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Opposite control of mesocortical and mesoaccumbal dopamine pathways by serotonin$_{2B}$ receptor blockade: Involvement of medial prefrontal cortex serotonin$_{1A}$ receptors

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ABSTRACT

Recent studies have shown that serotonin$_{2B}$ receptor (5-HT$_{2B}$R) antagonists exert opposite facilitatory and inhibitory effects on dopamine (DA) release in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc), respectively, thereby leading to the proposal that these compounds could provide an interesting pharmacological tool for treating schizophrenia. Although the mechanisms underlying these effects remain unknown, several data in the literature suggest that 5-HT$_{1A}$Rs located into the mPFC could participate in this interaction. The present study, using *in vivo* microdialysis and electrophysiological recordings in rats, assessed this hypothesis by means of two selective 5-HT$_{1A}$R (WAY 100635) and 5-HT$_{2B}$R (RS 127445) antagonists. WAY 100635, administered either subcutaneously (0.16 mg/kg, s.c) or locally into the mPFC (0.1 μM), blocked the changes of mPFC and NAc DA release induced by the intraperitoneal administration of RS 127445 (0.16 mg/kg, i.p.). The administration of RS 127445 (0.16 mg/kg, i.p.) increased both dorsal raphe nucleus (DRN) 5-HT neuron firing rate and 5-HT outflow in the mPFC. Likewise, mPFC 5-HT outflow was increased following the intra-DRN injection of RS 127445 (0.032 μg/0.2 μl). Finally, intra-DRN injection of RS 127445 increased and decreased DA outflow in the mPFC and the NAc, respectively, these effects being reversed by the intra-mPFC perfusion of WAY 100635. These results demonstrate the existence of a functional interplay between mPFC 5-HT$_{1A}$Rs and DRN 5-HT$_{2B}$Rs in the control of the DA mesocorticolimbic system, and highlight the clinical interest of this interaction, as both receptors represent an important pharmacological target for the treatment of schizophrenia.

1. Introduction

The central serotonin$_{2B}$ receptor (5-HT$_{2B}$R) is now well-established as a modulator of dopamine (DA) neuron function in the mammalian brain (Auclair et al., 2010; Devroye et al., 2015; Doly et al., 2008, 2009). Interestingly, recent findings have pointed out this receptor as a new pharmacological target for treating schizophrenia (Devroye et al., 2016). Indeed, 5-HT$_{2B}$R blockade reverses the cognitive deficit induced by phencyclidine (PCP) in the novel object recognition test and suppresses PCP-induced

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hyperlocomotion, two behavioral models classically used to predict the ability of antipsychotic drugs (APDs) to alleviate cognitive and positive symptoms of schizophrenia, respectively (Newman-Tancredi and Kleven, 2011; Porsolt et al., 2010). In keeping with the classical hypothesis that the cognitive and positive symptoms of schizophrenia result from a DA hypoactivity in the medial prefrontal cortex (mPFC) and a DA hyperactivity in the nucleus accumbens (NAc), respectively (Creese et al., 1976; Newman-Tancredi and Kleven, 2011; Svensson, 2000), the efficacy of 5-HT_{2B}R antagonists in these behavioral trials has been related to their ability to increase DA outflow in the mPFC and decrease it in the NAc (Auclair et al., 2010; Devroye et al., 2016).

However, the mechanisms underlying the 5-HT_{2B}R modulation of DA outflow remain unknown to date. The possibility of a local control has already been discarded by recent findings showing that intra-mPFC or intra-NAc perfusion of 5-HT_{2B}R antagonists does not alter DA outflow in these brain regions (Devroye et al., 2016). On the other hand, the fact that 5-HT_{2B}R blockade increases mPFC 5-HT outflow (Devroye et al., 2016) raises the possibility that 5-HT_{2B}R antagonist-induced changes of DA outflow could involve polysynaptic cortico-subcortical circuits driven by the stimulation of 5-HT_{1A}Rs located in the mPFC. Indeed, agonists of the 5-HT_{1A}R, a key pharmacological target in the therapeutic benefit of atypical APDs (Meltzer and Massey, 2011; Newman-Tancredi and Kleven, 2011), are known to increase mPFC DA outflow and decrease it in the NAc (Assié et al., 2005; Díaz-Mataix et al., 2005; Ichikawa and Meltzer, 2000; Ichikawa et al., 2001), likely *via* the stimulation of mPFC 5-HT_{1A}Rs (Lladó-Pelfort et al., 2012).

Thus, the present study, using *in vivo* intracerebral microdialysis and extracellular unitary recordings in rats, was aimed at assessing the role of mPFC 5-HT_{1A}Rs in the opposite effects of 5-HT_{2B}R antagonists on NAc and mPFC DA outflow, by using two selective 5-HT_{2B}R and 5-HT_{1A}R antagonists, RS 127445 and WAY 100635, respectively (Bonhaus et al., 1999; Müller et al., 2007). First, we assessed the influence of the systemic and intra-mPFC administration of WAY 100635 on RS 127445-induced changes of DA outflow in the mPFC and the NAc. Thereafter, as 5-HT_{2B}Rs are expressed in the dorsal raphe nucleus (DRN) (Bonaventure et al., 2002), which contains 5-HT neurons projecting to the mPFC (Azmitia and Segal, 1978), we investigated the effect of the systemic administration of RS 127445 on DRN 5-HT neuron firing rate, as well as the effect of its systemic and intra-DRN administration on mPFC 5-HT outflow. Finally, the interaction between WAY 100635 and RS 127445 on mPFC and NAc DA outflow was studied following their administration into the mPFC and the DRN, respectively.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (IFFA CREDO, Lyon, France) weighing 280–350 g were used. Animals, housed in individual plastic cages were kept at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a 12 h light/dark cycle (dark from 20:00 h), and had free access to water and food. For electrophysiological experiments, rats were housed two per cage and kept under standard laboratory conditions as above. Animals were acclimated to the housing conditions for at least one week prior to the start of the experiments. All experiments were conducted during the light phase of the light-dark cycle. Animal use procedures were approved by the local ethical committee of the University of Bordeaux (experimental protocol number 50120190-A) and conformed to the International European Ethical Standards (2010/63/EU) and the French National Committee (décret 87/848) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

The following compounds were used: the 5-HT_{1A}R antagonist WAY 100635.$C_4H_4O_4$ (*N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate), and the 5-HT_{2B}R antagonist RS 127445.HCl (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine hydrochloride), purchased from R&D Systems (Abingdon, UK); the nonsteroidal anti-inflammatory drug meloxicam (METACAM® 2 mg/ml), and the local anesthetic lidocaine (Lurocaine® 20 mg/ml) purchased from Centravet (Dinan, France). All other chemicals and reagents were the purest commercially available (VWR, Strasbourg, France; Sigma-Aldrich, Saint-Quentin Fallavier, France).

