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Serotonin2B receptor blockade in the rat dorsal raphe nucleus suppresses cocaine-induced hyperlocomotion through an opposite control of mesocortical and mesoaccumbens dopamine pathways

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ABSTRACT

Serotonin$_{2B}$ receptor (5-HT_{2B}R) antagonists inhibit cocaine-induced hyperlocomotion independently of changes of accumbal dopamine (DA) release. Given the tight relationship between accumbal DA activity and locomotion, and the inhibitory role of medial prefrontal cortex (mPFC) DA on subcortical DA neurotransmission and DA-dependent behaviors, it has been suggested that the suppressive effect of 5-HT_{2B}R antagonists on cocaine-induced hyperlocomotion may result from an activation of mPFC DA outflow which would subsequently inhibit accumbal DA neurotransmission. Here, we tested this hypothesis by means of the two selective 5-HT_{2B}R antagonists, RS 127445 and LY 266097, using a combination of neurochemical, behavioral and cellular approaches in male rats.

The intraperitoneal (i.p.) administration of RS 127445 (0.16 mg/kg) or LY 266097 (0.63 mg/kg) potentiated cocaine (10 mg/kg, i.p.)-induced mPFC DA outflow. The suppressant effect of RS 127445 on cocaine-induced hyperlocomotion was no longer observed in rats with local 6-OHDA lesions in the mPFC. Also, RS 127445 blocked cocaine-induced changes of accumbal glycogen synthase kinase (GSK) 3β phosphorylation, a post-synaptic cellular marker of DA neurotransmission. Finally, in keeping with the location of 5-HT_{2B}Rs on GABAergic interneurons in the dorsal raphe nucleus (DRN), the intra-DRN perfusion of the $GABA_A$R antagonist bicuculline (100 μM) prevented the effect of the systemic or local (1 μM, intra-DRN) administration of RS 127445 on cocaine-induced mPFC DA outflow. Likewise, intra-DRN bicuculline injection (0.1 μg/0.2 μl) prevented the effect of the systemic RS 127445 administration on cocaine-induced hyperlocomotion and GSK3β phosphorylation.

These results show that DRN 5-HT_{2B}R blockade suppresses cocaine-induced hyperlocomotion by potentiation of cocaine-induced DA outflow in the mPFC and the subsequent inhibition of accumbal DA neurotransmission.

1. Introduction

The serotonin$_{2B}$ receptor (5-HT_{2B}R) is the most recent addition to the brain 5-HT_2R family, which also includes the 5-HT_{2A}R and the 5-HT_{2C}R subtypes (Hannon and Hoyer, 2008). As the other members of this receptor family, the 5-HT_{2B}R has been shown to modulate dopamine (DA) neuron activity and forebrain DA release, by specifically controlling the mesocorticolimbic system. Accordingly, the 5-HT_{2B}R has been proposed as a new pharmacological target for treating DA-related neuropsychiatric disorders, such as schizophrenia or drug addiction (for review see Devroye et al., 2018). In this latter context, studies in rats have shown that 5-HT_{2B}R blockade inhibits cocaine-induced hyperlocomotion (Devroye et al., 2015), a behavioral response related to accumbal DA function (Dunnett and Robbins, 1992) which is classically assessed to

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predict the reinforcing properties of drugs of abuse (Bubar and Cunningham, 2008; Gancarz et al., 2011). The suppressive effect of 5-HT_{2B}R antagonists occurs independently of changes of accumbal and striatal DA outflow, and has been proposed to result from a direct modulation of DA neurotransmission in these brain regions (Devroye et al., 2015). This view is supported by the finding that 5-HT_{2B}R antagonists also suppress the late-onset hyperlocomotion induced by quinpirole, a behavioral response related to the direct stimulation of post-synaptic DA-D_2Rs (Benaliouad et al., 2009; Devroye et al., 2015).

Interestingly, the medial prefrontal cortex (mPFC) may be of particular relevance in this interaction, as previously suggested (Devroye et al., 2015). Indeed, this brain region is anatomically and functionally linked to the nucleus accumbens (NAc) and the striatum, and is known to participate in cocaine-induced behavioral responses (Filip and Cunningham, 2003; Leggio et al., 2009; Tzschentke, 2001). The mPFC contains a very large density of pyramidal neurons projecting to the NAc, located in the prelimbic and infralimbic subdivisions of the ipsi- and contralateral hemispheres (Gabbott et al., 2005). The abundant presence of DA-D_1Rs or DA-D_2Rs in pyramidal neurons of different layers in these subdivisions (Santana and Artigas, 2017) makes up the anatomical/neurochemical substrate for the mPFC-driven modulation of neuronal activity in the NAc. Hence, several studies have shown that mPFC DA activity exerts an inhibitory control on subcortical DA neurotransmission (Jaskiw et al., 1991; Kolachana et al., 1995; Louilot et al., 1989) and on behaviors depending on subcortical DA neurotransmission (Broersen et al., 1999; Lacroix et al., 2000; Vezina et al., 1991).

Thus, it has been suggested that mPFC DA could drive the suppressive effect of 5-HT_{2B}R antagonists on cocaine-induced hyperlocomotion *via* polysynaptic cortical-subcortical (i.e., mPFC-NAc) pathways afferent to the NAc and controlling DA neurotransmission in this brain region (Devroye et al., 2015). However, key information in support of this hypothesis is lacking as the impact of 5-HT_{2B}R antagonists on cocaine-induced DA outflow in the mPFC remains unknown to date.

The present study, combining *in vivo* intracerebral microdialysis, and behavioral and cellular approaches in rats, aimed at assessing this issue by studying (1) the effect of 5-HT_{2B}R antagonists on cocaine-induced DA outflow in the mPFC, (2) the role of mPFC DA in their inhibitory effect on cocaine-induced hyperlocomotion, (3) the impact of 5-HT_{2B}R antagonism on cocaine-induced changes of glycogen synthase kinase (GSK) 3β phosphorylation in the NAc, a cellular marker of accumbal DA-mediated neurotransmission related to cocaine-induced hyperlocomotion (Beaulieu et al., 2007; Kim et al., 2013; Zhao et al., 2016), and (4) the role of the dorsal raphe nucleus (DRN), the main site of action of 5-HT_{2B}Rs to control the mesocorticolimbic DA system activity (Cathala et al., 2019; Devroye et al., 2017), in the effect of 5-HT_{2B}R antagonists on cocaine-induced responses investigated in points 1 to 3.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (IFFA CREDO, Lyon, France) weighing 280–350 g were used (7–9 week aged; total number: 390 rats). 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. 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 antagonists RS 127445.HCl (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine hydrochloride) and LY 266097.HCl (1-[(2-Chloro-3,4-dimethoxyphenyl) methyl]-2,3,4,9-tetrahydro-6-methyl-1H-pyrido[3,4-b] indole hydrochloride), 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 $GABA_A$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), purchased from R&D Systems (Abingdon, UK); cocaine hydrochloride purchased from Cooper (Melun, France); desipramine hydrochloride (DMI) and 6-hydroxydopamine hydrochloride (6-OHDA) purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France); 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).

2.3. Pharmacological treatments

Cocaine was dissolved in NaCl 0.9% (saline), and administered intraperitoneally (i.p.) at 10 mg/kg. RS 127445 was dissolved in a 0.3% Tween 80 distilled water solution, and administered i.p. at 0.16 mg/kg. LY 266097, dissolved in distilled water, was injected i.p. at 0.63 mg/kg. SB 242084 was dissolved in distilled water and administered i.p. at 0.2, 0.5 and 1 mg/kg. MDL 100907, dissolved in distilled water, was injected i.p. at 0.2, 0.5 and 1 mg/kg. All these compounds were administered in a volume of 1 ml/kg. When administered locally into the DRN by reverse dialysis, RS 127445 was first dissolved in 0.3% Tween 80 distilled water solution to obtain a 500 μM concentration, and then further diluted to the required concentration (1 μM) with artificial cerebrospinal fluid (aCSF) just before use in microdialysis experiments.

Bicuculline was first dissolved in distilled water to obtain a 1 mM concentration and then further diluted to the required concentration (100 μM) with aCFS just before its intra-DRN administration by reverse dialysis. For microinjections into the DRN, bicuculline was dissolved in distilled water and injected at 0.1 μg/0.2 μl. DMI was dissolved in saline and injected i.p. at 25 mg/kg in a volume of 3 ml/kg.

