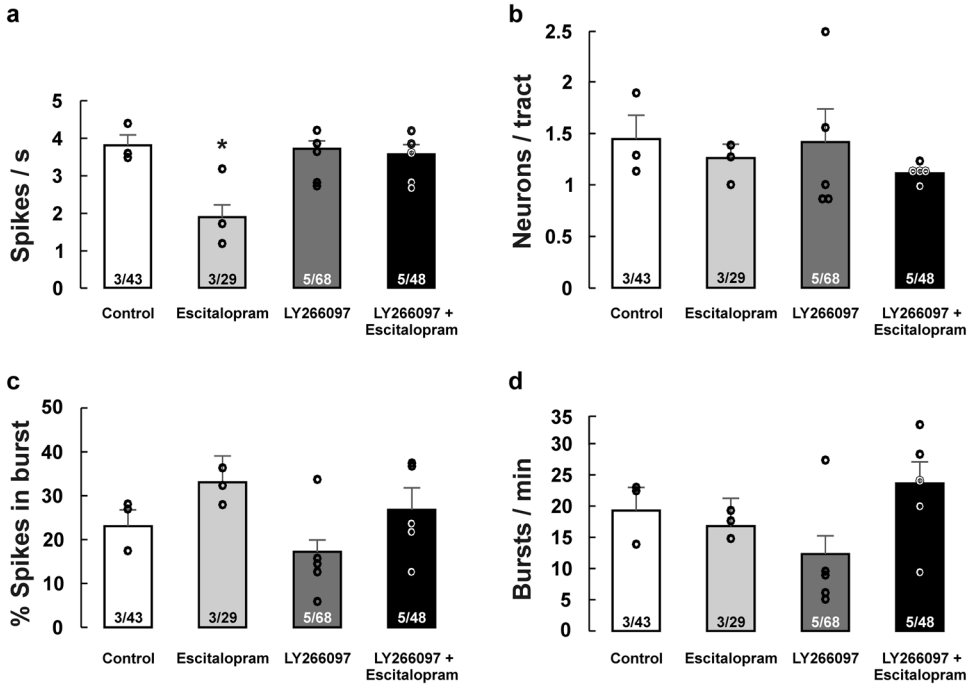

Fig. 2 Blockade of 5-HT2B receptors reverses escitalopram-induced inhibition of DA neurons. Firing rate (**a**) and population activity (**b**) of VTA DA neurons following a two-day administration of vehicle, escitalopram (10 mg/kg/day; s.c.), the 5-HT_{2B} antagonist LY266097 (0.6 mg/kg/day; i.p.) and their combination. DA neurons burst rate expressed as % spikes occurring in burst (**c**) or in number of bursts per min (**d**) after a two-day administration of vehicle, escitalopram (10 mg/kg/day; s.c.), the 5-HT_{2B} antagonist LY266097 (0.6 mg/kg/day; i.p.) and their combination. Data are presented as mean ± S.E.M. *$p < 0.05$ relative to control group. Numerators in the histogram represent the total number of rats used, and denominators represent the total number of neurons recorded. Open circles represent the mean of firing, population, and burst activity in each rat.

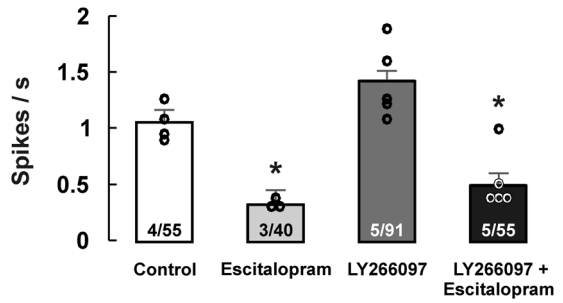

Fig. 3 Blockade of 5-HT2B receptors does not rescue escitalopram-induced inhibition of 5-HT neurons. Firing rate of DRN 5-HT neurons in rats following 2-day administration of vehicle, escitalopram (10 mg/kg/day; s.c.), the 5-HT_{2B} antagonist LY266097 (0.6 mg/kg/day; i.p.) and their combination. Data are presented as mean ± S.E.M. *$p < 0.05$ relative to control group. Numerators within the histogram represent the total number of rats used, and denominators represent the total number of neurons recorded. Open circles represent the mean of firing activity in each rat.

escitalopram-induced inhibition of DA neurons, hence possibly involving this receptor as a target in the antidepressant effect, particularly in the combination of 5-HT reuptake inhibition and 5-HT/DA partial agonists possessing high affinity for 5-HT_{2B} receptors such as aripiprazole.