2.3. Pharmacological treatments

WAY 100635 was dissolved in distilled water and administered subcutaneously (s.c.) at 0.16 mg/kg in a volume of 1 ml/kg; when administered locally into the mPFC by reverse dialysis, it was first dissolved in distilled water to obtain a 500 mM concentration, and then further diluted to the required concentration (0.1 μM) with artificial cerebrospinal fluid just before use. RS 127445 was dissolved in a 0.3% Tween 80 distilled water solution, and administered intraperitoneally (i.p.) at 0.16 mg/kg in a volume of 1 ml/kg, or locally injected into the DRN at 0.016 or 0.032 μg/0.2 μl. When studying their interaction, RS 127445 was administered systemically or into the DRN 30 min after the systemic injection of WAY 100635 or the beginning of its perfusion in the mPFC.

Doses and pretreatment administration time of WAY 100635 and RS 127445 were chosen according to their pharmacodynamic properties (Bonhaus et al., 1999; Laporte et al., 1994) and on the basis of previous studies reporting their efficacy to selectively block 5-HT_{1A}Rs and 5-HT_{2B}Rs, respectively (Assié et al., 2005; Auclair et al., 2010; Devroye et al., 2016; Ichikawa and Meltzer, 2000).

All drug doses were calculated as the free base. In each experimental group, animals received either drugs or their appropriate vehicle, according to a randomized design, except for electrophysiological experiments in which the neurons recorded before RS 127445 administration represented the control group.

2.4. Microdialysis

Surgery and perfusion procedures were performed as previously described (Auclair et al., 2010; Devroye et al., 2016) with minor modifications. In all experiments, microdialysis probes (CMA/11, cuprophan, 240 μm outer diameter, Carnegie Medicin, Phymep, France) were 4 mm length for the mPFC and 2 mm length for the NAc. Stereotaxic coordinates were chosen according to the atlas of Paxinos and Watson (2005).

For experiments performed in freely moving animals (see Fig. 1), rats were administered with meloxicam (1 mg/kg, s.c.) 30 min before surgery. Then, rats were anesthetized with 3% isoflurane (CSP, Cournon-d'Auvergne, France) and placed in a stereotaxic frame. A siliconized stainless guide-cannula (Carnegie Medicin, Phymep, Paris) was stereotaxically implanted, just above the right mPFC (coordinates of the lower extremity of the guide, in mm, relative to the interaural point: anteroposterior (AP) = 11.7, lateral (L) = 0.5, ventral (V) = 7.7) or the right NAc shell (AP = 10.7, L = 1.0, V = 4.0), so that the tip of the probe, once lowered through the guide-cannula, could reach a depth value of 3.7 mm (mPFC) or 2 mm (NAc shell) above the interaural point. Immediately after suture, lidocaine was administered (s.c.) around the site of surgery. The probe was inserted in the guide-cannula on the day of the

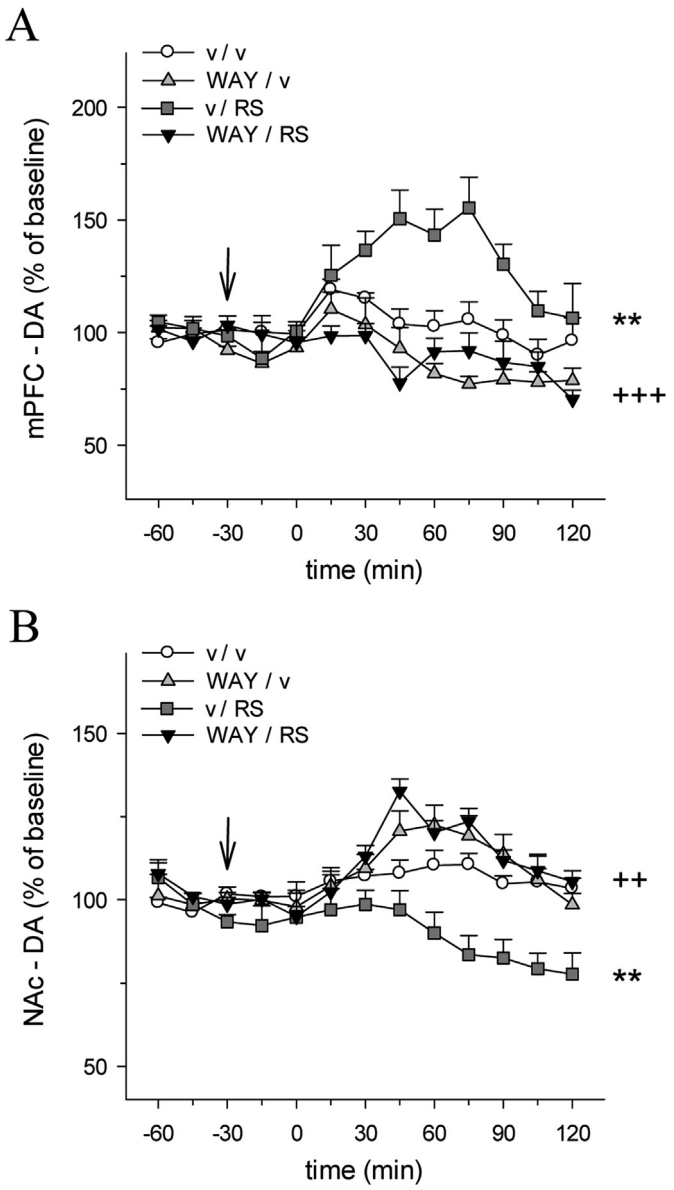

Fig. 1. Time course effect of the peripheral administration of WAY 100635 on RS 127445-induced changes of dopamine (DA) outflow in the medial prefrontal cortex (mPFC, A) and the nucleus accumbens (NAc, B). WAY 100635 (WAY, 0.16 mg/kg) was subcutaneously injected (vertical arrow) 30 min before the intraperitoneal administration (time zero) of RS 127445 (RS, 0.16 mg/kg). Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding drug administration (n = 4–6 and 5–6 animals/group for the mPFC and the NAc, respectively). Absolute basal levels of DA in dialysates collected in each brain region did not differ across the different experimental groups (mPFC: $F_{(3,15)} = 2.65$, NS; NAc: $F_{(3,18)} = 1.63$, NS, ANOVA) and were (mean ± SEM): 0.25 ± 0.03 nM for the mPFC (n = 19) and 1.28 ± 0.12 nM for the NAc (n = 22). $^{**}p < 0.01$ *versus* the corresponding v/v group and $^{++}p < 0.01$, $^{+++}p < 0.001$ *versus* the corresponding v/RS group (Newman-Keuls test).