Doses, concentrations and pretreatment administration time were chosen according to the pharmacodynamic properties of each drug (Andrew and Johnston, 1979; Audia et al., 1996; Bonhaus et al., 1999; Cussac et al., 2002; Kennett et al., 1997; Kramer et al., 2010) and on the basis of previous studies reporting its selectivity toward the targeted site (Auclair et al., 2010; Berg et al., 2008; Bimpisidis et al., 2013; Bonaccorso et al., 2002; Cathala et al., 2019; Cunningham et al., 2013; Devroye et al., 2015; Fletcher et al., 2002, 2006; Li et al., 2005; Navailles et al., 2004; 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. 6-OHDA lesion procedure

Lesion of mesocortical DA neurons was performed according to a previously described procedure (Bimpisidis et al., 2013) with minor modifications. Briefly, rats were administered with meloxicam (1 mg/kg, i.p.) 30 min before surgery, anesthetized with 3% isoflurane (CSP, Cournon-d'Auvergne, France), placed in a stereotaxic frame and lidocaine was administered subcutaneously (s.c.) around the site of surgery. 6-OHDA was dissolved in saline containing 0.2% ascorbic acid to obtain a final concentration of 4 μg/μl. 6-OHDA or vehicle were administered bilaterally into the mPFC at four different sites (1 μl/site

delivered at a constant flow rate of 0.2 μl/min) through a 30 gauge stainless steel cannula [coordinates, in mm, relative to the interaural point: anteroposterior (AP) = 11.7, lateral (L) = ± 0.7, ventral (V) =7.5 and AP = 12.2, L = ± 0.6, V = 5] (Paxinos and Watson, 2005; see Supplementary Fig. S4). Thirty minutes before each lesion, the animals were injected with desipramine (25 mg/kg, i.p.; 3 ml/kg) to prevent lesion of noradrenergic terminals in the targeted brain area. Immediately after suture, lidocaine was administered s.c. around the site of surgery. After 1 week of post-operative recovery, measurement of locomotor activity was performed.

2.5. Surgical implantation of cannulae and intra-DRN microinjection protocol

In experiments reported in Fig. 6, bicuculline microinjection into the DRN was performed according to a previously described procedure (Devroye et al., 2017; Leggio et al., 2009) with minor modifications. Rats were administered with meloxicam (1 mg/kg, i.p.) 30 min before surgery. Then, rats were anesthetized with 3% isoflurane (CSP) and placed in a stereotaxic frame. A stainless guide-cannula (26 G; Phymep, France) was stereotaxically implanted, just above the DRN (coordinates of the lower extremity of the guide, in mm, relative to the interaural point: 20° lateral from vertical, AP = 1, L = −1.5, V = 4.7, according to the atlas of Paxinos and Watson, 2005, see Supplementary Fig. S4) so that the tip of the injector (33 G), once lowered through the guide-cannula, could reach a depth value of 3.7 mm above the interaural point. Immediately after suture, lidocaine was administered (s.c.) around the site of surgery. Rats received a post-surgical treatment 24 h after surgery and were administered with meloxicam (1 mg/kg, i.p.) and saline (1 ml/kg, i.p.). The injector was inserted in the guide-cannula on the day of the experiment (5–7 days after surgery).

Drug or corresponding vehicle (see section 2.3. for details) was delivered into the DRN in a final volume of 0.2 μl at a constant flow rate of 0.1 μl/min with a 5 μl Hamilton syringe and a syringe pump (CMA 400, Carnegie Medicin, Phymep). After completion of the microinjection, the injector was left in place for an additional 3 min before withdrawal to allow diffusion from the tip and prevent reflux of the injected solution. After intra-DRN injection, locomotor activity test (see section 2.7) or NAc protein extraction for measurement of p-GSK3β were performed (see section 2.9).

2.6. Microdialysis and chromatographic analysis

Surgery and perfusion procedures were performed as previously described (Cathala et al., 2019; Devroye et al., 2017) with minor modifications. For all experiments, microdialysis probes (CMA/11, cuprophan, 240 μm outer diameter, Carnegie Medicin, Phymep) 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).

For experiments performed in freely moving animals (see Fig. 1 and Fig. 2), rats were administered with meloxicam (1 mg/kg, i.p.) 30 min before surgery. Then, rats were anesthetized with 3% isoflurane (CSP), placed in a stereotaxic frame and lidocaine was administered (s.c.) around the site of surgery. A siliconized stainless guide-cannula (Carnegie Medicin, Phymep) was stereotaxically implanted, just above the right mPFC (coordinates of the lower extremity of the guide, in mm, relative to the interaural point: AP = 11.7, L = 0.5, V = 7.7) so that the tip of the probe, once lowered through the guide-cannula, could reach a depth value of 3.7 mm above the interaural point (see Supplementary Fig. S4). 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 experiment (5–7 days after surgery).

For experiments performed in anesthetized animals (see Fig. 5 and Supplementary Figs. S2 and S3), rats were anesthetized with 3% isoflurane (CSP), 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) (see Supplementary Fig. S4). Only one microdialysis probe was implanted in

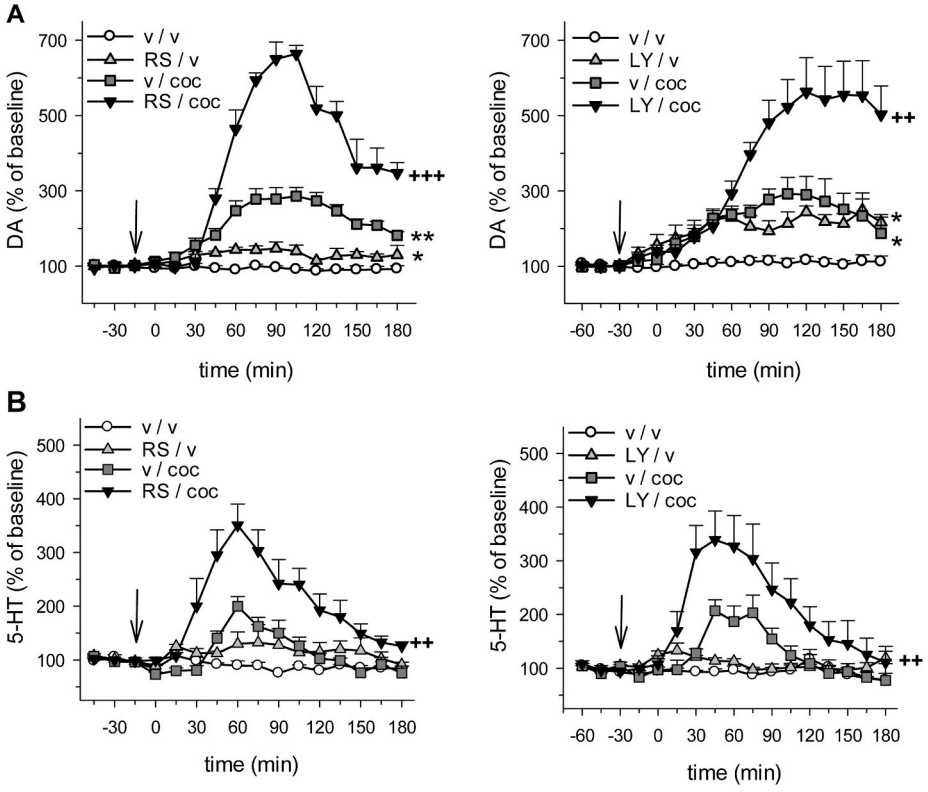

Fig. 1. Time course effect of the administration of RS 127445 and LY 266097 on cocaine-induced increase in (A) dopamine (DA) and (B) serotonin (5-HT) outflow in the medial prefrontal cortex (mPFC). RS 127445 (RS, 0.16 mg/kg) or LY 266097 (LY, 0.63 mg/kg) was intraperitoneally (i.p.) injected (vertical arrow) 15 or 30 min before the administration of cocaine (coc, 10 mg/kg, i.p., time zero), respectively. 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; B: n = 4–5 animals/group). Absolute basal levels of DA in dialysates did not differ across the different experimental groups (A, for RS experiment: $F_{(3, 17)} = 1.20$, NS; for LY experiment: $F_{(3, 16)} = 0.07$, NS, ANOVA) and were (mean ± SEM) for RS: 0.25 ± 0.02 nM (n = 21) and for LY: 0.25 ± 0.03 nM (n = 20). Absolute basal levels of 5-HT in dialysates did not differ across the different experimental groups (B, for RS experiment: $F_{(3, 15)} = 0.5$, NS, ANOVA; for LY experiment: $F_{(3, 14)} = 0.27$, NS, ANOVA) and were (mean ± SEM) for RS: 0.76 ± 0.15 nM (n = 19) and for LY: 0.70 ± 0.05 nM (n = 18). *p < 0.05, **p < 0.01 *versus* the corresponding v/v group and $^{++}$p < 0.01, $^{+++}$p < 0.001 *versus* the corresponding v/coc group (Newman-Keuls test).

