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Research paper

Emission of 50-kHz ultrasonic vocalizations stimulated by antiparkinsonian dopaminomimetic drugs in hemiparkinsonian rats is associated with neuronal activation in subcortical regions that regulate the affective state

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ABSTRACT

Dopamine replacement therapy (DRT) of Parkinson's disease (PD) may trigger non-motor complications, some of which affect hedonic homeostatic regulation. Management of iatrogenic alterations in the affective state in PD is unsatisfactory, partly because of the limitations in the experimental models that are used in the preclinical investigation of the neurobiology and therapy of these alterations. In this connection, we recently employed a new experimental approach consisting in measuring the emission of 50-kHz ultrasonic vocalizations (USVs), a marker of positive affect, in hemiparkinsonian rats treated with drugs used in the DRT of PD. To further strengthen our approach, we here evaluated how the acute and repeated (× 5, on alternate days) administration of apomorphine (2 mg/kg, i.p.) or L-3,4-dihydroxyphenilalanine (L-DOPA, 12 mg/kg, i.p.) modified the immunoreactivity for Zif-268, a marker of neuronal activation, in the nucleus accumbens (NAc), caudate-putamen (CPu) and medial prefrontal cortex (mPFC), which are brain regions that regulate emotional states and drugs' affective properties. Acute and repeated treatment with either apomorphine or L-DOPA stimulated the emission of 50-kHz USVs in hemiparkinsonian rats, and this effect was paired with increased Zif-268 immunoreactivity in the NAc and CPu, but not mPFC. These findings indicate that subcortical and cortical regions may differently regulate the emission of 50-kHz USVs in hemiparkinsonian rats treated with dopaminergic drugs used in the DRT of PD. Moreover, they provide further evidence that measuring 50-kHz USV emissions in hemiparkinsonian rats may be a relevant approach to investigate at the preclinical level the affective properties of antiparkinsonian drugs.

1. Introduction

Dopamine replacement therapy (DRT) is the standard medication for Parkinson's disease (PD) and utilizes L-3,4-dihydroxyphenilalanine (L-DOPA), the metabolic precursor of dopamine, and agonists of dopamine receptors to restore the deficient nigrostriatal dopaminergic tone. Although effective on motor disabilities, DRT may elicit motor and non-motor untoward effects. The typical motor untoward effects caused by DRT are dyskinesias, that are associated with a significant deterioration of therapeutic efficacy (Bastide et al., 2015). Non-motor untoward effects of DRT can be manifested at the autonomic level, consisting in symptoms like nausea and orthostatic hypotension, and at the central level, consisting in various manifestations like daytime sleepiness, delusions, hallucinations, and behavioral addictions (Bastide et al., 2015). Iatrogenic behavioral addictions in PD include impulse control disorders (ICDs) (e.g., hypersexuality, pathological gambling, pathological shopping) and dopamine dysregulation syndrome (DDS), consisting in the compulsive use of dopamine-replacing medications (Voon et al., 2017; Kelly et al., 2020). Although there is still debate on the existence of a causal link between the use of DRT and certain non-motor manifestations in PD patients (e.g., nausea, sleepiness, see Bastide et al., 2015), several lines of evidence suggest that the use of DRT could be a causal factor for the manifestation of hedonic homeostatic dysregulation and behavioral addictions in PD patients (Giovannoni et al., 2000; Weintraub et al., 2015; Voon et al., 2017; Kelly et al., 2020). Limited therapeutic options are available to manage hedonic homeostatic dysregulation and behavioral addictions in patients with PD medicated with DRT. A factor that may contribute to such a deficit situation is that

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the experimental models employed in the preclinical investigation of the affective/motivational properties of drugs used in the DRT of PD may display some limitations, such as the possible influence of the motor-stimulating effects of drugs on the behavioral readouts that are measured (Rokosik and Napier, 2012; Campbell et al., 2014; Floris et al., 2022). A new possibility for improving the preclinical study of hedonic homeostatic dysregulation elicited by drugs used in the DRT of PD may come from measuring the emission of ultrasonic vocalizations (USVs), a behavioral marker of arousal, affect and motivation, in dopamine-denervated rats treated with antiparkinsonian drugs (Simola et al., 2021).

Stimuli that bear affective valence may trigger the emission of USVs in rats (Simola and Granon, 2019). When exposed to pleasurable stimuli, rats emit the so-called "50-kHz USVs". These calls, which have high maximum frequency (up to 120 kHz) and short duration (less than 150 ms) and may display substantial frequency modulation (Simola and Brudzynski, 2018a; Lenell et al., 2021), are considered a marker of arousal and positive affect in rats (Pereira et al., 2014; Burgdorf et al., 2020; Premoli et al., 2023). Previous studies have found enduring elevations in the emission of 50-kHz USVs in rats repeatedly treated with addictive drugs and have suggested that this phenomenon may stem from a persistent elevation in affectivity triggered by repeated drug experience (Ahrens et al., 2009; Mu et al., 2009; Ma et al., 2010; Simola et al., 2014). Moreover, we have demonstrated that the repeated treatment with either apomorphine or L-DOPA persistently elevates the emission of 50-kHz USVs in hemiparkinsonian but not sham-operated rats (Simola et al., 2021), which may be consistent with earlier evidence indicating that denervation of the dopaminergic nigrostriatal system amplifies the affective properties of antiparkinsonian drugs (Riddle et al., 2012; Campbell et al., 2014; Zengin-Toktas et al., 2013). Accordingly, we suggest that measuring the emission of 50-kHz USVs may be a valuable approach to disclose how drugs used in the DRT of PD modify the affective state in hemiparkinsonian rats. This approach could also be relevant to gain further insight into the neurobiological underpinnings of the behavioral addictions that may be induced by antiparkinsonian drugs, which may depend on the presence of a iatrogenic hedonic homeostatic dysregulation (Giovannoni et al., 2000; Delpont et al., 2017; Terenzi et al., 2018).

The present study aimed at further elucidating the significance of the 50-kHz USVs emitted by hemiparkinsonian rats treated with drugs used in the DRT of PD. To this end, hemiparkinsonian rats received an acute or repeated (×5, every other day) administration of either apomorphine or L-DOPA and were assessed for calling behavior, rotational behavior, and Zif-268 immunoreactivity in the nucleus accumbens (NAc; shell, core), caudate-putamen (CPu; dorsolateral, ventromedial) and medial prefrontal cortex (mPFC). These brain regions were evaluated since they all regulate the emotional state and mediate the affective/motivational properties of drugs, and because we previously found that increased Zif-268 immunoreactivity occurred in the NAc, CPu and mPFC of neurologically intact rats that were repeatedly treated with amphetamine, a dopaminergic psychostimulant of abuse (Costa et al., 2015, 2020). Accordingly, we wanted to ascertain whether calling behavior elicited by apomorphine or L-DOPA in hemiparkinsonian rats was associated with patterns of neuronal activation similar to those previously observed in neurologically intact rats treated with a drug that markedly elevates the affective state and enduringly increases the emission of 50-kHz USVs.

2. Materials and methods

2.1. Animals

A total of 114 male Sprague-Dawley rats (Envigo, Italy) were included in the present study. Rats weighed 275–300 g (age 75–80 days) at the beginning of the experiments (i.e., on the day of surgeries) and were housed in groups of 4 under a 12-h light/dark cycle (lights on at 7:00 am) with ad libitum access to water and food, except during recordings (performed between 10:00 am and 4:00 pm). This study was conducted according to the EU directives for animal experimentation (2010/63/EU, L.276; 22/09/2010), and the guidelines issued by the Organization for Animal Welfare of the University of Cagliari (prot: 1236/2015-PR). All the appropriate procedures were followed to minimize animal discomfort and numbers of animals used.

2.2. Drugs

Different groups of hemiparkinsonian or sham-operated rats were treated with apomorphine (2 mg/kg), L-DOPA (12 mg/kg), or vehicle (distilled water). Rats that received L-DOPA were pretreated with benserazide, an inhibitor of peripheral decarboxylases (3 mg/kg, 30 min before L-DOPA), to maximize L-DOPA brain levels. All the rats that received 6-OHDA were pretreated with the noradrenaline transporter inhibitor desipramine (10 mg/kg), to reduce damage to noradrenergic neurons. 6-OHDA, apomorphine, benserazide, desipramine (all hydrochlorides) and L-DOPA (free base) were purchased from Sigma Aldrich/Merck Life Science (Milan, Italy). Apomorphine, benserazide, desipramine and L-DOPA were solubilized in distilled water and administered by the intraperitoneal (i.p.) route in a volume of 3 ml/kg. Vehicle was administered by the i.p. route (3 ml/kg). 6-OHDA (2 μg/μl) was solubilized in saline solution (0.9 % NaCl) + 0.05 % ascorbic acid (to prevent 6-OHDA autoxidation) and administered intracranially. The drug doses used in the present study were selected based on our previous investigation that evaluated the emission of 50-kHz USVs in hemiparkinsonian rats after acute and repeated administration of apomorphine or L-DOPA (Simola et al., 2021).

2.3. Dopaminergic nigrostriatal denervation

Denervation of the dopaminergic nigrostriatal system was performed according to Frau et al. (2013). Rats were anaesthetized with Equithesin (pentobarbital 0.97 g, $MgSO_4$ 2.1 g, chloral hydrate 4.25 g, propylene glycol 42.8 ml, ethanol 90 % 11.5 ml, to 100 ml with distilled water; administration volume: 5 ml/kg, i.p.). Afterwards, each rat was individually placed in a David–Kopf stereotaxic apparatus (Tujunga, CA, USA) and received a unilateral injection of 6-OHDA (2 μg/μl, total volume 4 μl) in the left medial forebrain bundle (MFB). The injection was performed by means of a stainless-steel cannula connected to a peristaltic pump by polyethylene tubing. The stereotaxic coordinates used were: anterior-posterior (AP) = −2.2, lateral, (L) = +1.5, relative to bregma, and vertical (V) = −7.8, relative to dura; the incisor bar was set at +5.0, according to the rat brain atlas of Pellegrino et al., 1979. A flow rate of 1 μl/min was used. Moreover, the cannula was left in place for 2 min after injection was completed, to ensure diffusion of 6-OHDA in the surrounding tissue. After surgical procedures were completed, rats were monitored until they completely recovered and were then returned to their home cages. Sham-operated rats underwent the same procedures described above, except that they received 0.9 % NaCl +0.05 % ascorbic acid instead of 6-OHDA.

2.4. Experimental plan

Fig. 1 shows the experimental plan, which was conceived based on our previous study that evaluated the emission of 50-kHz USVs in hemiparkinsonian rats repeatedly treated with apomorphine or L-DOPA (Simola et al., 2021). Rats received 5 min of handling for 2 days before surgical procedures. Fourteen days after the completion of surgeries, rats were evaluated in the cylinder test to score forelimb asymmetry as a parameter that estimates the magnitude of dopaminergic nigrostriatal denervation. Afterwards, 6-OHDA-lesioned rats were homogeneously distributed among the experimental groups according to the extent of forelimb asymmetry. Conversely, sham-operated rats were randomly distributed among the experimental groups, since they did not display

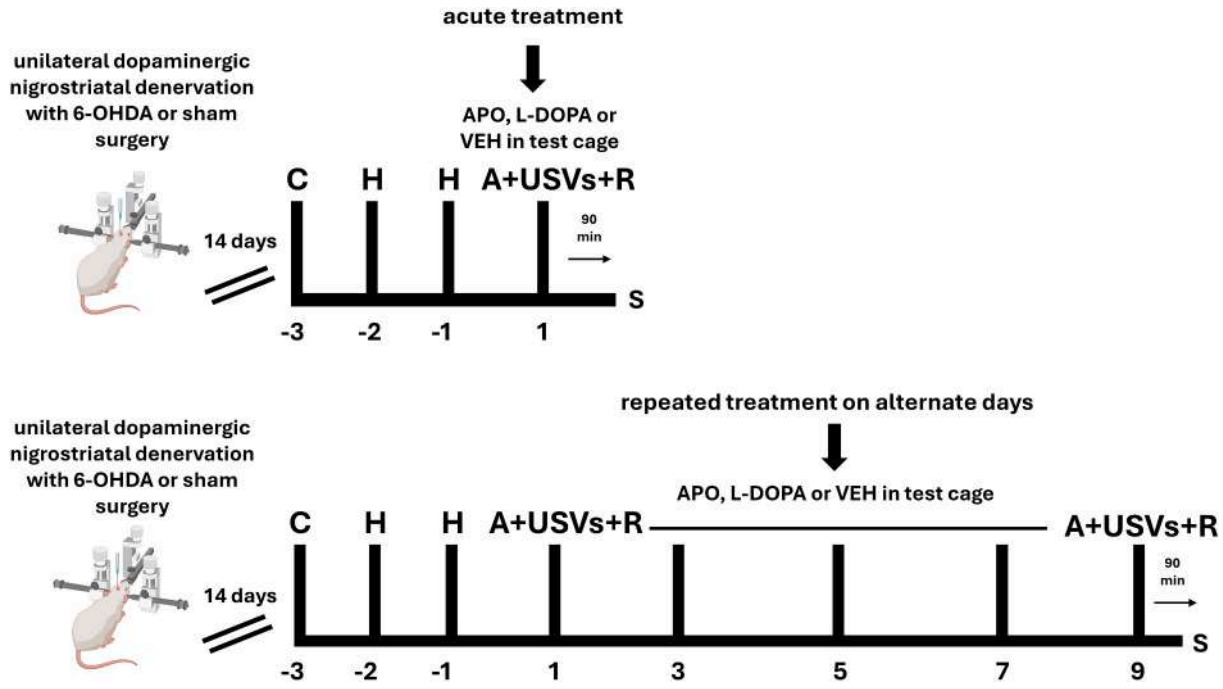


Fig. 1. Experimental protocol. Rats were subjected to either unilateral denervation of the nigrostriatal dopaminergic system with 6-hydroxydopamine or sham surgery. Fourteen days later, rats were first assessed for forelimb asymmetry in the cylinder test and then habituated to test cages for two consecutive days. Afterwards, hemiparkinsonian and sham-operated rats received apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.) in either acute or repeated (×5, on alternate days) administration. Emission of ultrasonic vocalizations and performance of rotational behavior were scored on days 1 of the experimental protocol in rats that received acute treatment, and on days 1 and 9 of the experimental protocol in rats that received repeated treatment. Rats were sacrificed 90 min after treatment on day 1 (acute treatment) or 9 (repeated treatment) of the experimental protocol, to evaluate immunoreactivity for tyrosine hydroxylase or Zif-268. 6-OHDA = 6-hydroxydopamine; A = administration; APO = apomorphine; C = cylinder test; H = test cage habituation; L-DOPA = L-3,4-dihydroxyphenylalanine; R = rotational behavior; S = sacrifice; USVs = ultrasonic vocalizations; VEH = vehicle. Image partly created with Biorender.com.