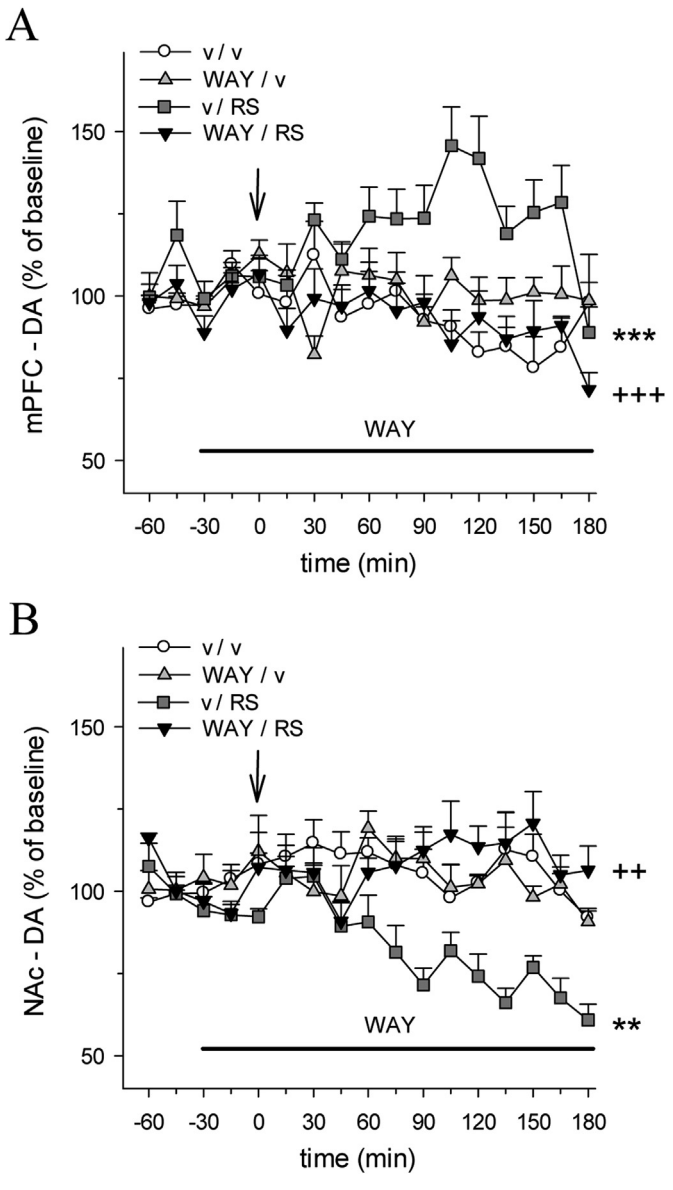

Fig. 2. Time course effect of the intra-medial prefrontal cortex (mPFC) administration of WAY 100635 on RS 127445-induced changes of dopamine (DA) outflow in the mPFC (A) and the nucleus accumbens (NAc, B). WAY 100635 (WAY, 0.1 μM) was applied by reverse dialysis for 210 min (horizontal bar); RS 127445 (RS, 0.16 mg/kg) was intraperitoneally administered 30 min after the beginning of the perfusion of WAY (time zero, vertical arrow). Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding drug administration (n = 4–7 and 4–6 animals/group for the mPFC and the NAc, respectively). Absolute basal levels of DA in dialysates collected in each brain region did not differ across the different experimental groups (mPFC: $F_{(3,17)} = 1.36$, NS; NAc: $F_{(3,16)} = 0.21$, NS, ANOVA) and were (mean ± SEM): 0.26 ± 0.02 nM for the mPFC (n = 21) and 0.70 ± 0.09 nM for the NAc (n = 20). $^{**}p < 0.01$, $^{***}p < 0.001$ *versus* the corresponding v/v group and $^{++}p < 0.01$, $^{+++}p < 0.001$ *versus* the corresponding v/RS group (Newman-Keuls test).

experiment (5–7 days after surgery).

For experiments performed in anesthetized animals, rats were anesthetized with 3% isoflurane, and placed in a stereotaxic frame. When assessing DA outflow (see Figs. 2 and 5), two microdialysis probes were simultaneously implanted in the right mPFC (coordinates, in mm, relative to the interaural point: 10° anterior from vertical, AP = 11.2, L = 0.5, V = 3.6) and the right NAc shell (AP = 10.7, L = 1, V = 2). When assessing 5-HT outflow (Fig. 4), one microdialysis probe was implanted in the right mPFC (coordinates, in mm, relative to the interaural point: AP = 11.7, L = 0.5, V = 3.7). After the surgery, the percentage of isoflurane was adjusted to 1.5% until the end of the experiment.

In all experiments, probes were perfused at a constant flow rate (1 μl/min), by means of a microperfusion pump (CMA 111, Carnegie Medicin, Phymep), with artificial cerebrospinal fluid containing (in mM): 147 NaCl, 4 KCl, 2.2 $CaCl_2$, pH 7.4.

Pharmacological treatments (see section 2.3 for details) were performed 120 min after the beginning of the perfusion (stabilization period). DA outflow was monitored during 120 or 180 min after the last drug injection; 5-HT outflow was monitored during

