Systems/Circuits

Preferential Modulatory Action of 5-HT$_{2A}$ Receptors on the Dynamic Regulation of Basal Ganglia Circuits

Lise Guilhemsang,[1,2,3] Amaia Gutierrez-Ceballos,[1] Mario Antonazzo,[1,4] Nicolas P. Mallet,[2,3] Luisa Ugedo,[1,4] and Teresa Morera-Herreras[1,4]

[1]Department of Pharmacology, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain, [2]Université de Bordeaux, Institut des Maladies Neurodégénératives, 33076 Bordeaux, France, [3]Institut des Maladies Neurodégénératives, CNRS UMR 5293, Université de Bordeaux 33076 Bordeaux, France, and [4]Neurodegenerative Diseases Group, Biocruces Health Research Institute, 48903 Barakaldo, Spain

In rodents, cortical information is transferred to the *substantia nigra pars reticulata* (SNr) through motor and medial prefrontal (mPF) basal ganglia (BG) circuits implicated in motor and cognitive/motivational behaviors, respectively. The serotonergic 5-HT$_{2A}$ receptors are located in both of these neuronal networks, displaying topographical differences with a high expression in the associative/limbic territories, and a very low expression in the subthalamic nucleus. This study investigated whether the stimulation of 5-HT$_{2A}$ receptors could have a specific signature on the dynamic regulation of BG circuits, preferentially modulating the mPF information processing through trans-striatal pathways. We performed *in vivo* single-unit extracellular recordings to assess the effect of the 5-HT$_{2A}$ agonist TCB-2 on the spontaneous and cortically evoked activity of lateral and medial SNr neurons in male rats (involved in motor and mPF circuits, respectively). TCB-2 (50–200 μg/kg, i.v.) increased the basal firing rate and enhanced the cortically evoked inhibitory response of medial SNr neurons (transmission through the direct striato-nigral pathway). A prior administration of the preferential 5-HT$_{2A}$ receptor antagonist MDL11939 (200 μg/kg, i.v.) did not modify any electrophysiological parameter, but occluded TCB-2-induced effects. In animals treated with the 5-HT synthesis inhibitor pCPA (4-chloro-DL-phenylalanine methyl ester hydrochloride), TCB-2 failed to induce the above-mentioned effects, thus suggesting the contribution of endogenous 5-HT. However, the mobilization of 5-HT induced by the acute administration of fluoxetine (10 mg/kg, i.p.) did not mimic the effects triggered by TCB-2. Overall, these data suggest that 5-HT$_{2A}$ receptors have a preferential modulatory action on the dynamic regulation of BG circuitry.

Key words: **5-HT$_{2A}$ receptor; basal ganglia circuits; fluoxetine; pCPA; serotonin; TCB-2**

Significance Statement

Motor and medial prefrontal (mPF) basal ganglia (BG) circuits play an important role in integrative brain functions like movement control or cognitive/motivational behavior, respectively. Although these neuronal networks express 5-HT$_{2A}$ receptors, the expression is higher in associative/limbic structures than in the motor ones. We show a topographical-dependent dissociation in the effects triggered by the 5HT$_{2A}$ agonist TCB-2, which specifically increases the medial *substantia nigra pars reticulata* neuron activity and has a preferential action on mPF information processing through the striato-nigral direct pathway. These are very likely to be 5-HT$_{2A}$ receptor-mediated effects that require mobilization of the endogenous 5-HT system. These findings provide evidence about the specific signature of 5-HT$_{2A}$ receptors on the dynamic regulation of BG circuits.

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Correspondence should be addressed to Teresa Morera-Herreras at teresa.morera@ehu.eus.

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Introduction

The basal ganglia (BG) form a highly organized network that integrates information from several cortical areas, conforming segregated anatomic circuits that process different functional domains: the motor circuits related to movement control, and the medial prefrontal (mPF) circuits involved in cognitive/motivational processes (Alexander et al., 1986; Parent and Hazrati, 1995; Middleton and Strick, 2000; Haber, 2003). In rodents, the electrical stimulation of the motor cortex (MC) or mPF cortex (mPFC) may evoke a triphasic response in lateral (motor) and medial (associative/limbic) territories of the *substantia nigra pars*

reticulata (SNr) neurons, respectively (Kolomiets et al., 2003; Aliane et al., 2009; Beyeler et al., 2010; Antonazzo et al., 2019, 2021). The characteristics of each individual component of this response allows the study of the cortico-nigral transmission through the hyperdirect [early excitation (EE)], direct [inhibition (I)], and indirect [late excitation (LE)] pathways (Maurice et al., 1999), providing relevant information about the dynamic network of circuits of BG.

Serotonin (5-HT) modulates the activity of the BG circuitry by acting on a large variety of 5-HT receptor subtypes (Di Matteo et al., 2008). The 5-HT$_{2A}$ receptor, a 5-HT G-protein-coupled receptor subtype, is expressed in both motor and associative/limbic territories of the nuclei of BG (for review, see Miguelez et al., 2018). Consequently, behavioral studies have pointed to the implication of the 5-HT$_{2A}$ receptor subtype in the regulation of motor, executive, and cognitive functions of BG as well as automatisms (Zhang and Stackman, 2015; Aznar and Hervig, 2016). In addition, 5-HT$_{2A}$ receptors may also play a role in many diseases associated with the dysfunction of BG. Nowadays, the 5-HT$_{2A}$ receptor is a pharmacological target in treatment development for neuropsychiatric disorders, such as schizophrenia (Miyamoto et al., 2005) or Parkinson's disease psychosis (Chang and Fox, 2016).

Interestingly, the 5-HT$_{2A}$ receptor distribution in nuclei of BG displays topographical differences, showing a higher expression in associative/limbic structures (medial striatum, nucleus accumbens, and ventral pallidum) than in the motor structures (Appel et al., 1990; Morilak et al., 1993; Ito et al., 1998; Cornea-Hébert et al., 1999; López-Giménez et al., 1999; Rodríguez et al., 1999; Pinborg et al., 2003; Riss et al., 2011). The subthalamic nucleus (STN) is the sole glutamatergic nucleus and plays a central role in the functional dynamics of BG networks. The 5-HT$_{2A}$ receptor expression in the STN is very low and does not seem to participate in the control of STN neuron activity (Pompeiano et al., 1994; Reznitsky et al., 2016). This is a differential anatomic and functional feature between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, which are closely related 5-HT G-protein-coupled receptors with a similar pharmacological profile and cellular signaling pathways. Thus, 5-HT$_{2C}$ receptors are highly expressed in the STN (Mengod et al., 1990; Pompeiano et al., 1994; Eberle-Wang et al., 1997), and their stimulation increases STN excitability (Stanford et al., 2005; Xiang et al., 2005; Aristieta et al., 2014). In line with these observations, Beyeler et al. (2010) described an enhancement in the late excitatory component of the triphasic response of SNr neurons to mPFC stimulation by the 5-HT$_{2C}$ agonist Ro-60–0175, suggesting that the 5HT$_{2C}$ receptors participate in the integrative properties of the BG circuitry involving the STN. However, the role of the 5-HT$_{2A}$ receptors in cortical-BG information processing is still not well understood.

The fact that 5HT$_{2A}$ receptors are more highly expressed in BG associative/limbic territories than in the motor territories and that they do not appear to alter STN functionality raises the hypothesis that the sole stimulation of 5-HT$_{2A}$ receptors could preferentially act on mPF circuits by modulating the cortico-nigral transmission through trans-striatal pathways. To address this hypothesis, *in vivo* single-cell extracellular recordings were performed to assess the effect of the 5-HT$_{2A}$ agonist TCB-2 and the antagonists MDL11939 and methiothepin on the spontaneous and cortically evoked activity in lateral and medial SNr neurons. Additionally, the role of endogenous 5-HT in the 5-HT$_{2A}$ receptor-mediated effects was studied by using the 5-HT synthesis inhibitor 4-chloro-DL-phenylalanine methyl ester hydrochloride (pCPA) and the selective serotonin reuptake inhibitor (SSRI) fluoxetine.

Materials and Methods

Animals. A total of 127 adult male Sprague Dawley rats (weight, 280–330 g) were used, and these were further divided into two subgroups for recording in either motor or mPF circuits. Rats were housed collectively (five per cage) under standard laboratory conditions (temperature, 22 ± 1°C; relative humidity, 55 ± 5%; and 12 h light/dark cycle) with food and water provided *ad libitum*. All animal procedures were conducted in accordance with the European Community Council Directive on "The Protection of Animals Used for Scientific Purposes" (2010/63/EU) and with the Spanish Law (RD 53/2013). The experimental protocols were reviewed and approved by the Local Ethical Committee for Animal Research of the University of the Basque Country (Universidad del Pais Vasco/Euskal Herriko Unibertsitatea, Comite de Ética en Experimentación Animal (CEEA) reference ES48/054000/6069). Every effort was made to minimize animal suffering and to use the minimum number of animals per group and experiment.