overt forelimb asymmetry. Experiments were made in 2 phases: 1) habituation to the test cage (15 min), twice a day × 2 days (total: 4 sessions); 2) acute or repeated (× 5, every other day) administration of either apomorphine or L-DOPA in the test cage. Two groups of hemiparkinsonian rats and two groups of sham-operated rats received vehicle in either acute or repeated administration and were used as controls in the respective experiments, to reduce the number of animals used.

2.5. Estimation of the magnitude of dopaminergic nigrostriatal denervation with the cylinder test

Rats were individually placed in transparent Plexiglas cylinders (height: 45 cm, diameter: 20 cm) and then videotaped for 3 min. Rats' behavior inside the cylinder consisted in rearing and vertical/lateral exploration of walls. To evaluate forelimb use, wall contacts made with the contralateral/homolateral forelimb and with both forelimbs were scored. Thereafter, the percentage of use of the contralateral to injection forelimb (i.e., the one impacted by the 6-OHDA lesion) was calculated by dividing the number of wall contacts made with the contralateral forelimb by the total number of wall contacts, according to Schallert et al. (2000). Rats lesioned with 6-OHDA were included in the study only if they showed a percentage of contralateral to injection forelimb use of no more than 20 %, indicative of a substantial depletion of dopamine (85 % or greater) in the nigrostriatal pathway (Schallert et al., 2000). Of a total of 60 rats that received 6-OHDA, 56 met the criterion for inclusion in the cylinder tests, and 4 were excluded from the study. The numbers of rats in each experimental group were as follows: 6-OHDA + vehicle, acute administration $n = 9$, repeated administration $n = 8$; 6-OHDA + apomorphine, acute administration $n = 10$, repeated administration n = 9; 6-OHDA + L-DOPA, acute administration n = 9, repeated administration $n = 11$; sham + vehicle, acute administration n = 9, repeated administration n = 10; sham + apomorphine, acute administration n = 11, repeated administration n = 9; sham + L-DOPA, acute administration n = 11, repeated administration $n = 8$.

2.6. Recording and analysis of ultrasonic vocalizations and recording of rotational behavior

Rats were individually placed in test cages that consisted of Plexiglas cylinders (height: 30 cm, diameter: 30 cm) and had one half totally painted black and the other half partially painted black to form alternating horizontal black and transparent stripes (Supplementary Material, Fig. S1). Cylinders were topped with lids endowed with ultrasonic microphones (CM16/CMPA, Avisoft, Berlin, Germany) that were connected to ultrasonic recorders (Ultrasound Gate 116 Hb, Avisoft). Microphones were located at an average distance of 27 cm from rats and intensity gain was kept at a constant level throughout recordings. Moreover, the bottom of cylinders was covered with sawdust, part of which coming from the home cage of each specific rat evaluated, in the attempt to facilitate the emission of 50-kHz USVs (Natusch and Schwarting, 2010). Calling behavior was recorded for 90 min after each administration of apomorphine, L-DOPA, or vehicle, starting immediately after injections. Moreover, the 50-kHz USVs recorded were categorized in flat, trill, and frequency modulated (FM). Categorization was done according to the criteria proposed by Wright et al. (2010) and to the procedure previously described (Simola et al., 2012). In the present study, the category of FM calls comprises all the categories of 50-kHz USVs other than flat and trill calls, as defined by Wright et al. (2010). Fig. 2 demonstrates examples of USVs recorded during the present study. The software SASLab Pro (Avisoft, Berlin, Germany) was used to convert the recordings of USVs into spectrograms and to count the number of USVs in each spectrogram. The numbers of 50-kHz USVs were scored in all the rats included in the study, and scoring was performed for all the 90 min of recording. Moreover, the acoustic parameters of

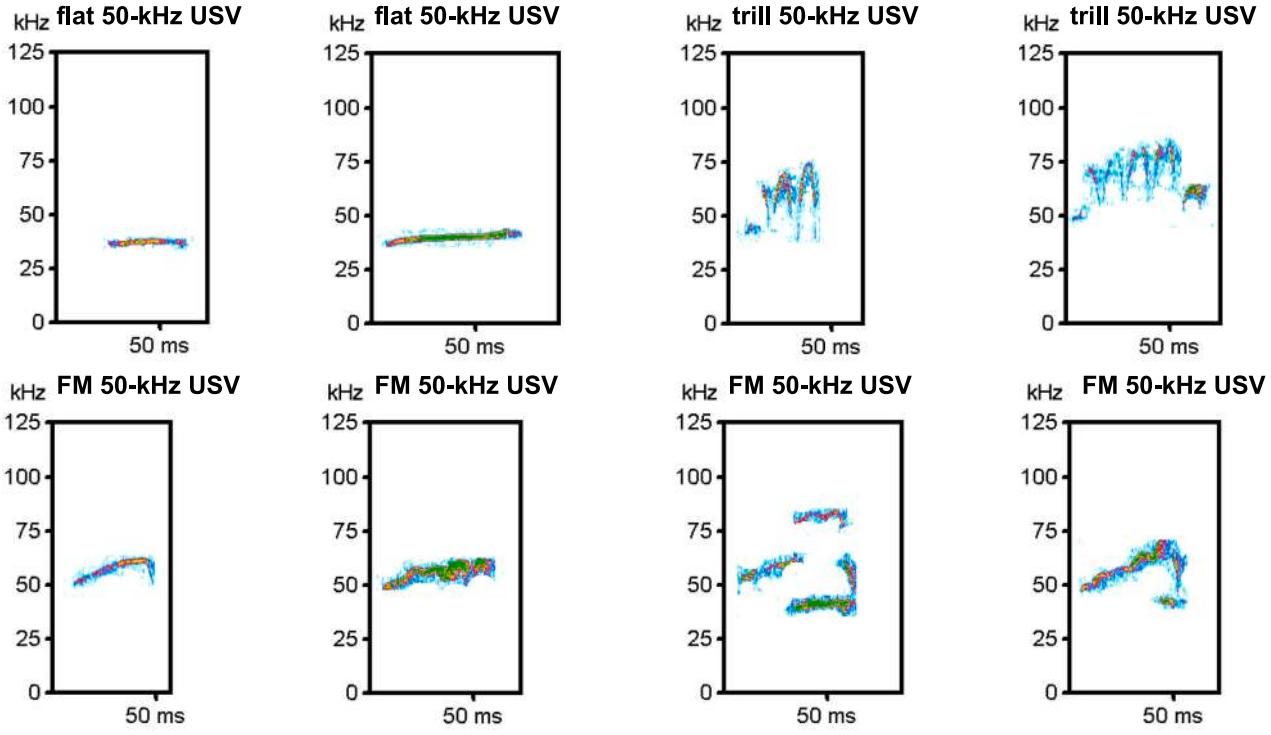


Fig. 2. Examples of ultrasonic vocalizations recorded during the present study. The figure demonstrates independent vocalizations emitted by different rats. FM = frequency modulated; USV = ultrasonic vocalization.

calls (i.e., bandwidth, duration and maximum frequency) were scored in a subset of rats (2 rats per experimental group) by analyzing the calls emitted during 1-min intervals every 10 min of recording. This additional analysis aimed at confirming that the calls recorded in the present study exhibited acoustic characteristics of authentic 50-kHz USVs.

Rotational behavior was recorded simultaneously with USVs in hemiparkinsonian rats. For that purpose, rats were videotaped, and the numbers of rotations (360°) directed towards the intact (contralateral rotational behavior) or dopamine-denervated (homolateral rotational behavior) brain hemisphere were subsequently scored for 90 min by an experimenter unaware of the treatment groups.

2.7. Quantification of dopaminergic denervation and Zif-268 immunoreactivity

2.7.1. Tissue preparation

Ninety minutes after the first (acute treatment, day 1) or the fifth (repeated treatment, day 9) administration of apomorphine, L-DOPA, or vehicle, rats were deeply anaesthetized with equithesin and transcardially perfused with saline, followed by 4 % paraformaldehyde in 0.1 M phosphate buffer (PB; pH = 7.4). Afterwards, brains were removed, postfixed overnight in the same solution at 4 °C and preserved in PB saline 1× (PBS) at 4 °C. The next day, brains were coronally cut on a vibratome (VT1000S, Leica Biosystems) to yield sections (thickness, 40 μm) suited for immunohistochemistry (IHC) processing. Three coronal sections were obtained for each rat, according to the following stereotaxic coordinates: 1) 2.70 mm to 2.20 mm, mPFC and NAc (core and shell); 2) 1.70 mm to 0.20 mm, CPu (dorsolateral and ventromedial); 3) 4.80 mm to −5.80 mm, SNc and VTA. All stereotaxic coordinates were relative to bregma, according to the rat brain atlas of Paxinos and Watson (1998). A set of sections from the CPu, mPFC, NAc, SNc, and VTA was processed to quantify the levels of tyrosine hydroxylase (TH), as marker of dopaminergic denervation. Another set of sections from the CPu, mPFC, and NAc was processed to quantify the levels of Zif-268, as marker of neuronal activation.

2.7.2. Reaction protocol

Free-floating sections were rinsed in 0.1 M PB, blocked in a solution containing 3 % normal goat serum (NGS, Vector, UK) and 0.3 % Triton X-100 in 0.1 M PB at room temperature (2 h). The diaminobenzidine (DAB) technique was used for the visualization of TH. To this end, sections were incubated overnight at 4 °C with the mouse monoclonal primary antibody anti-TH (1:1000, Merck, Italy, T1299). Thereafter, the biotinylated secondary antibody (goat anti-mouse IgG, Vector, UK, BA-9200) was added, followed by the avidin–biotin–peroxidase complex protocol (ABC, Vector, UK, PK-6100). Finally, sections were mounted onto glass slides coated with gelatine in Eukitt mounting medium for visualization. The immunofluorescence technique was used for the visualization of Zif-268. To this end, sections were incubated at 4 °C (48 h) with the rabbit monoclonal primary antibody anti-Zif-268 (1:1000, Cell Signaling, U.S.A., #4153), rinsed three times in 0.1 M PB, and then incubated with the secondary antibody, AlexaFluor® 594-labeled goat anti-rabbit IgG (1:400, Jackson ImmunoResearch Europe, UK, 111–585-003) in 0.1 M PB at room temperature (2 h). Afterwards, sections were rinsed and immediately mounted onto glass slides coated with gelatine in Mowiol mounting medium (Costa et al., 2019). The omission of the primary/secondary antibodies served as negative control in each reaction protocol and produced no labelling (data not shown).

2.7.3. Image acquisition and analysis

Images of single wavelength (14-bit depth) were obtained with a ZEISS Axio Scan Z1 slide scanner (Zeiss, Germany), as previously described (Tassan Mazzocco et al., 2024). Brain sections immunostained for TH or Zif-268 were captured at 20× magnification (Objective: Plan-Apochromat 20×/0.8 M27) to acquire: i) the whole prelimbic/infralimbic area of the mPFC; ii) the shell and core portions of the NAc; iii) two portions from the CPu (dorsolateral and ventromedial); iv) the whole SNc and VTA. Both hemispheres were acquired for each brain area analyzed. The ImageJ software (National Institutes of Health, USA) was used to quantify: i) the number of TH-positive neurons in the SNc and VTA; ii) the mean intensity of TH-positive fibers in the mPFC, NAc

(core and shell), and CPu; iii) the mean intensity of Zif-268-positive neurons in the mPFC, NAc (core and shell), and CPu (dorsolateral and ventromedial). The unilateral injection of 6-OHDA in the MFB leads to highly reproducible results in terms of denervation of the nigrostriatal dopaminergic system (Simola et al., 2007). Accordingly, the analysis of IHC for TH in hemiparkinsonian rats involved a within-group comparison of the signals obtained in the dopamine-denervated brain hemisphere and in the intact brain hemisphere. This approach was followed to minimize the use of antibodies and rat tissues. Conversely, the analysis of IHC for Zif-268 involved a between-group comparison in hemiparkinsonian and sham-operated rats. To quantify the number of TH-positive neurons, images were first converted to 8-bit, background adjusted using a constant value across all sections, and then neurons were manually counted by using the ImageJ multi-point tool. To quantify the mean intensity of TH- and Zif-268-immunostained sections, images were background adjusted using a constant value across all sections. Then, the pixels were converted into square micrometres using a suited calibration, to obtain the area occupied by a specific immunoreaction product. Analyses were always performed in a blinded manner in the three sections. No significant differences in TH and Zif-268 immunoreactivity were found among the three sections of a given area in each rat. Accordingly, for each area analyzed values from different levels were averaged, normalized with respect to the sham-operated rats treated with vehicle, and expressed as percentages.

