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Research paper

Serotonin$_{2B}$ receptors in the rat dorsal raphe nucleus exert a GABA-mediated tonic inhibitory control on serotonin neurons

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ABSTRACT

The central serotonin$_{2B}$ receptor (5-HT$_{2B}$R) is a well-established modulator of dopamine (DA) neuron activity in the rodent brain. Recent studies in rats have shown that the effect of 5-HT$_{2B}$R antagonists on accumbal and medial prefrontal cortex (mPFC) DA outflow results from a primary action in the dorsal raphe nucleus (DRN), where they activate 5-HT neurons innervating the mPFC. Although the mechanisms underlying this interaction remain largely unknown, data in the literature suggest the involvement of DRN GABAergic interneurons in the control of 5-HT activity. The present study examined this hypothesis using *in vivo* (intracerebral microdialysis) and *in vitro* (immunohistochemistry coupled to reverse transcription-polymerase chain reaction) experimental approaches in rats. Intraperitoneal (0.16 mg/kg) or intra-DRN (1 μM) administration of the selective 5-HT$_{2B}$R antagonist RS 127445 increased 5-HT outflow in both the DRN and the mPFC, these effects being prevented by the intra-DRN perfusion of the GABA$_A$ antagonist bicuculline (100 μM), as well as by the subcutaneous (0.16 mg/kg) or the intra-DRN (0.1 μM) administration of the selective 5-HT$_{1A}$R antagonist WAY 100635. The increase in DRN 5-HT outflow induced by the intra-DRN administration of the selective 5-HT reuptake inhibitor citalopram (0.1 μM) was potentiated by the intra-DRN administration (0.5 μM) of RS 127445 only in the absence of bicuculline perfusion. Finally, *in vitro* experiments revealed the presence of the 5-HT$_{2B}$R mRNA on DRN GABAergic interneurons. Altogether, these results show that, in the rat DRN, 5-HT$_{2B}$Rs are located on GABAergic interneurons, and exert a tonic inhibitory control on 5-HT neurons innervating the mPFC.

1. Introduction

The central serotonin$_{2B}$ receptor (5-HT$_{2B}$R), in keeping with its ability to modulate the activity of the mesocorticolimbic dopamine (DA) pathways, is currently considered as a promising pharmacological target for improved treatments of DA-related neuropsychiatric disorders, such as drug addiction or schizophrenia (Devroye et al., 2018). Recent electrophysiological and microdialysis studies in rats have shown that the opposite effect of 5-HT$_{2B}$R antagonists on accumbal and medial prefrontal cortex (mPFC) DA outflow results from a primary action at the level of the dorsal raphe nucleus (DRN), where they activate 5-HT neurons innervating the mPFC (Devroye et al., 2017). Indeed, the peripheral administration of the selective 5-HT$_{2B}$R antagonist, RS 127445, increases DRN 5-HT neuronal firing and 5-HT release in the mPFC, this latter effect being also observed following its intra-DRN administration (Devroye et al., 2017).

The mechanisms whereby 5-HT$_{2B}$Rs regulate the activity of 5-HT neurons in the DRN remain unknown to date. Interestingly, electrophysiological and neurochemical studies assessing the role of DRN postsynaptic 5-HTRs in the control of 5-HT neurons (Adell et al., 2002; Liu et al., 2000; Quérée et al., 2009; Sharp et al., 2007) raise the possibility that the facilitatory effect of 5-HT$_{2B}$R antagonists on 5-HT neuron activity involves an action on DRN GABAergic interneurons. Indeed, in addition to the self-inhibitory control exerted by somatodendritic 5-HT$_{1A}$ autoreceptors, DRN 5-HT neurons are regulated by a negative-feedback loop involving GABAergic interneurons (Adell et al.,

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2002; Liu et al., 2000; Sharp et al., 2007), which provide a $GABA_A$R-mediated tonic inhibitory input to 5-HT neurons (Celada et al., 2001; Gao et al., 1993; Liu et al., 2000; Tao et al., 1996). Also, GABAergic interneuron activity undergoes excitatory and inhibitory controls exerted respectively by 5-$HT_{2A/2C}$Rs and 5-HT_{1A}Rs expressed by these interneurons (Adell et al., 2002; Day et al., 2004; Liu et al., 2000; Monti, 2010; Serrats et al., 2005; Sharp et al., 2007). These observations raise the possibility that 5-HT_{2B}Rs, as the other members of the 5-HT_2R family, are located on DRN GABA interneurons. This would provide an additional fine-tuning local control of 5-HT activity, such as that observed for 5-HT_{1A}Rs (Liu et al., 2000). In agreement with this view, 5-HT_{2B}R blockade would then reduce a $GABA_A$R-mediated inhibition of DRN 5-HT neurons and enhance their firing activity and forebrain 5-HT release, as previously observed (Devroye et al., 2017).

The present study examined this hypothesis, using *in vivo* intracerebral microdialysis in rats, by studying the role of DRN GABAergic transmission in the effect of RS 127445 on 5-HT outflow in the DRN and the mPFC. A series of experiments involving local and systemic drug administration have been conducted in order to examine the local and distal modulation of 5-HT outflow by RS 127445, and its interaction with other DRN 5-HTRs known to control 5-HT neuron activity. Furthermore, immunohistochemistry coupled to reverse transcription-polymerase chain reaction (RT-PCR) experiments were performed to assess the presence of the 5-HT_{2B}R mRNA on DRN GABAergic interneurons.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (IFFA CREDO, Lyon, France) weighing 280–350 g were used. Animals, housed in collective 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. 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 A11356) 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_{2B}R antagonist RS 127445.HCl (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine hydrochloride), the $GABA_A$R antagonist (−)-bicuculline ([R-(R*,S*)]-5-(6,8-Dihydro-8-oxofuro[3,4-e]-1,3-benzodioxol-6-yl)-5,6,7,8-tetrahydro-6,6-dimethyl-1,3-dioxolo[4,5-*g*]isoquinolinium iodide), the 5-HT_{2A}R antagonist MDL 100907 (*R*)-(+)-α-(2,3-Dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperinemethanol, the 5-HT_{2C}R antagonist SB 242084.2HCl (6-chloro-5-methyl-1-[6-(2-methylpiridin-3-yloxy) pyridin-3-yl carbamoyl]indoline.dihydrochloride), 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 selective 5-HT reuptake inhibitor (SSRI) (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile hydrobromide), purchased from R&D Systems (Abingdon, UK); the non-steroidal 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

Bicuculline was first dissolved in distilled water to obtain a 1 mM concentration and then further diluted to the required concentration (100 μM) with artificial cerebrospinal fluid (aCFS) just before its intra-DRN administration by reverse dialysis. 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; 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 DRN by reverse dialysis, both compounds were first dissolved in distilled water to obtain a 500 mM concentration, and then further diluted to the required concentration (RS 127445: 0.5 or 1 μM; WAY 100635: 0.1 μM) with aCSF just before use. Citalopram was first dissolved in distilled water to obtain a 10 mM concentration, and then further diluted to the required concentration (0.1 μM) with aCSF just before its local administration into the DRN by reverse dialysis. SB 242084 and MDL 100907 were first dissolved in distilled water to obtain a 1 mM concentration, and then further diluted to the required concentration (0.1 or 1 μM) with aCSF just before their intra-DRN administration by reverse dialysis.