Pharmacology. The following drugs were used in this study: the 5-HT$_{2A}$ receptor agonist TCB-2, the preferential 5-HT$_{2A}$ receptor antagonist MDL11939 [α-phenyl-1-(2-phenylethyl)−4-piperidinemethanol], and the nonselective 5-HT receptor antagonist methiothepin were obtained from Tocris Bioscience (Spain); the tryptophan hydroxylase inhibitor pCPA and chloral hydrate from Sigma-Aldrich Quimica (Spain); and the SSRI fluoxetine from Biotrend. All drugs, TCB-2, MDL11939, methiothepin, pCPA, fluoxetine, and chloral hydrate, were dissolved in physiological saline (NaCl 0.9%) and freshly prepared before immediate use on the day of the experiment.

TCB-2 was chosen for its high affinity for 5-HT$_{2A}$ receptors because, to the best of our knowledge, no selective agonist for this receptor subtype has been synthesized to date. MDL11939 was used for being one of the most selective 5-HT$_{2A}$ receptor antagonists available for research use. Doses were selected on the basis of previous electrophysiological studies reporting their efficacy and selectivity (Venzi et al., 2016; Delicata et al., 2018).

pCPA and fluoxetine were used to address the role of endogenous 5-HT in the effects triggered by TCB-2. pCPA-treated animals ($n = 39$; motor circuits, $n = 21$; mPF circuits, $n = 18$) were injected intraperitoneally with the tryptophan hydroxylase inhibitor for 3 consecutive days at a daily dose of 300 mg/kg and recorded 24 h after the last administration. This pCPA dosage regimen has been shown to cause an almost complete depletion of 5-HT throughout the brain while leaving catecholamine levels intact (Koe and Weissman, 1966; Miczek et al., 1975; Edén et al., 1979; Näslund et al., 2013, 2015; Pettersson et al., 2016). As a control group, vehicle-treated animals were used, and received intraperitoneal daily injections of 0.9% saline for 3 consecutive days. Fluoxetine-treated rats ($n = 18$; motor circuits, $n = 9$; mPF circuits, $n = 9$) received an acute administration of the SSRI (10 mg/kg, i.p.). This acute dose of fluoxetine has been reported of being capable of inducing elevated stable brain levels of 5-HT for at least 6 h following the injection (Volle et al., 2018). For this reason, animals were recorded between 1 and 6 h following the fluoxetine administration. In this case, taking into account that data from vehicle-treated rats did not differ from those of control animals, this group was also used as a control for a group acutely injected with fluoxetine.

Electrophysiological procedures. As schematized in Figure 1*A*, electrophysiological recordings were performed as previously described (Antonazzo et al., 2019). Animals were anesthetized with chloral hydrate (420 mg/kg, i.p., for induction followed by continuous administration of chloral hydrate at a rate of 115 mg/kg, i.p. per hour using a peristaltic pump to maintain a steady level of anesthesia). The right jugular vein was cannulated for additional drug administration. The animal was placed in a stereotaxic frame with its head secured in a horizontal orientation, and the body temperature was maintained at ~37°C for the entire experiment with a heating pad connected to a rectal probe. The skull of each animal was exposed, and two 3 mm burr holes were drilled over the lateral or medial SNr and the ipsilateral MC or mPFC, respectively.

In vivo single-unit extracellular recordings were performed by an Omegadot single-glass micropipette, pulled with an electrode puller (model PE-2, Narishige Scientific Instrument Laboratory), broken back

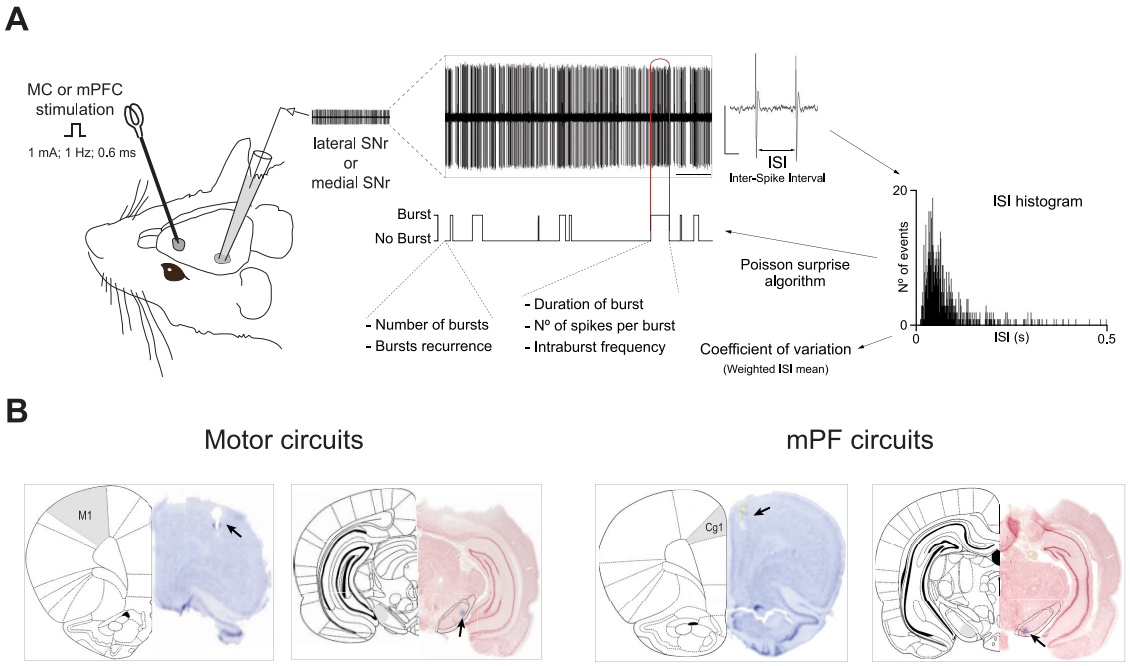

Figure 1. Schematic illustration of the electrophysiological recordings and firing pattern analysis of neurons from the lateral or medial subdivisions of the substantia nigra pars reticulata. ***A***, Left, Representation of the *in vivo* experimental setup for single-unit extracellular recordings of lateral and medial SNr neurons with simultaneous electrical stimulation (1 mA, 1 Hz, 0.6 ms) of the MC or mPFC in anesthetized rats. Middle, Magnified recording trace of an SNr neuron (top; scale bar, 5 s), and a trace showing the bursts detected by the Poisson surprise algorithm (bottom). From this, we could calculate the number of bursts and burst recurrences in the analyzed period as well as the duration, number of spikes, and frequency for each of these bursts. Right, Characteristic narrow spike from an SNr neuron (calibration: 0.5 V/10 ms), which indicates the interspike interval (ISI) that was used to assess the firing pattern and regularity of these neurons, with the Poisson surprise algorithm and the ISI coefficient of variation, respectively. ***B***, Representative coronal brain slices showing the placement of the stimulation and recording electrodes. Left, When studying the motor circuits, the stimulation electrode was placed in the MC (M1) and the recording site was in the lateral SNr, as shown by the deposition of pontamine sky blue. Right, When studying the mPF circuits, the stimulation electrode was placed in the mPFC (Cg1) and the recording site was in the medial SNr, as shown by the deposition of pontamine sky blue. Arrowheads indicate the place where these electrodes were placed.

to a tip diameter of 1–2.5 µm under a light microscope and filled with 2% pontamine sky blue in 0.5% sodium acetate. This electrode was lowered into the motor (lateral) or mPF (medial) SNr region [motor SNr coordinates: anteroposterior (AP), −5.6 mm caudal to bregma; mediolateral (ML), −2.2 mm from the midline; dorsoventral (DV), approximately −7.5 from the cortical surface; medial SNr coordinates: AP, −5.3 mm caudal to bregma; ML, −1.7 mm from the midline; DV, ∼7.5 from the cortical surface]. All SNr neurons were identified as GABAergic by their classically defined electrophysiological properties: a spike width of <2 ms and the ability to present relatively high-frequency discharges without a decrease in spike amplitude (for review, see Antonazzo et al., 2019). The signal from the electrode was amplified with a high-input impedance amplifier and then monitored on an oscilloscope and an audio monitor. Neuronal spikes were digitized using software (CED micro 1401 interface and Spike2 software, version 7, Cambridge Electronic Design).