2.8. Statistical analysis

Means ± S.E.M. were calculated for: i) the percentages of use of the limb contralateral to 6-OHDA injection in the cylinder test; ii) the numbers of 50-kHz USVs (total and categorized) and rotations recorded on days 1 (acute treatment, repeated treatment) and 9 (repeated treatment) of the experimental protocol; iii) the values of TH-positive neurons, TH-positive fibers and Zif-268-positive neurons. Behavioral results obtained on day 1 of repeated treatment were not included in the statistical analysis. D'Agostino-Pearson, Kolmogorov-Smirnov and Levene tests were performed to check for normality and homoscedasticity of data. When homoscedasticity was not present, data were log-transformed and a constant (+1) was added to all datasets subjected to transformation, to correct for null values. Group differences in the percentage of use of forelimbs during the cylinder test were analyzed by Mann Whitney test. Group differences in the values of TH-positive neurons and TH-positive fibers were analyzed by one-way ANOVA followed by Tukey's post-hoc test when data displayed homoscedasticity. In the case data did not display homoscedasticity after transformation, analysis was performed with Brown-Forsythe ANOVA followed by Dunnet's test or with Kruskal-Wallis test followed by Dunn's test. Group differences in the numbers of 50-kHz USVs emitted after acute or repeated pharmacological treatment were analyzed with Kruskal-Wallis test followed by Dunn's test. Due to the substantial lack of 50-kHz USV emissions observed from 40 to 90 min of recording, analysis of the time-course of calling behavior was limited to the first 30 min of recording. Group differences in the intensity of Zif-268 immunoreactivity after acute or repeated treatment were analyzed by one-way ANOVA followed by Tukey's post-hoc test, or by Brown-Forsythe ANOVA followed by Dunnet's test in the case data did not display homoscedasticity after transformation. Group differences in the numbers of contralateral rotations performed by hemiparkinsonian rats after acute or repeated treatment were analyzed by Mann-Whitney test. Spearman's test was used to test for correlations in hemiparkinsonian rats between: i) the numbers of 50-kHz USVs emitted and of contralateral rotations performed; ii) the numbers of 50-kHz USVs emitted and the levels of TH or Zif-268 immunoreactivity in each brain area analyzed in the dopamine-denervated hemisphere; and iii) the levels of Zif-268 immunoreactivity in each brain area analyzed in the dopamine-denervated hemisphere. For the sake of clarity and conciseness, figures show raw data and statistical main effects are reported only for statistically significant results. Statistical analyses were performed with Statistica (StatSoft, Tulsa, OK, USA), Prism (GraphPad, La Jolla, CA, USA), and QI Macros (KnowWare International, Denver, CO, USA) for Windows. Significance was set at $p < 0.05$ for all analyses.

3. Results

3.1. Dopaminergic denervation

Unilateral injection of 6-OHDA (8 μg) in the left MFB led to a near complete depletion of TH-positive neurons in the left SNc and of TH-positive fibers in the left NAc and CPu, homolateral to the injection (Figs. 3 and 4). Moreover, unilateral injection of 6-OHDA in the left MFB caused a partial, albeit significant, depletion of TH-positive neurons in the left VTA and of TH-positive fibers in the left mPFC, homolateral to the injection (Figs. 3 and 4). Supplementary material reports further details on the magnitude of dopaminergic denervation in the different brain regions analyzed.

3.2. Emission of 50-kHz ultrasonic vocalizations

Acute treatment with apomorphine significantly stimulated the emission of 50-kHz USVs in both hemiparkinsonian and sham-operated rats, although the former emitted higher numbers of calls. Conversely, repeated treatment with apomorphine, acute treatment with L-DOPA and repeated treatment with L-DOPA all significantly stimulated the emission of 50-kHz USVs in hemiparkinsonian rats only.

Kruskal-Wallis test revealed a significant effect of treatment on the total number of 50-kHz USVs emitted in rats that received apomorphine (2 mg/kg, i.p.) or L-DOPA (12 mg/kg, i.p.) in acute (apomorphine, $H = 23.17$, $p < 0.01$; L-DOPA, $H = 20.19$, $p < 0.01$) or repeated (apomorphine, $H = 21.66$, $p < 0.01$; L-DOPA, $H = 23.64$, $p < 0.01$) administration. Dunn's test showed that hemiparkinsonian rats treated with either apomorphine or L-DOPA emitted higher numbers of 50-kHz USVs after acute and repeated administration, compared with vehicle-treated hemiparkinsonian rats (acute apomorphine, $p = 0.0003$; acute L-DOPA, $p = 0.0094$; repeated apomorphine and repeated L-DOPA, $p < 0.0001$) (Fig. 5A, C, E, G). Moreover, Dunn's test showed that hemiparkinsonian rats acutely treated with L-DOPA emitted higher numbers of 50-kHz USVs compared with sham-operated rats acutely treated with L-DOPA ($p = 0.0001$) (Fig. 5C) and that hemiparkinsonian rats repeatedly treated with either apomorphine ($p = 0.0151$) or L-DOPA ($p = 0.0051$) emitted higher numbers of 50-kHz USVs compared with sham-operated rats repeatedly treated with apomorphine or L-DOPA, respectively (Fig. 5E, G). Furthermore, Dunn's test revealed that sham-operated rats acutely treated with apomorphine emitted higher numbers of 50-kHz USVs, compared with sham-operated rats acutely treated with vehicle ($p = 0.042$) (Fig. 5A). Finally, multiple Kruskal-Wallis tests followed by Dunn's test performed for each 5-min time bin within the first 30 min after treatment revealed significant increases in the emission of 50-kHz USVs in hemiparkinsonian rats: i) at 5–30 min after treatment, in rats that received acute apomorphine (Fig. 5B); ii) at 10 and 15 min after treatment, in rats that received acute L-DOPA (Fig. 5D); iii) at 5–30 min after the last (fifth) drug administration, in rats that received repeated apomorphine (Fig. 5F); iv) at 10–25 min after the last (fifth) drug administration, in rats that received repeated L-DOPA (Fig. 5H). Statistical values of Kruskal-Wallis and Dunn's test for the time course analysis of the emission of 50-kHz USVs are demonstrated in Tables S1 and S2.

3.2.1. Emission of categorized ultrasonic vocalizations and acoustic parameters of calls

Acute treatment with apomorphine significantly stimulated the emission of flat and FM 50-kHz USVs in both hemiparkinsonian and sham-operated rats. Conversely, acute treatment with L-DOPA significantly stimulated the emission of FM 50-kHz USVs in hemiparkinsonian

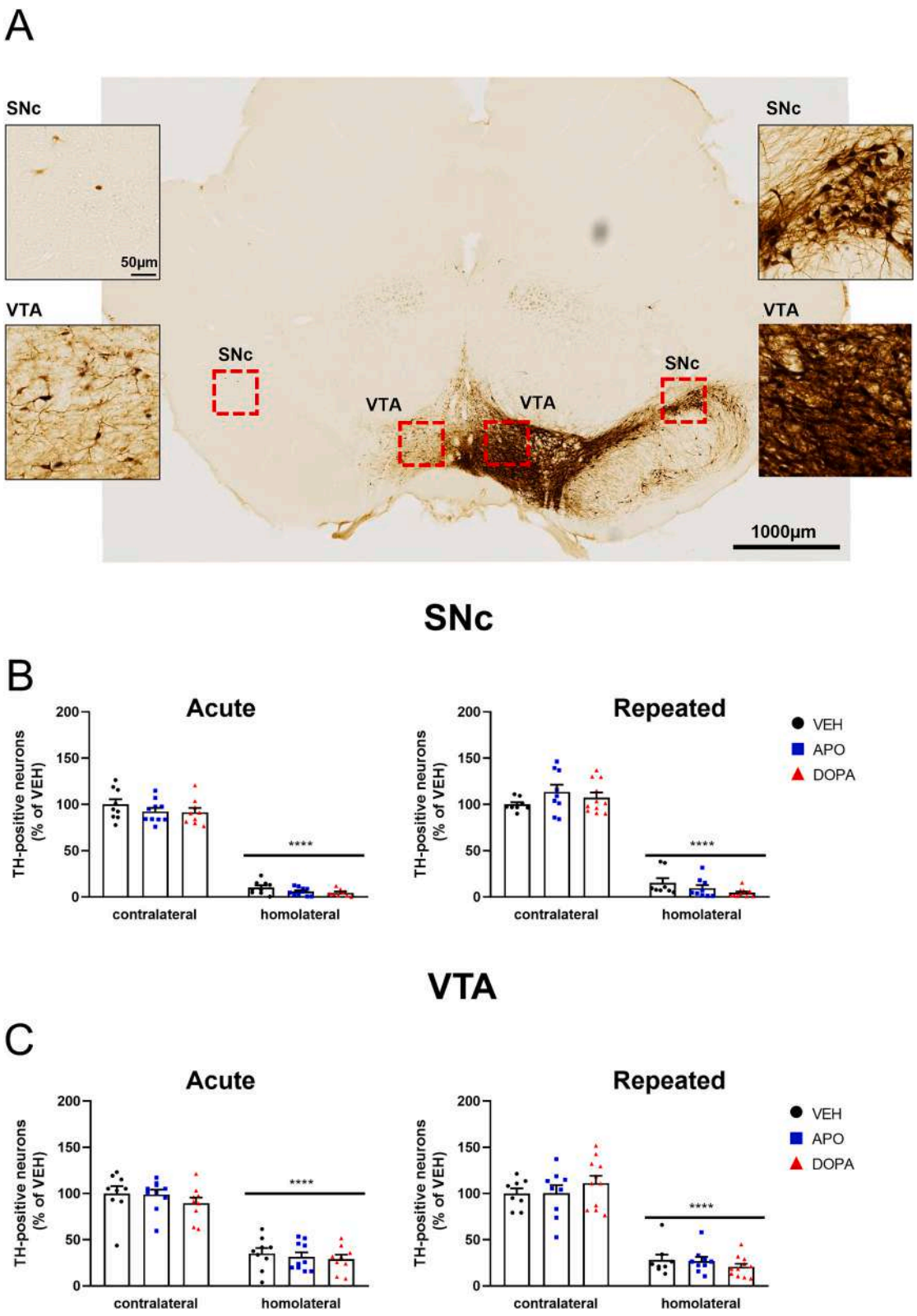
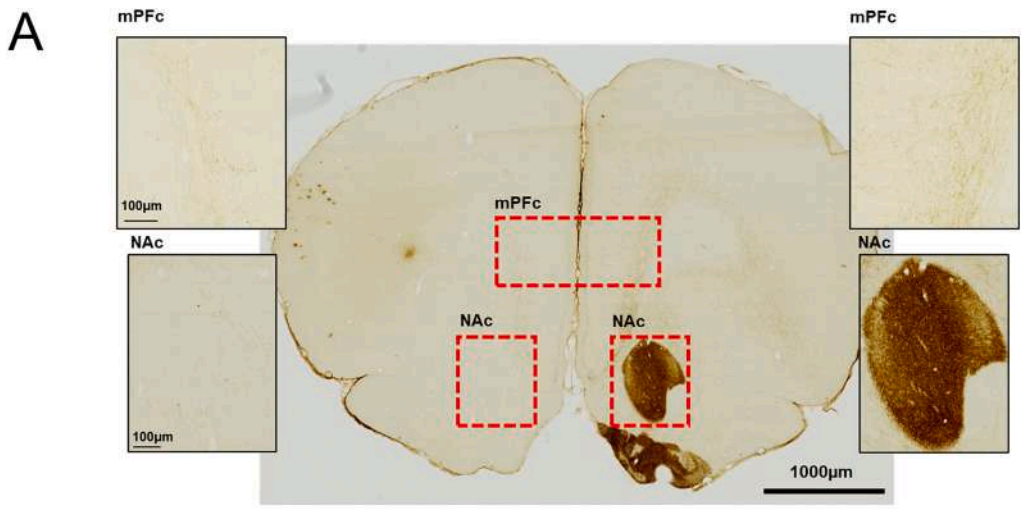
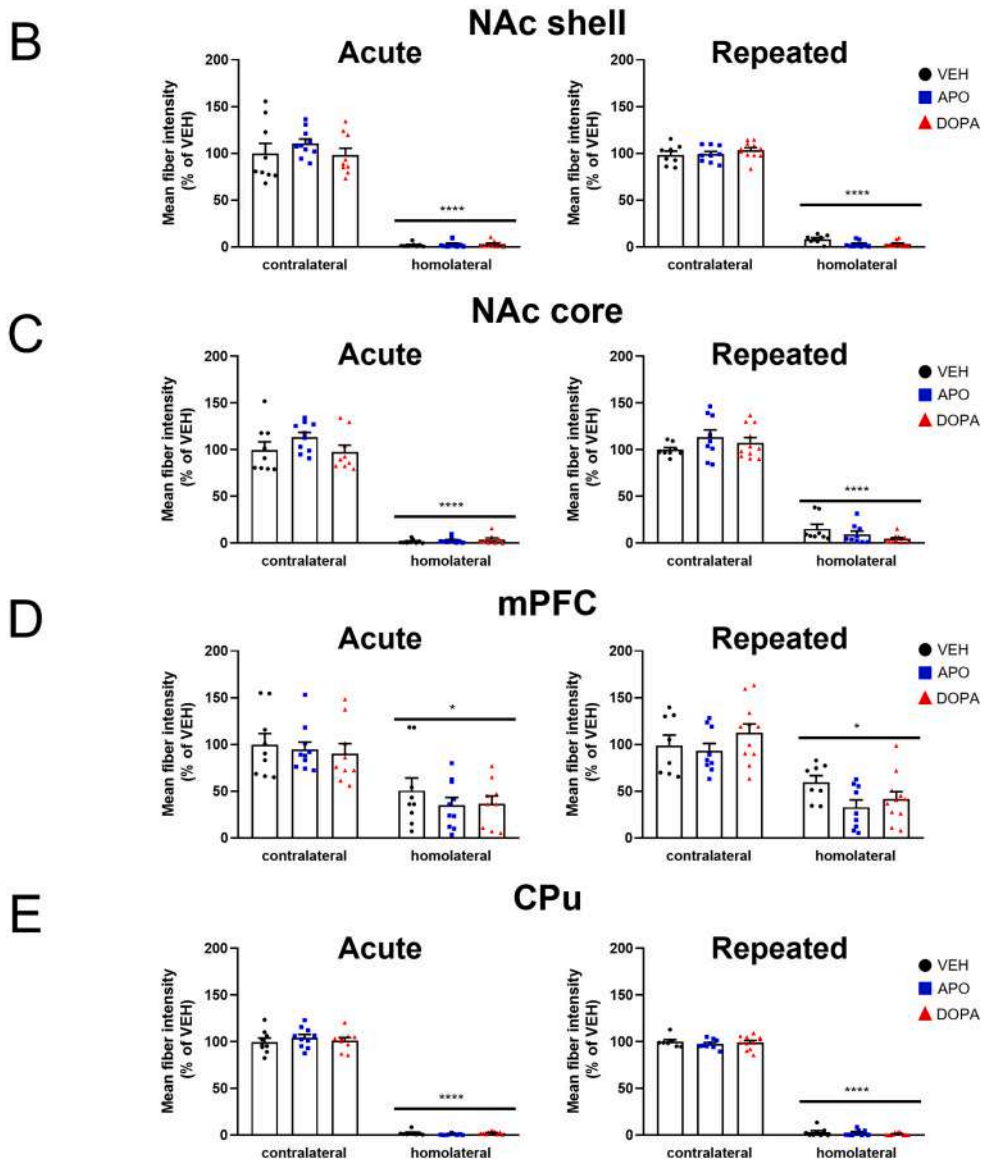