Doses, concentrations and pretreatment administration time were chosen according to the pharmacodynamic properties of each drug (Andrews and Johnston, 1979; Bonhaus et al., 1999; Fletcher et al., 1996; Kennett et al., 1997; Kramer et al., 2010; Sur et al., 1998) and on the basis of previous studies reporting its selectivity towards the targeted site (Assié et al., 2005; Auclair et al., 2010; Cunningham et al., 2012; Devroye et al., 2018; Ichikawa and Meltzer, 2000; Müller et al., 2007; Navailles et al., 2008; Tao et al., 2000; Tao and Auerbach, 2002).

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.

2.4. Microdialysis and 5-HT assay

Surgery and perfusion procedures were performed as previously described (Devroye et al., 2017) with minor modifications. Microdialysis probes (CMA/11, cuprophan, 240 μm outer diameter, Carnegie Medicin, Phymep, France) were 4 mm length for the mPFC and 1 mm length for the DRN. Stereotaxic coordinates were chosen according to the atlas of Paxinos and Watson (2005). Rats were anesthetized with 3% isoflurane, and placed in a stereotaxic frame. Two microdialysis probes were simultaneously implanted in the right mPFC (coordinates, in mm, relative to the interaural point: AP = 11.7, L = 0.5, V = 3.7) and in the DRN (20° lateral from vertical, AP = 1, L = −1.5, V = 4.1). After the surgery, the percentage of isoflurane was adjusted to 1.5% until the end of the experiment. Probes were perfused at a constant flow rate (0.5 μl/min), by means of a microperfusion pump (CMA 111, Carnegie Medicin, Phymep), with aCSF 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). 5-HT outflow was monitored during 120 min after the last drug injection. Dialysates were collected in a refrigerated fraction collector (MAB 85 Microbiotech, Phymep) every 20 min.

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.

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

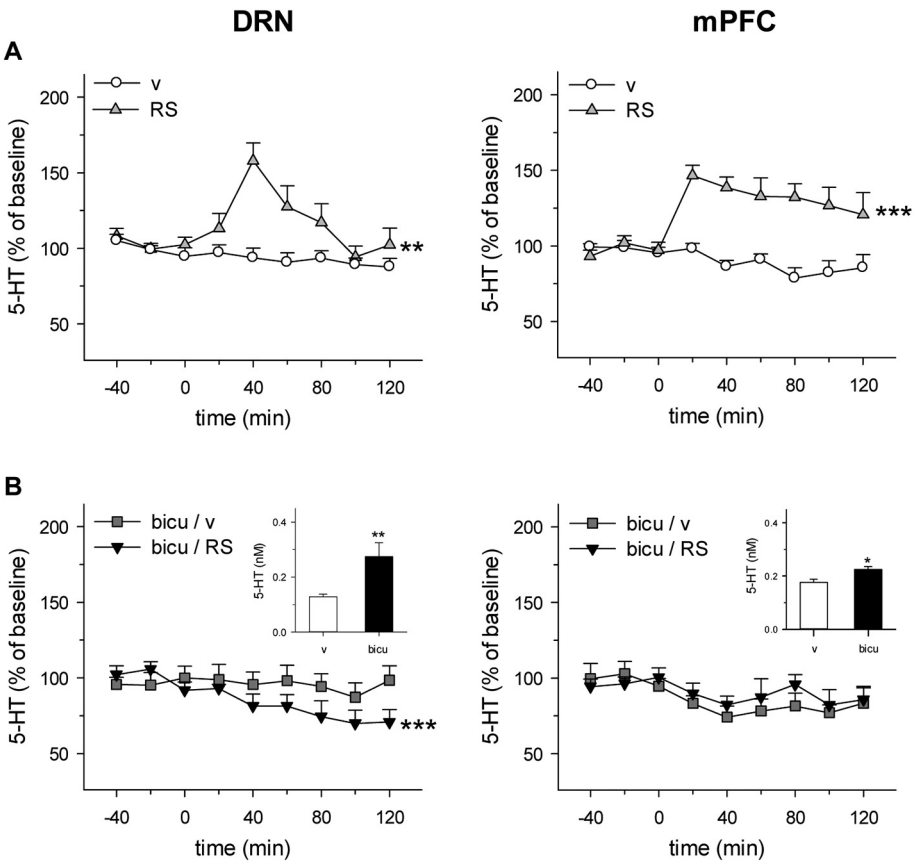

Fig. 1. Time course effect of RS 127445 on 5-HT outflow in the dorsal raphe nucleus (DRN) and the medial prefrontal cortex (mPFC) following its administration alone (A) or in the presence of bicuculline (B). RS 127445 (RS, 0.16 mg/kg) was administered intraperitoneally at time zero. Bicuculline (bicu, 100 µM) was applied into the DRN by reverse dialysis at the beginning of the perfusion (stabilization period) and maintained during the entire experimental period. Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding RS administration (A: n = 6–7 animals/group; B: n = 5–6 animals/group). Absolute basal levels of 5-HT in dialysates collected in each brain region did not differ across the different experimental groups (A: in the DRN, $F_{(1, 12)} = 0.25$, NS and in the mPFC, $F_{(1, 10)} = 0.06$, NS; B: in the DRN, $F_{(1, 8)} = 3.32$, NS and in the mPFC, $F_{(1, 8)} = 0.64$, NS, ANOVA) and were (mean ± SEM) for A: 0.13 ± 0.01 nM (n = 14) in the DRN and 0.19 ± 0.01 nM (n = 12) in the mPFC; for B: 0.30 ± 0.1 nM (n = 10) in the DRN and 0.24 ± 0.02 nM (n = 12) in the mPFC. **p < .01 ***p < .001 *versus* the corresponding v group (Newman-Keuls test). Insets (Fig. 1B). Effect of bicuculline infusion into the DRN on basal 5-HT levels in the DRN and the mPFC. Histograms represent the mean ± SEM absolute basal levels of 5-HT averaged over 60 min monitoring prior to drug injection (DRN: n = 10–15 and mPFC: n = 9–11 animal/group). *p < .05, **p < .01 *versus* the corresponding v group (ANOVA).

described (Devroye et al., 2017). The mobile phase [containing (in mM) 100 phosphoric acid, 100 citric acid, 0.1 EDTA.2H_2O, 1.1 octanesulfonic acid.NaCl plus 6% acetonitrile, adjusted to pH 6.0 with NaOH solution (50%)] was delivered at 0.070 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 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 time for 5-HT was 4–4.5 min. and the sensitivity was 50 pM with a signal/noise ratio of 3:1. 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.

2.5. Immunohistochemistry and laser microdissection and pressure catapulting technique (LMPC)-combined experiments

Rats were deeply anesthetized (pentobarbital, 100 mg/kg, CEVA) and transcardially perfused with phosphate-buffered solution (PBS) containing 4% paraformaldehyde. Dissected brains were cryoprotected in PBS with 30% sucrose and stored at −80 °C. Coronal sections of the DRN (12 µm thickness) were made using a CM3050 S cryostat (Leica Biosystem, Wetzlar, Germany) and mounted on polyethylene-naphthalate membrane glass slides (P.A.L.M. Microlaser Technologies AG, Zeiss, Germany) pretreated with RNasezap (Thermo Fisher Scientific, Walthman, USA) and ultraviolet light to inactivate RNase. After 30 min in a blocking solution (10% goat serum, 1 U/µl RNase inhibitor in PBS) at 4 °C, sections were incubated with a previously described (Botcher et al., 2014) mouse monoclonal anti-glutamic acid decarboxylase 67 (GAD67) antibody (1/500 in 10% goat serum, 1 U/µl RNase inhibitor in PBS, MAB5406, Millipore, Burlington, USA), overnight at 4 °C. After 3 washes (PBS), sections were incubated for 90 min at 4 °C with Alexa 488-conjugated goat anti-mouse (1/1500, 10% goat serum, 1 U/µl RNase inhibitor in PBS). Immediately after 3 rinses in PBS, laser microdissection was performed, as previously described (Maitre et al., 2011), using a P.A.L.M. MicroBeam microdissection system version 4.0–1206 equipped with P.A.L.M. ROBOSOFTWARE (P.A.L.M. Microlaser Technologies AG, Zeiss, Germany). Briefly, at the cellular level, after having delimited the DRN at 5× magnification on each brain slice, green cells were visualized using filter set 10 (Excitation BP 450–490 nm, Emission BP 515-565 nm) at 63× magnification and microdissection was performed. Samples were collected in adhesive caps (P.A.L.M. Microlaser Technologies AG, Bernried,Germany). These cells were resuspended in RNA proteinase K digestion buffer and stored immediately at −80 °C until processing.