After identifying a stable baseline frequency of discharge of an SNr neuron (variation of the firing rate less than or equal to ±10%), the basal firing rate was recorded for 3–5 min. Next, the protocol of stimulation was performed. The MC (AP, +3.5 mm anterior to bregma; ML, +3.2 mm to the midline; DV, −1.6 mm ventral to the dura mater) or the mPFC (AP, +2.9 mm anterior to bregma; ML, +0.6 mm to the midline; DV, −1.7 mm ventral to the dura mater) ipsilateral to the recording site was electrically stimulated (pulse width, 600 µs; frequency, 1 Hz; intensity, 1 mA) using coaxial stainless steel electrodes (diameter, 250 µm; tip diameter, 100 µm; tip-to-barrel distance, 300 µm; Cibertec). Our previous electrophysiological and histologic verifications demonstrated no cortical tissue damage or plasticity phenomena after this stimulation protocol (Antonazzo et al., 2019). As we previously described, cortical stimulation evoked different patterns of responses consisting of an I preceded or not by an EE and followed or not by an LE (Antonazzo et al., 2019). Only SNr neurons responding to cortical stimulation were analyzed in this study. The cortically evoked response was recorded for 3 min.

Thereafter, in the pharmacological assays, the outcome of the spontaneous and cortically evoked response of the SNr neuron was studied before and after the administration of the corresponding drug (TCB-2, MDL11939, or methiothepin). For this purpose, after the initial baseline characterization, the drug was administered intravenously, and, after 5 min, the spontaneous activity and stimulation-evoked activity were recorded again (for 5 and 3 min, respectively). This protocol was repeated for different doses of the same drug or for consecutive administration of different drugs (MDL11939 + TCB-2). Multiple neurons were recorded in each animal to characterize spontaneous and cortically evoked activity in both motor and mPF circuits; however, only one SNr cell responding to cortical stimulation was pharmacologically studied per animal. When possible, drugs were tested on neurons presenting a triphasic response, to fully characterize the effects of the drugs over cortico-nigral transmission.

In a set of experiments, at the end of the SNr recordings, histologic verification of the cortical stimulation site (MC or mPFC) and recording place within lateral or medial SNr was conducted (Fig. 1*B*). For verification of the stimulation electrode, 40-µm-thick coronal sections of the cortex were Nissl stained, revealing the track of the stimulation electrode under the microscope. For verification of the recording electrode position, a discrete spot of pontamine sky blue was deposited at the recording site by a cathodal current of 10 µA over 10 min. Afterward, 40-µm-thick coronal sections were stained with 1% neutral red for 10 min to microscopically verify the anatomic position of the blue point, indicating the recording site.

Data analysis. Firing parameters of SNr neurons, such as firing rate and coefficient of variation (CV) were analyzed offline using the Spike2 software (version 7). The CV consisted of the ratio, expressed as a percentage, between the SD and the mean of the interspike interval histogram of the neuron. This histogram was made with 1 ms bins, and all the interspike intervals <0.5 s were considered, given the relatively high firing rate of SNr neurons. The existence of burst firing in neurons was

determined through a Spike2 script ("surprise.s2s"), based on the Poisson surprise algorithm, and the following parameters were obtained: number and duration of bursts, number of spikes per burst, recurrence of bursts and intraburst frequency. These parameters were analyzed during time epochs of 150 s under basal conditions or for 120 s once the drug was administered (Fig. 1*A*).

To analyze the cortically evoked response in SNr neurons, peristimulus time histograms (PSTHs) were generated from 180 stimulation trials using 1 ms bins. The criterion used to determine the existence of an excitatory response was a twofold increase of the SD plus the mean number of spikes during the prestimulus period, for at least three consecutive bins. The amplitude of excitatory responses was quantified by calculating the difference between the mean number of spikes evoked within the excitation time window and the mean number of spikes occurring spontaneously during the prestimulus period. The duration of an inhibitory response corresponded to the time interval without spikes for at least three consecutive bins (see Fig. 3*A*).

Statistics. Experimental data proceeding only from stimulation-responding neurons were analyzed using the computer program GraphPad Prism (version 5.01; GraphPad Software). To explore differences in pCPA-treated and fluoxetine-treated groups on the firing rate, CV, and cortically evoked responses in lateral and medial SNr neurons, one-way ANOVA followed by *post hoc* comparisons using Bonferroni's *post hoc* test was used. The χ^2 test was used to determine differences in neurons exhibiting burst firing patterns and changes in the occurrence of cortically evoked responses, as well as in the different patterns occurring after cortical stimulation. Burst-related parameters were analyzed using the Kruskal–Wallis test, except for the intraburst frequency, which was analyzed using ANOVA, followed by Bonferroni's *post hoc* test.

To assess the effect of TCB-2, MDL11939, and methiothepin in motor and mPF circuits, repeated-measures one-way ANOVA was used followed by Bonferroni's *post hoc* test to compare the mean values of the firing rate, CV, and parameters related to cortically evoked responses before and after drug application. To evaluate the effect of a single dose of TCB-2 (100 µg/kg, i.v.) on cortically evoked responses, a paired two-tailed Student's *t* test was used. Chi-squared tests before and after drug application were also used to study the effects of these drugs on burst activity. The level of statistical significance was set at $p < 0.05$. Data are presented as the group mean ± SEM of *n* neurons.

Results

Effects of 5-HT$_{2A}$ receptor stimulation/blockade on spontaneous and cortically evoked activity of SNr neurons

To study the functional role of the 5-HT$_{2A}$ receptor subtype on SNr neuron activity, we first examined the effect of the 5-HT$_{2A}$ receptor agonist TCB-2 on the electrical activity of lateral and medial SNr neurons from vehicle-treated animals. To do so, SNr neurons were separately recorded from the lateral and medial territories of the nucleus [Fig. 1*B* (see also Fig. 3*A*)]. All recorded cells exhibited typical electrophysiological characteristics of GABAergic SNr neurons, including a narrow spike waveform and a relatively high firing rate (>7 Hz) with a regular pattern of discharge. To ensure the SNr cells belong to their corresponding BG circuit, only those responding to cortical stimulation were used in the analysis (success rate: motor circuits, 98.7%; mPF circuits, 48.6%). Data on spontaneous and cortically evoked activity did not differ from those obtained in the control animals of this study (data not shown).

The systemic administration of TCB-2 (50–200 µg/kg, i.v., doubling doses; one cell per rat) did not modify the basal firing rate, the coefficient of variation, or the firing pattern of lateral SNr neurons (Fig. 2*A–C*, left). In contrast, in medial SNr neurons, TCB-2 produced a significant increase in the basal firing rate in a dose-dependent manner (maximal increment of the firing rate was 32% of the basal value; $F_{(3,20)} = 12.33$, $p = 0.0003$, repeated-measures one-way ANOVA; Fig. 2*D*, left), while the regularity and the firing pattern remained unaltered (Fig. 2*E*, *F*, left). In medial SNr neurons, the effects induced by TCB-2 on the basal firing rate were effectively blocked by the previous administration of the preferential 5-HT$_{2A}$ receptor antagonist MDL11939 (200 µg/kg i.v; $p > 0.05$, repeated-measures one-way ANOVA; Fig. 2*D*, right). Additionally, no changes in the spontaneous activity of medial or lateral SNr cells were found after the administered dose of the 5-HT$_{2A}$ antagonist (Fig. 2*A–F*, right).

We next investigated the effect of the 5-HT$_{2A}$ receptor activation on cortically evoked responses of lateral and medial SNr neurons. As we previously showed, stimulation of the MC or mPFC evoked characteristic triphasic responses in SNr neurons (30.1% of the recorded cells in the lateral SNr and 29.0% in the medial SNr) that consisted of an early excitation followed by an inhibition and a late excitation (Antonazzo et al., 2019). As illustrated in Figure 3*A*, early excitation is attributable to the activation of the "hyperdirect" cortico-subthalamo-nigral pathway, inhibition to the activation of the "direct" cortico-striato-nigral pathway, and late excitation to the activation of the "indirect" cortico-striato-pallido-subthalamo-nigral pathway (Maurice et al., 1999). Furthermore, different patterns of responses could be observed in both SNr territories. In addition to triphasic responses, biphasic and monophasic cortically evoked responses were recorded in both SNr territories from the activation of different pathways along the circuits. For the percentage of occurrence of such patterns of responses in lateral and medial SNr neurons, see Figure 5, *C* and *D*.

To accurately analyze cortically evoked responses, the dose of TCB-2 was carefully selected from the results described above with the view to minimize any effects on the firing activity of SNr neurons, which could make the analysis of cortically evoked responses difficult. In lateral SNr neurons, systemic administration of TCB-2 (100 µg/kg, i.v.) diminished the duration of the late excitation ($t_{(6)} = 3.118$, $p = 0.0206$, paired Student's *t* test). However, no significant modifications in the early excitation or inhibition were observed (Fig. 3*B*, top, *C*, left). In medial SNr neurons, TCB-2 significantly increased the duration of the inhibition ($t_{(4)} = 3.352$, $p = 0.0285$, paired Student's *t* test), whereas it reduced the duration of the late excitation ($t_{(4)} = 3.206$, $p = 0.0327$, paired Student's *t* test; Fig. 3*D*, top, *E*, left).