Fig. 3. Immunoreactivity for tyrosine hydroxylase in the substantia nigra pars compacta and ventral tegmental area of rats subjected to unilateral injection of 6-hydroxydopamine in the medial forebrain bundle and to subsequent acute or repeated (×5) treatment with apomorphine, L-DOPA, or vehicle. Upper panel (A): representative coronal section immunostained for tyrosine hydroxylase taken from a hemiparkinsonian rat acutely treated with vehicle. Lower panels (B-C): histograms showing the mean numbers of neurons positive to tyrosine hydroxylase in the substantia nigra pars compacta (B) and ventral tegmental area (C) in the brain hemispheres contralateral and homolateral to 6-hydroxydopamine injection of rats subjected to acute or repeated treatment with apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.). **** $p < 0.0001$ compared with the contralateral brain hemisphere within the respective experimental group. Values are expressed as a percentage of the contralateral/VEH group. Symbols within each bar indicate the values of individual rats ($N = 8$–11 per group). Scale bar: 1000 µm (composed image), 50 µm (zoomed images). APO = apomorphine; DOPA = L-3,4- dihydroxyphenylalanine; SNc = substantia nigra *pars compacta*; TH = tyrosine hydroxylase; VEH = vehicle; VTA = ventral tegmental area.





(caption on next page)

Fig. 4. Immunoreactivity for tyrosine hydroxylase in the shell and core of the nucleus accumbens, medial prefrontal cortex, and caudate-putamen of rats subjected to unilateral injection of 6-hydroxydopamine in the medial forebrain bundle and to subsequent acute or repeated (×5) treatment with apomorphine, L-DOPA , or vehicle. Upper panel (A): representative coronal section immunostained for tyrosine hydroxylase taken from a hemiparkinsonian rat acutely treated with vehicle. Lower panels (B-E): histograms showing the mean intensities of fibers positive to tyrosine hydroxylase in the shell (B) and core (C) portions of the nucleus accumbens, medial prefrontal cortex (D), and caudate-putamen (E, representative coronal section not shown) in the brain hemispheres contralateral and homolateral to 6-hydroxydopamine injection of rats subjected to acute or repeated treatment with apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.). * $p < 0.05$, **** $p < 0.0001$, compared with the contralateral brain hemisphere within the respective experimental group. Values are expressed as a percentage of the contralateral/VEH group. Symbols within each bar indicate the values of individual rats (N = 8–11 per group). Scale bar: 1000 μm (composed image), 50 μm (zoomed images). APO = apomorphine; DOPA = L-3,4-dihydroxyphenylalanine; CPu = caudate-putamen nucleus; mPFC = medial prefrontal cortex; NAc = nucleus accumbens; VEH = vehicle.

rats only. Moreover, repeated treatment with either apomorphine or L-DOPA significantly stimulated the emission of flat, trill and FM 50-kHz USVs in hemiparkinsonian rats only. The analysis of the acoustic parameters of a subset of calls yielded values of duration, maximum frequency, and bandwidth in the range of those previously reported for 50-kHz USVs (Simola and Brudzynski, 2018a). Supplementary material reports further details on the emission of categorized 50-kHz USVs and on the acoustic parameters of calls (Figs. S2-S4, Table S3).

3.3. Rotational behavior in hemiparkinsonian rats

Both apomorphine and L-DOPA significantly stimulated the performance of contralateral rotational behavior in hemiparkinsonian rats after acute and repeated administration (Supplementary Material, Fig. S5). Neither apomorphine nor L-DOPA elicited homolateral rotational behavior in hemiparkinsonian rats (data not shown).

3.4. Effects of apomorphine and L-DOPA on Zif-268 immunoreactivity

Apomorphine and L-DOPA significantly elevated Zif-268 immunoreactivity in the brain hemisphere homolateral to 6-OHDA injection of hemiparkinsonian rats, after acute (Figs. 6, 7) and repeated administration (Figs. 8, 9). Specifically, increased Zif-268 immunoreactivity was detected in the shell and core of the NAc, in the dorsolateral and ventromedial CPu, but not in the mPFC. Apomorphine and L-DOPA did not modify Zif-268 immunoreactivity in the brain of sham-operated rats (Supplementary Material, Fig. S6).

3.4.1. Nucleus accumbens shell

Kruskal-Wallis test revealed a significant effect of treatment on Zif-268 immunoreactivity in the NAc shell of rats that received the acute administration of apomorphine (2 mg/kg, i.p., H = 13.76; $p = 0.0032$). Dunn's test showed higher Zif-268 immunoreactivity in the dopamine-denervated NAc shell in hemiparkinsonian rats acutely treated with apomorphine compared with hemiparkinsonian rats acutely treated with vehicle ($p = 0.0359$), and compared with the NAc shell of sham-operated rats acutely treated with apomorphine ($p = 0.043$) (Fig. 6, 10A). Brown-Forsythe ANOVA revealed a significant effect of treatment on Zif-268 immunoreactivity in the NAc shell of rats that received acute L-DOPA (12 mg/kg, i.p., $F_{3.0,12.80} = 4.6$; $p = 0.021$). However, Dunnet's test showed no significant group differences in Zif-268 immunoreactivity, although a trend towards an increase was observed in the dopamine-denervated NAc shell of hemiparkinsonian rats acutely treated with L-DOPA (Fig. 6, 10A).

One-way ANOVA for the repeated administration of either apomorphine (2 mg/kg, i.p., $F_{3,31} = 11.95$; $p < 0.001$) or L-DOPA (12 mg/kg, i.p., $F_{3,32} = 26.61$; $p < 0.001$) revealed a significant effect of treatment on Zif-268 immunoreactivity in the NAc shell. Tukey's test showed a significant increase in Zif-268 immunoreactivity in the dopamine-denervated NAc shell of hemiparkinsonian rats repeatedly treated with either apomorphine or L-DOPA compared with the dopamine-denervated NAc shell of hemiparkinsonian rats repeatedly treated with vehicle, and compared with the NAc shell of sham-operated rats repeatedly treated with either apomorphine or L-DOPA, respectively ($p < 0.0001$ for all comparisons) (Fig. 8, 10A).

3.4.2. Nucleus accumbens core

Kruskal-Wallis test revealed a significant effect of treatment on Zif-268 immunoreactivity in the NAc core of rats that received the acute administration of either apomorphine (2 mg/kg, i.p., H = 14.93; $p = 0.0019$) or L-DOPA (12 mg/kg, i.p., H = 15.15; $p = 0.0017$). Dunn's test showed higher Zif-268 immunoreactivity in the dopamine-denervated NAc core of hemiparkinsonian rats acutely treated with either apomorphine or L-DOPA, compared with the dopamine-denervated NAc core of hemiparkinsonian rats acutely treated with vehicle (apomorphine, $p = 0.0038$; L-DOPA, $p = 0.0017$), and compared with the NAc core of sham-operated rats acutely treated with either apomorphine or L-DOPA, respectively (apomorphine, $p = 0.0097$; L-DOPA, $p = 0.045$) (Fig. 6, 10B).

One-way ANOVA for the repeated administration of either apomorphine (2 mg/kg, i.p., $F_{3,32} = 25.53$; $p < 0.001$) or L-DOPA (12 mg/kg, i.p., $F_{3,33} = 43.94$; $p < 0.001$) revealed a significant effect of treatment on Zif-268 immunoreactivity in the NAc core. Tukey's test showed a significant increase in Zif-268 immunoreactivity in the dopamine-denervated NAc core of hemiparkinsonian rats repeatedly treated with either apomorphine or L-DOPA, compared with the dopamine-denervated NAc core of hemiparkinsonian rats repeatedly treated with vehicle, and compared with the NAc core of sham-operated rats repeatedly treated with either apomorphine or L-DOPA, respectively ($p < 0.0001$ for all comparisons) (Fig. 8, 10B).

3.4.3. Medial prefrontal cortex

One-way ANOVA for the acute and repeated administration of either apomorphine (2 mg/kg, i.p.) or L-DOPA (12 mg/kg, i.p.) revealed no significant effects of treatment on Zif-268 immunoreactivity in the mPFC (Figs. 6, 8, 10C).

3.4.4. Dorsolateral caudate-putamen

One-way ANOVA for the acute administration of apomorphine (2 mg/kg, i.p.) revealed a significant effect of treatment on Zif-268 immunoreactivity in the DL CPu ($F_{3,34} = 20.53$, $p < 0.0001$). Tukey's test showed a significant increase in Zif-268 immunoreactivity in the dopamine-denervated DL CPu of hemiparkinsonian rats acutely treated with apomorphine compared with the dopamine-denervated DL CPu of hemiparkinsonian rats acutely treated with vehicle, and compared with the DL CPu of sham-operated rats acutely treated with apomorphine ($p < 0.0001$ for both comparisons) (Fig. 7, 11A). Brown-Forsythe ANOVA for the acute administration of L-DOPA (12 mg/kg, i.p.) revealed a significant effect of treatment on Zif-268 immunoreactivity in the DL CPu ($F_{3.0,9.014} = 8.35$; $p = 0.0057$). Dunnet's test showed no significant group differences in Zif-268 immunoreactivity, although a trend towards an increase was observed in the dopamine-denervated DL CPu of hemiparkinsonian rats acutely treated with L-DOPA (Fig. 7, 11A).