A previous study has shown that blockade of 5-HT_{2B} receptors located on rat DRN GABA neurons by RS127445 [12], results in a significant increase of 5-HT neuronal firing activity [49]. Although an increase of similar magnitude (40%) was observed with sub-acute administration of LY266097, herein, this did not reach significance. However, the addition of LY266097 did not rescue escitalopram-induced inhibition of 5-HT neuronal firing activity. Similar combination composed of RS127445 and citalopram significantly enhanced levels of 5-HT around DRN 5-HT neurons [12], hence triggering the negative feedback exerted by the 5-HT_{1A} autoreceptor. Therefore, the lack of effect of LY266097 addition to escitalopram may be due to the predominance of the activity of the autoreceptor over the 5-HT_{2B} receptor in modulating 5-HT neuronal firing. It is thus possible that the desensitization of the autoreceptor would unmask the reversing effect mediated by 5-HT_{2B} receptor blockade. Nevertheless, the overall serotonergic effects of combination of RS127445 and citalopram on 5-HT concentrations in projection areas such as the mPFC were found to be augmented [12]. It is noteworthy that, in mice however, similar regimen combining RS127445 and paroxetine did not increase 5-HT levels in the hippocampus [17]. This apparent discrepancy may stem from the fact that 5-HT_{2B} receptors are located on DRN 5-HT neurons in mice [19], but on GABA neurons that inhibit the firing of 5-HT neurons in the rat DRN [12]. Thus, in rats, RS127445 would block 5-HT_{2B} receptors located on GABA neurons, hence resulting in a disinhibition of 5-HT neurons and an increase in 5-HT levels in DRN and mPFC [12]. In mice, RS127445 would antagonize excitatory 5-HT_{2B} receptors on 5-HT neurons, which results in dampening 5-HT concentrations in the hippocampus [17]. While several findings show that 5-HT neurons are under complex regulation by 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{6}, 5-HT_{7} receptors [50–53], these results, altogether, point to a role for 5-HT_{2B} receptors in this modulation and consequently the antidepressant response. This also shows that blocking 5-HT_{2B} receptors does not blunt reuptake inhibition by an SSRI in DRN.

It was shown that activation of the 5-HT_{2B} receptor by the 5-HT_{2B} receptor agonist BW723c86 induces a hyperphosphorylation of 5-HTTs [20] that was abolished in the presence of LY266097, thus confirming the role of the 5-HT_{2B} receptor in post-translational modification of the 5-HTT. This hyperphosphorylated state was postulated to decrease the capacity of SSRIs to bind to