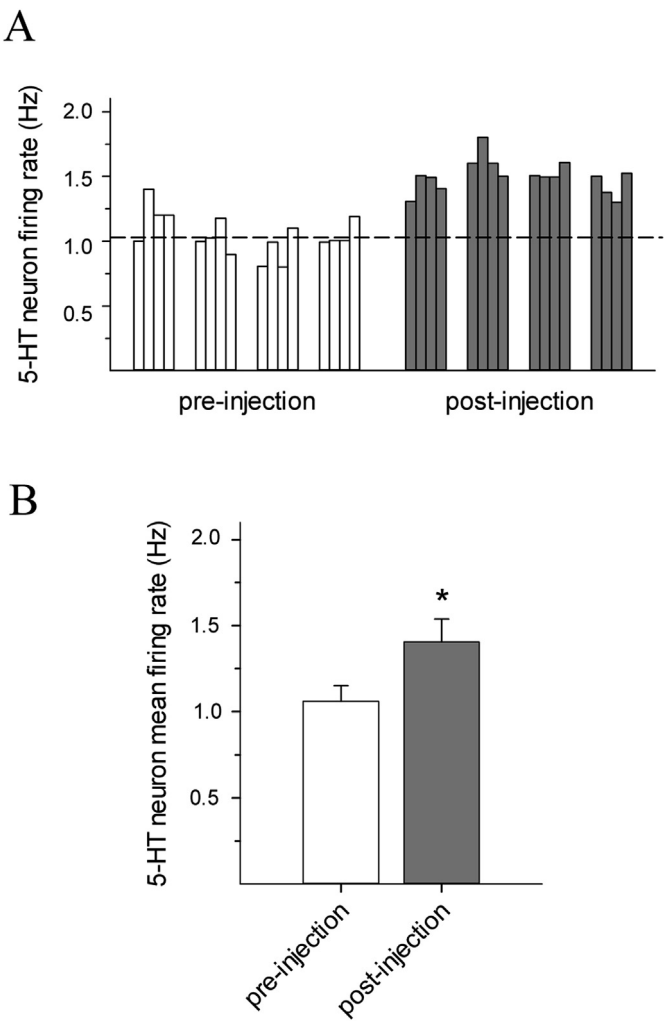

Fig. 3. Effect of RS 127445 on serotonin (5-HT) neuronal firing rate in the dorsal raphe nucleus (DRN). DRN 5-HT neuron firing rate was recorded before and 30 min after the intraperitoneal administration of RS 127445 (RS, 0.16 mg/kg). (A) Integrated firing rate histograms showing 8 representative samples of 5-HT neurons recorded from descents performed along the DRN before (pre-injection group) or after (post-injection group) RS administration. Each cluster of bars represents the electrical activity of one neuron, 1 bar representing the average number of recorded action potentials per 10 s. The dash line illustrates the mean firing rate of all the neurons recorded before RS administration (n = 36). (B) Histograms representing the mean ± SEM firing rate of DRN 5-HT neurons recorded in 6 rats before or after RS administration (n = 36 and 34 neurons for pre- and post-injection groups, respectively). $^{*}p < 0.05$ *versus* the pre-injection group (ANOVA).

120 min after drug administration. Dialysates were collected in a refrigerated fraction collector (MAB 85 Microbiotech, Phymep) every 15 min.

2.5. Surgical implantation of cannulae and microinjection protocol

In the experiments reported in Figs. 4B and 5, drug applications were performed after the stabilization of 5-HT or DA levels in the perfusate (see section 2.3), as described previously (Leggio et al., 2009). Briefly, a stainless steel cannula (30 G) was stereotaxically lowered into the DRN through a previously drilled hole, just before drug injections, according to the atlas of Paxinos and Watson (2005) (coordinates, in mm, relative to the interaural point, 20° lateral from vertical, AP = 1.0, L = −1.3, V = 3.3). Drug or vehicle was delivered into the DRN (see section 2.3 for details) in a final volume of 0.2 μl at a constant flow rate of 0.1 μl/min by a 5 μl Hamilton syringe (Sigma-Aldrich) and a syringe pump (Pico plus elite, Phymep). After completion of the microinjection, the injection cannula was left in place for an additional 5 min before withdrawal to allow diffusion from the tip and prevent reflux of the solution injected.

2.6. Histology

At the end of each experiment, the animal was deeply anesthetized with a pentobarbital overdose (100 mg/kg, CEVA, Libourne, France), and its brain was removed and fixed in NaCl (0.9%)/paraformaldehyde solution (10%). Probe or injection cannula location into the targeted region was determined histologically on serial coronal sections (60 μm) stained with cresyl violet, and only data obtained from rats with correctly implanted probes were included in the results.

2.7. Chromatographic analysis

After collection, dialysate samples were immediately analyzed with a high-performance liquid chromatography apparatus (Alexys UHPLC/ECD Neurotransmitter Analyzer, Antec, The Netherlands), equipped with an autosampler (AS 110 UHPLC cool 6-PV, Antec), as previously described (Devroye et al., 2016). The mobile phase [containing (in mM) 100 phosphoric acid, 100 citric acid, 0.1 EDTA.2H_2O, 4.6 octanesulfonic acid.NaCl plus 4.5% or 6% acetonitrile for the measurement of DA or 5-HT, respectively, adjusted to pH 6.0 with NaOH solution (50%)] was delivered at 0.065 ml/min flow rate with a LC 110S pump (Antec) through an Acquity UPLC BEH column (C_{18}; 1 × 100 mm, particle size 1.7 μm; Waters, Saint-Quentin en Yvelynes, France). Detection of DA or 5-HT was carried out with an electrochemical detector (DECADE II, Antec) with a VT-03 glassy carbon electrode (Antec) set at +460 mV *versus* Ag/AgCl. Output signals were recorded on a computer (Clarity, Antec). Under these conditions, the retention times for DA and 5-HT were 4–4.5 and 8–8.5 min, respectively, and the sensitivity was 50 pM with a signal/noise ratio of 3:1. DA and 5-HT content in each sample was expressed as the percentage of the average baseline level calculated from the three fractions preceding the first drug administration. Data correspond to the mean ± S.E.M. of the percentage obtained in each experimental group.

2.8. Extracellular recordings of 5-HT neurons

Surgery and extracellular recording procedures were performed as previously described (Mnie-Filali et al., 2006) with minor modifications. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus. Extracellular recordings were performed with single-barreled glass micropipettes preloaded with fiberglass filaments in order to facilitate filling. The tip was broken back to 2–4 μm and filled with a 0.5 M NaCl solution saturated with blue Chicago dye to verify the electrode position. Presumed DRN 5-HT neurons were located over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct, according to the atlas of Paxinos and Watson (2005). These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials. To determine possible changes in the spontaneous firing activity of presumed 5-HT neurons induced by RS 127445 injection, 3–4 successive descents were performed along the DRN in each rat to find about 4–6 neurons before and/or 4–6 neurons 30 min after the injection (Etiévant et al., 2015). At the end of each experiment, the animal was deeply anesthetized with a pentobarbital overdose (100 mg/kg, CEVA, Libourne, France), and its brain was removed, snap-frozen in isopentane (Sigma-Aldrich) and stored at −40 °C. Electrode