One-way ANOVA for the repeated administration of either apomorphine (2 mg/kg, i.p.) or L-DOPA (12 mg/kg, i.p.) revealed a significant effect of treatment on Zif-268 immunoreactivity in the DL CPu (apomorphine, $F_{3,32} = 24.29$; $p < 0.001$; L-DOPA, $F_{3,33} = 135.9$; $p < 0.001$). Tukey's test showed a significant increase in Zif-268 immunoreactivity in the dopamine-denervated DL CPu of hemiparkinsonian rats repeatedly treated with either apomorphine or L-DOPA, compared with the dopamine-denervated DL CPu of hemiparkinsonian rats repeatedly

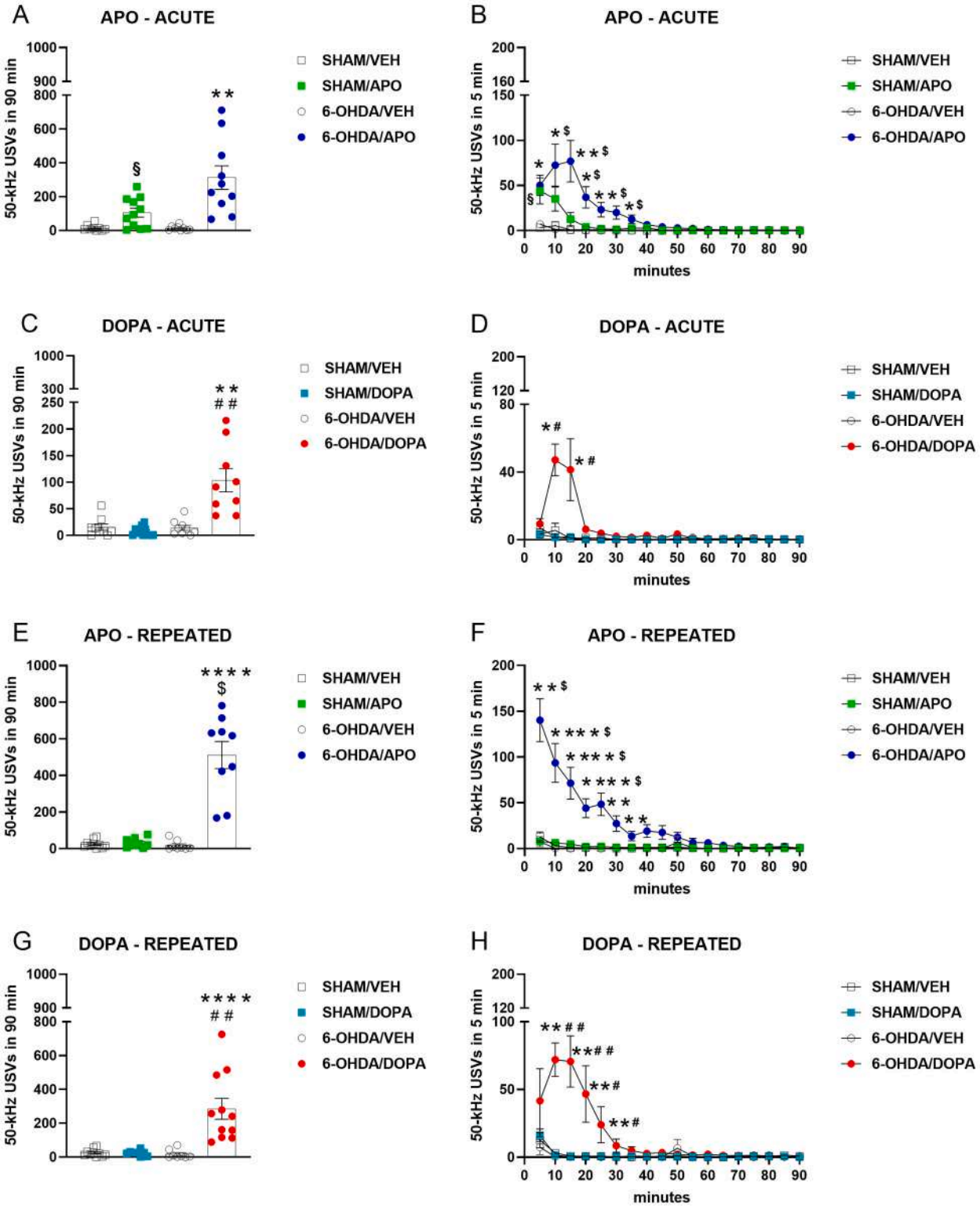


Fig. 5. Total numbers of 50-kHz ultrasonic vocalizations emitted in hemiparkinsonian and sham-operated rats. Rats received apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.) in acute or repeated (×5) administration. The figure demonstrates the emission of 50-kHz ultrasonic vocalizations recorded for 90 min after acute treatment (A-D) and after the last (fifth) drug administration of repeated treatment (*E*-H). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ compared with 6-OHDA/VEH. $ $p < 0.05$ compared with SHAM/APO. # $p < 0.05$, ## $p < 0.01$ compared with SHAM/DOPA. § $p < 0.05$ compared with SHAM/VEH. N = 8–11 rats for each experimental group. 6-OHDA = 6-hydroxydopamine; APO = apomorphine; DOPA = L-3,4-dihydroxyphenylalanine; USVs = ultrasonic vocalizations; VEH = vehicle.

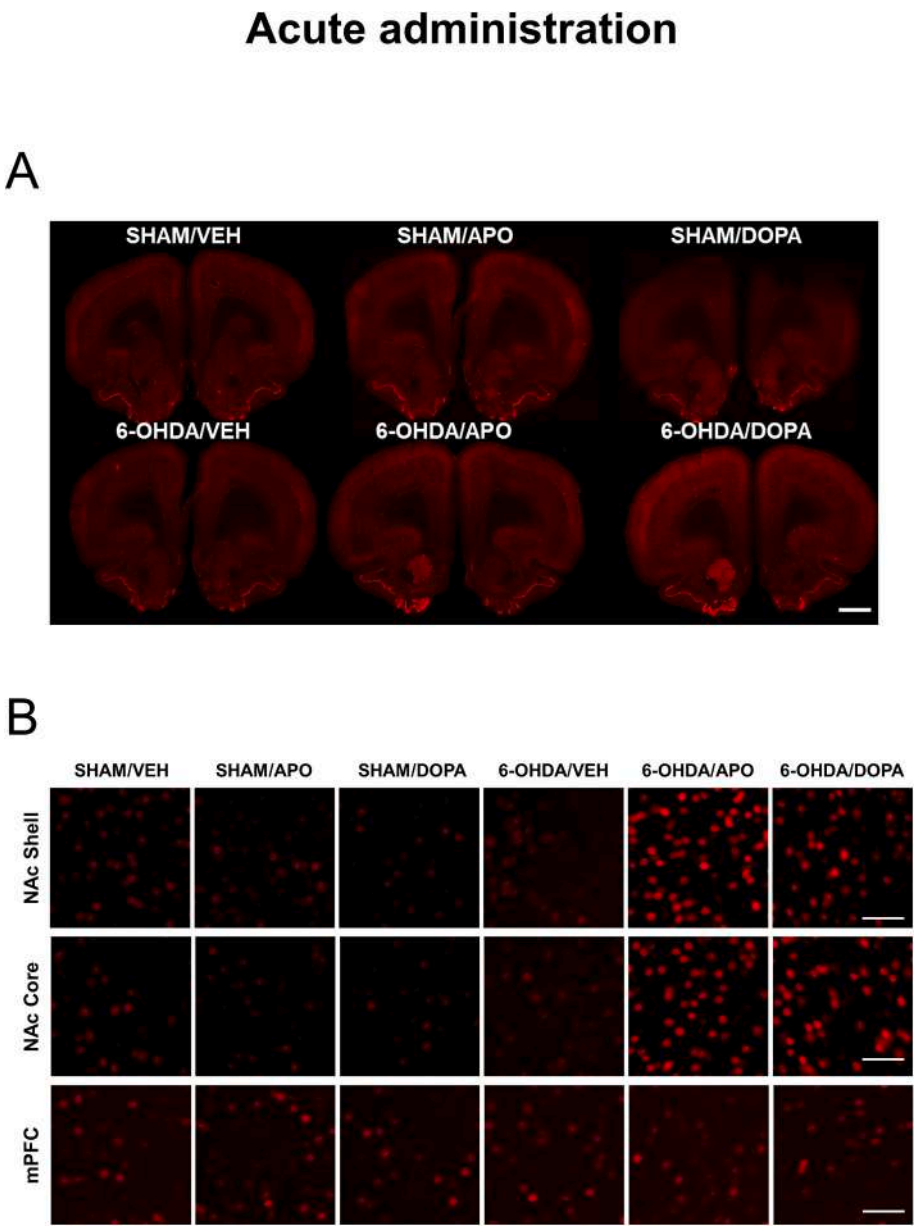


Fig. 6. Images demonstrating the modifications in Zif-268 immunoreactivity in the nucleus accumbens and medial prefrontal cortex after acute treatment. Upper panel (A): representative coronal brain sections immunostained for Zif-268 taken from hemiparkinsonian and sham-operated rats treated with apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.). Lower panel (B): representative sections demonstrating Zif-268 immunoreactivity in the shell and core portions of the nucleus accumbens, and in the medial prefrontal cortex. Sections in panel B were taken from the brain hemisphere homolateral to either 6-hydroxydopamine injection or sham surgery. 6-OHDA = 6-hydroxydopamine; APO = apomorphine; DOPA = L-3,4-dihydroxyphenylalanine; mPFC = medial prefrontal cortex; NAc = nucleus accumbens; VEH = vehicle. Scale bar: 1000 μm (composed image), 50 μm (zoomed images).

treated with vehicle, and compared with the DL CPu of sham-operated rats repeatedly treated with either apomorphine or L-DOPA, respectively ($p < 0.0001$ for all comparisons) (Fig. 9, 11A).

3.4.5. Ventromedial caudate-putamen

Kruskal-Wallis test revealed a significant effect of treatment on Zif-268 immunoreactivity in the VM CPu of rats that received the acute administration of apomorphine (2 mg/kg, i.p., $H = 16.85$; $p = 0.0008$). Dunn's test showed higher Zif-268 immunoreactivity in the dopamine-denervated VM CPu of hemiparkinsonian rats acutely treated with apomorphine compared with the dopamine-denervated VM CPu of hemiparkinsonian rats acutely treated with vehicle ($p = 0.0003$) (Fig. 7, 11B). One-way ANOVA for the acute administration of L-DOPA (12 mg/kg, i.p.) revealed a significant effect of treatment on Zif-268 immunoreactivity in the VM CPu ($F_{3,34} = 3.488$, $p = 0.0265$). Tukey's test showed a significant increase in Zif-268 immunoreactivity in the dopamine-denervated VM CPu of hemiparkinsonian rats acutely treated with L-DOPA compared with the dopamine-denervated VM CPu of hemiparkinsonian rats acutely treated with vehicle ($p = 0.0271$) (Fig. 7, 11B).

Brown-Forsythe ANOVA revealed a significant effect of treatment on Zif-268 immunoreactivity in the VM CPu of rats that received the repeated administration of either apomorphine (2 mg/kg, i.p., $F_{3.0,10.09} = 15.86$; $p = 0.004$) or L-DOPA (12 mg/kg, i.p., $F_{3.0,14.44} = 39.56$; $p < 0.0001$). Dunnet's test showed a significant increase in Zif-268 immunoreactivity in the dopamine-denervated VM CPu of hemiparkinsonian rats repeatedly treated with either apomorphine or L-DOPA compared with the dopamine-denervated VM CPu of hemiparkinsonian rats

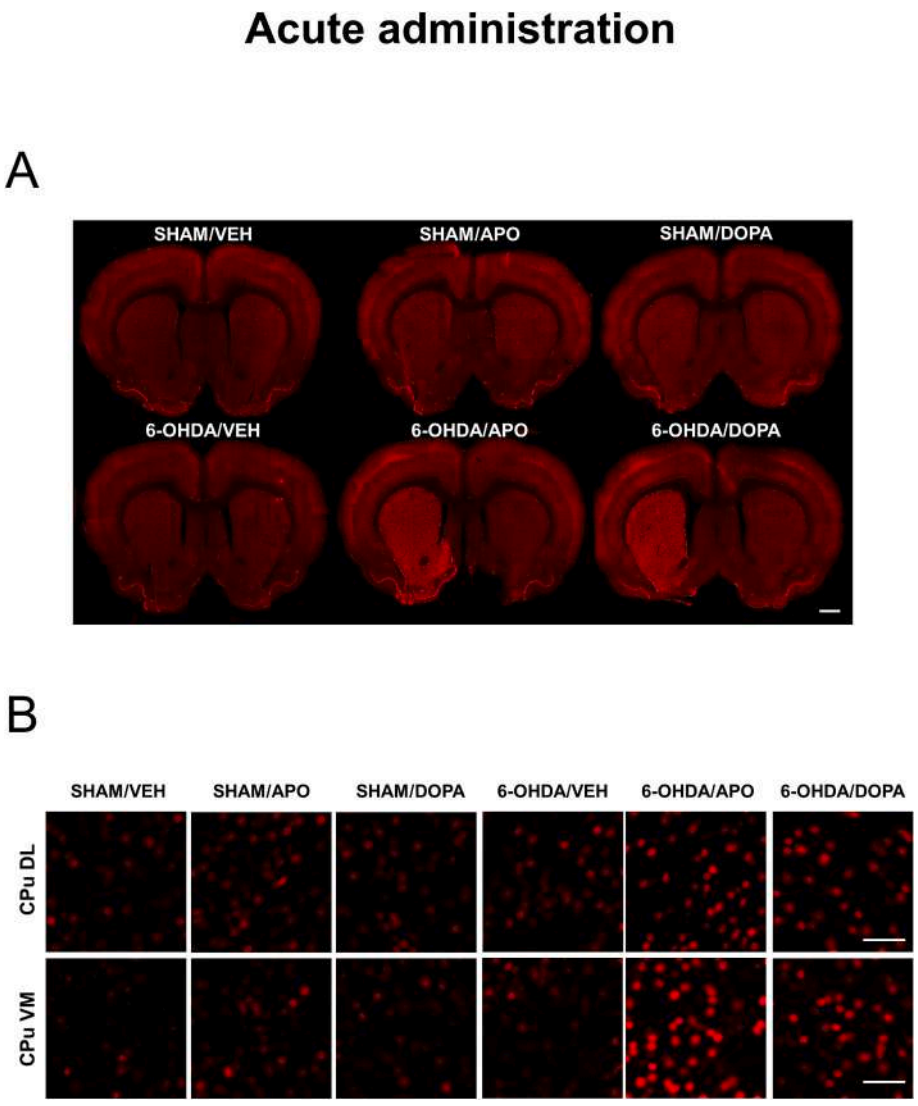


Fig. 7. Images demonstrating the modifications in Zif-268 immunoreactivity in the dorsolateral and ventromedial caudate-putamen after acute treatment. Upper panel (A): representative coronal brain sections immunostained for Zif-268 taken from hemiparkinsonian and sham-operated rats treated with apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.). Lower panel (B): representative sections demonstrating Zif-268 immunoreactivity in the dorsolateral and ventromedial portions of the caudate-putamen. Sections in panel B were taken from the brain hemisphere homolateral to either 6-hydroxydopamine injection or sham surgery. 6-OHDA = 6-hydroxydopamine; APO = apomorphine; DOPA = L-3,4-dihydroxyphenylalanine; CPu = caudate-putamen; DL = dorsolateral; VM = ventromedial; VEH = vehicle. Scale bar: 1000 μm (composed image), 50 μm (zoomed images).