In all the lateral and medial SNr recorded neurons, the previous administration of the preferential 5-HT$_{2A}$ antagonist MDL11939 (200 µg/kg, i.v.) blocked the effects induced by TCB-2 administration (100 µg/kg, i.v.) on the cortico-nigral transmission through the motor and mPF BG circuits (Fig. 3*B–E*). Moreover, MDL11939 did not alter the cortically evoked responses ($p > 0.05$, repeated-measures one-way ANOVA for early excitation, inhibition, and late excitation in both circuits; Fig. 3*C*,*E*).

In another set of experiments, to confirm the lack of effect of the 5-HT$_{2A}$ antagonist MDL11939, presumably suggesting that endogenous 5-HT does not exert a tonic control on SNr neuron electrical activities via 5-HT$_{2A}$ receptors, the effect of the nonselective 5-HT receptor antagonist methiothepin was evaluated on the spontaneous and cortically evoked activity of SNr neurons. Systemic administration of cumulative low doses of methiothepin (25–100 µg/kg, i.v.; doubling doses) did not produce any significant modifications in lateral and medial SNr neuron activity or in cortico-nigral transmission through motor and mPF circuits (Fig. 4*A*,*B*).

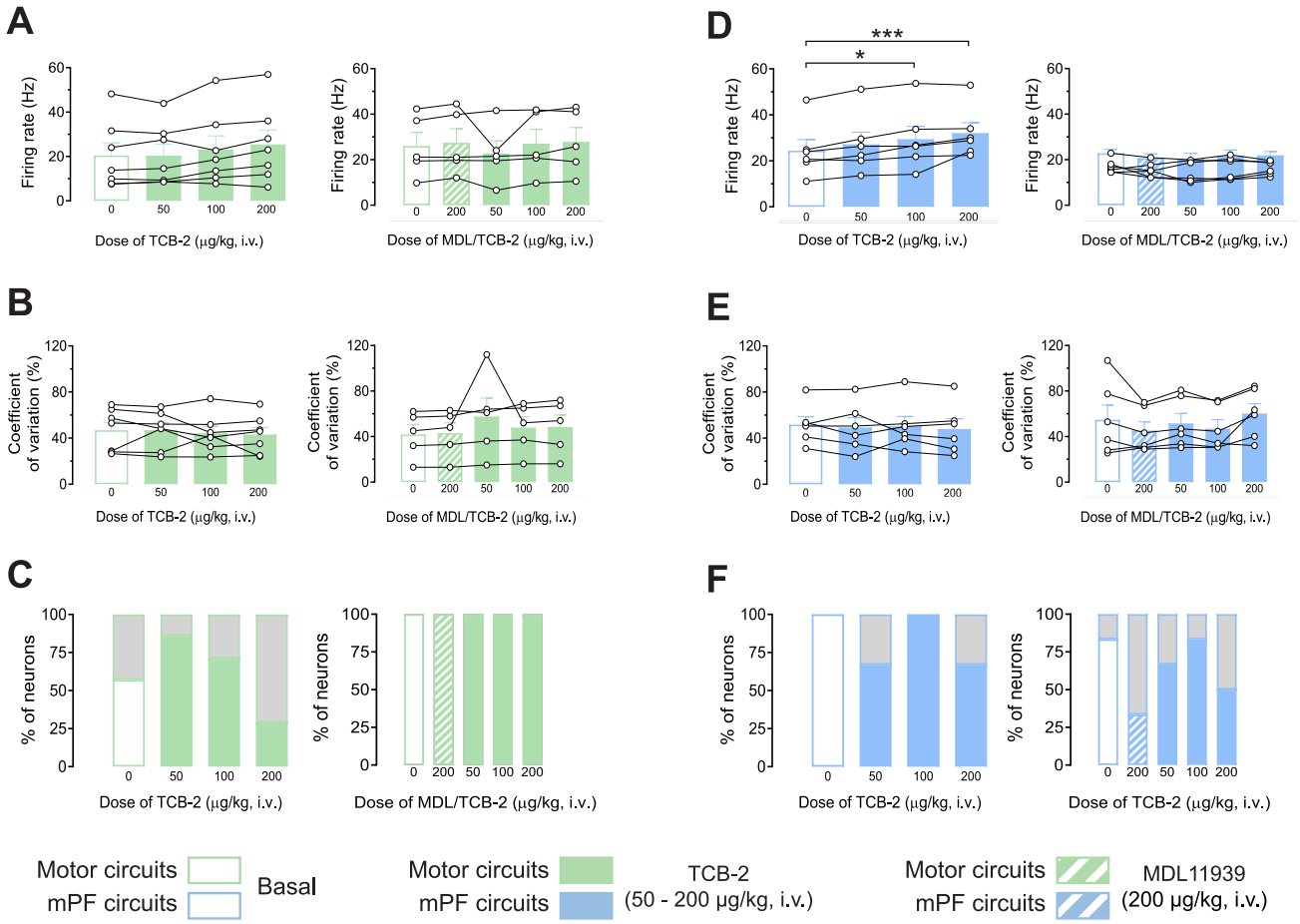

Figure 2. Effect of systemic administration of cumulative doses of the 5-HT$_{2A}$ receptor agonist TCB-2, and its blockade by the preferential 5-HT$_{2A}$ receptor antagonist MDL11939, on electrophysiological parameters of lateral and medial substantia nigra pars reticulata neurons. ***A–F***, Histograms illustrating the effect of cumulative doses of TCB-2 (50–200 µg/kg, i.v.) on the mean firing rate, and the mean coefficient of variation and firing pattern of SNr neurons [percentage of bursting (color) vs nonbursting (gray)] from the motor (***A–C***, left) and from the mPF (***D–F***, left) circuits recorded in anesthetized rats 24 h after the last vehicle injection. Administration of TCB-2 increased the basal firing rate of medial SNr neurons. Administration of MDL11939 (200 µg/kg, i.v.) did not show any effect by itself in the mean firing rate, and mean coefficient of variation and firing pattern of SNr neurons [percentage of bursting (color) vs nonbursting (gray)] from the motor (***A–C***, right) and the mPF (***D–F***, right) circuits recorded in control anesthetized rats. However, this dose of MDL11939 was able to block the effects observed after TCB-2 administration in the firing rate of medial SNr neurons. Each bar represents the mean ± SEM of *n* neurons. Each dot represents a single neuron. $*p < 0.05$, $***p < 0.001$ versus basal (Bonferroni's *post hoc* test).

Role of the endogenous 5-HT in the effects triggered by TCB-2

Next, to study the contribution of the endogenous 5-HT in the TCB-2-mediated effects, we administered the drug to animals treated with the 5-HT synthesis inhibitor pCPA (300 mg/kg, i.p., for 3 d).

First, the spontaneous electrophysiological parameters and the response to cortical stimulation were recorded in lateral and medial territories of the SNr from pCPA-treated animals (57 lateral neurons from $n = 15$ rats and 46 medial SNr neurons from $n = 12$ rats), and the results were compared with those obtained in their respective vehicle-treated animals (74 lateral neurons from $n = 20$ rats; 35 medial SNr neurons from $n = 10$ rats). As illustrated in Figure 5*A*, compared with the vehicle-treated group, the mean basal firing rate of lateral SNr neurons was significantly higher in pCPA-treated animals ($F_{(2,189)} = 12.82$, $p < 0.001$, one-way ANOVA), whereas it remained unchanged in medial SNr neurons from these animals (Fig. 5*B*). Moreover, no differences were found in the rest of the analyzed parameters.

pCPA treatment did not induce any changes in the percentage of neurons displaying different patterns of response in any of the SNr subterritories studied (Fig. 5*C*,*D*). However, regarding the electrophysiological parameters of the cortically evoked responses in SNr neurons, in pCPA-treated animals, we observed an enhancement in the duration of the inhibition in medial SNr neurons (related to the direct pathway in mPF circuits; $F_{(2,66)} = 5.812$, $p = 0.0047$, one-way ANOVA; Fig. 5*E*,*F*).

Next, we studied the effect of systemic administration of TCB-2 (50–200 µg/kg, i.v.), which did not modify the firing rate, the coefficient of variation, or the firing pattern of lateral SNr neurons in pCPA-treated rats ($n = 6$; Fig. 6*A*). In medial SNr neurons of these animals ($n = 6$), TCB-2 (50–200 µg/kg, i.v.) also did not induce any changes in the basal firing rate or in the coefficient of variation, but the minimal dose of 5-HT$_{2A}$ receptor agonist (50 µg/kg, i.v.) significantly reduced the number of neurons exhibiting a burst-firing pattern ($\chi^2 = 8.0$, $p = 0.0460$, χ^2 test; Fig. 6*B*). In addition, in contrast to the outcome observed in the vehicle-treated group, the administration of TCB-2 (100 µg/kg, i.v.) in pCPA-treated animals did not modify any of the studied parameters of the cortically evoked responses of lateral or medial SNr neurons (Fig. 6*C*,*D*).