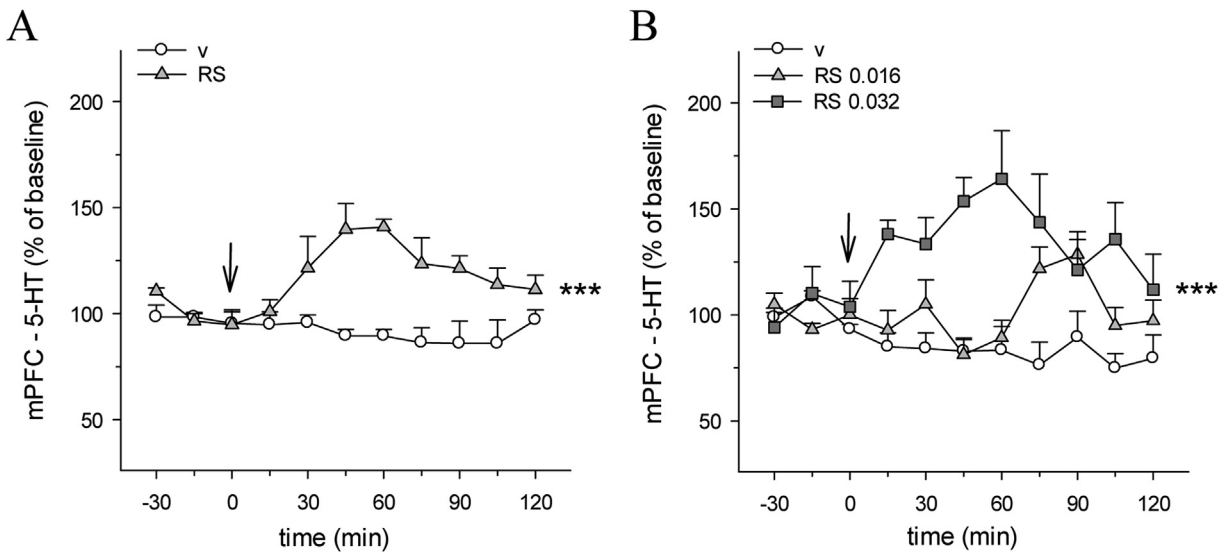

Fig. 4. Time course effect of RS 127445 administration on serotonin (5-HT) outflow in the medial prefrontal cortex (mPFC). RS 127445 (RS) was administered at time zero (vertical arrow) either intraperitoneally (0.16 mg/kg, A) or into the dorsal raphe nucleus (0.016 or 0.032 μg/0.2 μl, B). Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding drug administration (n = 4 animals/group). Absolute basal levels of 5-HT in dialysates collected in the mPFC did not differ across the different experimental groups (A: $F_{(1,6)} = 1.33$, NS; B: $F_{(2,11)} = 1.10$, NS, ANOVA) and were (mean ± SEM): 0.24 ± 0.03 nM (n = 8) and 0.18 ± 0.01 nM (n = 14) for A and B, respectively. $^{***}p < 0.001$ *versus* the corresponding v group (A: ANOVA; B: Dunnett test).

location into the targeted region was determined histologically on serial coronal sections (60 μm), and only data obtained from rats with correctly implanted electrodes were included in the results. Neuronal firing was calculated over 1–2 min periods. Data correspond to the mean ± S.E.M. of neuronal firing obtained prior or after RS 127445 administration.

2.9. Statistics

Statistical analysis was carried out by Statistica 8.0 for Windows (Statsoft, Maisons-Alfort, France). In microdialysis experiments, the ability of WAY 100635 (pretreatment) to modulate the effect of RS 127445 (treatment) on DA outflow was analyzed by a multifactorial ANOVA with pretreatment and treatment as the between-subject factors, and time as the within-subject factor (including values from time −30 to time 120 or 180 min). The effect of the systemic or intra-DRN administration of RS 127445 (treatment) on mPFC 5-HT outflow was analyzed by a multifactorial ANOVA with treatment as the between-subject factor, and time as the within-subject factor (including values from time 0 to time 120 min). In each microdialysis experiment, statistical differences in basal DA or 5-HT values among groups were assessed by a one-way ANOVA using group as a main factor. The impact of RS 127445 on the mean DRN 5-HT neuron firing rate was analyzed by a one-way ANOVA, using group as a main factor. Finally, in all experiments, when multifactorial ANOVA results were significant ($p < 0.05$), the Newman-Keuls or the Dunnett (for dose-response studies) *post-hoc* test was performed to allow adequate multiple comparisons between groups.

3. Results

3.1. Effect of WAY 100635 on RS 127445-induced effects on mPFC and NAc DA outflow

Fig. 1 illustrates the effect of WAY 100635 on RS 127445-induced effects on DA outflow in the mPFC (Fig. 1A) and the NAc (Fig. 1B).

In the mPFC (Fig. 1A), statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,15)} = 4.6$, $p < 0.05$; $F_{WAY \times RS \times time\ (10,150)} = 3.54$, $p < 0.001$). Post-hoc analysis revealed that, as reported previously (Auclair et al., 2010; Devroye et al., 2016), RS 127445 produced an overall significant increase in DA outflow ($p < 0.01$, *versus* the vehicle/vehicle group), reaching 155% of baseline. WAY 100635, with no effect on basal DA outflow (NS, *versus* the vehicle/vehicle group), reduced RS 127445-induced increased DA outflow ($p < 0.001$, *versus* the vehicle/RS group).

In the NAc (Fig. 1B), statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,18)} = 9.23$, $p < 0.01$; $F_{WAY \times RS \times time\ (10,180)} = 2.82$, $p < 0.01$). Post-hoc analysis revealed that, as reported previously (Auclair et al., 2010; Devroye et al., 2016), RS 127445 produced an overall significant decrease in DA outflow ($p < 0.01$, *versus* the vehicle/vehicle group), reaching 76% of baseline. WAY 100635, with no effect on basal DA outflow (NS, *versus* the vehicle/vehicle group), reversed RS 127445-induced decreased DA outflow ($p < 0.01$, *versus* the vehicle/RS group).

3.2. Effect of the intra-mPFC administration of WAY 100635 on RS 127445-induced effects on mPFC and NAc DA outflow

Fig. 2 illustrates the effect of the intra-mPFC perfusion of WAY 100635 on RS 127445-induced effects on DA outflow in the mPFC (Fig. 2A) and the NAc (Fig. 2B).

In the mPFC (Fig. 2A), statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,17)} = 30.78$, $p < 0.001$; $F_{WAY \times RS \times time\ (14,238)} = 2.54$, $p < 0.01$). Post-hoc analysis revealed that, as previously observed (Auclair et al., 2010; Devroye et al., 2016), RS 127445 produced an overall significant increase in DA outflow ($p < 0.001$, *versus* the vehicle/vehicle group), reaching 146% of baseline. Intra-mPFC perfusion of WAY 100635, with no effect on basal DA outflow (NS, *versus* the vehicle/vehicle group), reduced RS 127445-induced increased DA outflow ($p < 0.001$, *versus* the vehicle/RS group).