repeatedly treated with vehicle (apomorphine, $p = 0.0117$; L-DOPA, $p = 0.0001$), and compared the VM CPu of sham-operated rats repeatedly treated with either apomorphine or L-DOPA, respectively (apomorphine, $p = 0.023$; L-DOPA, $p = 0.0002$) (Fig. 9, 11B).

3.5. Correlation analysis of calling behavior, rotational behavior and Zif-268 immunoreactivity

Spearman's test showed that in hemiparkinsonian rats acutely or repeatedly treated with either apomorphine (2 mg/kg, i.p.) or L-DOPA (12 mg/kg, i.p.) the numbers of 50-kHz USVs emitted were not significantly correlated with the numbers of contralateral rotations performed. In hemiparkinsonian rats acutely treated with apomorphine significant correlations in Zif-268 immunoreactivity were observed between the NAc shell and the DL CPu ($r = 0.8666$, $p = 0.0022$) and between the DL CPu and the mPFC ($r = 1.000$, $p = 0.0167$). In hemiparkinsonian rats repeatedly treated with L-DOPA a significant correlation was observed between the numbers of 50-kHz USVs emitted and Zif-268 immunoreactivity in the DL CPu ($r = 0.6818$, $p = 0.0251$). In the same rats, significant correlations in Zif-268 immunoreactivity were observed between the NAc shell and the NAc core ($r = 0.6909$, $p = 0.0226$), the NAc shell and the VM CPu ($r = 0.8727$, $p = 0.0009$), the NAc core and the VM CPu ($r = 0.6273$, $p = 0.0440$). Supplementary material reports further details on the results of correlation analysis (Figs. S7-S10, Tables S4-S7).

4. Discussion

The present study evaluated the emission of 50-kHz USVs and Zif-268 immunoreactivity in the NAc, CPu and mPFC of hemiparkinsonian and sham-operated rats that were acutely or repeatedly treated with either apomorphine or L-DOPA, two drugs used in the DRT of PD. Both apomorphine and L-DOPA markedly stimulated calling behavior in hemiparkinsonian rats and elevated Zif-268 immunoreactivity in the dopamine-denervated NAc and CPu of the same rats. These results indicate that the emission of 50-kHz USVs in hemiparkinsonian rats treated with either apomorphine or L-DOPA is associated with increased neuronal activation in subcortical regions of the dopamine-denervated brain hemisphere.

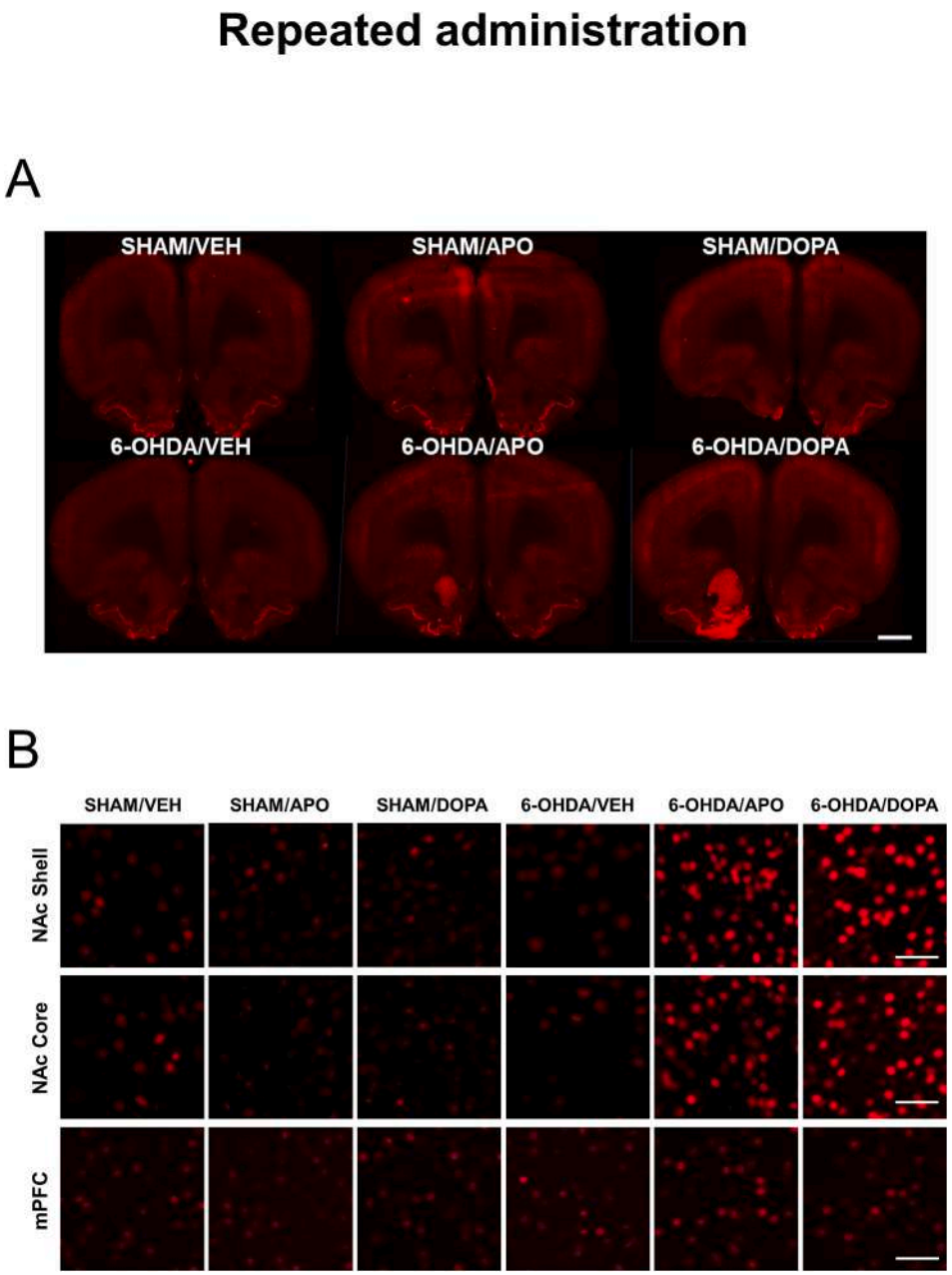


Fig. 8. Images demonstrating the modifications in Zif-268 immunoreactivity in the nucleus accumbens and medial prefrontal cortex after repeated treatment. Upper panel (A): representative coronal brain sections immunostained for Zif-268 taken from hemiparkinsonian and sham-operated rats treated with apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.). Lower panel (B): representative sections demonstrating Zif-268 immunoreactivity in the shell and core portions of the nucleus accumbens, and in the medial prefrontal cortex. Sections in panel B were taken from the brain hemisphere homolateral to either 6-hydroxydopamine injection or sham surgery. 6-OHDA = 6-hydroxydopamine; APO = apomorphine; DOPA = L-3,4-dihydroxyphenylalanine; mPFC = medial prefrontal cortex; NAc = nucleus accumbens; VEH = vehicle. Scale bar: 1000 μm (composed image), 50 μm (zoomed images).

Consistent with our previous findings (Simola et al., 2021), apomorphine elicited the emission of a higher number of 50-kHz USVs in hemiparkinsonian than sham-operated rats, whereas L-DOPA stimulated calling behavior in hemiparkinsonian rats only. We here adopted an extended recording time of 90 min to better clarify the time course of calling behavior stimulated by either apomorphine or L-DOPA, and we found that the emission of 50-kHz USVs began 5–10 min after drug administration and that significant levels of calling behavior were detectable up to 25–30 min after drug administration. Moreover, in both apomorphine- and L-DOPA-treated hemiparkinsonian rats the time course of general calling behavior (i.e., the total number of 50-kHz USVs emitted) substantially overlapped with the time course of the emission of categorized 50-kHz USVs. The findings on the time course of calling behavior obtained here are consistent with our earlier results collected by using a shorter (30 min) recording time (Simola et al., 2021). This could further substantiate the possibility that apomorphine and L-DOPA rapidly elicit positive affect in hemiparkinsonian rats after their administration. Moreover, the relatively short-lived emission of 50-kHz USVs observed here could indicate that apomorphine and L-DOPA induce a transient positive affect in hemiparkinsonian rats.

Acute or repeated treatment with either apomorphine or L-DOPA increased Zif-268 immunoreactivity in the dopamine-denervated brain hemisphere of hemiparkinsonian rats, but neither in the intact brain hemisphere of hemiparkinsonian rats nor in the brain of sham-operated rats. More specifically, an elevation in Zif-268 immunoreactivity was detected in the shell and core of the NAc as well as in the dorsolateral

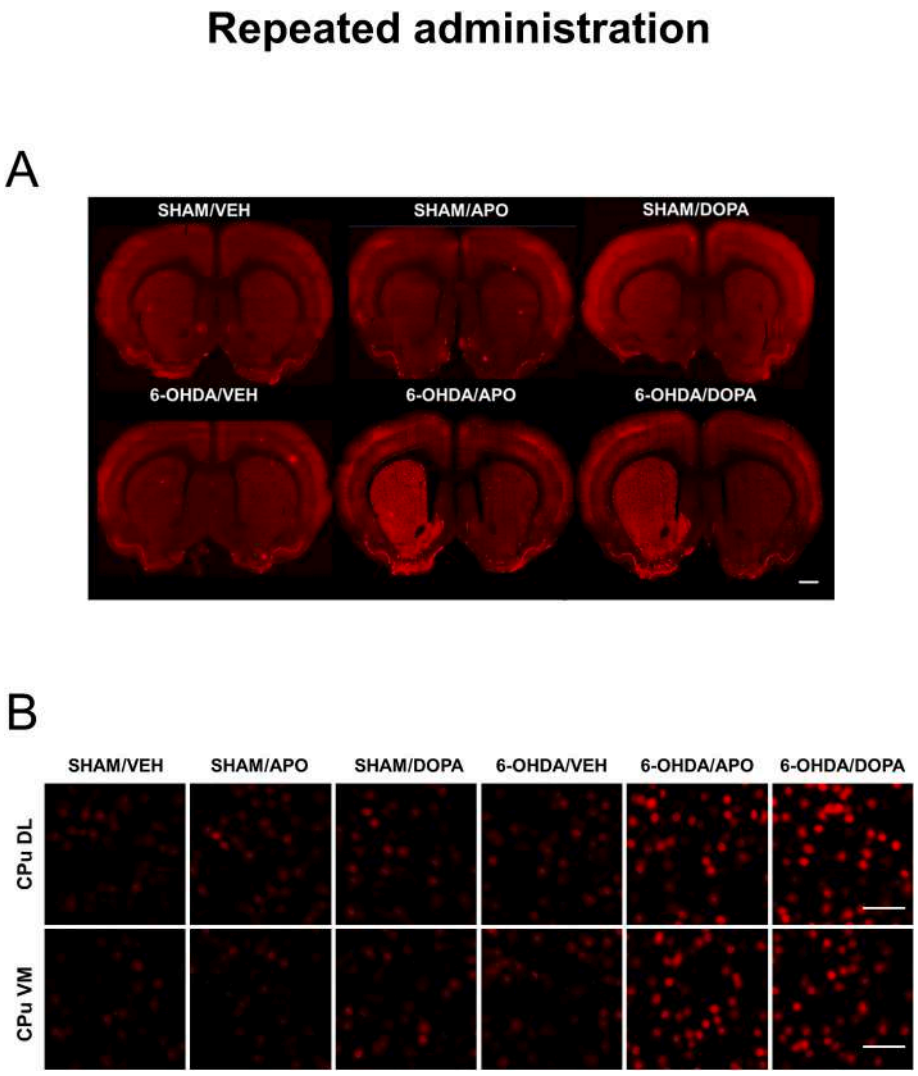


Fig. 9. Images demonstrating the modifications in Zif-268 immunoreactivity in the dorsolateral and ventromedial caudate-putamen after repeated treatment. Upper panel (A): representative coronal brain sections immunostained for Zif-268 taken from hemiparkinsonian and sham-operated rats treated with apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.). Lower panel (B): representative sections demonstrating Zif-268 immunoreactivity in the dorsolateral and ventromedial portions of the caudate-putamen. Sections in panel B were taken from the brain hemisphere homolateral to either 6-hydroxydopamine injection or sham surgery. 6-OHDA = 6-hydroxydopamine; APO = apomorphine; DOPA = L-3,4-dihydroxyphenylalanine; CPu = caudate-putamen; DL = dorsolateral; VM = ventromedial; VEH = vehicle. Scale bar: 1000 μm (composed image), 50 μm (zoomed images).