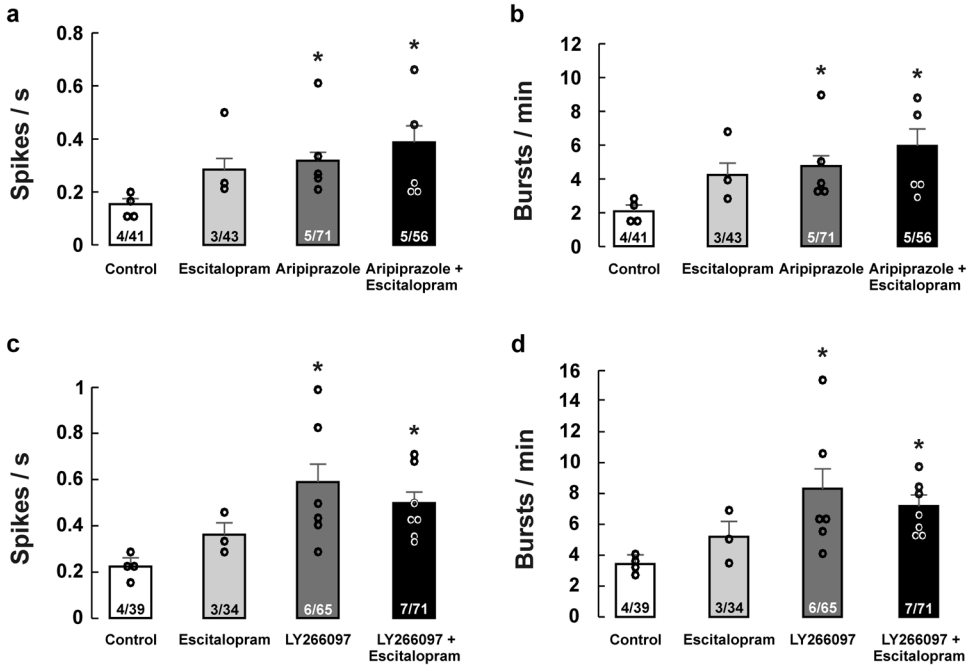

Fig. 4 Blockade of 5-HT2B receptors increases mPFC pyramidal neurons firing and burst activity. Firing (**a**) and burst rate (**b**) of mPFC pyramidal neurons following 14-day administration of vehicle, escitalopram (10 mg/kg/day; s.c.), aripiprazole (2 mg/kg/day; s.c.) and their combination. **c** Firing and burst rate (**d**) of pyramidal neurons in rats administered vehicle, escitalopram for 14 days (10 mg/kg/day; s.c.). LY266097 was added for the last 3 days of saline or escitalopram treatment, which lasted for 14 days. Data are presented as mean ± S.E.M. *$p <$ 0.05, relative to control group. Numerators within the histogram represent the total number of rats used, and denominators within the same histogram represent the total number of neurons recorded. Open circles represent the mean firing and burst activity in each rat.

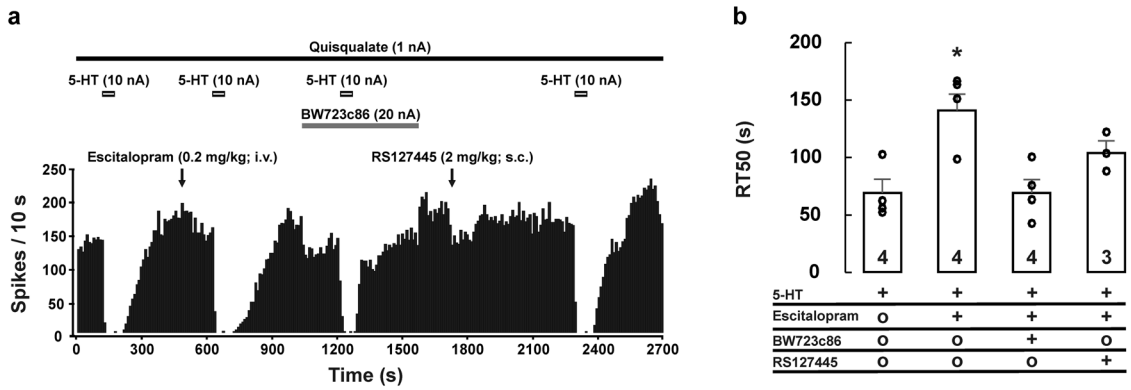

Fig. 5 5-HT2B receptor agonism but not antagonism impairs 5-HTT function. a Integrated firing rate histogram of a representative pyramidal neuron in the CA3 region of the hippocampus, illustrating the effect of i.v. injection of escitalopram (0.2 mg/kg) on the inhibitory effect of microiontophoretic application of 5-HT on firing activity. Note that the duration of 5-HT-induced inhibition was prolonged in the presence of escitalopram. While 5-HTTs were still blocked, the 5-HT_{2B} receptor agonist BW723c86 was co-ejected with 5-HT. Note that BW723c86 blunted the prolongation induced by escitalopram. The 5-HT_{2B} receptor antagonist RS127445 was then administered s.c. and reversed the effect of BW723c86 on 5-HT uptake. **b** RT-50 values in seconds as a measure of 5-HTT activity following the microiontophoretic application of 5-HT in the presence of the aforementioned treatments. Data are presented as mean ± S.E.M. *$p < 0.05$, relative to baseline. Numbers within the histograms represent the total number of neurons recorded. Only one neuron was recorded per rat. Open circles represent the RT-50 values in each rat.

the 5-HTT [20, 54]. In the present in vivo study, the iontophoretic application of BW723c86 reversed the escitalopram-induced blockade of 5-HTT in the hippocampus, which was blocked by RS127445. This provided further evidence that 5-HT_{2B} receptor activation may disrupt SSRI binding to the 5-HTT. On the contrary, escitalopram-induced inhibition of 5-HT reuptake was unaltered in the presence of 5-HT_{2B} receptor blockade when aripiprazole was added [7]. This confirms that in the presence of 5-HT_{2B} receptor antagonism, reuptake inhibition by an SSRI was not impaired in the hippocampus, as was the case in the DRN.