In the NAc (Fig. 2B), statistical analysis revealed a significant and

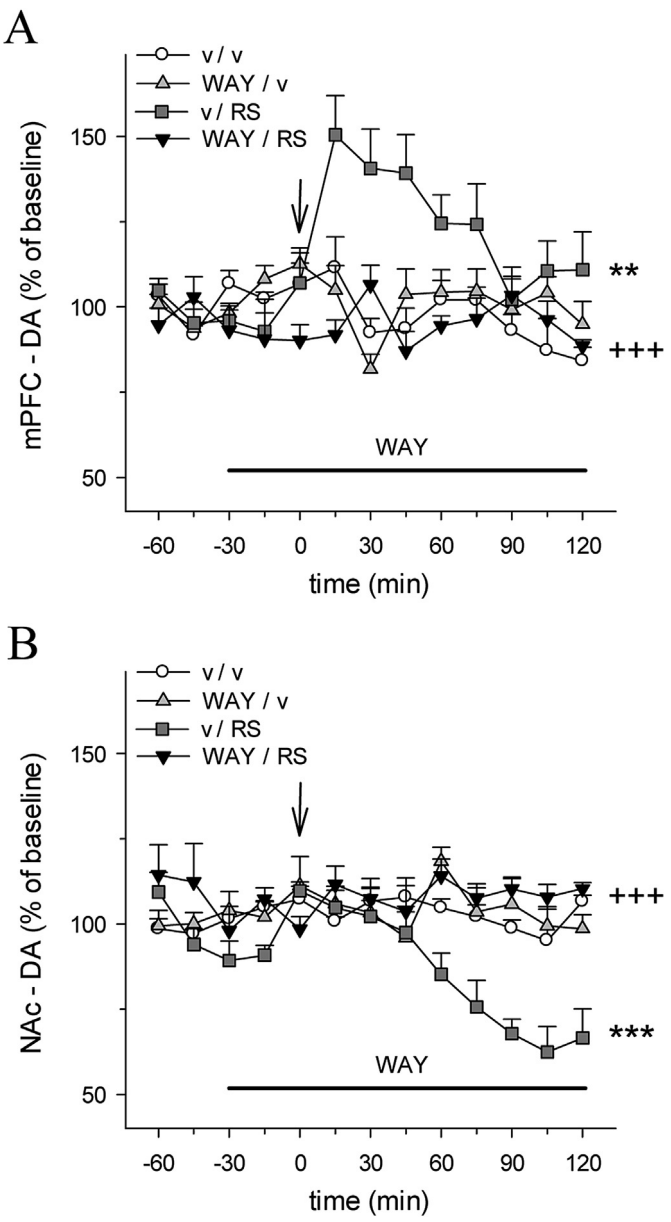

Fig. 5. Time course effect of the intra-medial prefrontal cortex (mPFC) administration of WAY 100635 on the changes of dopamine (DA) outflow in the mPFC (A) and the nucleus accumbens (NAc, B) elicited by RS 127445 injection into the dorsal raphe nucleus (DRN). WAY 100635 (WAY, 0.1 μM) was applied into the mPFC by reverse dialysis for 150 min (horizontal bar); RS 127445 (RS, 0.032 μg/0.2 μl) was injected into the DRN 30 min after the beginning of the perfusion of WAY (time zero, vertical arrow). Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding drug administration (n = 5–6 and 4–7 animals/group for the mPFC and the NAc, respectively). Absolute basal levels of DA in dialysates collected in each brain region did not differ across the different experimental groups (mPFC: $F_{(3,17)} = 2.57$, NS; NAc: $F_{(3,18)} = 0.78$, NS, ANOVA) and were (mean ± SEM): 0.27 ± 0.03 nM for the mPFC (n = 21) and 0.76 ± 0.06 nM for the NAc (n = 22). $^{**}p < 0.01$, $^{***}p < 0.001$ *versus* the corresponding v/v group and $^{+++}p < 0.001$ *versus* the corresponding v/RS group (Newman-Keuls test).

time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,16)} = 12.13$, $p < 0.01$; $F_{WAY \times RS \times time\ (14,224)} = 2.35$, $p < 0.01$). Post-hoc analysis revealed that, as reported previously (Auclair et al., 2010; Devroye et al., 2016), RS 127445 produced an overall significant decrease in DA outflow ($p < 0.01$, *versus* the vehicle/vehicle group), reaching 61% of baseline. WAY 100635, with no effect on basal DA outflow (NS, *versus* the vehicle/vehicle group), reversed RS 127445-induced decreased DA outflow ($p < 0.01$, *versus* the vehicle/RS group).

3.3. Effect of RS 127445 on 5-HT neuron firing rate in the DRN

Fig. 3 illustrates the effect of RS 127445 on the firing rate of presumed 5-HT neurons in the DRN. The mean spontaneous firing activity of DRN 5-HT neurons before RS 127445 injection was 1.06 ± 0.09 Hz (n = 36 neurons recorded in 6 animals). Statistical analysis revealed that the systemic administration of RS 127445 produced a significant increase (32%) of the spontaneous firing activity of DRN 5-HT neurons ($F_{RS\ (1,68)} = 4.74$, $p < 0.05$, *versus* the pre-injection group).

3.4. Effect of RS 127445 on mPFC 5-HT outflow

Fig. 4 illustrates the effect of RS 127445 on mPFC 5-HT outflow following its systemic (Fig. 4A) or intra-DRN (Fig. 4B) administration.

In Fig. 4A, statistical analysis revealed a significant and time-dependent effect of treatment ($F_{RS\ (1,6)} = 51.40$, $p < 0.001$; $F_{RS \times time\ (8,48)} = 2.59$, $p < 0.05$). Indeed, the peripheral administration of RS 127445 produced an overall significant increase in 5-HT outflow, reaching 141% of baseline ($p < 0.001$, *versus* the vehicle group).

In Fig. 4B, statistical analysis demonstrated a significant and time-dependent effect of treatment ($F_{RS\ (2,11)} = 19.46$, $p < 0.001$; $F_{RS \times time\ (16,88)} = 2.02$, $p < 0.05$). Post-hoc analysis revealed that the dose of 0.032 μg/0.2 μl (but not 0.016 μg/0.2 μl) of RS 127445 produced an overall significant increase in 5-HT outflow, reaching 164% of baseline ($p < 0.001$, *versus* the vehicle group).

3.5. Effect of the intra-mPFC administration of WAY 100635 on the changes of mPFC and NAc DA outflow elicited by the intra-DRN injection of RS 127445

Fig. 5 illustrates the influence of the intra-mPFC perfusion of WAY 100635 on the effects of the intra-DRN injection of RS 127445 on DA outflow, in the mPFC (Fig. 5A) and the NAc (Fig. 5B).