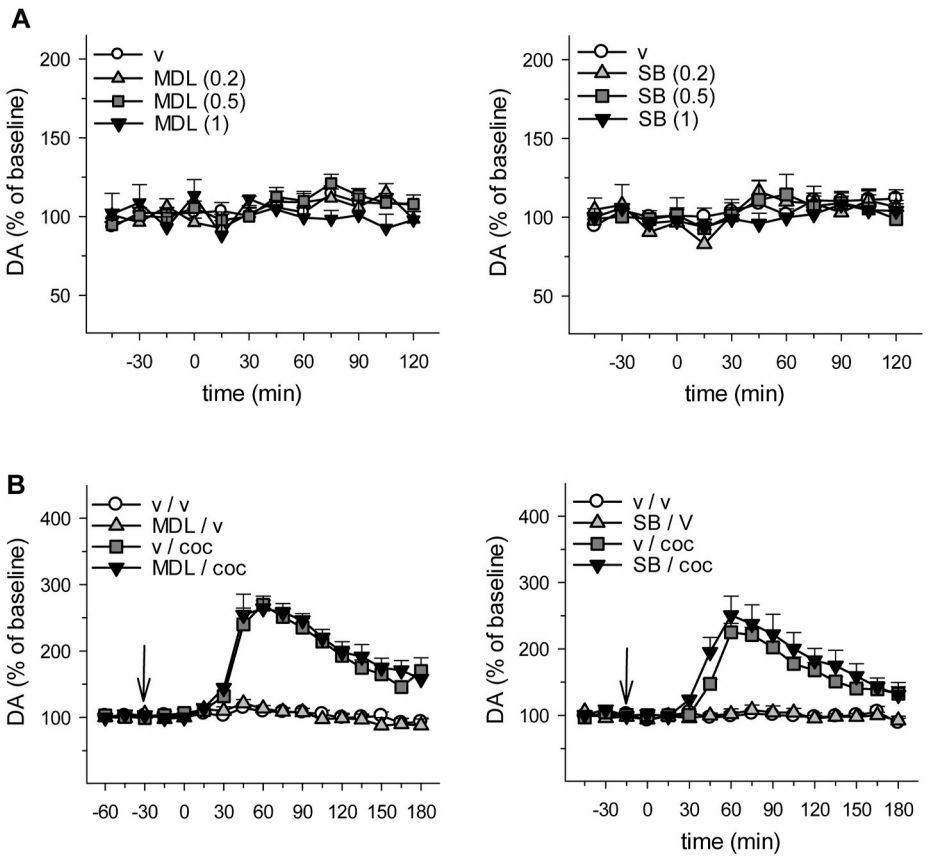

Fig. 2. Time course effect of MDL 100907 and SB 242084 on basal and cocaine-increased dopamine (DA) outflow in the medial prefrontal cortex (mPFC). (A) Dose response effect of the intraperitoneal (i.p.) administration (time zero) of MDL 100907 (MDL; 0.2, 0.5, 1 mg/kg) and SB 242084 (SB; 0.2, 0.5, 1 mg/kg) on mPFC basal DA outflow. (B) Effect of MDL and SB on cocaine-induced increase in mPFC DA outflow. MDL (0.5 mg/kg) or SB (1 mg/kg) was administered i.p. (vertical arrow) 30 or 15 min before the administration of cocaine (coc, 10 mg/kg, i.p., time zero), respectively. Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding the first drug administration (n = 4–6 animals/group). Absolute basal levels of DA in dialysates collected did not differ across the different experimental groups (A: for MDL, $F_{(3, 18)} = 2.49$, NS and for SB, $F_{(3, 14)} = 1.22$, NS; B: for MDL, $F_{(3, 17)} = 0.72$, NS and for SB, $F_{(3, 13)} = 0.94$, NS, ANOVA) and were (mean ± SEM) A: 0.22 ± 0.02 nM (n = 22) for MDL and 0.24 ± 0.02 nM (n = 18) for SB; B: 0.29 ± 0.02 nM (n = 21) for MDL and 0.24 ± 0.02 nM (n = 17) for SB.

the right mPFC in the experiment reported in the Supplementary Fig. 2. 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 aCSF (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 or 5-HT outflow was monitored during 120 or 180 min after the last drug injection. Dialysates were collected in a refrigerated fraction collector (MAB 85 Microbiotech, Phymep) every 15 min.

At the end of each experiment, the animal was deeply anesthetized with a pentobarbital overdose (Exagon, 200 mg/kg, Centravet), and its brain was removed and fixed in NaCl (0.9%)/paraformaldehyde solution (10%). Probe 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 (HPLC) apparatus (Alexys UHPLC/ECD Neurotransmitter Analyzer, Antec, The Netherlands), equipped with an autosampler (AS 110 UHPLC cool 6-PV, Antec), as previously described (Cathala et al., 2019; Devroye et al., 2017). The mobile phase [containing (in mM) 100 phosphoric acid, 100 citric acid, 0.1 EDTA.$2H_2O$, 4.6 or 1.15 octanesulfonic acid.NaCl for DA and 5-HT respectively, 5% acetonitrile and 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 DA or 5-HT was carried out with an electrochemical detector (DECADE II, Antec) with a VT-03 glassy carbon electrode (Antec) set at +300 mV or +460 mV *versus* Ag/AgCl for measurement of DA and 5-HT, respectively. Output signals were recorded on a computer (Clarity, Antec). Under these conditions, the retention time for DA and 5-HT was 4–4.5 min and 5–5.5 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.

2.7. Measurement of locomotor activity

As described previously (Devroye et al., 2015, 2016), locomotor activity was measured in a circular corridor equipped with four photoelectric cells placed at the two perpendicular axes of the apparatus to automatically record horizontal locomotion. The apparatus were placed in a light- and sound-attenuated chamber. All rats were habituated to the test environment for 3 h/day on each of the three days before the start of the experiment. Drug injections (see section 2.3. for details) were performed outside the testing room. After the last injection, rats were placed into the circular corridor, and locomotor activity was recorded for a period of 120 min. Data are presented as mean ± S.E.M. total horizontal activity counts.

2.8. Tissue dissection and measurement of tissue levels of biogenic amines

Immediately after behavioral test, rats were sacrificed and brains were then quickly dissected and snap-frozen in isopentane (Sigma-Aldrich) and stored at −80 °C before to be subjected to laser microdissection and pressure catapulting (LMPC) technique. Brain sections obtained from LMPC were prepared using a procedure previously described (Cathala et al., 2019; Maitre et al., 2011). Briefly, laser-assisted microdissection of the mPFC was performed using a P.A.L.M. MicroBeam microdissection system (P.A.L.M. Microlaser Technologies AG, Zeiss, Germany) on 60 μm thick cresyl violet counterstained

coronal sections. Tissue sections from the mPFC were homogenized in 400 µl of 0.1 M $HClO_4$ using the homogenizer Precellys 24 (Bertin Technologies, Montigny-le-Bretonneux, France) and centrifuged at 10000 r.p.m. for 30 min at 4 °C. Supernatants were filtered (Ultrafree-MC, 0.22 µm, Merck-Millipore, France) and injected in an HPLC system (for details see section 2.4). The mobile phase [containing (in mM) 100 phosphoric acid, 100 citric acid, 0.1 EDTA.$2H_2O$, 4,6 octanesulfonic acid.NaCl plus 6% acetonitrile, adjusted to pH 6.0 with NaOH solution (50%)] was delivered at 0.070 ml/min flow rate. Detection of noradrenaline (NA), DA and 5-HT was carried out with a VT-03 glassy carbon electrode (Antec) set at +460 mV *versus* Ag/AgCl. Under these conditions, the retention time for NA, DA and 5-HT was 1.5, 4, and 10 min respectively, and the sensitivity was 50 pM with a signal/noise ratio of 3:1 for all compounds. Results are expressed as the mean ± S.E.M. mg/mm^2 of microdissected surface.

2.9. Protein extraction and quantitation by AlphaLISA analysis

Protein preparation and quantification were performed as previously described (Zanese et al., 2020) with minor modifications. On the test day, in the first experiment (Fig. 4), rats were randomly assigned to four groups and each group received a pretreatment with RS 127445 (0.16 mg/kg; i.p.) or vehicle, 15 min prior to treatment with vehicle or cocaine (10 mg/kg; i.p.). In a second experiment (Fig. 6B), each group received a pretreatment with bicuculline (0.1 µg/0.2 µl) or vehicle into the DRN 5 min before treatment with vehicle or RS 127445 (0.16 mg/kg; i.p.); all animals received a cocaine injection (10 mg/kg; i.p.) 15 min after treatment. Animals were quickly sacrificed by decapitation 10 min after the last injection. This sacrifice time-point was chosen on the basis of a previous experiment assessing the time-dependency of the effect of cocaine on GSK3β^{Ser9} phosphorylation (see Supplementary Fig. S1). To preserve phosphorylation, the NAc including both the shell and the core subregions (see Supplementary Fig. S4) was quickly dissected on ice, and stored at −80 °C in Precellys tubes (Bertin Technologies) before protein extraction (Revest et al., 2010). Total proteins from the NAc were extracted in AlphaLISA SureFire Ultra lysis buffer supplemented with protease and phosphatase inhibitors (#P8340 and #P0044, Sigma, USA) using the tissue grinder Precellys 24 homogenizer (Bertin Technologies). The homogenizing protocol of brain samples in lysis buffer was two cycles of 30 s at 2655 G-force with a 10-sec break between the two cycles using ceramic CK14 beads (#03961-1-0032, Bertin Technologies). After homogenization, samples were centrifuged three times at 10.621 G-force, 10 min at 4 °C, and total proteins in the supernatants were quantified using a Direct Detect X spectrometer (Merck Millipore). Then protein samples were stored at −80 °C until use.