2.6. RT-PCR

Preliminary experiments performed in whole DRN extracts revealed a very low expression of 5-HT_{2B}R mRNA. This result prompted us to elaborate another strategy using immunofluorescence and LCMP coupled to real time RT-PCR after pre-amplification.

Total RNA was purified using the Qiagen RNeasy FFPE Kit (Valencia, CA, USA) according to the manufacturer's instructions. Total RNA yield and concentration were quantified using the NanoDrop 1000 (Thermo Scientific, Waltham, MA, USA). RNA integrity was checked using capillary electrophoresis performed on Agilent Bioanalyzer 2100, with RNA 6000 Pico Assay (Agilent Technologies). cDNA was generated using the qScript XLT cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA) following the manufacturer's instructions. Nested pre-amplification PCR was then performed on the LightCycler® 480 (Roche Applied Science, Manheim, Germany) using PerfeCTa PreAmp SuperMix (Quanta Biosciences) with template cDNA and the

outer primers (0.5 μM) (see supplementary Table S1). The reaction mixture was subjected to 17 PCR cycles consisting of an initial denaturation step at 95 °C for 2 min followed by 17 cycles of 95 °C for 15 s, and 60 °C for 3 min. Unincorporated primers were removed by Exonuclease (NEB, Ipswich, MA, USA) digestion. The second amplification was performed, using LightCycler® 480 SYBR Green I Master (Roche Applied Science) on amplified cDNA in a reaction of 10 μl, using the inner primers (0.6 μM, see supplementary Table S1). An initial denaturation step at 95 °C for 5 min was followed by 45 cycles of 95 °C for 15 s, and 60 °C for 30 s. PCR products were ran in an automated electrophoretic DNA separation system (Labchip GX II, Caliper life sciences, MA, USA). For each primer pair a no-template-control without cDNA was also tested, and no PCR products were observed.

2.7. Statistics

Statistical analysis was carried out by Statistica 8.0 for Windows (Statsoft, Maisons-Alfort, France). The effect of bicuculline on the mean DRN and mPFC 5-HT absolute basal values was analyzed by a one-way ANOVA, using group as a main factor (insets in Fig. 1B). The effect of the systemic or intra-DRN administration of RS 127445 (treatment) on 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), (Figs. 1 and 2). As well, the effect of the intra-DRN administration of SB 242084 or MDL 100907 (treatment) on DRN 5-HT outflow was analyzed by a multifactorial ANOVA with treatment as the between-subject factors, and time as the within-subject factor (including values from time 0 to 60 min and 61 to 120 min for 0.1 μM and 1 μM concentrations, respectively) (Fig. 3). When assessing the interaction between WAY 100635 (pretreatment) and RS 127445 (treatment) (Fig. 4) as well as between RS 127445 (pretreatment) and citalopram (treatment) (Fig. 5) on 5-HT outflow, data were 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 − 20 to time 120 min). In each microdialysis experiment, statistical differences in basal 5-HT values among groups were assessed by a one-way ANOVA using group as a main factor. Finally, in all experiments, when multifactorial ANOVA results were significant ($p < .05$), the Newman-Keuls post-hoc test was performed to allow adequate multiple comparisons between groups.

3. Results

3.1. Influence of bicuculline on RS 127445-induced effects on DRN and mPFC 5-HT outflow

Fig. 1 illustrates the effect of RS 127445 on DRN and mPFC 5-HT outflow following its intraperitoneal administration alone (Fig. 1A) or in the presence of bicuculline in the DRN (Fig. 1B).

Statistical analysis revealed a significant and time-dependent effect of treatment ($F_{RS\ (1,\ 12)} = 17.05$, $p < .01$; $F_{RS\ \times\ time\ (6,\ 72)} = 3.39$, $p < .01$) in the DRN (Fig. 1A). Indeed, RS 127445 produced an overall significant increase in 5-HT outflow reaching 158% of baseline ($p < .01$ *versus* the vehicle group). As well, in the mPFC (Fig. 1A), statistical analysis demonstrated a significant and time-dependent effect of treatment ($F_{RS\ (1,\ 10)} = 73.80$, $p < .001$; $F_{RS\ \times\ time\ (6,\ 60)} = 2.39$, $p < .05$). As expected (Devroye et al., 2017), RS 127445 produced an overall significant increase in 5-HT outflow reaching 147% of baseline ($p < .001$ *versus* the vehicle group).

When the effect of RS 127445 on 5-HT outflow was examined in the presence of bicuculline in the DRN (Fig. 1B), statistical analysis revealed a significant and time-independent effect of treatment in the DRN ($F_{bicu/RS\ (1,\ 8)} = 51.55$, $p < .001$; $F_{bicu/RS\ \times\ time\ (6,\ 48)} = 0.30$, NS) and no significant effect of treatment in the mPFC ($F_{bicu/RS\ (1,\ 8)} = 1.08$,