In the mPFC (Fig. 5A), statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,17)} = 17.51$, $p < 0.001$; $F_{WAY \times RS \times time\ (10,170)} = 1.97$, $p < 0.05$). Post-hoc analysis revealed that the intra-DRN administration of RS 127445 produced an overall significant increase in DA outflow ($p < 0.01$, *versus* the vehicle/vehicle group), reaching 150% of baseline. Intra-mPFC perfusion of WAY 100635, with no effect on basal DA outflow (NS, *versus* the vehicle/vehicle group), reduced RS 127445-induced increased DA outflow ($p < 0.001$, *versus* the vehicle/RS group).

In the NAc (Fig. 5B), statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,18)} = 13.63$, $p < 0.01$; $F_{WAY \times RS \times time\ (10,180)} = 2.92$, $p < 0.01$). Post-hoc analysis revealed that the intra-DRN administration of RS 127445 produced an overall significant decrease in DA outflow ($p < 0.001$, *versus* the vehicle/vehicle group), reaching 62% of baseline. WAY 100635, with no effect on basal DA outflow (NS, *versus* the vehicle/vehicle group), reversed RS 127445-induced decreased DA outflow ($p < 0.001$, *versus* the vehicle/RS group).

4. Discussion

The present study demonstrates that mPFC 5-HT_{1A}Rs participate in the 5-HT_{2B}R-mediated control of the mesocortical and mesoaccumbal DA pathway activity, and provides the first evidence of the functional role of a specific 5-HT_{2B}R population in the rat brain.

In particular, the ability of 5-HT_{2B}R antagonists to increase and decrease DA outflow in the mPFC and the NAc, respectively, appears to be mediated by an increase of DRN 5-HT activity which subsequently translates into the activation of mPFC 5-HT_{1A}Rs and the opposite modulation of DA outflow in the mPFC (increase) and the NAc (decrease) (see Fig. 6). These findings provide evidence for the existence of a functional interplay between DRN 5-HT_{2B}Rs and mPFC 5-HT_{1A}Rs, and highlight its interest in APD development.

The effects of 5-HT_{2B}R and 5-HT_{1A}R blockade were assessed by using two potent and selective antagonists, RS 127445 and WAY 100635, respectively, which have been well-characterized *in vitro* and *in vivo* (Auclair et al., 2010; Devroye et al., 2016; Müller et al., 2007). Both compounds possess high affinity for the corresponding receptor (RS 127445, pKi = 9.50; WAY 100635, pKi = 9.02) and at least 1000-fold (RS 127445) and 100-fold (WAY 100635) selectivity for the 5-HT_{2B}R and the 5-HT_{1A}R, respectively, over other receptors (Bonhaus et al., 1999; Fletcher et al., 1996).

In a first group of experiments, using microdialysis in freely moving rats, we assessed the effect of WAY 100635 on RS 127445-induced changes of mPFC and NAc DA outflow, following their systemic administration. In agreement with previous studies (Auclair et al., 2010; Devroye et al., 2016), we found that RS 127445 was able to increase and decrease DA outflow in the mPFC and the NAc, respectively. The effects of RS 127445 were suppressed by WAY 100635 pretreatment, which *per se* had no effect on basal DA outflow, as previously shown (Assié et al., 2005; Ichikawa and Meltzer, 2000). These findings support the involvement of 5-HT_{1A}Rs in the 5-HT_{2B}R-mediated control of mesocorticolimbic DA pathways.

A second group of experiments aimed at exploring the specific involvement of 5-HT_{1A}Rs located in the mPFC. This brain region contains a large population of layer V pyramidal glutamatergic neurons projecting to the VTA, mostly in the prelimbic and infralimbic subdivisions (Gabbott et al., 2005), where 5-HT_{1A}Rs are expressed by pyramidal neurons and GABAergic interneurons (Santana et al., 2004). In particular, the stimulation of 5-HT_{1A}Rs on GABAergic interneurons has been shown to inhibit their activity, thereby leading to the disinhibition of mPFC pyramidal neurons which send excitatory projections to the VTA (Lladó-Pelfort et al., 2012), where they provide a direct and a GABA-mediated input to DA neurons projecting to the mPFC and to the NAc, respectively (Sesack et al., 2003; Svensson, 2000). Interestingly, we found that the intra-mPFC perfusion of WAY 100635 prevented the changes in mPFC and NAc DA outflow elicited by the systemic administration of RS 127445, thereby confirming the specific involvement of mPFC 5-HT_{1A}Rs in this interaction. In keeping with the ability of RS 127445 to increase mPFC 5-HT outflow (Devroye et al., 2016; present study), the effects of 5-HT_{2B}R blockade on DA outflow are likely triggered by the stimulation of 5-HT_{1A}Rs localized to mPFC GABA interneurons. Also, the present findings are in line with previous studies demonstrating the role of GABA interneurons in the 5-HT_{1A}R agonist-induced increase in mPFC DA outflow (Díaz-Mataix et al., 2005; Lladó-Pelfort et al., 2012), and suggest that this receptor population participates in the 5-HT_{1A}R agonist-induced decrease in DA outflow in the NAc (Ichikawa and Meltzer, 2000; Sesack et al., 2003).

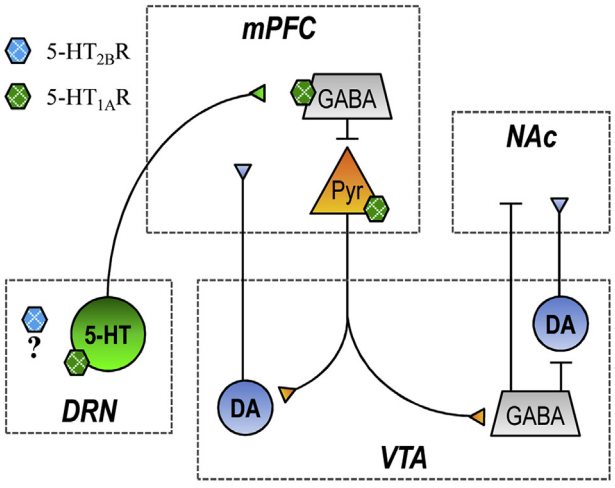

Fig. 6. Schematic representation of the possible circuits involved in the functional interplay between 5-HT_{2B}Rs and 5-HT_{1A}Rs driving the opposite effects of 5-HT_{2B}R antagonists on DA outflow in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). The 5-HT_{2B}R is expressed in the dorsal raphe nucleus (DRN; Bonaventure et al., 2002), which sends 5-HT projections to the mPFC (Azmitia and Segal, 1978). The 5-HT_{1A}R is expressed by DRN 5-HT neurons (Pazos and Palacios, 1985; Pompeiano et al., 1992), as well as by mPFC GABA interneurons and pyramidal glutamatergic neurons (Santana et al., 2004). In the ventral tegmental area (VTA), mPFC pyramidal glutamate neurons provide a direct excitatory and a GABA-mediated inhibitory control on the mesocortical and mesoaccumbal DA ascending pathways, respectively (Sesack et al., 2003). Blockade of 5-HT_{2B}Rs in the DRN induces an increase in mPFC 5-HT outflow, which could trigger the stimulation of 5-HT_{1A}Rs expressed by GABA interneurons in this brain region. Subsequent disinhibition of mPFC pyramidal neurons could respectively stimulate and inhibit the activity of the mesocortical and mesoaccumbal DA pathways, thereby leading to increased DA outflow in the mPFC and decreased DA outflow in the NAc.