In the mPFC, an acute systemic low dose of aripiprazole, previously shown to increase DA levels [25, 55], elicited an increase in the number of bursts per minute but not the mean firing activity of pyramidal neurons. Long-term drug administration regimens, however, are more relevant to the therapeutic effects of drugs since their beneficial actions take place after repeated treatment in the clinic. Long-term administration of escitalopram did not induce any change in the firing and burst activity of pyramidal neurons, which is in line with previous results [36]. However, sustained administration of aripiprazole alone or in

combination with escitalopram increased firing and burst activity of pyramidal neurons herein. Since 5-HT_{2B} receptors are expressed on pyramidal neurons [15] and the mixed 5-$HT_{2B/2C}$ receptor agonist meta-chlorophenylpiperazine (mCPP) [56] inhibited their firing activity [57], it is, thus, possible that this enhancement in neuronal activity involved blockade of these receptors. This is strengthened by the present results showing that blockade of 5-HT_{2B} receptors by the selective 5-HT_{2B} receptor antagonist, LY266097, alone or when added to escitalopram resulted in an increase of firing and burst activity with comparable magnitude to that induced by aripiprazole administration alone and in combination with escitalopram. Similarly, a 3-week administration of olanzapine, which has strong affinity for 5-HT_{2B} receptors [9, 58], increased the basal firing activity of pyramidal neurons and reversed fluoxetine-induced inhibition of these neurons [59]. These data show that 5-HT_{2B} receptor blockade plays a role in the augmenting effects of aripiprazole alone or when added to 5-HTT inhibition. As is the case for 5-HT neurons, mPFC pyramidal neurons are modulated by several 5-HT receptor subtypes with the best characterized being the inhibitory 5-HT_{1A} and the excitatory 5-HT_{2A} receptors [60]. While several medications used in the treatment of MDD engage these receptors, 5-HT_{2B} receptor blockade may thus also be a relevant target for antidepressant drugs.

Although a role of 5-HT_{2B} receptors in the antidepressant-like response was already reported, its involvement in the augmenting effect stemming from both blockade of 5-HT_{2B} receptors and 5-HTTs was never investigated. Indeed, the present results add the following: 1) 5-HT_{2B} receptor antagonism reversed an SSRI-induced decrease in DA but not 5-HT neuron activity, and 2) antagonism of 5-HT_{2B} receptors alone or in combination with an SSRI increased mPFC pyramidal neuron activity. 3) Lastly, it was shown herein that 5-HT_{2B} receptor activation appears to interfere with the 5-HT reuptake process, while their blockade does not [7]. The present experiments have thus unveiled the potential role of 5-HT_{2B} receptor antagonism in commonly used augmentation treatment strategies in patients with MDD having an inadequate response to first-line medications. It is noteworthy to mention that besides aripiprazole, three other medications used as adjuncts in TRD have a higher affinity for the 5-HT_{2B} receptor than the D_2 receptor (namely olanzapine, quetiapine, and ziprasidone [9]). Whether antagonism at 5-HT_{2B} receptors by itself is sufficient to induce a beneficial therapeutic effect remains to be elucidated.

FUNDING AND DISCLOSURE

This research was conducted as part of the Canadian Biomarker Integration Network for Depression (CAN-BIND) program through the Ontario Brain Institute, an independent non-profit corporation, funded partially by the Ontario Government. The opinions, results, and conclusions are those of the authors and no endorsement by the Ontario Brain Institute is intended or should be inferred. RH and EIM declare no conflict of interest. PB received grant funding and/or honoraria for lectures, expert testimony, and/or participation in advisory boards from Allergan, Bristol Myers Squibb, Eli Lilly, Janssen, Lundbeck, Otsuka, Pfizer, Pierre Fabre Médicaments, Takeda, and Valeant.

AUTHOR CONTRIBUTIONS

RH contributed to the conception, acquisition, analysis, and interpretation of data, as well as drafting and revisions of the paper. MEM contributed to the conception and interpretation of data, as well as drafting, revisions, and final approval of the paper. PB contributed to the conception and interpretation of data, as well as drafting, revisions, and final approval of the paper.