and ventromedial CPu, but not in the mPFC. These findings may indicate that apomorphine and L-DOPA stimulate the emission of 50-kHz USVs in hemiparkinsonian rats by acting on striatal regions. Moreover, they may further support the earlier evidence demonstrating that the denervation of the nigrostriatal dopaminergic system boosts the affective/motivational properties of drugs used in the DRT of PD. In fact, the NAc shell is a key region where drugs act to elicit a state of reward (Di Chiara et al., 2004). Besides, the NAc core and the dorsolateral and ventromedial CPu are major regions where drugs act to elicit associative learning and to increase motivation for their use (Di Chiara et al., 2004; Everitt and Robbins, 2013; Stamos et al., 2022). In this regard, it is also noteworthy that apomorphine and L-DOPA, in general, led to higher Zif-268 immunoreactivity after repeated treatment than after acute treatment. This finding may suggest that progressive neuroadaptive facilitatory changes occurred in the NAc and CPu of the dopamine-denervated brain hemisphere in hemiparkinsonian rats repeatedly treated with either apomorphine or L-DOPA, which could have resulted in a persistent elevation in the affective/motivational properties of those drugs. In this connection, the lack of an increase in Zif-268 immunoreactivity in the dopamine-denervated mPFC of apomorphine- and L-DOPA-treated hemiparkinsonian rats may appear an unexpected finding. Indeed, similar to the NAc and CPu, the mPFC is a brain region where drugs act to elicit associative learning and to increase motivation for their use (Koob and Volkow, 2010; Robbins and Everitt, 2002; Pintori et al., 2021). Moreover, a previous study of our group found an increased Zif-268 immunoreactivity in the mPFC of neurologically intact rats that developed a sensitized emission of 50-kHz USVs in response to repeated treatment with amphetamine, a drug that markedly elevates the affective/motivational state in rats (Costa et al., 2015).

Regarding the mechanisms that could lead to the effects of apomorphine and L-DOPA observed in the present study, we may propose some hypotheses based on the neurobiology of 50-kHz USVs and on the characteristics of the experimental model of nigrostriatal dopaminergic denervation used. First, it is noteworthy that the dopaminergic system plays a pivotal role in the emission of 50-kHz USVs. Indeed, studies that evaluated calling behavior in rats either treated with amphetamine or exposed to social stimuli have demonstrated that the emission of 50-kHz USVs is initiated by the activation of dopamine transmission in the NAc shell and is suppressed by the antagonism of dopamine receptors (Burgdorf et al., 2001; Thompson et al., 2006;

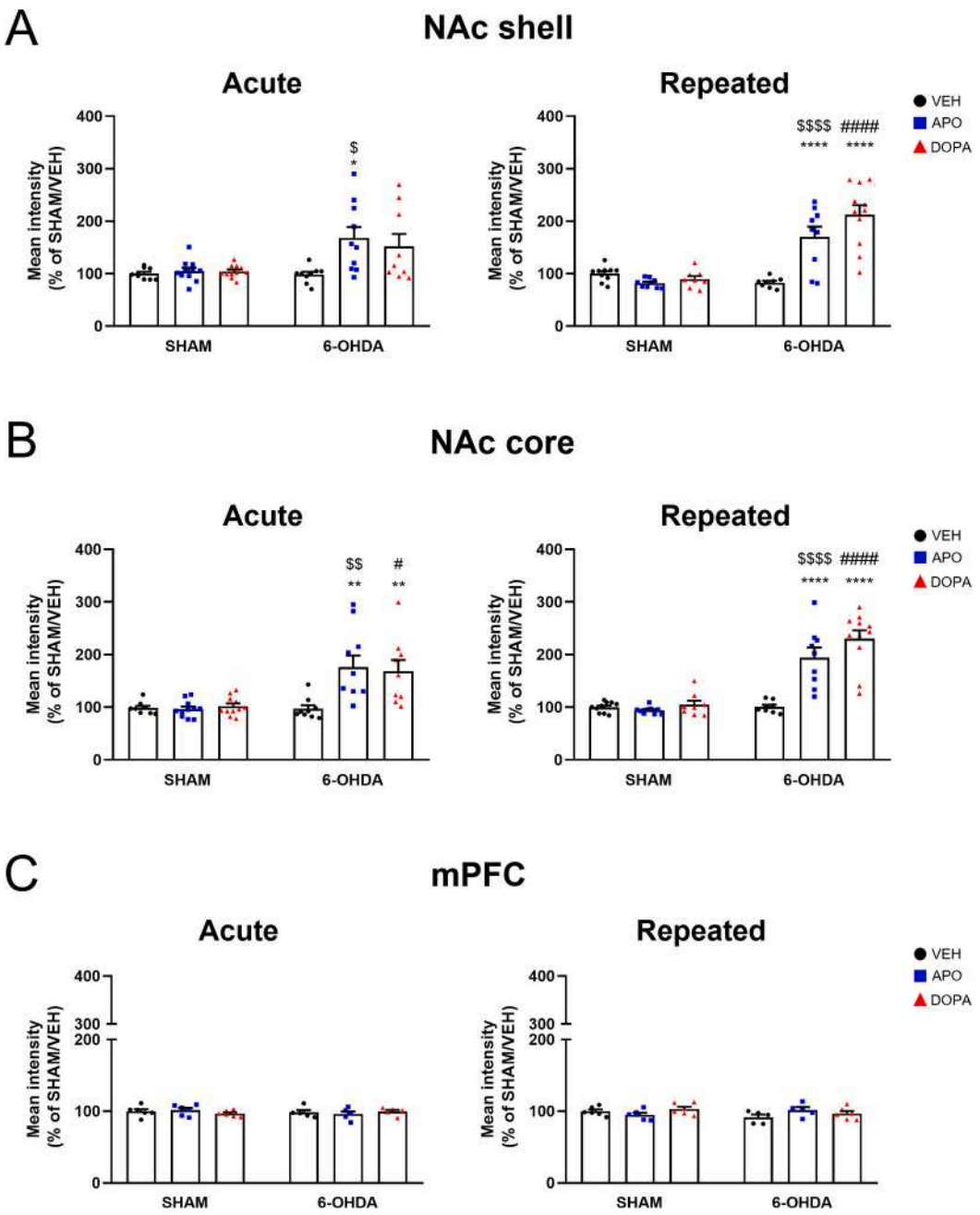


Fig. 10. Histograms demonstrating the modifications in Zif-268 immunoreactivity in the nucleus accumbens and medial prefrontal cortex. Rats received apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.) in acute or repeated (×5) administration. Histograms indicate the mean intensity of Zif-268 immunoreactivity in the shell (A) and core (B) portions of the nucleus accumbens and in the medial prefrontal cortex (C). The values of Zif-268 immunoreactivity shown in the figure were measured in the brain hemisphere homolateral to either 6-hydroxydopamine injection or sham surgery. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ compared with 6-OHDA/VEH. $ $p < 0.05$, $$ $p < 0.01$, $$$$ $p < 0.0001$ compared with SHAM/APO. # $p < 0.05$, #### $p < 0.0001$ compared with SHAM/DOPA. N = 8–11 for NAc shell and core; *N* = 5–6 for mPFC. Values are expressed as a percentage of the respective SHAM/VEH group for each brain region evaluated. Symbols within each bar indicate the values of Zif-268 immunoreactivity in individual rats. 6-OHDA = 6-hydroxydopamine; APO = apomorphine; DOPA = L-3,4-dihydroxyphenylalanine; mPFC = medial prefrontal cortex; NAc = nucleus accumbens; VEH = vehicle.

Wright et al., 2013; Shimoju and Shibata, 2021; Serra et al., 2024). Moreover, other studies that evaluated the emission of 50-kHz USVs in rats treated with amphetamine have demonstrated that calling behavior can be modulated by dopamine transmission in the CPu, NAc core and mPFC (Costa et al., 2020; Czarna et al., 2020; Mulvihill and Brudzynski, 2020). In addition, earlier investigations in rats have consistently demonstrated that 6-OHDA injection in either the SNc or the VTA induces supersensitivity of dopamine receptors in the dopamine-denervated efferent regions, and that this phenomenon is most evident in the CPu than in the mPFC (Tassin et al., 1982; Marshall et al., 1989; Gerfen et al., 1990, 2002; Gerfen, 2003; Simola et al., 2007). On these bases, a hypothesis that could explain our findings is that the occurrence of dopamine receptor supersensitivity in the 6-OHDA-lesioned brain hemisphere is requisite for the full manifestation of the effects that apomorphine and L-DOPA elicit on calling behavior and Zif-268 immunoreactivity in hemiparkinsonian rats. In fact, the results of TH-immunoreactivity showed that dopaminergic denervation was nearly complete in the NAc and CPu, but only partial in the mPFC. This, in turn, may have resulted in high levels of dopamine receptor supersensitivity in the CPu and low levels of dopamine receptor supersensitivity in the

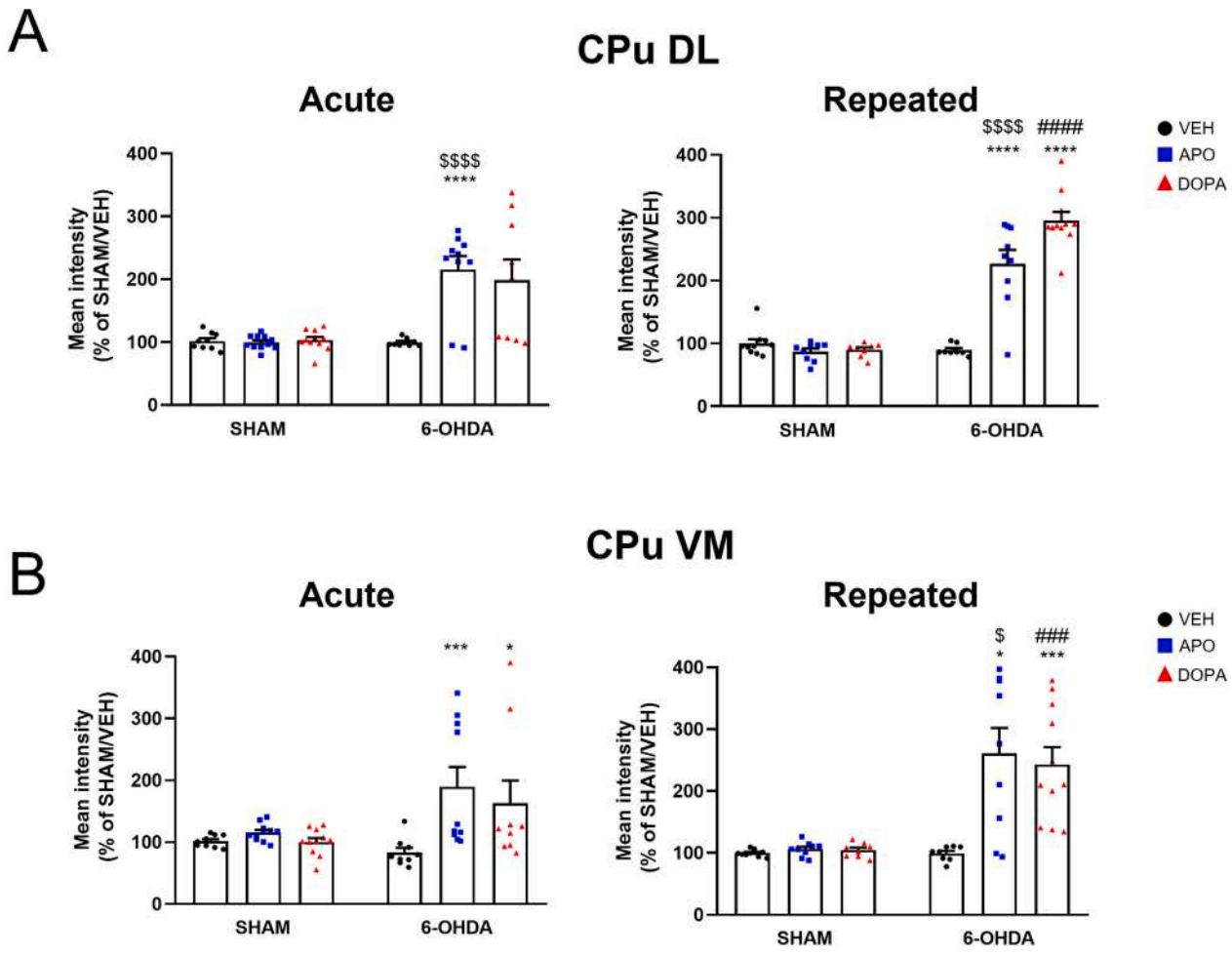


Fig. 11. Histograms demonstrating the modifications in Zif-268 immunoreactivity in the caudate-putamen nucleus. Rats received apomorphine (2 mg/kg, i.p.), L-DOPA (12 mg/kg, i.p.), or vehicle (i.p.) in acute or repeated (×5) administration. Histograms indicate the mean intensity of Zif-268 immunoreactivity in the dorsolateral (A) and ventromedial (B) portions of the caudate putamen nucleus. The values of Zif-268 immunoreactivity shown in the figure were measured in the brain hemisphere homolateral to either 6-hydroxydopamine injection or sham surgery. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ compared with 6-OHDA/VEH. $ $p < 0.05$, $$$$ $p < 0.0001$ compared with SHAM/APO. ### $p < 0.001$, #### $p < 0.0001$ compared with SHAM/DOPA. N = 8–11 rats for each experimental group. Values are expressed as a percentage of the respective SHAM/VEH group for each brain region evaluated. Symbols within each bar indicate the values of Zif-268 immunoreactivity in individual rats. 6-OHDA = 6-hydroxydopamine; APO = apomorphine; CPu = caudate-putamen nucleus; DL = dorsolateral; DOPA = L-3,4-dihydroxyphenylalanine; VEH = vehicle; VM = ventromedial.