Cofilin (ALSU-TCOF-A500) and p-GSK3β^{Ser9} (ALSU-PGS3B-A500) levels were quantified using AlphaLISA SureFire Ultra kits according to PerkinElmer's instructions. Briefly, the samples were diluted in lysis buffer at the desired concentration: 0.125 and 0.250 µg/µL were used to measure GSK3β^{Ser9} and Cofilin respectively. Then, 10 µl/well of diluted protein samples were transferred to an OptiPlate-384 white opaque microplate (PerkinElmer, USA) and 5 µl of acceptor mix were added to wells. The plate was sealed with a clear adhesive film and incubated for 1 h at room temperature. Then 5 µl of donor mix were added to wells under subdued light (<100 lux). The plate was sealed again with a clear adhesive film, covered with foil and incubated for 1 h at room temperature in the dark. Finally, the plate was read with an Alpha technology-compatible plate reader (EnSpire Alpha plate reader, PerkinElmer) using default AlphaLISA settings. Alpha measurements were performed in duplicates: each sample was systematically transferred in two independent wells and the mean Alpha signal value of both wells was used. Raw data represent the luminescence intensity of the Alpha signal expressed as a number of counts. Results are represented as the mean ± SEM of the ratio between p-GSK3β Alpha signal and cofilin Alpha signal.

2.10. Statistics

Statistical analysis was carried out by Statistica 8.0 for Windows (Statsoft, Maisons-Alfort, France).

In microdialysis experiments, the effect of the systemic administration of RS 127445, SB 242084 or MDL 100907 (pretreatment) on cocaine (treatment)-induced DA (Figs. 1A and 2B) or 5-HT (Fig. 1B) outflow in the mPFC 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 −15 or −30 to time 120 min or 180min). The effect of the systemic administration of SB 242084 or MDL 100907 (treatment) on mPFC DA 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 0–120 min) (Fig. 2A). When assessing the interaction between intra-DRN administration of RS 127445 (pretreatment) and cocaine (treatment) on mPFC DA outflow in the presence or absence of bicuculline, 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 −15 to time 120 min) (Fig. 5). In each microdialysis experiment, statistical differences in basal DA and 5-HT values among groups were assessed by a one-way ANOVA using group as a main factor.

In behavioral experiments, the ability of RS 127445 or bicuculline (pretreatment) to modify the effect of cocaine (treatment) on locomotor activity was analyzed by a multifactorial ANOVA with pretreatment and treatment as the between-subject factors (Fig. 3 and Fig. 6A).

As well, in cellular biology experiments, the ability of RS 127445 or bicuculline (pretreatment) to modify the effect of cocaine (treatment) on p-GSK3β activity was analyzed by a multifactorial ANOVA with pretreatment and treatment as the between-subject factors (Figs. 4 and 6B). In all experiments, when multifactorial ANOVA results were significant ($p < 0.05$), the *post-hoc* Newman-Keuls test was performed to allow adequate multiple comparisons between groups.

Finally, differences in tissue neurotransmitter levels (NA, DA and 5-HT) between sham and 6-OHDA-lesioned groups (Table 1) were analyzed by a one-way ANOVA using group as a main factor.

3. Results

3.1. Effect of RS 127445 and LY 266097 on cocaine-induced DA and 5-HT outflow in the mPFC

Fig. 1 shows the potentiation of cocaine-induced DA (Fig. 1A) and 5-HT (Fig. 1B) outflow in the mPFC by the systemic administration of the selective 5-HT_{2B}R antagonists RS 127445 and LY 266097.

When examining the interaction between RS 127445 and cocaine on DA outflow, statistical analysis revealed a significant and time-dependent effect of pretreatment (RS) x treatment (coc) interaction ($F_{RS\ x\ coc\ (1,17)} = 39.28$, $p < 0.001$; $F_{RS\ x\ coc\ x\ time\ (13,221)} = 13.59$, $p < 0.001$). *Post-hoc* analysis revealed that, as reported previously (Devroye et al., 2016; Tanda et al., 1997), RS 127445 and cocaine produced an overall significant increase in DA outflow ($p < 0.01$ and $p < 0.05$, *versus* the vehicle/vehicle group respectively), reaching 147% and 286% of baseline, respectively. Cocaine-induced DA outflow was significantly potentiated by RS 127445 pretreatment ($p < 0.001$ *versus* the vehicle/cocaine group). Likewise, when assessing the interaction between LY 266097 and cocaine on DA outflow (Fig. 1A), statistical analysis revealed a significant time-dependent effect of pretreatment (LY) x treatment (coc) interaction ($F_{LY\ x\ coc\ (1,16)} = 0.76$, NS; $F_{LY\ x\ coc\ x\ time\ (14,224)} = 5.99$, $p < 0.001$). *Post-hoc* analysis revealed that, as reported previously (Devroye et al., 2016; Tanda et al., 1997), LY 266097 and cocaine produced an overall significant increase in DA outflow ($p < 0.05$ *versus* the vehicle/vehicle group for both compounds), reaching 224% and 293% of baseline, respectively. Also, cocaine-induced DA outflow was significantly potentiated by LY 266097 pretreatment ($p < 0.01$

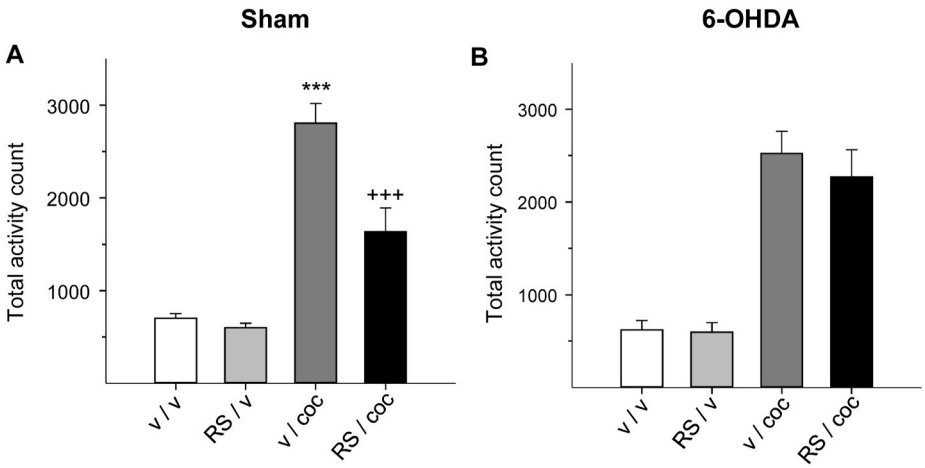

Fig. 3. Effect of RS 127445 on cocaine-induced hyperlocomotion in (A) sham or (B) 6-hydroxydopamine (6-OHDA)-lesioned rats. RS 127445 (RS, 0.16 mg/kg) was intraperitoneally (i.p.) injected 15 before the administration of cocaine (coc, 10 mg/kg, i.p.). Histograms represent the mean ± SEM horizontal activity counts over a 2-h test period (n = 6–7 and n = 7–8 animals/group for sham and 6-OHDA experiments respectively). ***p < 0.001 *versus* the corresponding v/v group and $^{+++}$p < 0.001 *versus* the corresponding v/coc group (Newman-Keuls test).

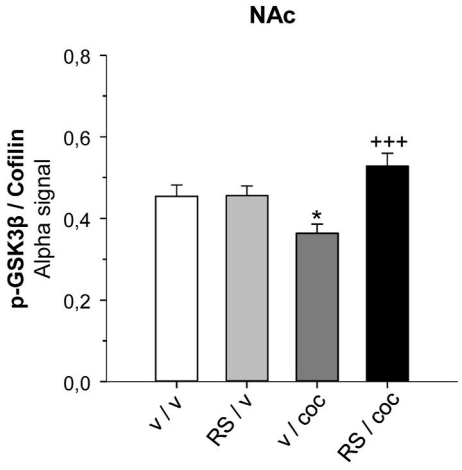

Fig. 4. Effect of RS 127445 on cocaine-induced changes of glycogen synthase kinase (GSK) 3β phosphorylation in the nucleus accumbens (NAc). RS 127445 (RS, 0.16 mg/kg) was intraperitoneally (i.p.) injected 15 before the administration of cocaine (coc, 10 mg/kg, i.p.). Histograms represent the mean ± SEM ratio p-GSK3β/cofilin Alpha signal (n = 7–8 animals/group). *p < 0.05 *versus* the corresponding v/v group and $^{+++}$p < 0.001 *versus* the corresponding v/coc group (Newman-Keuls test).

versus the vehicle/cocaine group).