The mobilization of the endogenous 5-HT system with fluoxetine does not mimic the specific responses triggered by TCB-2

The absence of modulation of the SNr activity observed in pCPA-treated animals following 5-HT$_{2A}$ receptor activation suggests that

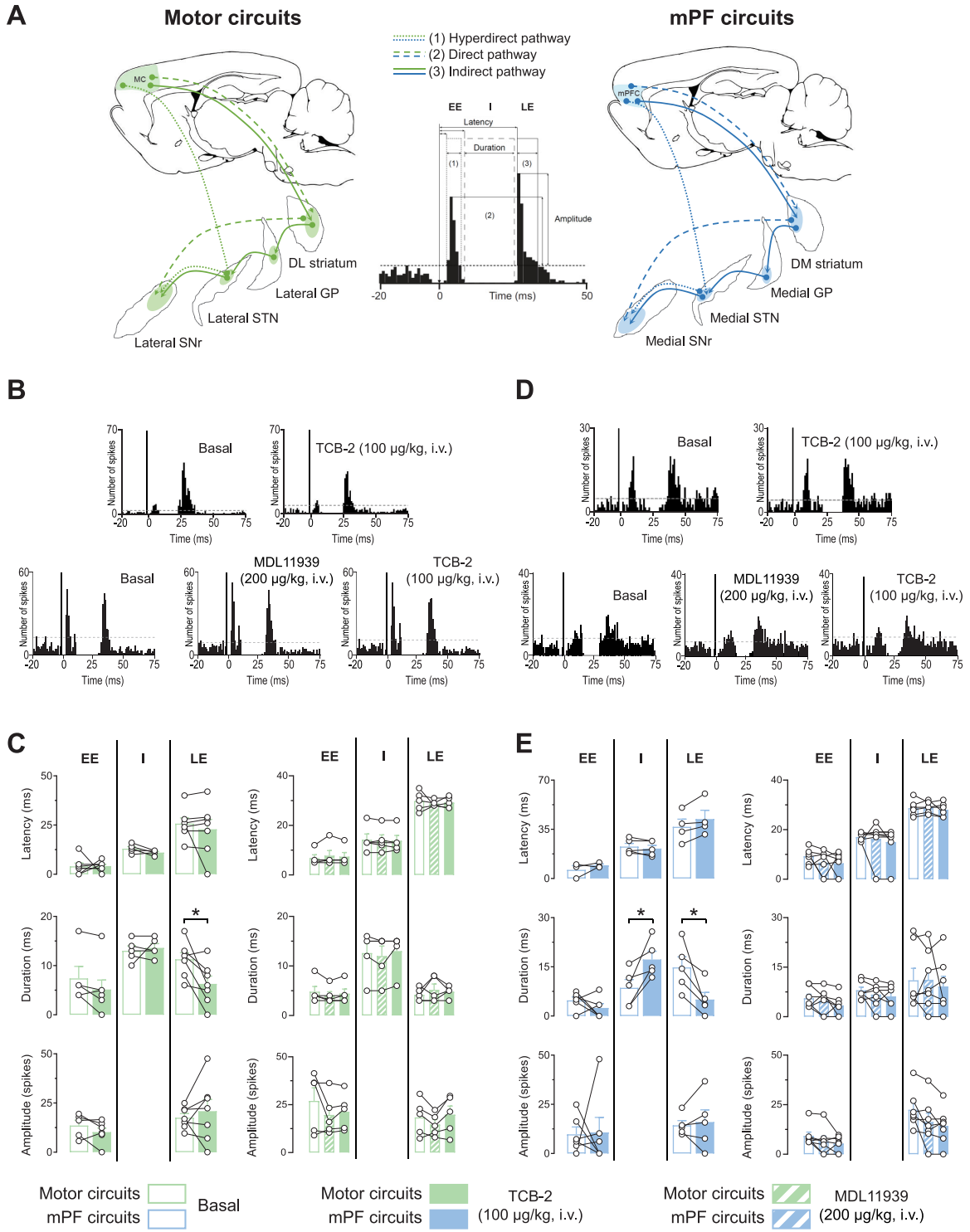

Figure 3. Effect of systemic administration of cumulative doses of the 5-HT$_{2A}$ receptor agonist TCB-2, and its blockade by the preferential 5-HT$_{2A}$ receptor antagonist MDL11939, on cortically evoked responses in lateral and medial substantia nigra pars reticulata neurons. ***A***, Schematic anatomic diagram illustrating the motor (left) and mPF (right) cortico-BG circuits within the rat brain. Neurons from the MC or mPFC project to the lateral or medial STN, respectively. STN neurons then reach the SNr, constituting the hyperdirect pathway [dotted line (1)]. Additionally, MC and mPF cortical neurons send projections to the dorsolateral (DL) or dorsomedial (DM) striatum, respectively. From the striatum, there are two main projections: the direct pathway to the lateral or medial part of the SNr [dashed line (2)] and the indirect pathway connecting the DL/DM striatum to the lateral/medial globus pallidus (GP), the lateral/medial STN, and finally the lateral/medial SNr [solid line (3)]. Middle, PSTHs showing the characteristic triphasic response evoked in the SNr after MC or mPFC stimulation, consisting of EE [hyperdirect pathway (1)], I [direct pathway (2)], and LE [indirect pathway (3)], and the definition of these responses. The dashed line represents the threshold dividing excitatory and inhibitory responses; those above the threshold were considered excitatory. Duration indicates how long a response occurred, latency represents the time period between the occurrence of stimulation and response, and the amplitude indicates the magnitude of the excitations. ***B***, Representative PSTHs illustrating the effect of the administration of the 5HT$_{2A}$ agonist TCB-2 (100 µg/kg, i.v.) on the cortically evoked activity of SNr neurons from the motor circuits recorded in vehicle-treated anesthetized rats (top), and its blockade by previous administration of the preferential 5-HT$_{2A}$ receptor antagonist MDL11939 (200 µg/kg, i.v.; bottom). ***C***, Histograms illustrating the effect of the administration of TCB-2 (100 µg/kg, i.v.) in vehicle-treated anesthetized rats (left) on the different components (i.e., EE, I, and LE) of the cortically evoked activity of lateral SNr neurons, and the different electrophysiological parameters analyzed (i.e., latency, duration, and amplitude). Administration of TCB-2