Given that 5-HT_{2B}Rs are expressed in the DRN (Bonaventure et al., 2002), which is known to send 5-HT projections to the mPFC (Azmitia and Segal, 1978), the next step of our investigations aimed at exploring the role of the DRN in the effects of 5-HT_{2B}R antagonists. We found that the systemic administration of RS 127445 increased DRN 5-HT neuron firing rate, an effect that could account for increased 5-HT outflow in the mPFC (present study). Of note, RS 127445-induced increase in mPFC 5-HT outflow was previously reported in freely moving rats (Devroye et al., 2016), thereby showing that, as for DA neurons (Auclair et al., 2010; Devroye et al., 2015, 2016; Navailles et al., 2008), volatile (halothane, isoflurane) anesthesia does not alter 5-HT neuron reactivity. Interestingly, we found that mPFC 5-HT outflow was also increased following the intra-DRN administration of RS 127445. As discussed above, this effect may trigger the stimulation of mPFC 5-HT_{1A}Rs located onto GABAergic interneurons, leading to the activation of pyramidal glutamatergic neurons projecting to the VTA, thereby driving opposite changes in mPFC and NAc DA outflow (see Fig. 6). In support of this hypothesis, we found that the intra-DRN administration of RS 127445 was able to increase and decrease DA outflow in the mPFC and the NAc, respectively, and that both effects were abolished by the intra-mPFC perfusion of WAY 100635.

These results, altogether, support the existence of a functional interplay between DRN 5-HT_{2B}Rs and mPFC 5-HT_{1A}Rs driving the effects of 5-HT_{2B}R antagonists on DA outflow (see Fig. 6), and provide the first evidence of a functional role exerted by a specific 5-HT_{2B}R population in the rat brain. Noteworthy, the mechanisms whereby 5-HT_{2B}Rs control the activity of DRN 5-HT neurons remain to be established. In this context, the only data available in the literature with respect to the cellular localization and the functional role of DRN 5-HT_{2B}Rs are provided by *in vitro* experiments and studies performed in mice. Indeed, 5-HT_{2B}Rs have been shown to be expressed by 5-HT neurons in the mouse DRN (Diaz et al., 2012). Furthermore, studies in primary neuronal cultures of mouse embryonic raphe nucleus suggest that 5-HT_{2B}Rs may regulate extracellular 5-HT levels *via* an interaction with the 5-HT transporter (SERT; Launay et al., 2006). It is therefore tempting to suggest that blockade of DRN 5-HT_{2B}Rs may decrease local 5-HT outflow *via* a SERT-dependent interaction, which would then reduce the 5-HT_{1A} autoreceptor-mediated self-inhibition of 5-HT neurons, thereby

leading to an increase of 5-HT outflow at terminals. However, caution must be taken when transposing data from mice to rats, in particular in light of some species-dependent differences related to the 5-HT_{2B}R-mediated control of the DA network activity (Auclair et al., 2010; Devroye et al., 2016; Doly et al., 2009; Pitychoutis et al., 2015). Alternatively, the modulation of astrocytic function by 5-HT_{2B}Rs (Sandén et al., 2000) and the involvement of astrocytes in the control of excitatory neurotransmission (Perea and Araque, 2007; Perea et al., 2009) raise the possibility that 5-HT_{2B}R blockade may enhance excitatory inputs onto DRN 5-HT cells. Thus, further studies are warranted to confirm the expression of the 5-HT_{2B}R in rat DRN 5-HT neurons, as well as its role in the control of DRN 5-HT neuron activity.

Finally, the present findings deserve some considerations from a therapeutic point of view. It has been recently shown that 5-HT_{2B}R antagonists induce a suppressive effect in behavioral models classically used to reflect the cognitive and positive symptoms of schizophrenia, suggesting that they could be useful for the development of new atypical APDs (Devroye et al., 2016; Newman-Tancredi and Kleven, 2011; Porsolt et al., 2010; Svensson, 2000). Cognitive and positive symptoms being related to a hypoDA and hyperDA activity in the mesocortical and mesoaccumbal DA pathways, respectively (Newman-Tancredi and Kleven, 2011; Svensson, 2000), 5-HT_{2B}R antagonist ability to alleviate these symptoms may result from their differential control of mPFC and NAc DA outflow. Thus, it is tempting to suggest that 5-HT_{1A}Rs would enable the efficacy of 5-HT_{2B}R antagonists in these behavioral paradigms. Also, the existence of a functional interplay between 5-HT_{2B}Rs and 5-HT_{1A}Rs suggests that drugs exhibiting both antagonist and agonist properties towards the 5-HT_{2B}Rs and the 5-HT_{1A}Rs, respectively, would represent a useful pharmacological tool for improved treatments of schizophrenia. In line with this hypothesis, many atypical APDs already combine these two pharmacological features (Abbas et al., 2009; Kiss et al., 2010; Shahid et al., 2009; Shapiro et al., 2003). Furthermore, the 5-HT_{1A}R is known to play a critical role in the therapeutic benefit of atypical APDs (Meltzer and Massey, 2011; Newman-Tancredi and Kleven, 2011). In this context, additional investigations are needed to evaluate the significance of the functional interplay between 5-HT_{1A}Rs and 5-HT_{2B}Rs in the therapeutic benefit of atypical APDs.

In conclusion, the present study provides the first evidence of a functional interplay between 5-HT_{2B}Rs and 5-HT_{1A}Rs, which accounts for the opposite effects exerted by 5-HT_{2B}R antagonists on DA outflow in the mPFC and the NAc. Specifically, the tonic inhibitory and excitatory controls of mesocortical and mesoaccumbal DA pathways result from DRN-5-HT_{2B}R blockade which increases 5-HT activity and mPFC 5-HT outflow which, in turn, leads to the stimulation of mPFC 5-HT_{1A}Rs located on GABA interneurons. Finally, our findings support the therapeutic relevance of atypical APDs combining 5-HT_{2B}R antagonist and 5-HT_{1A}R agonist properties for improved treatment of schizophrenia.

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