When assessing the interaction between RS 127445 and cocaine on 5HT outflow (Fig. 1B), statistical analysis revealed a significant and time-dependent effect of pretreatment (RS) x treatment (coc) interaction ($F_{RS\ x\ coc\ (1,15)} = 4.86$, $p < 0.05$; $F_{RS\ x\ coc\ x\ time\ (13,195)} = 1.79$, $p < 0.05$). As reported previously (Cathala et al., 2019; Devroye et al., 2016; Pum et al., 2007), RS 127445 and cocaine *per se* elicited an overall increase in 5-HT outflow reaching 149% and 200% of baseline, respectively ($F_{RS\ (1,15)} = 16.07$, $p < 0.01$; $F_{RS\ x\ time\ (13,195)} = 3.76$, $p < 0.001$; $F_{coc\ (1,15)} = 14.88$, $p < 0.01$; $F_{coc\ x\ time\ (13,195)} = 13.13$, $p < 0.001$). However, these effects, because of their small size, did not reach statistical significance in the context of our statistical analysis (*post-hoc* Newman-Keuls test). Finally, *post-hoc* analysis revealed that cocaine-induced 5-HT outflow was significantly potentiated by RS 127445 pretreatment ($p < 0.01$ *versus* the vehicle/cocaine group).

Similarly, when examining the interaction between LY 266097 and cocaine on 5-HT outflow (Fig. 1B), statistical analysis revealed a significant time-dependent effect of pretreatment (LY) x treatment (coc) interaction ($F_{LY\ x\ coc\ (1,14)} = 4.81$, $p < 0.05$; $F_{LY\ x\ coc\ x\ time\ (14,196)} = 2.57$, $p < 0.01$). As in the case of RS 127445/cocaine interaction described above, the increases in 5-HT outflow induced by LY 266097 or cocaine

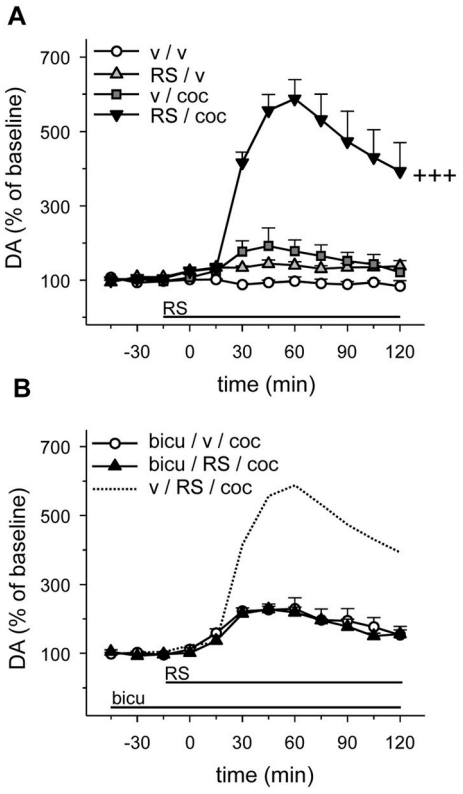

Fig. 5. Time course effect of the intra-dorsal raphe nucleus (DRN) administration of RS 127445 on cocaine-induced increase in dopamine (DA) outflow in the medial prefrontal cortex (mPFC) assessed in (A) the absence or (B) in the presence of bicuculline. RS 127445 (RS, 1 μM) was perfused into the DRN by reverse dialysis for 135 min (time −15, horizontal bar). Cocaine (coc, 10 mg/kg) was administered intraperitoneally at time zero. As illustrated in panel B, 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. To illustrate the influence of bicuculline on the effect of the intra-DRN administration of RS 127445 on cocaine-increased mPFC DA outflow, data of the RS 127445/cocaine interaction are replotted from panel A and are shown without error bars (dotted line). Data are represented as the mean ± SEM percentages of the baseline calculated from the three samples preceding the first drug administration (n = 4–6 animals/group). Absolute basal levels of DA in dialysates collected did not differ across the different experimental groups (A: $F_{(3,16)} = 0.55$, NS; B: $F_{(1,8)} = 0.39$, NS, ANOVA) and were (mean ± SEM) for A: 0.27 ± 0.03 nM (n = 20) and for B: 0.28 ± 0.02 nM (n = 10). $^{+++}$p < 0.001 *versus* the corresponding v/coc group (Newman-Keuls test).

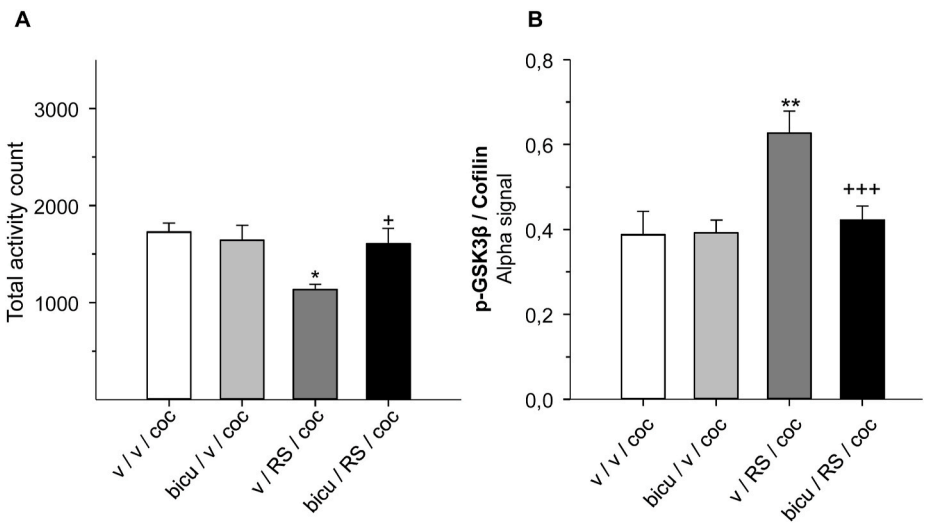

Fig. 6. Influence of the intra-dorsal raphe nucleus (DRN) microinjection of bicuculline on the effect of RS 127445 on (A) cocaine-induced hyperlocomotion and (B) cocaine-induced inhibition of glycogen synthase kinase (GSK) 3β phosphorylation in the nucleus accumbens (NAc). A: bicuculline (bicu) was injected into the DRN (0.1 μg/0.2 μl) 5 min before the intraperitoneal (i.p.) administration of RS 127445 (RS, 0.16 mg/kg). Cocaine (coc, 10 mg/kg, i.p.) was administered i.p 15 min after RS administration. Histograms represent the mean ± SEM horizontal activity counts over a 2-h test period (n = 6–8 animals/group). *p < 0.05 *versus* the corresponding v/v/coc group and $^{+}$p < 0.05 *versus* the corresponding v/RS/coc group (Newman-Keuls test). B: bicuculline (bicu) was injected into the DRN (0.1 μg/0.2 μl) 5 min before the intraperitoneal administration of RS (0.16 mg/kg). Cocaine (coc, 10 mg/kg) was administered i.p 15 min after RS administration. Histograms represent the mean ± SEM ratio p-GSK3β/cofilin Alpha signal (n = 7–8 animals/group). **p < 0.01 *versus* the corresponding v/v/coc group and $^{+++}$p < 0.001 *versus* the corresponding v/RS/coc group (Newman-Keuls test).

Table 1
Effect of 6-OHDA lesion on tissue levels of noradrenaline, dopamine and serotonin in the medial prefrontal cortex.

Group	Noradrenaline	Dopamine	Serotonin
Sham	8.38±0.47	16.38±1.43	2.40±0.34
6-OHDA	6.57±0.61*	5.16±0.66***	2.22±0.22
Percentage depletion	22%	68%	8%

Tissue levels (mg/mm^2 micro-dissected surface) and percentage of depletion of noradrenaline, dopamine and serotonin in the medial prefrontal cortex of randomly chosen 6-hydroxydopamine (6-OHDA) (n = 7) and sham (n = 7) operated rats. Value are means ± SEM; *p < 0.05, ***p < 0.001 *versus* the sham group (Newman-Keuls test).

per se ($F_{LY\ (1,\ 14)} = 10.05$, $p < 0.01$; $F_{LY\ x\ time\ (14,\ 196)} = 2.87$, $p < 0.001$; $F_{coc\ (1,\ 14)} = 18.34$, $p < 0.001$; $F_{coc\ x\ time\ (14,\ 196)} = 14.82$, $p < 0.001$), because of their small size (133% and 203% of baseline) (Devroye et al., 2016; Pum et al., 2007), failed to reach statistical significance in the context of our analysis (*post-hoc* Newman-Keuls test). Finally, *post-hoc* analysis revealed that cocaine-induced 5-HT outflow was significantly potentiated by LY 266097 pretreatment (p < 0.01 *versus* the vehicle/cocaine group).