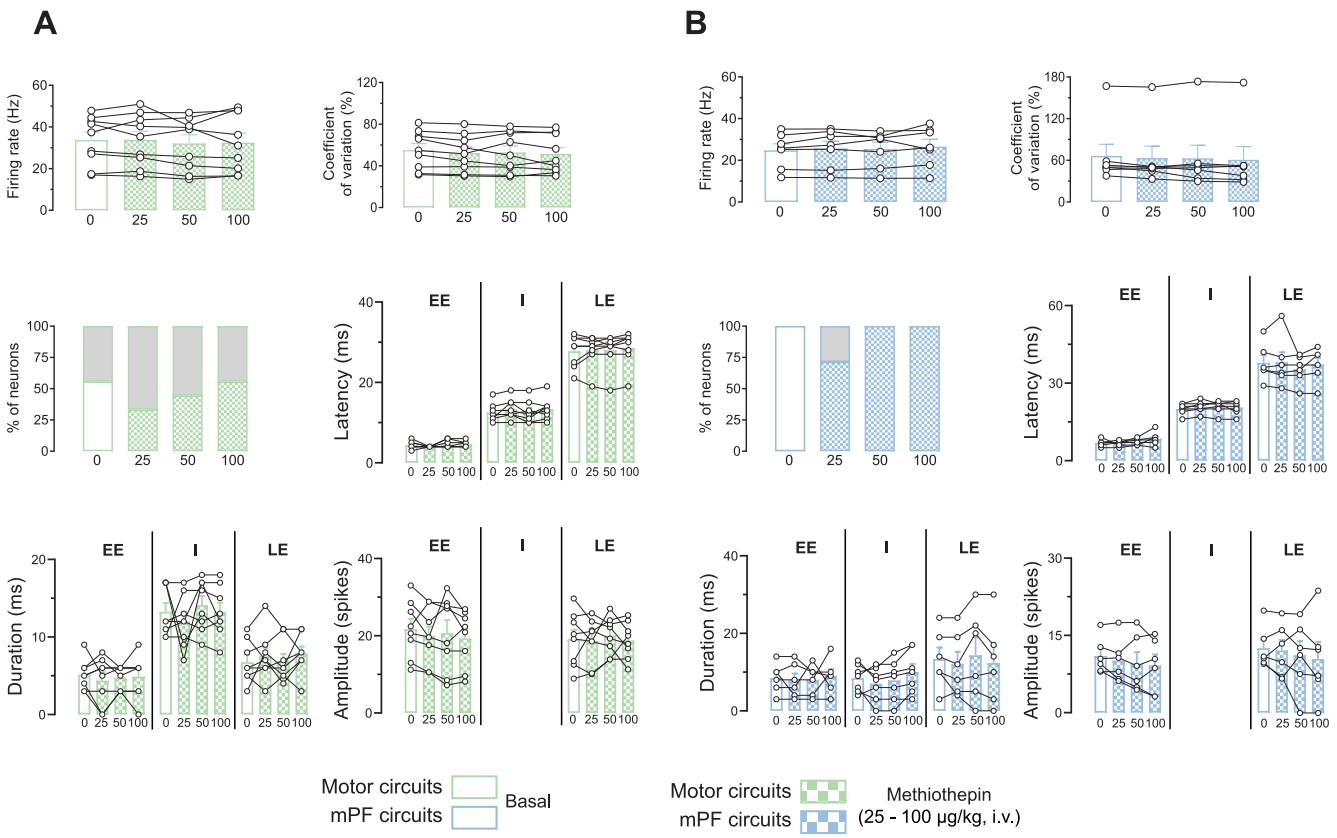

Figure 4. Effects of the systemic administration of cumulative doses of the nonselective 5-HT receptor antagonist methiothepin on the spontaneous and cortically evoked activity of lateral and medial substantia nigra pars reticulata neurons. ***A***, ***B***, Mean firing rate, coefficient of variation, firing pattern [percentage of bursting (color) vs nonbursting (gray)], and cortically evoked activity of lateral (***A***) and medial (***B***) SNr neurons recorded in anesthetized control animals. Cumulative doses of the nonselective 5-HT receptor antagonist methiothepin (25–100 µg/kg, i.v.) induced no changes in either of the analyzed spontaneous electrophysiological parameters or in those regarding cortically evoked responses observed in lateral and medial SNr neurons. Each bar represents the mean ± SEM of *n* neurons. Each dot represents a single neuron.

the effects triggered by TCB-2 could require the contribution of endogenous 5-HT. To test this hypothesis, we used an acute administration of the SSRI fluoxetine (10 mg/kg, i.p.) to elevate endogenous 5-HT brain levels and study whether, under these conditions, we could mimic TCB-2-induced effects. Thus, lateral and medial SNr neurons were recorded in fluoxetine-injected animals (62 lateral SNr neurons from n = 9 rats; 48 medial SNr neurons from n = 9 rats) from 1 to 6 h after the administration.

Compared with the vehicle-treated group, the SNr neurons recorded in the lateral territory in fluoxetine-injected rats showed a significantly reduced mean basal firing rate while displaying no differences in their firing pattern ($F_{(2,189)}$ = 12.82, $p < 0.001$, one-way ANOVA; Fig. 5*A*). In medial SNr cells, fluoxetine did not modify the basal firing rate but reduced the number of neurons exhibiting a burst firing pattern (χ^2 = 16.57, $p = 0.0003$, χ^2 test; Fig. 5*B*).

Next, we studied the cortically evoked SNr activity. The administration of fluoxetine increased the number of monophasic responses consisting of a late excitation in lateral SNr neurons (χ^2 = 6.455, $p = 0.0397$, χ^2 test; Fig. 5*C*). Moreover, fluoxetine reduced the proportion of medial SNr neurons displaying triphasic responses (χ^2 = 6.076, $p = 0.0479$, χ^2 test; Fig. 5*D*) and greatly reduced early excitation occurrence (χ^2 = 6.192, $p = 0.0452$, χ^2 test; Fig. 5*B*, bottom, right). Regarding the electrophysiological parameters of the cortically evoked responses in SNr neurons, fluoxetine modified the characteristics of the early and late excitatory responses in both territories (Fig. 5*E*,*F*). In medial SNr neurons, fluoxetine decreased early excitation amplitude ($F_{(2,58)}$ = 5.822, $p = 0.0049$, one-way ANOVA) and decreased late excitation latency ($F_{(2,89)}$ = 11.20, $p < 0.0001$, one-way ANOVA). Lateral SNr cells from fluoxetine-treated animals showed an increase in early excitation duration ($F_{(2,106)}$ = 4.859, $p = 0.0096$, one-way ANOVA) and a diminished late excitation amplitude ($F_{(2,127)}$ = 6.703, $p = 0.0017$, one-way ANOVA).

Discussion

The present study tested the hypothesis that the stimulation of 5HT$_{2A}$ receptors could have a specific signature on the dynamic regulation of BG circuitry, preferentially acting on associative/

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(100 µg/kg, i.v.) caused a reduction in the duration of the LE. Previous administration of MDL11939 (200 µg/kg, i.v.) blocked the TCB-2-induced effects on the duration of the LE. MDL11939 caused no effect by itself on the cortically evoked activity of these neurons (right). ***D***, Representative peristimulus time histograms illustrating the effect of the administration of TCB-2 (100 µg/kg, i.v.) on the cortically evoked activity of medial SNr neurons recorded in vehicle-treated anesthetized rats (top), and its blockade by previous administration of MDL11939 (200 µg/kg, i.v.; bottom). ***E***, Histograms illustrating the effect of the administration of TCB-2 (100 µg/kg, i.v.) in vehicle-treated anesthetized rats (left) on the different components (i.e., EE, I, and LE) of the cortically evoked activity of medial SNr neurons, and the different electrophysiological parameters analyzed (i.e., latency, duration, and amplitude). Administration of TCB-2 (100 µg/kg, i.v.) caused an increase in the duration of the I and reduced the duration of the LE. Previous administration of MDL11939 (200 µg/kg, i.v.) blocked the TCB-2-induced effects on the duration of the I and LE. MDL11939 caused no effect by itself on the cortically evoked activity of these neurons (right). Each bar represents the mean ± SEM of *n* neurons. Each dot represents a single neuron. $*p < 0.05$, versus basal (Bonferroni's *post hoc* test).