ADDITIONAL INFORMATION

REFERENCES

1. Trivedi MH, Fava M, Wisniewski SR, Thase ME, Quitkin F, Warden D, et al. Medication augmentation after the failure of SSRIs for depression. N. Engl J Med. 2006;354:1243–52.
2. Zohar J, Stahl S, Moller HJ, Blier P, Kupfer D, Yamawaki S, et al. A review of the current nomenclature for psychotropic agents and an introduction to the neuroscience-based nomenclature. Eur Neuropsychopharmacol 2015;25:2318–25.
3. Luan S, Wan H, Zhang L, Zhao H. Efficacy, acceptability, and safety of adjunctive aripiprazole in treatment-resistant depression: a meta-analysis of randomized controlled trials. Neuropsychiatr Dis Treat. 2018;14:467–77.
4. Chernoloz O, El Mansari M, Blier P. Electrophysiological studies in the rat brain on the basis for aripiprazole augmentation of antidepressants in major depressive disorder. Psychopharmacol (Berl). 2009;206:335–44.
5. Haj-Dahmane S. D2-like dopamine receptor activation excites rat dorsal raphe 5-HT neurons in vitro. Eur J Neurosci. 2001;14:125–34.
6. Aman TK, Shen RY, Haj-Dahmane S. D2-like dopamine receptors depolarize dorsal raphe serotonin neurons through the activation of nonselective cationic conductance. J Pharm Exp Ther. 2007;320:376–85.
7. Ebrahimzadeh M, El Mansari M, Blier P. Synergistic effect of aripiprazole and escitalopram in increasing serotonin but not norepinephrine neurotransmission in the rat hippocampus. Neuropharmacology 2019;146:12–18.
8. Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, et al. Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. Neuropsychopharmacology 2003;28:1400–11.
9. Shahid M, Walker GB, Zorn SH, Wong EHF. Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. J Psychopharmacol. 2009;23:65–73.
10. Devroye C, Cathala A, Piazza PV, Spampinato U. The central serotonin 2B receptor as a new pharmacological target for the treatment of dopamine-related neuropsychiatric disorders: rationale and current status of research. Pharm Ther. 2018;181:143–55.
11. Bonaventure P, Guo H, Tian B, Liu X, Bittner A, Roland B, et al. Nuclei and subnuclei gene expression profiling in mammalian brain. Brain Res. 2002;943:38–47.
12. Cathala A, Devroye C, Drutel G, Revest JM, Artigas F, Spampinato U. Serotonin2B receptors in the rat dorsal raphe nucleus exert a GABA-mediated tonic inhibitory control on serotonin neurons. Exp Neurol. 2019;311:57–66.
13. Doly S, Quentin E, Eddine R, Tolu S, Fernandez SP, Bertran-Gonzalez J, et al. Serotonin 2B receptors in mesoaccumbens dopamine pathway regulate cocaine responses. J Neurosci. 2017;37:1354–17.
14. Duxon MS, Flanigan TP, Reavley TAC, Baxter TGS, Blackburn TP, Fone KCF. Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. Lett Neurosci. 1997;76:323–9.
15. Niebert M, Vogelgesang S, Koch UR, Bischoff AM, Kron M, Bock N, et al. Expression and function of serotonin 2A and 2B receptors in the mammalian respiratory network. PLoS ONE 2011;6:e21395.
16. Diaz SL, Maroteaux L. Implication of 5-HT2B receptors in the serotonin syndrome. Neuropharmacology 2011;61:495–502.
17. Diaz SL, Doly S, Narboux-Neme N, Fernández S, Mazot P, Banas SM, et al. 5-HT2B receptors are required for serotonin-selective antidepressant actions. Mol Psychiatry 2012;17:154–63.
18. Diaz SL, Narboux-Nême N, Boutourlinsky K, Doly S, Maroteaux L. Mice lacking the serotonin 5-HT2B receptor as an animal model of resistance to selective serotonin reuptake inhibitors antidepressants. Eur Neuropsychopharmacol. 2016;26:265–79.
19. Belmer A, Quentin E, Diaz SL, Guiard BP, Fernandez SP, Doly S, et al. Positive regulation of raphe serotonin neurons by serotonin 2B receptors. Neuropsychopharmacology 2018;43:1–10.
20. Launay J-M, Schneider B, Lorie S, Da Prada M, Kellermann O. Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT2B receptor signaling in serotonergic neuronal cells. FASEB J. 2006;20:1843–54.
21. Kennett GA, Bright F, Trail B, Baxter GS, Blackburn TP. Effects of the 5-HT2B receptor agonist, BW 723C86, on three rat models of anxiety. Br J Pharm. 1996;117:1443–8.
22. Bonhaus DW, Flippin LA, Greenhouse RJ, Jaime S, Rocha C, Dawson M, et al. RS-127445: a selective, high affinity, orally bioavailable 5-HT2B receptor antagonist. Br J Pharm. 1999;127:1075–82.