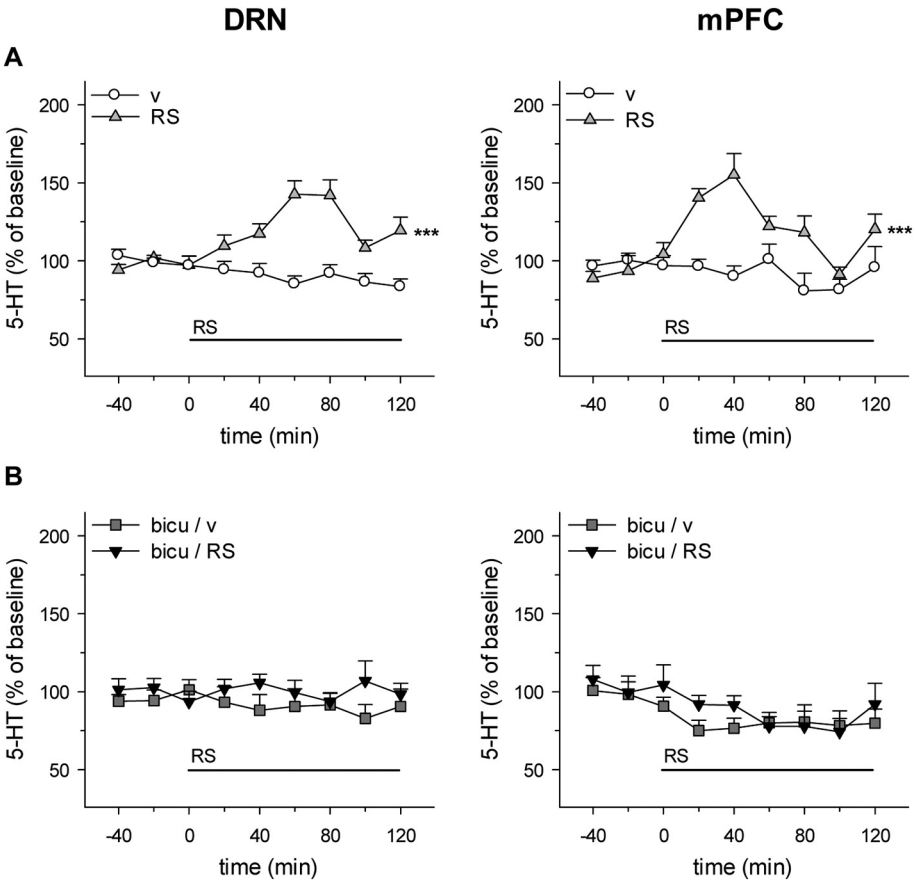

Fig. 2. Time course effect of RS 127445 on 5-HT outflow in the dorsal raphe nucleus (DRN) and the medial prefrontal cortex (mPFC) administered into the DRN alone (A) or in the presence (B) of bicuculline. RS 127445 (RS, 1 μM) was perfused into the DRN by reverse dialysis for 120 min (time zero, horizontal bar). Bicuculline (bicu, 100 μM) was applied into the DRN by reverse dialysis at the beginning of the perfusion and maintained during the entire experimental period. Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding RS administration (A: n = 4–7 animals/group, B: 5–7 animals/group). Absolute basal levels of 5-HT in dialysates collected in each brain region did not differ across the different experimental groups (A: in the DRN, $F_{(1,\ 11)} = 0.61$, NS, and in the mPFC: $F_{(1,\ 7)} = 1.23$, NS; B: in the DRN, $F_{(1,\ 11)} = 0.03$, NS, and in the mPFC: $F_{(1,\ 9)} = 0.20$, NS, ANOVA), and were (mean ± SEM) for A: 0.13 ± 0.01 nM (n = 13) in the DRN, and 0.18 ± 0.0.1 nM (n = 9) in the mPFC); for B: 0.19 ± 0.01 nM (n = 13) in the DRN, and 0.21 ± 0.01 nM (n = 11) in the mPFC). ***p < .001 *versus* the corresponding v group (Newman-Keuls test).

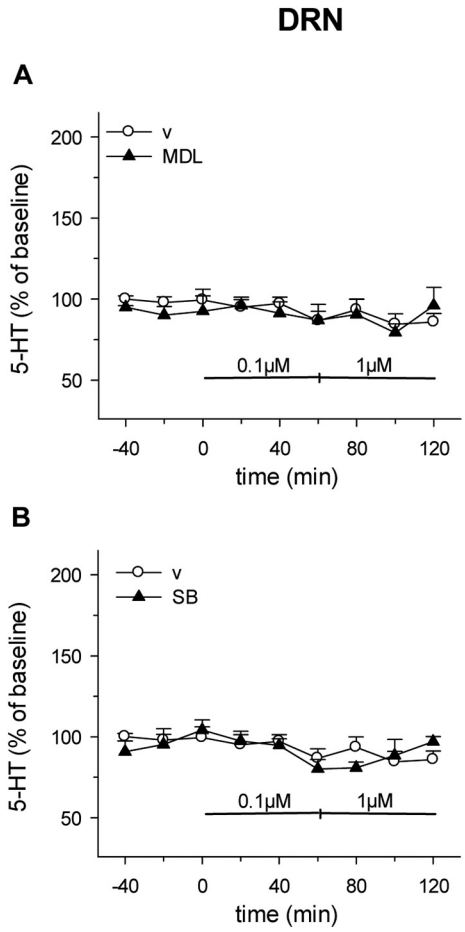

Fig. 3. Time course effect of the intra-dorsal raphe nucleus (DRN) administration of MDL 100907 (A) and SB 242084 (B) on serotonin (5-HT) outflow in the DRN.

MDL 100907 (MDL) or SB 242084 (SB) were applied into the DRN by reverse dialysis at two concentrations (0.1 µM: from 0 to 60 min; 1 µM from 61 to 120 min; horizontal bars). Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding drug administration (A and B: n = 5–6 animals/group). Absolute basal levels of 5-HT in dialysates collected in the DRN did not differ across the different experimental groups (A: $F_{(1,\ 10)} = 1.17$, NS; B: $F_{(1,\ 10)} = 0.09$, NS, ANOVA) and were (mean ± SEM): 0.15 ± 0.01 nM (A, n = 12) and 0.14 ± 0.01 nM (B, n = 12).

NS; $F_{bicu/RS \times time\ (6,\ 48)} = 0.14$, NS). Indeed, at variance with the mPFC, where 5-HT extracellular levels were not significantly different between groups, in the DRN, 5-HT extracellular levels in the bicu/RS group were significantly lower than those in the bicu/v group ($p < .001$ *versus* the bicu/v group). Thus, in both brain regions, bicuculline prevented the facilitatory effect of RS 127445 on 5-HT outflow.

Insets in Fig. 1B show the effect of bicuculline on the absolute basal levels of 5-HT in the DRN and mPFC. In agreement with previous findings (Tao and Auerbach, 2002), intra-DRN infusion of bicuculline produced a significant increase in absolute basal 5-HT extracellular levels in both the DRN ($F_{bicu\ (1,\ 23)} = 11.47$, $p < .01$ *versus* the vehicle group) and the mPFC ($F_{bicu\ (1,\ 18)} = 6.53$, $p < .05$ *versus* the vehicle group). Indeed, absolute basal 5-HT levels in the v group were lower (mean ± SEM: 0.13 ± 0.01 nM, n = 10 animals/group in the DRN and 0.18 ± 0.01 nM, n = 9 animals/group in the mPFC) than in the bicu group (mean ± SEM: 0.27 ± 0.05 nM, n = 15 animals/group in the DRN and 0.22 ± 0.01 nM, n = 11 animals/group in the mPFC). In addition, the effect of bicuculline on basal 5-HT outflow was persistent and stable over time (see time-course effect in Fig. 1B).

Fig. 2 illustrates the effect of RS 127445 on DRN and mPFC 5-HT outflow following its intra-DRN perfusion alone (Fig. 2A) or in the presence of bicuculline (Fig. 2B).

In both the DRN and the mPFC (Fig. 2A), statistical analysis revealed a significant and time-dependent effect of treatment (DRN: $F_{RS\ (1,\ 11)} = 43.15$, $p < .001$; $F_{RS \times time\ (6,\ 66)} = 5.42$, $p < .001$; mPFC: $F_{RS\ (1,\ 7)} = 60.63$, $p < .001$; $F_{RS \times time\ (6,\ 42)} = 3.61$, $p < .01$). Indeed, as following its intraperitoneal injection (Fig. 1B), the intra-DRN administration of RS 127445 produced an overall significant increase in 5-HT outflow reaching 143 and 155% of baseline in the DRN ($p < .001$, *versus* the vehicle group) and in the mPFC ($p < .001$ *versus* the vehicle group), respectively.

When looking at the effect of RS 127445 on 5-HT outflow in the presence of bicuculline (Fig. 2B), statistical analysis revealed no significant effect of treatment in both the DRN ($F_{bicu/RS\ (1,\ 10)} = 1.33$, NS; $F_{bicu/RS \times time\ (6,\ 60)} = 0.86$, NS) and the mPFC ($F_{bicu/RS\ (1,\ 9)} = 1.19$, NS; $F_{bicu/RS \times time\ (6,\ 54)} = 0.69$, NS). Thus, in both brain regions, as observed after its systemic administration (Fig. 1B), the increase in 5-HT outflow induced by the intra-DRN administration of RS 127445 was prevented by the intra-DRN perfusion of bicuculline.