3.2. Effect of SB 242084 and MDL 100907 on basal and cocaine-induced DA outflow in the mPFC

Fig. 2 illustrates the dose-response effect of the 5-HT_{2A}R antagonist MDL 100907 and the 5-HT_{2C}R antagonist SB 242084 on basal DA outflow (Fig. 2A), and the effect of a single dose of each antagonist on cocaine-induced DA outflow in the mPFC (Fig. 2B).

As shown in Fig. 2A, both compounds failed to modify basal DA outflow at any of the administered doses (MDL 100907: 0.2, 0.5, 1 mg/kg; SB 242084: 0.2, 0.5, 1 mg/kg). Indeed, statistical analysis revealed no significant and time-dependent effect of either MDL 100907 ($F_{MDL\ (3,18)} = 1.07$, NS; $F_{MDL\ x\ time\ (24,144)} = 1.07$, NS) or SB 242084 ($F_{SB\ (3,14)} = 0.23$, NS; $F_{SB\ x\ time\ (24,112)} = 0.67$, NS).

As well, as shown in Fig. 2B, MDL 100907 (0.5 mg/kg) and SB 2042084 (1 mg/kg) failed to modify cocaine-increased DA outflow. Indeed statistical analysis revealed no significant and time-dependent effect of pretreatment (MDL or SB) x treatment (coc) interaction ($F_{MDL\ x\ coc\ (1,17)} = 0.41$, NS; $F_{MDL\ x\ coc\ x\ time\ (14,238)} = 0.31$, NS; $F_{SB\ x\ coc\ (1,13)} = 0.66$, NS; $F_{SB\ x\ coc\ x\ time\ (13,169)} = 0.54$, NS).

3.3. 6-OHDA lesions

Table 1 shows the NA, DA and 5-HT tissue levels and their percentage depletion in the mPFC of sham and 6-OHDA lesioned rats.

6-OHDA lesions produced a significant decrease in DA concentration (68% depletion, $F_{Group\ (1,\ 12)} = 51.06$, $p < 0.001$) and, to a lesser extent, in NA concentration (22% depletion, $F_{Group\ (1,\ 12)} = 5.46$, $p < 0.05$) but did not produce a statistically significant effect on 5-HT levels (8%, $F_{Group\ (1,\ 12)} = 0.21$, NS).

In the same animals, mPFC 6-OHDA lesion did not significantly alter DA, NA and 5-HT concentrations in the NAc (in mg/mm^2 of micro-dissected surface; DA: sham 120.95 ± 11.87 and 6-OHDA 126.46 ± 11.35, $F_{Group\ (1,\ 12)} = 0.11$, NS; NA: sham 16.57 ± 2.42 and 6-OHDA 17.20 ± 0.68, $F_{Group\ (1,\ 12)} = 0.06$, NS; 5-HT: sham 3.63 ± 0.42 and 6-OHDA 4.16 ± 0.39, $F_{Group\ (1,\ 12)} = 0.85$, NS).

3.4. Influence of mPFC 6-OHDA lesion on the effect of RS 127445 on cocaine-induced hyperlocomotion

Fig. 3 illustrates the influence of mPFC DA lesion on the effect of RS 127445 on cocaine-induced hyperlocomotion. For the sham group (Fig. 3A), statistical analysis revealed a significant pretreatment (RS) x treatment (coc) interaction ($F_{RS\ x\ coc\ (1,23)} = 6.82$, $p < 0.05$). *Post-hoc* analysis revealed that, as reported previously (Devroye et al., 2015), cocaine produced a significant increase in total locomotor counts recorded over the 120 min test session, reaching about 399% of basal activity (p < 0.001, *versus* the vehicle/vehicle group). Cocaine-induced hyperlocomotion was significantly reduced by about 42% by RS 127445 pretreatment (p < 0.001, *versus* the vehicle/cocaine group). In 6-OHDA lesioned rats (Fig. 3B), statistical analysis revealed no significant pretreatment (RS) x treatment (coc) interaction ($F_{RS\ x\ coc\ (1,\ 25)} = 0.29$, NS), showing that RS 127445-induced inhibition of cocaine-increased locomotion was prevented by mPFC 6-OHDA lesions. Finally, in both sham and 6-OHDA lesioned animals, administration of RS 127445 did not significantly alter basal locomotor activity (p > 0.05, *versus* the vehicle/vehicle group).

3.5. Effect of RS 127445 on cocaine-induced changes of GSK3β phosphorylation in the NAc

In keeping with the time-dependent regulation (Kim et al., 2013; Salles et al., 2013) of GSK3β phosphorylation in rodent brain, a

time-course study of the effect of cocaine was performed (see Supplementary Fig. S1). Statistical analysis demonstrated a significant effect of treatment (coc) 10 min, but not 20 and 30 min, after its injection ($F_{coc\ (1,\ 10)} = 9.89$, $p < 0.05$, ANOVA), showing that cocaine produced an overall significant decrease in p-GSK3β in the NAc.

On the basis of these results, the effect of RS 127445 on cocaine-induced decrease in GSK3β phosphorylation was studied 10 min after cocaine administration (Fig. 4). Statistical analysis revealed a significant pretreatment (RS) x treatment (coc) interaction ($F_{RS\ x\ coc\ (1,26)} = 9.24$, $p < 0.05$). *Post-hoc* analysis showed that, as reported previously (Kim et al., 2013; Zhao et al., 2016), cocaine produced a significant decrease by 20% in p-GSK3β in the NAc ($p < 0.05$, *versus* the vehicle/vehicle group). RS 127445, without effect by itself ($p > 0.05$ *versus* the vehicle/vehicle group) significantly blocked the suppressant effect of cocaine on p-GSK3β in the NAc ($p < 0.001$, *versus* the vehicle/cocaine group).

3.6. Effect of RS 127445 on cocaine-induced DA outflow in the mPFC in anesthetized rats

The effect of the systemic administration of RS 127445 on cocaine-induced DA outflow in the mPFC in anesthetized rats is illustrated in the Supplementary Fig. S2. Of note, this experiment allowed us to confirm the ability of RS 127445 to potentiate cocaine-increased DA outflow in anesthetized animals, an experimental condition used in subsequent experiments requiring a second probe implantation in the DRN (see section 3.7).

Statistical analysis revealed a significant and time-dependent effect of pretreatment (RS) x treatment (coc) interaction ($F_{RS\ x\ coc\ (1,17)} = 57,67$, $p < 0.001$; $F_{RS\ x\ coc\ x\ time\ (9,153)} = 42.77$, $p < 0.001$). *Post-hoc* analysis revealed that, as reported previously (Tanda et al., 1997), cocaine produced an overall significant increase in DA outflow ($p < 0.01$ *versus* the vehicle/vehicle group). As reported previously (Devroye et al., 2016), RS 127445 *per se* elicited an overall increase in DA outflow reaching 124% of baseline ($F_{RS\ (1,\ 17)} = 75.74$, $p < 0.001$; $F_{RS\ x\ time\ (9,\ 153)} = 34.99$, $p < 0.001$; $F_{coc\ (1,\ 17)} = 187.64$, $p < 0.001$; $F_{coc\ x\ time\ (9,\ 153)} = 58.38$, $p < 0.001$). However, this effect, because of its small size, did not reach statistical significance in the context of our analysis (*post-hoc* Newman-Keuls test). Finally, *post-hoc* analysis revealed that, as previously observed in freely moving animals (see Fig. 1A), cocaine-induced DA outflow was significantly potentiated by RS 127445 pretreatment ($p < 0.001$ *versus* the vehicle/cocaine group).

3.7. Influence of the intra-DRN perfusion of bicuculline and RS 127445 on cocaine-induced DA outflow in mPFC

Fig. 5 illustrates the effect of the intra-DRN administration of RS 127445 on cocaine-induced increase in mPFC DA outflow, alone (Fig. 5A) or co-perfused with bicuculline into the DRN (Fig. 5B).

In Fig. 5A, statistical analysis revealed a significant and time-dependent effect of pretreatment (RS) x treatment (coc) interaction ($F_{RS\ x\ coc\ (1,\ 16)} = 18.05$, $p < 0.001$; $F_{RS\ x\ coc\ x\ time\ (9,\ 144)} = 10.05$, $p < 0.001$). *Post-hoc* analysis revealed that, RS 127445, potentiated cocaine-increased DA outflow ($p < 0.001$, *versus* the vehicle/cocaine group).