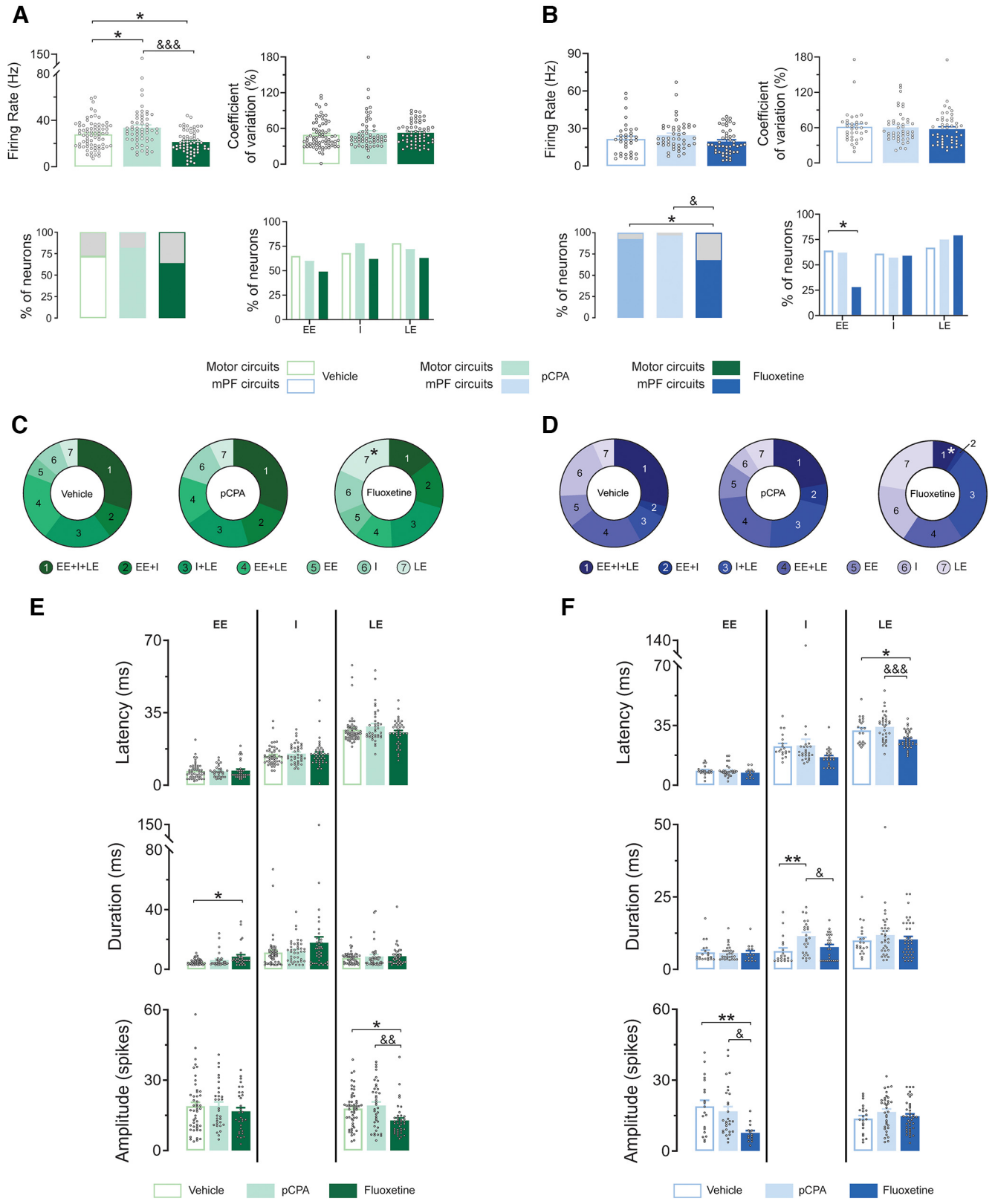

Figure 5. Effects of the administration of the 5-HT synthesis inhibitor pCPA (300 mg/kg/d, i.p., 3 d) or the selective 5-HT reuptake inhibitor fluoxetine (10 mg/kg, i.p.) on the spontaneous and cortically evoked electrophysiological activity of lateral and medial substantia nigra pars reticulata neurons from anesthetized rats. ***A***, ***B***, Histograms illustrating the effect on the mean firing rate, mean coefficient of variation, firing pattern [percentage of bursting (color) vs nonbursting (gray)] and on the different patterns of response displayed by neurons of the administration of vehicle or pCPA (300 mg/kg/d, i.p., 3 d) in lateral (***A***) and medial (***B***) SNr neurons recorded 24 h after the last injection, and of the acute dose of fluoxetine (10 mg/kg, i.p.) during the 6 h after its administration. pCPA administration increased the mean firing rate of lateral SNr neurons, while fluoxetine decreased their firing rate when compared with vehicle-treated rats. In the case of medial SNr neurons, their firing pattern changed after fluoxetine administration. The percentage of neurons displaying an EE, an I, or an LE is shown in ***A*** and ***B*** (bottom, right). Fluoxetine administration caused a decrease in the number of medial SNr neurons displaying an EE. After cortical stimulation, single SNr neurons would display different patterns of response consisting of

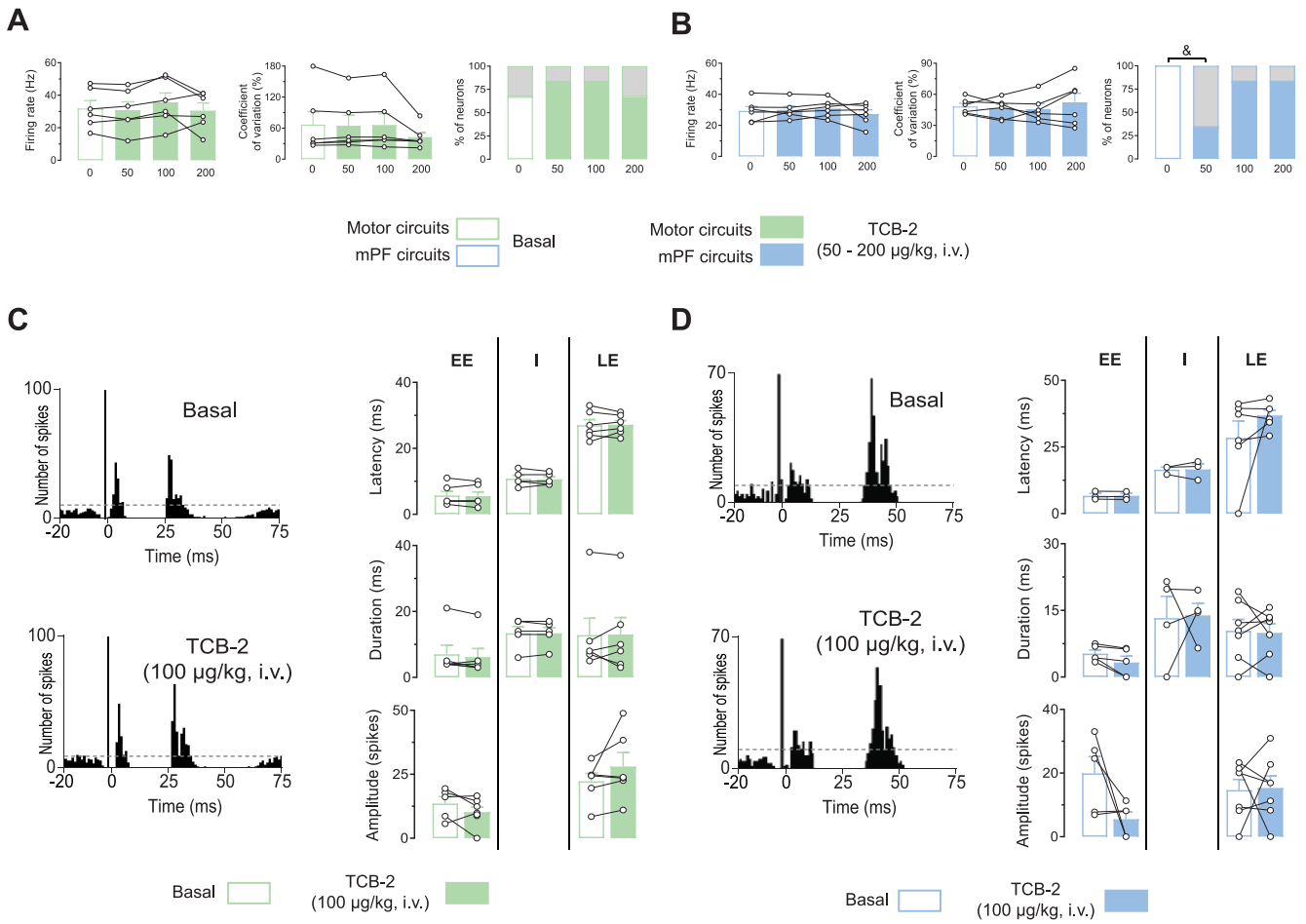

Figure 6. Effect of the systemic administration of cumulative doses of the 5-HT$_{2A}$ receptor agonist TCB-2 on the spontaneous activity and cortically evoked responses of lateral and medial substantia nigra pars reticulata neurons from pCPA-treated anesthetized rats. ***A***, ***B***, Histograms illustrating the effect on the mean firing rate, the mean coefficient of variation, and the firing pattern [percentage of bursting (color) vs nonbursting (gray)] of the administration of TCB-2 (50–200 µg/kg, i.v.) in lateral (***A***) and medial (***B***) SNr neurons recorded in pCPA-treated rats (300 mg/kg/d, i.p., 3 d) 24 h after the last injection. In neurons from the medial SNr, the administration of TCB-2 at the lowest dose (50 µg/kg, i.v.) was able to reduce the percentage of bursting neurons with no effect at higher doses (100–200 µg/kg, i.v.). ***C***, ***D***, Histograms illustrating the effect on the electrophysiological parameters analyzed from cortically evoked responses in lateral (***C***) and medial (***D***) SNr neurons of the administration of TCB-2 (50–200 µg/kg, i.v.) in pCPA-treated rats (300 mg/kg/d, i.p., 3 d) 24 h after the last injection. In pCPA-treated rats, TCB-2 (100 µg/kg, i.v.) was unable to alter cortico-nigral information transmission through any of the pathways that constitute the motor and mPF circuits. Each bar represents the mean ± SEM of *n* neurons. Each dot represents a single neuron. &$p < 0.05$ versus basal (neurons exhibiting burst firing pattern, χ^2 test).

limbic territories of the nuclei conforming those networks. In accordance with this hypothesis, here we show a topographical-dependent dissociation in the effects triggered by the 5HT$_{2A}$ agonist TCB-2, which specifically increases the medial SNr neuron activity and is able to enhance the mPF-BG transmission through the direct pathway. These are very likely to be 5-HT$_{2A}$ receptor-mediated effects that require mobilization of the endogenous 5-HT system.

Data from positron emission tomography studies performed with specific radioligands ([^{11}C]-MDL100907, [^{125}I]-(±) 2,5-dimethoxy-4-iodoamphetamine (DOI), [^{3}H]-ketanserin, or [^{18}F]-altanserin) and those from autoradiography or immunohistochemical assays agree on the topographical differences in the distribution of 5-HT$_{2A}$ receptors among BG nuclei (Appel et al., 1990; Morilak et al., 1993; Ito et al., 1998; Cornea-Hébert et al., 1999; López-Giménez et al., 1999; Rodríguez et al., 1999; Pinborg et al., 2003; Riss et al., 2011). These studies describe relatively moderate levels of expression in associative/limbic structures, such as the medial striatum and the ventral pallidum (with the exception of the *nucleus accumbens*, which has the highest expression of these receptors) and lower expression in most of the BG nuclei. In agreement with this distribution, the present *in vivo* electrophysiological study provides evidence that 5-HT$_{2A}$ receptors have a preferential regulatory action on limbic/cognitive territories of the SNr and on mPF BG circuitry.