3.2. Effect of MDL 100907 and SB 242084 on DRN 5-HT outflow

Fig. 3 illustrates the effect of the intra-DRN perfusion of MDL 100907 (Fig. 3A) and SB 242084 (Fig. 3B) on DRN 5-HT outflow. As shown in Fig. 3A, MDL 100907 failed to modify basal 5-HT outflow at both the perfused concentrations ($F_{MDL\ 0.1\mu M\ (1,\ 10)} = 0.87$, NS; $F_{MDL\ 1\mu M\ (1,\ 10)} = 0.01$, NS, $F_{MDL\ 0.1\mu M \times time\ (1,\ 10)} = 0.124$, NS; $F_{MDL\ 1\mu M \times time\ (3,\ 30)} = 1.34$, NS). In a similar manner, basal 5-HT outflow remained unaltered following the perfusion of 0.1 or 1 µM SB 242084 ($F_{SB\ 0.1\mu M\ (1,\ 10)} = 0.49$, NS; $F_{SB\ 1\mu M\ (1,\ 10)} = 0.08$, NS, $F_{SB\ 0.1\mu M \times time\ (3,\ 30)} = 0.32$, NS; $F_{SB\ 1\mu M \times time\ (3,\ 30)} = 1.83$, NS, Fig. 3B).

3.3. Effect of WAY 100635 on RS 127445-induced increase in 5-HT outflow in the DRN and the mPFC

The effect of WAY 100635 on RS 127445-induced increase in 5-HT outflow was assessed following their systemic (Fig. 4A) or intra-DRN (Fig. 4B) administration.

In the DRN (Fig. 4A), statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,\ 15)} = 6.70$, $p < .05$; $F_{WAY \times RS \times time\ (6,\ 90)} = 2.36$, $p < .05$). Post-hoc analysis revealed that, as observed previously (Fig. 1B), RS 127445 produced an overall significant increase in 5-HT outflow ($p < .01$, *versus* the vehicle/vehicle group) reaching 155% of baseline. WAY 100635, with no effect on basal 5-HT outflow (NS, *versus* the vehicle/vehicle group), reduced RS 127445-induced increase in 5-HT outflow ($p < .05$, *versus* the vehicle/RS group). As well, in the mPFC (Fig. 4A), statistical analysis revealed a significant effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,\ 16)} = 22.46$, $p < .001$; $F_{WAY \times RS \times time\ (6,\ 96)} = 1.08$, NS). Post-hoc analysis revealed that, as reported previously (Devroye et al., 2017), RS 127445 produced an overall significant increase in 5-HT outflow ($p < .001$, *versus* the vehicle/vehicle group) reaching 147% of baseline. WAY 100635, with no effect on basal 5-HT outflow (NS, *versus* the vehicle/vehicle group), reversed RS 127445-induced increase in 5-HT outflow ($p < .001$, *versus* the vehicle/RS group).

When looking at the interaction between WAY 100635 and RS 127445 in the DRN following their local perfusion (Fig. 4B), statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{WAY \times RS\ (1,\ 26)} = 41.15$, $p < .001$; $F_{WAY \times RS \times time\ (8,\ 208)} = 2.47$, $p < .05$). Post-hoc analysis revealed that RS 127445 produced an overall significant increase in 5-HT outflow ($p < .001$, *versus* the vehicle/vehicle group) reaching 135% of baseline. WAY 100635, with no effect on basal 5-HT outflow (NS, *versus* the vehicle/vehicle group), inhibited RS 127445-induced increase in 5-HT outflow ($p < .001$, *versus* the vehicle/RS group). As well, in the

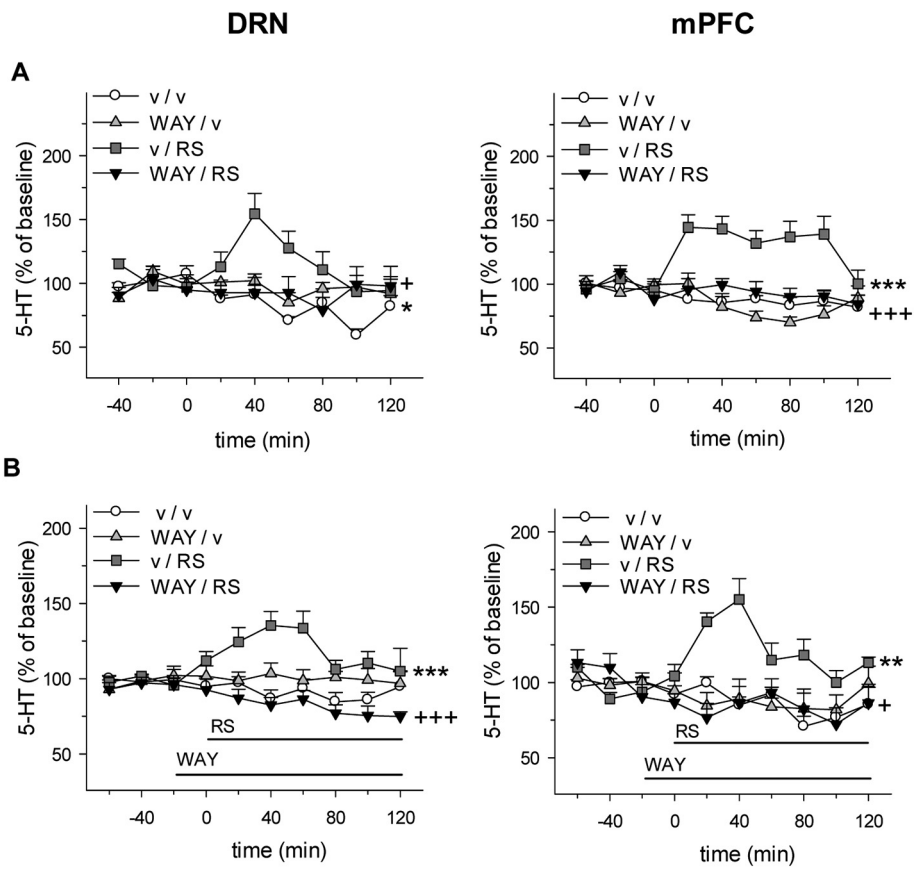

Fig. 4. Time course effect of WAY 100635 on RS 127445-induced increase in serotonin (5-HT) outflow in the dorsal raphe nucleus (DRN) and the medial prefrontal cortex (mPFC), following their systemic (A) or intra-DRN (B) administration. (A) WAY 100635 (WAY, 0.16 mg/kg) was subcutaneously injected immediately prior to the intraperitoneal administration of RS 127445 (time zero, RS, 0.16 mg/kg). (B) WAY (0.1 μM) and RS (1 μM) were applied into the DRN by reverse dialysis for 140 min and 120 min (horizontal bars), respectively, the RS 127445 perfusion starting (time zero) 20 min after that of WAY 100635. Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding the first drug administration (A: n = 4–7 animals/group, B: n = 4–9 animals/group). Absolute basal levels of 5-HT in dialysates collected in each brain region did not differ across the different experimental groups (A: in the DRN, $F_{(3, 15)} = 1.90$, NS, and in the mPFC, $F_{(3, 17)} = 0.40$, NS; B: in the DRN, $F_{(3, 26)} = 0.74$, NS, and in the mPFC, $F_{(3, 16)} = 1.20$, NS, ANOVA) and were (mean ± SEM): for A: 0.11 ± 0.01 nM (n = 18) in the DRN, and 0.17 ± 0.01 nM (n = 21) in the mPFC; for B: 0.13 ± 0.01 nM in the DRN (n = 30), and 0.23 ± 0.02 nM in the mPFC (n = 20). $^{**}p < .01$, $^{***}p < .001$ *versus* the corresponding v/v group and $^{+}p < .05$, $^{+++}p < .001$ *versus* the corresponding v/RS group (Newman-Keuls test).

mPFC (Fig. 4B), statistical analysis revealed a significant effect of pretreatment × treatment interaction ($F_{WAY \times RS (1, 16)} = 4.79$, $p < .05$; $F_{WAY \times RS \times time (7, 112)} = 1.30$, NS). Post-hoc analysis revealed that, as reported previously (Devroye et al., 2017), intra-DRN perfusion of RS 127445 produced an overall significant increase in 5-HT outflow ($p < .01$, *versus* the vehicle/vehicle group) reaching 155% of baseline. WAY 100635, with no effect on basal 5-HT outflow (NS, *versus* the vehicle/vehicle group), prevented RS 127445-induced increase in 5-HT outflow ($p < .05$, *versus* the vehicle/RS group).