As reported previously (Devroye et al., 2017; Tanda et al., 1997), RS 127445 and cocaine *per se* elicited an overall increase in DA outflow reaching 144% and 192% of baseline, respectively ($F_{RS\ (1,\ 16)} = 35.70$, $p < 0.001$; $F_{RS\ x\ time\ (9,\ 144)} = 13.02$, $p < 0.001$; $F_{coc\ (1,\ 16)} = 42.26$, $p < 0.001$; $F_{coc\ x\ time\ (9,\ 144)} = 20.24$, $p < 0.001$). However, this effect, because of its small size, did not reach statistical significance in the context of our statistical analysis (*post-hoc* Newman-Keuls test).

When looking at the interaction between the intra-DRN administration of RS 127445 and cocaine in the presence of bicuculline (Fig. 5B), statistical analysis revealed no significant effect of pretreatment (bicuculline) x treatment (RS) interaction ($F_{bicu/RS\ x\ coc\ (1,\ 8)} = 0.28$, NS; $F_{bicu/RS\ x\ coc\ x\ time\ (9,\ 72)} = 0.25$, NS). Thus, bicuculline prevented RS 127445-induced potentiation of cocaine-increased DA outflow.

3.8. Influence of the intra-DRN injection of bicuculline on the effect of RS 127445 on cocaine-induced hyperlocomotion and inhibition of GSK3β phosphorylation in the NAc

Fig. 6 illustrates the impact of bicuculline injection into the DRN on the effect of the systemic administration of RS 127445 on cocaine-induced hyperlocomotion (Fig. 6A) and GSK3β phosphorylation in the NAc (Fig. 6B).

Of note, the dose of bicuculline was established on the basis of a previous experiment showing its efficacy to prevent the potentiating effect of RS 127445 on cocaine-induced DA outflow in the mPFC (see Supplementary Fig. S3). When assessing the interaction between the systemic administration of RS 127445 and cocaine in the presence of bicuculline in the DRN, statistical analysis revealed no significant effect of bicuculline (pretreatment) x RS (treatment) interaction ($F_{bicu/RS\ x\ coc\ (1,\ 7)} = 0.02$, NS; $F_{bicu/RS\ x\ coc\ x\ time\ (10,\ 70)} = 1.14$, NS).

As shown in Fig. 6A, statistical analysis revealed a significant pretreatment (bicuculline) x treatment (RS) interaction ($F_{bicu\ x\ RS\ x\ coc\ (1,24)} = 4.62$, $p < 0.05$). *Post-hoc* analysis revealed that, as reported previously (Devroye et al., 2015), RS 127445 produced a significant decrease by about 34% in cocaine-induced hyperlocomotion recorded over the 120 min test session ($p < 0.05$, *versus* the vehicle/vehicle/cocaine group). The intra-DRN administration of bicuculline, without effect by itself ($p > 0.05$ *versus* the vehicle/vehicle/cocaine group), completely reversed the suppressant effect of RS 127445 on cocaine-induced hyperlocomotion ($p < 0.05$, *versus* the vehicle/RS 127445/cocaine group).

In Fig. 6B, statistical analysis revealed a significant pretreatment (bicuculline) x treatment (RS) interaction ($F_{bicu\ x\ RS\ x\ coc\ (1,25)} = 5.92$, $p < 0.05$). *Post-hoc* analysis showed that, as observed previously (present study, Fig. 4), RS 127445 significantly blocked the suppressant effect of cocaine on p-GSK3β ($p < 0.01$, *versus* the vehicle/vehicle/cocaine group). The intra-DRN administration of bicuculline, without effect by itself ($p > 0.05$ *versus* the vehicle/vehicle/cocaine group), completely reversed the effect of RS 127445 on cocaine-induced inhibition of GSK3β phosphorylation in the NAc ($p < 0.001$, *versus* the vehicle/RS 127445/cocaine group).

4. Discussion

The present study, encompassing neurochemical, behavioral and cellular approaches, shows that the inhibitory effect of 5-HT_{2B}R antagonists on cocaine-induced hyperlocomotion originates at the level of the DRN and depends on their ability to potentiate mPFC DA outflow which, in turn, leads to the concurrent inhibition of DA neurotransmission in the NAc, as assessed by GSK3β phosphorylation, a cellular marker of DA activity (Beaulieu et al., 2005, 2007). Indeed, the inhibitory control exerted by RS 127445 on cocaine-induced hyperlocomotion is no longer observed in animals with a 6-OH-DA lesion of the mesocortical DA pathway. Also, RS 127445 inhibits DA neurotransmission in the NAc, as revealed by its ability to reverse cocaine-induced dephosphorylation of GSK3β in this brain region. Finally, in keeping with the location of 5-HT_{2B}Rs on GABAergic interneurons in the DRN controlling 5-HT activity (Cathala et al., 2019), the intra-DRN administration of the $GABA_A$R antagonist bicuculline prevents the effect of RS 127445 on cocaine-induced mPFC DA outflow, as well as on cocaine-induced hyperlocomotion and GSK3β phosphorylation. Altogether, these findings, while confirming the major role of the DRN in mediating the effects of 5-HT_{2B}R blockade on rat DA mesocorticolimbic pathway, support the view that 5-HT_{2B}R antagonists are able to control cocaine-induced responses by acting at both pre- (DA outflow) and post- (DA neurotransmission) synaptic levels.

As in our previous studies (Cathala et al., 2019; Devroye et al., 2018), the role of 5-HT_{2B}Rs in the control of cocaine-induced responses was

assessed using two selective, brain penetrant and potent 5-HT_{2B}R antagonists, RS 127445 and LY 266097, which have been well characterized *in vitro* and *in vivo* (Auclair et al., 2010; Audia et al., 1996; Bonhaus et al., 1999; Devroye et al., 2018).

In agreement with previous studies, we found that cocaine (Pum et al., 2007; Tanda et al., 1997), as well as the 5-HT_{2B}R antagonists RS 127445 and LY 266097 (Devroye et al., 2016, 2017), increase DA and 5-HT outflow in the mPFC. Interestingly, LY 266097 and RS 127445, while having a small effect by themselves, were able to markedly potentiate the effect of cocaine on mPFC DA outflow. This potentiating effect likely results from an action of 5-HT_{2B}R antagonists at the level of the DRN where 5-HT_{2B}Rs located on GABAergic interneurons activate 5-HT neurons innervating the mPFC, thereby leading to the stimulation of mPFC DA outflow (Cathala et al., 2019). This view is supported by the finding that 5-HT_{2B}R antagonists are also able to potentiate cocaine-increased 5-HT outflow in the mPFC, which is the first step of the complex polysynaptic network leading to the activation of the mesocortical DA pathway (Cathala et al., 2019). Moreover, the potentiation of cocaine-increased mPFC DA outflow by RS 127445 is also observed following its intra-DRN administration, an effect prevented by the local perfusion of bicuculline, which blocks the local $GABA_A$R-mediated inhibitory control of serotonergic activity (Celada et al., 2001). An additional aspect to be considered is that mesencephalic DA neurons projecting to the mPFC do not possess functional somatodendritic DA-D_2 autoreceptors (Lammel et al., 2008). Thus, it is tempting to suggest that the lack of negative auto-regulation, making mesocortical DA neurons more reactive, would contribute to the observed potentiation of cocaine-induced mPFC DA outflow by 5-HT_{2B}R antagonists.

To further characterize the specificity of the effects elicited by 5-HT_{2B}R antagonists, we pursued our investigations by studying the impact of MDL 100907 and SB 242084, two selective antagonists of 5-HT_{2A} and 5-HT_{2C}R respectively (Kennett et al., 1997; Kramer et al., 2010), on basal and cocaine-stimulated DA outflow. Indeed, both 5-HT_{2A} and 5-HT_{2C}Rs are known to control ascending DA pathway activity (Alex and Pehek, 2007; Berg et al., 2008) as well as the neurochemical and behavioral responses of cocaine (Devroye et al., 2013; Filip et al., 2012; Howell and Cunningham, 2015; Sholler et al., 2019), yet their effect on cocaine-increased DA outflow in the mPFC remained unknown. We found that, at difference with 5-HT_{2B}R antagonists, MDL 100907 and SB 242084, administered at effective doses (Berg et al., 2008; Bonaccorso et al., 2002; Fletcher et al., 2002; Li et al., 2005), had no influence on basal and cocaine-increased DA outflow in the mPFC. The absence of effect of MDL 100907 is not surprising as it is generally accepted that 5-HT_{2A}Rs exert a state-dependent facilitatory control on DA outflow associated with increased DA synthesis and/or DA firing (Alex and Pehek, 2007; Lucas et al., 2000; Porras et al., 2002; Schmidt et al., 1992), and this is not the case of cocaine, whose effect on DA outflow is associated with an inhibition of these parameters (Nielsen et al., 1983; Pitts and Marwah, 1988). In line with these considerations, selective blockade of 5-HT_{2A}Rs by SR 46349B has been shown to have no effect on cocaine-increased DA outflow in the NAc and the striatum (unpublished results). As reported previously (Li et al., 2005), selective 5-HT_{2C}R blockade has no effect on basal DA outflow in the mPFC, at variance with subcortical DA regions (Berg et al., 2008). An increase in mPFC DA outflow has been reported following the administration of SB 242084 at doses much higher (Gobert et al., 2000; Pozzi et al., 2002) than the efficient doses used in other studies (Li et al., 2005; present study), so a non-specific effect of SB 242084 cannot be ruled out. The 5-HT_{2C}R has been also shown to control activated DA exocytosis at both cortical and subcortical levels (Berg et al., 2008; Li et al., 2005). However, once again at variance with subcortical DA regions (Navailles et al., 2004), SB 242084 has no effect on cocaine-increased DA outflow in the mPFC, which could be related to the absence of a tonic inhibitory control by 5-HT_{2C}Rs on mesoprefrontal DA neurons. These results, on their whole, confirm and extend previous findings pointing out the unique profile of action of 5-HT_{2B}R antagonists on DA network activity with respect to the other members of the 5-HT_2R family (Devroye et al., 2018).