23. Devroye C, Cathala A, Haddjeri N, Rovera R, Vallée M, Drago F, et al. Differential control of dopamine ascending pathways by serotonin2B receptor antagonists: new opportunities for the treatment of schizophrenia. Neuropharmacology 2016;109:59–68.
24. Banas SM, Doly S, Boutourlinsky K, Diaz SL, Belmer A, Callebert J, et al. Deconstructing antiobesity compound action: Requirement of serotonin 5-HT2B receptors for dexfenfluramine anorectic effects. Neuropsychopharmacology 2011;36:423–33.
25. Tanahashi S, Yamamura S, Nakagawa M, Motomura E, Okada M. Dopamine D2 and serotonin 5-HT1A receptors mediate the actions of aripiprazole in mesocortical and mesoaccumbens transmission. Neuropharmacology 2012;62:765–74.
26. Audia JE, Evrard DA, Murdoch GR, Droste JJ, Nissen JS, Schenck KW, et al. Potent, selective tetrahydro-β-carboline antagonists of the serotonin 2B (5HT2B) contractile receptor in the rat stomach fundus. J Med Chem. 1996;39:2773–80.
27. El Mansari M, Sanchez C, Chouvet G, Renaud B, Haddjeri N. Effects of acute and long-term administration of escitalopram and citalopram on serotonin neurotransmission: an In Vivo electrophysiological study in rat brain. Neuropsychopharmacology 2005;30:1269–77.
28. Chenu F, Shim S, El Mansari M, Blier P. Role of melatonin, serotonin 2B, and serotonin 2C receptors in modulating the firing activity of rat dopamine neurons. J Psychopharmacol. 2014;28:162–7.
29. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th edn. Radarweg, AM: Academic Press; 2007.
30. Grace AA, Bunney BS. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons-1. Identif Charact Neurosci. 1983;10:301–15.
31. Ungless MA, Grace AA. Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons. Trends Neurosci. 2012;35:422–30.
32. Vandermaelen CP, Aghajanian GK. Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. Brain Res. 1983;289:109–19.
33. Hajós M, Allers KA, Jennings K, Sharp T, Charette G, Sík A, et al. Neurochemical identification of stereotypic burst-firing neurons in the rat dorsal raphe nucleus using juxtacellular labelling methods. Eur J Neurosci. 2007;25:119–26.
34. Santana N, Bortolozzi A, Serrats J, Mengod G, Artigas F. Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. Cereb Cortex 2004;14:1100–9.
35. Santana N, Mengod G, Artigas F. Quantitative analysis of the expression of dopamine D1 and D2 receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. Cereb Cortex 2009;19:849–60.
36. Riga MS, Teruel-Martí V, Sánchez C, Celada P, Artigas F. Subchronic vortioxetine treatment –but not escitalopram– enhances pyramidal neuron activity in the rat prefrontal cortex. Neuropharmacology 2017;113:148–55.
37. Laviolette SR, Lipski WJ, Grace AA. A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine d4 receptor-dependent basolateral amygdala input. J Neurosci. 2005;25:6066–75.
38. Stumm RK, Zhou C, Schulz S, Höllt V. Neuronal Types Expressing μ- and δ-Opioid Receptor mRNA in the Rat Hippocampal Formation. J Comp Neurol. 2004;469:107–18.
39. Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, et al. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 2006;51:455–66.
40. Ranck JB. Behavioral correlates and firing repertoires of neurons in the dorsal hippocampal formation and septum of unrestrained rats. Boston, MA: The Hippocampus. Springer; 1975.
41. de Montigny C, Wang RY, Reader TA, Aghajanian GK. Monoaminergic denervation of the rat hippocampus: Microiontophoretic studies on pre- and postsynaptic supersensitivity to norepinephrine and serotonin. Brain Res. 1980;200:363–76.
42. Piñeyro G, Blier P, Dennis T, De Montigny C. Desensitization of the neuronal 5-HT carrier following its long-term blockade. J Neurosci. 1994;14:3036–47.