3.4. Influence of bicuculline on the effect of RS 127445 on citalopram-induced increase in DRN 5-HT outflow

Fig. 5 illustrates the interaction between RS 127445 and citalopram following their intra-DRN administration alone (Fig. 5A) or in the presence of bicuculline (Fig. 5B).

In Fig. 5A, statistical analysis revealed a significant and time-dependent effect of pretreatment × treatment interaction ($F_{RS \times CIT (1, 20)} = 8.70$, $p < .01$; $F_{RS \times CIT \times time (7, 140)} = 2.19$, $p < .05$). Post-hoc analysis revealed that, as expected (Romero and Artigas, 1997; Tao et al., 2000), citalopram produced an overall significant increase in DRN 5-HT outflow reaching 454% of baseline ($p < .01$, *versus* the vehicle/vehicle group). RS 127445, with no effect by itself on basal 5-HT outflow (NS, *versus* the vehicle/vehicle group), potentiated citalopram-increased 5-HT outflow ($p < .001$, *versus* the vehicle/RS group).

When looking at the interaction between RS 127445 and citalopram in the presence of bicuculline (Fig. 5B), statistical analysis revealed no significant effect of pretreatment × treatment interaction ($F_{bicu/RS \times CIT (1, 19)} = 1.00$, NS; $F_{bicu/RS \times CIT \times time (7, 133)} = 1.12$, NS). Indeed, bicuculline prevented RS 127445-induced potentiation of citalopram-increased 5-HT outflow.

3.5. Identification of the 5-HT_{2B}R mRNA in DRN GABA interneurons

Fig. 6 illustrates the mRNA expression of the 5-HT_{2B}R in DRN GAD67 immuno-positive cells, identified using immunofluorescence and LCMP coupled to real time RT-PCR. Immunofluorescence labeling of GAD67 cells revealed the presence of GABAergic neurons throughout the DRN (Fig. 6B), predominantly in the lateral wings in agreement with previous anatomical data (Monti, 2010). Approximately 500 GAD67 immuno-positive neurons were collected for analysis. As depicted in the electrophoresis gel (Fig. 6C), the expression of the 5-HT_{2B}R gene was detected in DRN GAD67 immuno-positive cells (Fig. 6C), establishing for the first time the presence of this receptor in DRN GABA interneurons. Of note, in the visual cortex, a brain structure in which the expression of the 5-HT_{2B}R gene has not been described (Duxon et al., 1997; Bonaventure et al., 2002), we found no amplification and no PCR products of the 5-HT_{2B}R mRNA (Fig. 6C), thereby consolidating our findings within the DRN. The full untruncated image of the electrophoresis gel is provided in the supplementary Fig. S1.

4. Discussion

The present study, combining *in vivo* intracerebral microdialysis and immunohistochemical and molecular approaches, shows that, in the rat DRN, 5-HT_{2B}Rs located on GABA interneurons exert a tonic inhibitory control on the activity of 5-HT neurons projecting to the mPFC. Indeed, intra-DRN perfusion of the $GABA_A$R antagonist bicuculline prevented the increase in DRN and mPFC 5-HT outflow induced by the systemic or intra-DRN administration of RS 127445, as well as its potentiating effect on the increase in DRN 5-HT outflow induced by the local perfusion of citalopram. Also, immunohistochemistry coupled to RT-PCR experiments confirmed the presence of the 5-HT_{2B}R on DRN GABA cells. These findings, while confirming that the DRN is the main site of action of 5-HT_{2B}R antagonists, provide the first cellular localization of the 5-

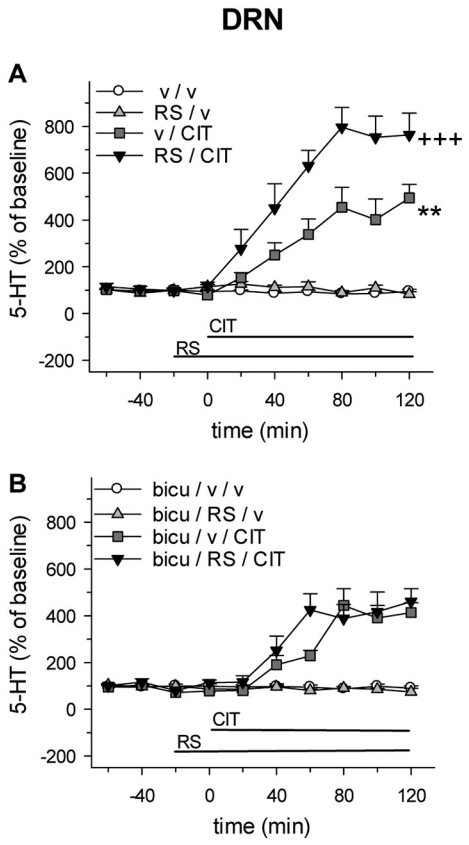

Fig. 5. Time course effect of RS 127445 on citalopram-induced increase in serotonin (5-HT) outflow in the dorsal raphe nucleus (DRN) assessed in the absence (A) or in the presence (B) of bicuculline. All drugs were applied locally into the DRN by reverse dialysis. RS 127445 (RS, 0.5 μM) and citalopram (CIT, 0.1 μM) were perfused for 140 min and 120 min (horizontal bar), respectively, the perfusion of CIT (time zero) starting 20 min after that of RS. Bicuculline (bicu, 100 μM) administration started at the beginning of the perfusion and was maintained during the entire experimental period (280 min including the 120 min stabilization period). Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding RS administration (A: n = 5–7 animals/group; B: 5–8 animals/group). Absolute basal levels of 5-HT in dialysates collected in the DRN did not differ across the different experimental groups (A: $F_{(3,\ 20)} = 2.37$, NS; B: $F_{(3,\ 20)} = 0.79$, NS; ANOVA) and were (mean ± SEM): 0.10 ± 0.01 nM (n = 24) and 0.25 ± 0.01 nM (n = 24) for A and B, respectively. $^{**}p < .01$ *versus* the corresponding v/v group and $^{+++}p < .001$ *versus* the corresponding v/CIT group (Newman-Keuls test).

$HT_{2B}R$ in a rat brain region, and show that 5-HT_{2B}Rs participate in the control of the local, negative-feedback loop regulating 5-HT neuron activity *via* GABA interneurons (see Fig. 7).

Likewise in our previous investigations (Devroye et al., 2016, 2017), in the present study the functional role of DRN 5-HT_{2B}Rs was assessed using the potent and selective 5-HT_{2B}R antagonist RS 127445, which has been well-characterized *in vitro* and *in vivo* (Auclair et al., 2010; Bonhaus et al., 1999; Devroye et al., 2018).