In vehicle-treated animals, systemic administration of TCB-2 dose-dependently stimulated medial SNr neuron activity but did not modify the lateral SNr neuronal electrical parameters. The increased firing rate of medial SNr neurons induced by TCB-2 was completely blocked by the previous administration of

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different combinations of EE and/or I and/or LE. ***C***, ***D***, Fluoxetine was able to alter these patterns of response, producing a decrease in the number of lateral SNr neurons displaying LE (***C***), and in the number of medial SNr neurons displaying triphasic responses (EE + I + LE; ***D***). ***E***, ***F***, Histograms illustrating the effect of the treatment with vehicle or pCPA (300 mg/kg/d, i.p., 3 d), and fluoxetine (10 mg/kg, i.p.) on the electrophysiological parameters analyzed from cortically evoked responses in lateral (***E***) and medial (***F***) SNr neurons. In lateral SNr neurons, fluoxetine was able to increase the duration of the EE and decrease the amplitude of the LE. Fluoxetine was also able to decrease the latency of the LE and the amplitude of the EE in neurons recorded in the medial SNr. In these neurons, pCPA increased the duration of the I. Each bar represents the mean ± SEM of *n* neurons. Each dot represents a single neuron. *$p < 0.05$, **$p < 0.01$ versus vehicle; &$p < 0.05$, &&$p < 0.01$, &&&$p < 0.001$ versus pCPA (firing rate, latency, duration and amplitude, Bonferroni's *post hoc* test; neurons exhibiting burst firing pattern, and patterns of response, χ^2 test).

MDL11939, thus indicating that it was mediated by 5-HT_{2A} receptors. The fact that neither this preferential antagonist nor the nonselective antagonist methiothepin caused any changes in the basal activity of SNr neurons additionally suggests that 5-HT_{2A} receptors do not exert any tonic control on them. Previous *in vitro* electrophysiological data had reported mixed results, showing that 5-HT_{2A} receptor activation had no effect (Rick et al., 1995) or participated in the inhibitory effect produced by 5-HT on SNr neurons (Góngora-Alfaro et al., 1997). The discrepancies between *in vitro* and *in vivo* studies may be because of the participation of $5HT_{2A}$ receptors located in other nuclei that project to the SNr when the agonist is systemically applied or may reflect the different selectivity of the drugs applied.

Our data are in agreement with behavioral studies performed in rodents showing that the acute or chronic administration of TCB-2 does not produce changes in locomotor activity, sensorimotor gating, or exploratory/stereotyped behavior, but is able to induce other behavioral abnormalities, such as the suppression of feeding in food-deprived animals (Fox et al., 2010; Tsybko et al., 2020). Food motivation and intake are associated with the anatomic circuit involving the mPFC and limbic parts of the BG (Sgambato-Faure et al., 2016); and dysfunctions of this circuit could be related to eating disorders (Stefano et al., 2013). Thus, the medial distribution of the effects of TCB-2 could be involved in the observations at the behavioral level.

According to the described effects on SNr spontaneous activity, we observed that mPFC-nigral transmission was also more affected by the TCB-2 administration than the cortico-BG information processing through motor circuits. Thus, in mPF circuits, activation of 5-HT_{2A} receptors resulted in a notable imbalance between the direct and indirect trans-striatal pathways, with an enhancement in information transfer through the direct pathway (increased inhibition) accompanied by a reduced transmission via the indirect pathway (decreased late excitation). The effects triggered by TCB-2 on cortico-BG transmission were blocked by the previous administration of MDL11939, confirming once again the implication of 5-HT_{2A} receptors. Intracellular recordings performed in rat brain slices have demonstrated that 5-HT depolarizes nucleus accumbens neurons via a 5-HT_{2A} receptor-mediated mechanism, as this effect was blocked by the antagonists ketanserin and mianserin, but not by other 5-HT receptor subtype antagonists (North and Uchimura, 1989). This stimulatory effect may explain the favored transmission through the direct pathway in mPF circuits, since this receptor is also present in the dorsomedial striatum. Furthermore, this receptor does not seem to participate in STN excitability (Xiang et al., 2005), which would explain why the hyperdirect pathway was not affected.

To the best of our knowledge, this work is the first demonstration that 5-HT_{2A} receptors have a specific regulatory role on BG circuit functionality, which differs from the previously reported role of 5-HT_{2C} receptors. Beyeler et al. (2010) demonstrated that the 5-HT_{2C} agonist Ro-60-0175 also did not affect the spontaneous or cortically activated lateral SNr activity, but was able to increase the late excitatory response (indirect pathway transmission) of medial SNr neurons. More recently, it has been shown that the 5-HT_{2C} antagonists SB243213 and SB242084 are able to abolish the enhancement in the cortico-nigral transmission though the hyperdirect pathway (early excitatory response) in medial SNr neurons induced by the $D_{2/3}$ receptor agonist quinpirole (Lagière et al., 2020). Although activation of both receptor subtypes acts preferentially on the associative/limbic territories of the BG, the 5-HT_{2C} receptor appears to regulate cortico-BG information processing via trans-subthalamic pathways, whereas 5-HT_{2A} does so through trans-striatal pathways. This hypothesis would be in agreement with the high expression of $5HT_{2C}$ receptors and the very low expression of $5HT_{2A}$ receptors in the STN (Pompeiano et al., 1994; Reznitsky et al., 2016).

The absence of modulation of the SNr neuron activity following TCB-2 administration in pCPA-treated animals highlights the participation of endogenous 5-HT in the observed effects. In mPF circuits, 5-HT depletion induced by pCPA treatment enhanced the cortico-BG transmission through the direct pathway by itself (i.e., it produced the same effect elicited by TCB-2 administration). Therefore, this effect could be indirectly related to the decrease in 5-HT neuron activity, as it has already been reported for the 5-HT_{2A} agonists LSD (lysergic acid diethylamide) and DOI (Aghajanian et al., 1970; De Montigny and Aghajanian, 1977; Martín-Ruiz et al., 2001). Supporting this idea, we recently have shown that the activation of the inhibitory 5-HT_{1A} receptors by buspirone induces an increase of the inhibitory component of the triphasic response in SNr neurons (Vegas-Suárez et al., 2022).

In addition, we further investigated whether the mobilization of the endogenous 5-HT system by injecting the SSRI fluoxetine was able to mimic the specific effects triggered by TCB-2. However, the acute challenge of fluoxetine specifically decreased the basal firing rate of lateral SNr neurons and consistently induced an increase in early excitation, together with a reduction in late excitation in lateral SNr neurons, while inducing a decrease in early excitation in neurons from the medial SNr. The discrepancies between the TCB-2-induced and fluoxetine-induced effects could be explained by the activation of 5-HT receptor subtypes (other than 5-HT_{2A} receptors located in the BG circuitry) after the elevation of 5-HT levels, such as the 5-HT_{1B} receptors in striato-pallidal and subthalamo-nigral synapses (Querejeta et al., 2005; Rav-Acha et al., 2008; Ding et al., 2013) or the 5-HT_{1A} receptors located in globus pallidus neurons (Rav-Acha et al., 2008).

There are some limitations in the present study. First, the off-targets of the used drugs. For instance, it should be noted that the full pharmacological profile of TCB-2 is unknown, and recent evidence indicates some affinity to 5-HT_{2C} and 5-HT_{1A} receptors (Di Giovanni and De Deurwaerdère, 2018). Furthermore, although it has a preferential action for 5-HT_{2A} receptors, MDL11939 can also behave as an antagonist of 5-HT_{2C} receptors. Similarly, fluoxetine, in addition to being a nonselective serotonin transporter blocker, can also act as a 5-HT_{2A}, 5-HT_{2B}, or 5-HT_6 receptor antagonist, a norepinephrine reuptake blocker, and a σ1 receptor ligand. Second, although the acute dose of fluoxetine used in our experimental design has been reported as being able to induce stable elevated brain 5-HT levels for at least 6 h after injection (Volle et al., 2018), this finding has not been evidenced by other authors (Rogóż and Gołembiowska, 2010). Finally, we restricted the study to male animals; however, sex differences have been evidenced in BG and cortical activities (Wilson, 1993; Nikulin and Brismar, 2005). Additional experiments in female rats would therefore be required to assess a possible sexual dimorphism in the modulatory role of 5-HT_{2A} on the functionality of BG circuits.

In summary, the present study allows us to better understand the role of the 5-HT_{2A} receptor on the dynamic regulation of BG circuits and highlights its preferential modulatory action enhancing mPF information processing through the direct pathway. In addition, these findings point out a therapeutic potential of the 5-HT_{2A} receptor in the treatment of disorders linked to abnormal activity on the associative/limbic BG circuits.

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