43. Oosterhof NN, Oosterhof CA. BurstiDAtor: A lightweight discharge analysis program for neural extracellular single unit recordings. Github. https://github.com/nno/burstiDAtor. 2013.
44. Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E. Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin(2C) receptors. Brain Res. 2000;865:85–90.
45. Gobert A, Rivet J-M, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas J-P, et al. Serotonin-2C receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: A combined dialysis and electrophysiological analysis in the rat. Synapse 2000;36:205–21.
46. Kennett GA, Trail B, Bright F. Anxiolytic-like actions of BW 723C86 in the rat Vogel conflict test are 5-HT2B receptor mediated. Neuropharmacology 1998;37:1603–10.
47. Arborelius L, Chergui K, Murase S, Nomikos GG, Hk BB, Chouvet G, et al. The 5-HTIA receptor selective ligands, (R)-8-OH-DPAT and (S)-UH-301, differentially affect the activity of midbrain dopamine neurons. Naunyn Schmiedebergs Arch Pharm. 1993;347:353–62.
48. Dremencov E, El Mansari M, Blier P. Effects of sustained serotonin reuptake inhibition on the firing of dopamine neurons in the rat ventral tegmental area. J Psychiatry Neurosci. 2009;34:223–9.
49. Devroye C, Haddjeri N, Cathala A, Rovera R, Drago F, Piazza PV, et al. Opposite control of mesocortical and mesoaccumbal dopamine pathways by serotonin2-Breceptor blockade: Involvement of medial prefrontal cortex serotonin1Ar-eceptors. Neuropharmacology 2017;119:91–99.
50. Davidson C, Stamford JA. Evidence that 5-hydroxytryptamine release in rat dorsal raphé nucleus is controlled by 5-HT1A, 5-HT1B and 5-HT1D autoreceptors. Br J Pharm. 1995;114:1107–9.
51. Boothman LJ, Allers KA, Rasmussen K, Sharp T. Evidence that central 5-HT 2A and 5-HT 2B/C receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat. Br J Pharm. 2003;139:998–1004.
52. Brouard JT, Schweimer JV, Houlton R, Burnham KE, Quérée P, Sharp T. Pharmacological Evidence for 5-HT6 Receptor Modulation of 5-HT Neuron Firing in Vivo. ACS Chem Neurosci. 2015;6:1241–7.
53. Mnie-Filali O, Faure C, Lambás-Sẽas L, El Mansari M, Belblidia H, Gondard E, et al. Pharmacological blockade of 5-HT 7 receptors as a putative fast acting antidepressant strategy. Neuropsychopharmacology 2011;36:1275–88.
54. Zhang YW, Rudnick G. Serotonin transporter mutations associated with obsessive-compulsive disorder and phosphorylation alter binding affinity for inhibitors. Neuropharmacology 2005;49:791–7.
55. Li Z, Ichikawa J, Dai J, Meltzer HY. Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. Eur J Pharm. 2004;493:75–83.
56. Cussac D, Newman-Tancredi A, Quentric Y, Carpentier N, Poissonnet G, Parmentier JG, et al. Characterization of phospholipase C activity at h5-HT2C compared with h5-HT2B receptors: Influence of novel ligands upon membrane-bound levels of [3H]phosphatidylinositols. Naunyn Schmiedebergs Arch Pharm. 2002;365:242–52.
57. Bergqvist PBF, Dong J, Blier P. Effect of atypical antipsychotic drugs on 5-HT2 receptors in the rat orbito-frontal cortex: an in vivo electrophysiological study. Psychopharmacol (Berl). 1999;143:89–96.
58. Wainscott B, Nelson L, Lilly E. Pharmacologic characterization of the human 5-HT2B receptor: evidence for species differences. J Pharm Exp Ther. 1996;276:720–7.
59. Gronier BS, Rasmussen K. Electrophysiological effects of acute and chronic olanzapine and fluoxetine in the rat prefrontal cortex. Neurosci Lett. 2003;349:196–200.
60. Celada P, Puig MV, Amargós-Bosch M, Adell A, Artigas F. The therapeutic role of 5-HT1A and 5-HT2A receptors in depression. J Psychiatry Neurosci. 2004;29:252–65.