In a first group of experiments, we investigated the effect of bicuculline on the changes of mPFC and DRN 5-HT outflow induced by the systemic administration of RS 127445. As previously observed in freely moving rats (Devroye et al., 2016), we found that RS 127445 increased 5-HT outflow in the mPFC. A similar effect was observed in the DRN, in keeping with the presence, within this brain region, of a substantial number of axon collaterals emitted by 5-HT neurons (Adell et al., 2002). The effect of RS 127445 on DRN and mPFC 5-HT outflow was prevented by the intra-DRN perfusion of bicuculline, which *per se* elicited an increase in basal 5-HT outflow in both brain regions, in agreement with previous studies showing the existence of a tonic GA-BA_AR-mediated inhibitory control on DRN 5-HT neurons (Tao et al., 1996; Tao and Auerbach, 2002). These findings support the involvement of DRN GABA transmission in the 5-HT_{2B}R-mediated control of DRN 5-HT neurons projecting to the mPFC.

The next step of our investigations aimed at assessing the specific involvement of 5-HT_{2B}Rs located within the DRN in the control of 5-HT neuron activity, as suggested by our previous biochemical and electrophysiological studies (Devroye et al., 2017). As observed following its systemic administration, the intra-DRN perfusion of RS 127445 was able to increase 5-HT outflow in both the DRN and the mPFC, these effects being prevented once again by the intra-DRN perfusion of bicuculline. These findings confirm our previous proposal that the DRN is the main site of action of 5-HT_{2B}R antagonists to activate 5-HT outflow in the mPFC and elicit consequent changes of mPFC and NAc DA outflow (Devroye et al., 2017). Furthermore, they demonstrate that, in the DRN, 5-HT_{2B}Rs exert a tonic inhibitory control on 5-HT activity, likely by activating local GABA inhibitory inputs onto 5-HT neurons. Noteworthy, this effect is solely related to the 5-HT_{2B}R, as we found that the intra-DRN perfusion of effective concentrations of selective 5-HT_{2A} and 5-HT_{2C}R antagonists (Navailles et al., 2008; Schmidt et al., 1994) has no influence on basal DRN 5-HT outflow. These latter results, strengthening the selectivity of RS 127445 towards the 5-HT_{2B}R following its local perfusion, are in line with previous electrophysiological and neurochemical studies reporting that neither 5-HT_{2A}Rs nor 5-HT_{2C}Rs exert a tonic regulation of DRN 5-HT neuron activity. Indeed, under basal conditions, DRN 5-HT cell firing and extracellular 5-HT levels remain unaltered following 5-HT_{2A}R and 5-HT_{2C}R antagonist administration (Boothman et al., 2003, 2006; Liu et al., 2000; Quérée et al., 2009; Sharp et al., 2007; Sotty et al., 2009).

In the next group of experiments, given the role of DRN post-synaptic 5-HT_{1A}Rs in the control of GABA interneuron activity (Liu et al., 2000; Sharp et al., 2007), we studied the effect of WAY 100635 on RS 127445-induced increase in DRN and mPFC 5-HT outflow, following their systemic and intra-DRN administration. Interestingly, we found that, under both experimental conditions, the facilitatory effect of RS 127445 on 5-HT outflow was suppressed by WAY 100635 pretreatment, which *per se* had no effect on basal 5-HT outflow, as previously shown (Adell et al., 2002; Lladó-Pelfort et al., 2010). These results cannot be related to an action of WAY 100635 on somatodendritic 5-HT_{1A} autoreceptors, as this would be expected to enhance the effect of RS 127445 on 5-HT outflow (Adell et al., 2002). More likely, as indicated by *in vitro* electrophysiological studies (Liu et al., 2000), the inhibitory effect of WAY 100635 on RS 127445-increased 5-HT outflow could result from a preferential action at postsynaptic 5-HT_{1A}Rs located on GABA interneurons involved in the local, negative-feedback loop regulating DRN 5-HT neurons. Indeed, 5-HT has been shown to exert opposite 5-HT_{1A} inhibitory and 5-$HT_{2A/2C}$ excitatory effects on DRN GABA interneurons (Liu et al., 2000; Sharp et al., 2007, see Fig. 7). Thus, in keeping with our findings suggesting that 5-HT_{2B}Rs also exert an excitatory control on GABA interneurons, the ability of WAY 100635 to prevent the increase in 5-HT outflow induced by RS 127445 could result from their opposite effects on GABA interneuron activity. These findings, showing a functional interaction between postsynaptic 5-HT_{1A}Rs and 5-HT_{2B}Rs in the DRN, provide additional support to the involvement of GABA interneurons in mediating the effect of 5-HT_{2B}R antagonists on 5-HT neuron activity, and point out the 5-HT_{2B}R as a new modulator of the local, negative-feedback loop regulating 5-HT neurons.

We pursued our investigations by assessing the influence of bicuculline on the effects of RS 127445 on citalopram-induced increase in DRN 5-HT outflow following their local infusion in the DRN, to explore a possible direct action of 5-HT_{2B}Rs on 5-HT neurons (Belmer et al., 2018; Diaz et al., 2012; Launay et al., 2006). As expected (Romero and Artigas, 1997; Tao et al., 2000), we found that citalopram enhanced DRN 5-HT outflow. RS 127445, perfused at a concentration without effect *per se*, potentiated citalopram-induced increase in DRN 5-HT