The next group of experiments aimed at assessing the functional role of mPFC DA in the RS 127445-dependent control of cocaine-induced hyperlocomotion by using a protocol of 6-OH-DA lesion of the mPFC (Bimpisidis et al., 2013) coupled with the measurement of locomotion. In line with previous findings (Bimpisidis et al., 2013), quantification of the mPFC tissue content of NA, DA and 5-HT showed a high reduction in mPFC DA levels, along with small changes in mPFC NA and 5-HT levels and no modifications of the three monoamine levels in the NAc, in the lesioned group with respect to the sham operated animals. This pattern of effect indicates that the results obtained under these experimental conditions are attributable to the disruption of DA transmission in the mPFC. Interestingly, we found that the inhibitory effect of RS 127445 on cocaine-induced hyperlocomotion, observed in sham-lesioned or non-operated rats (Devroye et al., 2015), is no longer observed in animals bearing a lesion of the mesocortical DA pathway. These findings indicate that mPFC DA plays a permissive role for the expression of the inhibitory effect of RS 127445 on cocaine-induced hyperlocomotion. In this context, it is important to remind that the mPFC is anatomically and functionally linked to the NAc and the striatum (see introduction), and participates in cocaine-induced behavioral responses (Filip and Cunningham, 2003; Leggio et al., 2009; Tzschentke, 2001). Indeed, compelling evidence shows that mPFC DA activity exerts an inhibitory control on subcortical DA neurotransmission (Jaskiw et al., 1991; Kolachana et al., 1995; Louilot et al., 1989) and on behaviors depending on subcortical DA neurotransmission (Broersen et al., 1999; Lacroix et al., 2000; Vezina et al., 1991). Given the tight relationship between accumbal DA function and locomotor activity (Dunnett and Robbins, 1992), it is likely that potentiated mPFC DA outflow drives the suppressive effect of 5-HT_{2B}R blockade on cocaine-induced hyperlocomotion *via* glutamatergic and/or GABAergic polysynaptic cortico-subcortical pathways afferent to the NAc (Azmitia and Segal, 1978; Gabbott et al., 2005; Sesack et al., 2003).

In the last decade, much attention has been devoted at investigating the role of GSK3β, a serine/threonine kinase involved in the regulation of DA signaling pathways (Beaulieu et al., 2005, 2007), in the behavioral responses of psychotropic drugs (Beaulieu et al., 2009; Del'Guidice et al., 2011). Specifically, compelling evidence have shown that GSK3β phosphorylation state in the NAc provides a useful cellular marker of DA neurotransmission related to cocaine-induced hyperlocomotion (Beaulieu et al., 2007; Kim et al., 2013; Miller et al., 2009; Zhao et al., 2016). Thus, we evaluated the impact of 5-HT_{2B}R antagonism on cocaine-induced changes of GSK3β phosphorylation in the NAc. We found that, as previously reported (Beaulieu et al., 2007; Kim et al., 2013), cocaine reduces phosphorylation levels of GSK3β in the NAc. Interestingly, RS 127445 is able to reverse this inhibitory effect. These results indicate that 5-HT_{2B}R blockade inhibits cocaine-induced hyperlocomotion by regulating cocaine-induced GSK3β phosphorylation, thereby supporting our hypothesis that the suppressant effect of 5-HT_{2B}R antagonists is related to the inhibition of accumbal DA neurotransmission, and occurs independently of changes of accumbal DA outflow (Devroye et al., 2015).

The next step of our investigations aimed at going further into the mechanisms underlying the effects of 5-HT_{2B}R antagonists on cocaine-induced neurochemical, behavioral and cellular responses, by focusing on the role of the DRN. Indeed, recent studies have shown 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 (Cathala et al., 2019). They have also shown that the DRN is the main site of action of 5-HT_{2B}R antagonists to modulate the mesocortical DA pathway activity (Cathala et al., 2019; Devroye et al., 2018). Thus, we studied the effect of the intra-DRN administration of RS 127445 and bicuculline, a $GABA_A$R antagonist, on cocaine-induced DA outflow in the mPFC. Requiring a dual probe implantation, these microdialysis experiments were performed in the anesthetized animal, in which the systemic

administration of RS 127445 potentiates cocaine-induced mPFC DA outflow as in the freely-moving rat (see Fig. S2). We found that the intra-DRN administration of RS 127445 potentiated cocaine-induced mPFC DA outflow. Interestingly, this effect was prevented by the intra-DRN perfusion of bicuculline, consistent with the presence of 5-HT_{2B}R on local GABA interneurons (Cathala et al., 2019). Furthermore, in agreement with the existence of a functional interplay between DRN 5-HT_{2B}Rs and mPFC 5-HT_{1A}Rs in the modulation of mPFC DA outflow (Devroye et al., 2017), the potentiating effect induced by the intra-DRN perfusion of RS 1277445 was also prevented by the intra-mPFC perfusion of the 5-HT_{1A}R antagonist WAY 100635 (unpublished results). Likewise, the intra-DRN injection of bicuculline prevented the effect of RS 127445 on cocaine-induced hyperlocomotion and GSK3β dephosphorylation in the NAc. Overall, these results confirm and extend previous studies (Cathala et al., 2019; Devroye et al., 2017) supporting the DRN as a key structure in the control exerted by 5-HT_{2B}Rs on mesocorticolimbic DA pathway activity. Also, they indicate that 5-HT_{2B}R blockade inhibits cocaine-induced hyperlocomotion by acting at the level of DA neurotransmission in the NAc, this effect being elicited by increased mPFC DA outflow and occurring independently of subcortical DA release (Devroye et al., 2015).

One limitation of the present study is the use of only male rats. Whilst this enabled us to make relevant comparisons with previous studies in this same field (for review see Devroye et al., 2018; Cathala et al., 2019), it leaves open the question of possible gender differences in female rats. Further studies should therefore address this issue by comparing male and female rats under similar conditions.

In conclusion, the present study, while confirming the importance of the DRN in mediating the central effects of 5-HT_{2B}R antagonists, provides additional knowledge into the regulation of the mesocorticolimbic DA pathway by the 5-HT_2R family and, in particular, into the mechanisms underlying the 5-HT_{2B}R-dependent control of cocaine-induced responses. Specifically, we demonstrate that 5-HT_{2B}R antagonists control the effects of cocaine on the mesocorticolimbic DA pathway at both pre- and post-synaptic levels, the potentiation of DA outflow in the mPFC leading to the inhibition of accumbal DA neurotransmission and, consequently, to the suppression of cocaine-evoked hyperlocomotion. Furthermore, from a therapeutic point of view, bearing in mind the key role of the mesocorticolimbic DA pathway in drug addiction (Wise, 2009) and that cocaine-induced hyperlocomotion is classically considered to be predictive of the reinforcing properties of drugs of abuse (Bubar and Cunningham, 2008; Gancarz et al., 2011), our findings, in line with recent studies in mice (Doly et al., 2017), highlight the potential of 5-HT_{2B}R antagonists for treating cocaine abuse and dependence. In this context, additional *in vivo* studies on the effects of 5-HT_{2B}R blockade on long-term cocaine administration are warranted to increase the translational significance of our findings.

CRediT authorship contribution statement

Adeline Cathala: Conceptualization, Data curation, Formal analysis, Writing - original draft. **Céline Devroye:** Data curation, Formal analysis, Writing - review & editing. **Éléa Robert:** Data curation, Formal analysis. **Monique Vallée:** Data curation, Formal analysis. **Jean-Michel Revest:** Resources. **Francesc Artigas:** Conceptualization, Data curation, Writing - review & editing. **Umberto Spampinato:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing, Supervision.

Declaration of competing interest

All authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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