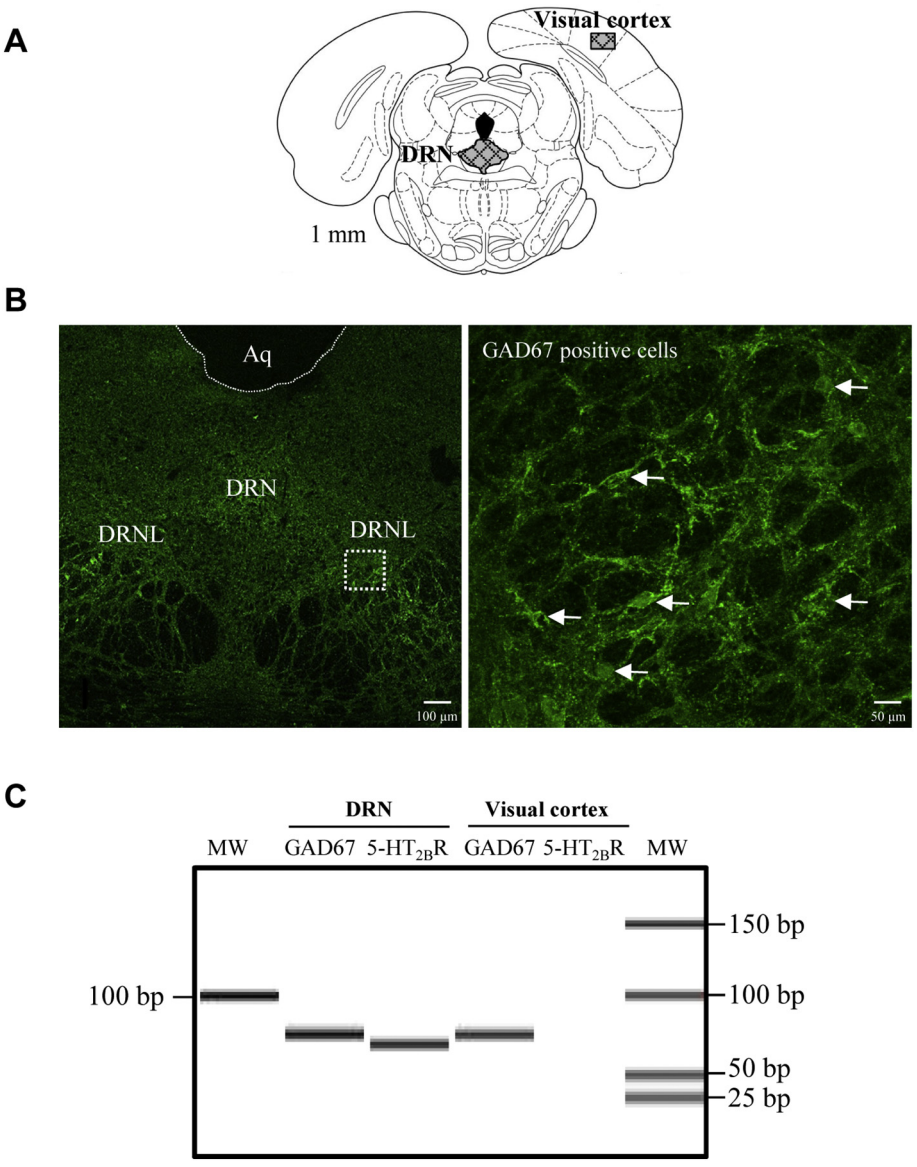

Fig. 6. Cellular location of the gene expression of the serotonin$_{2B}$ receptor (5-HT$_{2B}$R) in the rat dorsal raphe nucleus (DRN). (A) Schematic diagram, taken from the Paxinos and Watson atlas (2005), showing a representative picture of the DRN and the visual cortex. The number beside plate corresponds to millimeter from interaural point. (B) Photographs illustrate glutamic acid decarboxylase 67 (GAD67) fluorescent staining: in the left panel the entire DRN (Aq: Aqueduct; DRNL: dorsal raphe nucleus lateral part; scale bar = 100 µm), white square indicates a representative area where cells were collected; in the right panel the DRNL at higher magnification (scale bar = 50 µm). Horizontal arrows indicate representative GAD67 immuno-positive cells which were collected by laser microdissection and pressure catapulting (LCMP). (C) Electrophoretic gel showing the polymerase chain reaction products obtained from DRN and visual cortex GAD67 immuno-positive cells collected by LCMP: GAD67 and 5-HT$_{2B}$R gene expression. MW: molecular weight; bp: base pair.

outflow, this latter effect being prevented by bicuculline. It is likely that the potentiating effect of RS 127445 is related to the ability of citalopram to increase 5-HT endogenous tone at 5-HT$_{2B}$Rs, thereby leading to an increased responsiveness of GABA-mediated regulatory control of 5-HT neurons to 5-HT$_{2B}$R blockade. These results, while discarding a direct action of 5-HT$_{2B}$Rs on 5-HT transporter-dependent regulation of 5-HT neuron activity previously suggested by studies in mice (Diaz et al., 2012; Launay et al., 2006), provide additional evidence that 5-HT$_{2B}$Rs exert an indirect GABAergic-mediated tonic inhibitory control on 5-HT neurons.

Given the poor selectivity of the available antibodies towards the 5-HT$_{2B}$R (Diaz et al., 2012), we used a combined immunohistochemical and molecular assessment coupled to LMPC (Maitre et al., 2011) to investigate the presence of the 5-HT$_{2B}$R mRNA in DRN GABA cells, identified by the presence of GAD67. Interestingly, these experiments allowed us to visualize the presence of the 5-HT$_{2B}$R mRNA in DRN GABA interneurons, thereby providing an anatomical support to our *in vivo* findings. Noteworthy, this is the first report identifying a specific cellular location of the 5-HT$_{2B}$R in a rat brain region, thus confirming and extending previous findings showing the presence of 5-HT$_{2B}$R mRNA in the rat DRN (Bonaventure et al., 2002).

Altogether, the results obtained in the present study provide compelling anatomo-functional evidence confirming our working hypothesis that 5-HT$_{2B}$Rs afford a GABA-mediated inhibitory control on DRN 5-HT neurons innervating the mPFC (see Fig. 7). Of course, more complex regulations enabling GABAergic inhibitory input to 5-HT cells, such as sequential interactions between multiple interneurons, the involvement of glial cells or glutamatergic afferences to GABA interneurons (Adell et al., 2002; Sharp et al., 2007), cannot be excluded and deserve additional investigations.

Finally, it has to be mentioned that the conclusion offered by the present work contrasts with recent studies in mice showing that 5-HT$_{2B}$Rs are located on 5-HT neurons and exert a direct positive control on 5-HT neuron activity (Belmer et al., 2018). As discussed elsewhere (Devroye et al., 2018), these discrepant findings may result from species related anatomo-functional differences. Additional comparative studies between rats and mice are warranted to identify possible differences in the brain cellular distribution of the 5-HT$_{2B}$R. In this context, the recent development of fluorescent probes targeting the 5-HT$_{2B}$R may allow significant advances in the future (Azuaje et al., 2017).

In conclusion, the present study shows that, in the DRN, 5-HT$_{2B}$Rs located on GABA interneurons afford a tonic inhibitory control on 5-HT neurons innervating the mPFC. Thus, it provides the first evidence of

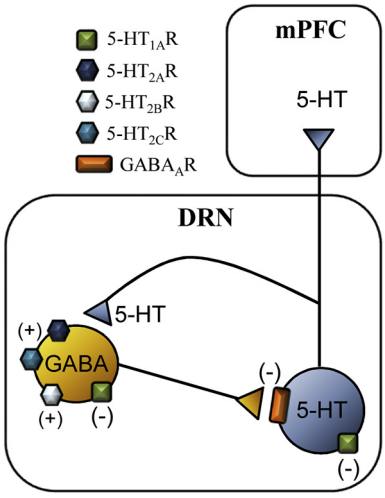

Fig. 7. Schematic representation of the location of serotonin receptors (5-HTRs) implicated in the feedback control of 5-HT neurons in the rat dorsal raphe nucleus (DRN). In addition to the autoinhibitory control exerted by somato-dendritic 5-HT_{1A} autoreceptors, 5-HT neurons are regulated by a local negative-feedback circuit involving GABA interneurons, which express the 5-HT_{1A}R and the different 5-HT_2R subtypes. GABA interneurons undergo opposite inhibitory 5-HT_{1A} and excitatory 5-HT_2R regulatory controls, and provide inhibitory inputs to 5-HT cells *via* $GABA_A$Rs. Blockade of 5-HT_{2B}Rs, by reducing GABA inhibitory tone, leads to increased activity of 5-HT neurons, and consequent increase in 5-HT outflow in the DRN and the medial prefrontal cortex (mPFC).

the location of the 5-HT_{2B}R on a specific cell population in the rat brain, and points out its participation in the control of the negative-feedback loop regulating DRN 5-HT neuron activity, together with the 5-$HT_{2A/2C}$R and the 5-HT_{1A}R (Liu et al., 2000; Serrats et al., 2005; Sharp et al., 2007). It is worth mentioning that, in this context, the 5-HT_{2B}R is the only one providing a tonic control on 5-HT neurons. Finally, from a functional point of view, it confirms the importance of the DRN in mediating the effects of 5-HT_{2B}R antagonists and provides additional information on the mechanisms underlying their effect on the meso-corticolimbic DA system, which has been shown to result from their ability to increase the activity of DRN 5-HT neurons projecting to the mPFC (Devroye et al., 2017). Likewise, given the enhancement of the activity of DRN-mPFC pathway by 5-HT_{2B}R antagonists, these agents may prove useful in the treatment of psychiatric disorders associated to a deficient 5-HT neurotransmission, such as major depressive disorder. The direct stimulation of 5-HT neuron activity by 5-HT_{2B}R antagonists may avoid the autoreceptor-mediated self-inhibitory mechanisms that limit the clinical effects of SSRIs.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2018.09.015.

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