mPFC, which were possibly not sufficient to trigger a significant elevation in Zif-268 immunoreactivity in that region after apomorphine/L-DOPA administration. This hypothesis may be further supported by the finding of the present study that no significant elevation in Zif-268 immunoreactivity occurred in sham-operated rats, as well as by the evidence from earlier investigations performed in rats treated with psychostimulants which showed that that D_1-like receptors are critically involved in the expression of *zif-268* in striatal regions (Wang and McGinty, 1995; Yano et al., 2006; Li et al., 2016). In this connection, we may speculate that the occurrence of dopamine D_1-like receptor supersensitivity in dopamine-denervated striatal regions may have facilitated the expression of *zif-268*, and the subsequent elevation in Zif-268 immunoreactivity, in hemiparkinsonian rats treated with either apomorphine or L-DOPA.

In addition to the occurrence of dopamine receptor supersensitivity in dopamine-denervated brain regions, other mechanisms may possibly underlie the effects of apomorphine and L-DOPA observed here and explain why we found no association between 50-kHz USV emission and activation of the mPFC, differently from what previously reported in neurologically intact rats treated with amphetamine (Costa et al., 2015; Mulvihill and Brudzynski, 2020). Among these mechanisms could be the pharmacodynamic actions of apomorphine and L-DOPA, that differ from those of amphetamine. Indeed, apomorphine is a direct dopamine receptor agonist and L-DOPA is a metabolic precursor of dopamine (Simola et al., 2010), whereas amphetamine is a dopaminergic psychostimulant that releases dopamine from presynaptic terminals (Sulzer et al., 2005; Jiao et al., 2015). Moreover, apomorphine, L-DOPA and amphetamine also differ with regard to their actions at the level of non-dopaminergic neurotransmission. Indeed, apomorphine and dopamine derived from L-DOPA can regulate neuronal firing by acting on presynaptic dopamine receptors located on dopaminergic and non-dopaminergic terminals (Godukhin et al., 1984; Picconi et al., 2004; Nevalainen et al., 2014). Conversely, amphetamine can directly stimulate the release of certain non-dopaminergic neurotransmitters, such as noradrenaline and glutamate (Sulzer et al., 2005). In this connection, it is noteworthy that the emission of 50-kHz USVs, although primarily initiated by the activation of dopamine receptors in the NAc shell, may be modulated by diverse non-dopaminergic neurotransmitters (Wright et al., 2012; Costa et al., 2015; Wöhr et al., 2015; Simola et al., 2016; Mulvihill and Brudzynski, 2019; Hoffmeister et al., 2022). Furthermore, non-dopaminergic neurotransmitters may influence the activation of immediate early genes, such as *zif-268*, in cortical and striatal regions of the rat brain (Bing et al., 1991; Wang, 1998; Li et al., 2016). All these things considered further studies are warranted to ascertain the

reciprocal role of dopaminergic and non-dopaminergic mechanisms in the effects that apomorphine and L-DOPA elicit on the emission of 50-kHz USVs and on the associated activation of striatal and cortical regions in dopamine-denervated rats.

Considering that 50-kHz USVs are a marker of positive affect in rats and that the NAc and CPu belong to the neurocircuits that regulate affective and motivational states and the effects that drugs elicit on those states, the results of the present study may further support the earlier experimental evidence indicating that the denervation of the dopaminergic nigrostriatal system boosts the affective/motivational properties of drugs used in the DRT of PD (Zengin-Toktas et al., 2013; Campbell et al., 2014). Nevertheless, the present study has some potential limitations that should be considered. Similar to what reported by previous investigations performed in neurologically intact rats treated with psychoactive drugs (Pereira et al., 2014; Wöhr et al., 2015; Simola and Costa, 2018), hemiparkinsonian rats treated with either apomorphine or L-DOPA displayed interindividual variability in the total numbers of 50-kHz USVs and in the numbers of categorized calls emitted after drug administration. In addition, the emission of 50-kHz USVs and Zif-268 immunoreactivity are perhaps markers that do not allow to fully discriminate between the effects that drugs elicit on the emotional state and the behavioral effects of drugs that stem from the presence of dopamine receptor supersensitivity (Williams and Undieh, 2016; Costa et al., 2020; Schwarting, 2023; Serra et al., 2024). Moreover, as discussed above, no significant elevation in Zif-268 immunoreactivity was observed in sham-operated rats after acute apomorphine administration, in spite of a significant elevation in the emission of drug-stimulated 50-kHz USVs in those rats, though of lower magnitude than that observed in hemiparkinsonian rats. This finding could suggest that significant elevations in Zif-268 immunoreactivity may occur only in the brain of rats that emit high numbers of 50-kHz USVs. Additionally, we could speculate that the full manifestation of the effects that dopaminomimetic drugs elicit on Zif-268 immunoreactivity requires conditions of dopamine receptor supersensitivity, like the ones that may occur after dopaminergic denervation (Gerfen, 2003) or after repeated treatment with psychostimulants of abuse like amphetamine (Seeman, 2009). Furthermore, it is noteworthy that the activation of the *zif-268* cascade in dopamine-denervated striatal regions of hemiparkinsonian rats treated with dopaminomimetic drugs may as well be a correlate of drug-induced rotational behavior (Frau et al., 2013; Pinna et al., 2016). Finally, it has to be acknowledged that in the present study we did not perform a detailed analysis of dyskinetic-like abnormal involuntary movements (AIMs) due to the experimental setup used (i.e., use of cylinders with black-painted walls), that did not allow a reliable quantification of AIMs (see Simola et al., 2021). The lack of AIMs quantification may somehow limit our understanding of the interplay between drug-stimulated 50-kHz USVs and motor responses in hemiparkinsonian rats treated with apomorphine or L-DOPA.

Based on the above considerations, it could be speculated that the increased Zif-268 immunoreactivity observed in the dopamine-denervated brain hemisphere of hemiparkinsonian rats treated with either apomorphine or L-DOPA may reflect both the presence of a drug-induced elevation in the affective/motivational state and the performance of drug-stimulated motor responses. Earlier investigations performed in neurologically intact rats have demonstrated that the emission of 50-kHz USVs is directly linked to the occurrence of drug-induced modifications in the affective/motivational state and is not dependent on the effects that drugs elicit on motor circuits (Burgdorf et al., 2001; Burgdorf and Panksepp, 2006; Browning et al., 2011; Maier et al., 2012; Simola and Morelli, 2015; Kuchniak et al., 2019; Costa et al., 2015; Simola et al., 2021). In our previous study, we have extended those earlier findings to hemiparkinsonian rats treated with either apomorphine or L-DOPA, since we observed a dissimilar time course of drug-stimulated calling behavior and rotational behavior (Simola et al., 2021). In the present study, we have confirmed the latter results by demonstrating that apomorphine and L-DOPA had rapid and transient effects on the emission of 50-kHz USVs (up to 30 min from administration), while they stimulated the performance of rotational behavior for a longer period of time (up to 90 from administration). Moreover, by using an extended recording time of 90 min, in the present study we found no significant correlations between the numbers of 50-kHz USVs emitted and of contralateral rotations performed in hemiparkinsonian rats treated with either apomorphine or L-DOPA. Conversely, in our earlier investigation that employed a shorter (30 min) recording time (Simola et al., 2021) we found some significant correlations between calling behavior and rotational behavior, although only in certain administration days of the experimental protocol. Taken together, these findings suggest that a dissociation may exist between the affective and motor stimulating properties of apomorphine and L-DOPA in hemiparkinsonian rats. In particular, we may propose that the emission of 50-kHz USVs reflects the presence of early onset and transient effects of apomorphine/L-DOPA on the emotional state of hemiparkinsonian rats, that could be manifested before the peak of motor-stimulating effects. Accordingly, 50-kHz USVs may be envisioned as a valuable behavioral marker to be used in studies about the affective properties of antiparkinsonian dopaminergic drugs in rat models of PD. Indeed, measuring the emission of 50-kHz USVs could not only disclose the effects that antiparkinsonian dopaminomimetic drugs elicit on the emotional state, but also be an approach that may allow reducing the influence that drug-induced motor stimulation could have on other behavioral readouts that are conventionally measured to evaluate these effects (see Rokosik and Napier, 2012; Campbell et al., 2014; Floris et al., 2022).

The results of the present study may pave the path for additional investigations aimed at further clarifying the relationship between changes in the affective state and emission of 50-kHz USVs in dopamine-denervated rats treated with drugs used in the DRT of PD. Importantly, in the present study we investigated apomorphine and L-DOPA, both of which have been associated with the manifestation of behavioral addictions in PD patients (Todorova et al., 2015; Weintraub et al., 2015). Moreover, we used an experimental model of repeated drug administration in rats bearing a near complete nigrostriatal dopaminergic denervation to reproduce repeated exposure to dopaminomimetic antiparkinsonian drugs and late-stage dopaminergic nigrostriatal degeneration, two conditions that have been suggested as risk factors for the development of hedonic homeostatic dysregulation and behavioral addictions in PD patients medicated with DRT (Eisinger et al., 2019). At the same time, we acknowledge that the experimental approach used here could not clarify how striatal brain regions interacted among them and with other brain regions to stimulate the emission of 50-kHz USVs in hemiparkinsonian rats treated with either apomorphine or L-DOPA.

Interestingly we found a significant correlation between the emission of 50-kHz USVs and the increase in Zif-268 immunoreactivity in the DL CPu of hemiparkinsonian rats repeatedly treated with L-DOPA, which also displayed significant correlations in the intensity of Zif-268 immunoreactivity between the NAc shell and the NAc core, as well as between either portion of the NAc and the VM CPu. Similarly, hemiparkinsonian rats acutely treated with apomorphine displayed significant correlations in Zif-268 immunoreactivity between the NAc shell and the DL CPu and between the DL CPu and the mPFC. Although these positive correlations do not allow to causally implicate the NAc, CPu and mPFC in the results of the present study, they may suggest that these regions are connected in a circuit that regulates the affective and motivational state, and then the emission of 50-kHz USVs, in dopamine-denervated rats treated with dopaminomimetic antiparkinsonian drugs. In this connection, again, it is noteworthy that the NAc is key nucleus in hedonic homeostatic regulation (Di Chiara et al., 2004). Moreover, dorsal and ventral striatal regions have been implicated in the manifestation of impulsive behaviors in experimental rats treated with dopaminomimetic antiparkinsonian drugs (Miller and Abercrombie, 1999; Jiménez-Urbieta et al., 2019; Floris et al., 2022; Magnard et al., 2024), as well as of behavioral addictions in PD patients, although the causal

implications of specific brain regions remains to be determined (see Kelly et al., 2020). Therefore, the results of the present study may lay the foundation for further investigations aimed at elucidating the neurocircuits that mediate the emission of 50-kHz USVs in dopamine-denervated rats treated with antiparkinsonian drugs. Future studies shall also clarify if measuring the emission of 50-kHz USVs can be a relevant complementary approach to investigate how hedonic homeostatic dysregulation induced by antiparkinsonian drugs may influence behavioral readouts that reproduce in dopamine-denervated rats the clinical manifestations of specific behavioral addictions that can occur in PD patients medicated with DRT.

5. Conclusions

The present study demonstrates that hemiparkinsonian rats treated with either apomorphine or L-DOPA display a heightened emission of 50-kHz USVs associated with an increased Zif-268 immunoreactivity in the NAc and CPu, two striatal regions where drugs act to modify the affective/motivational state. Since the emission of drug-stimulated 50-kHz USVs may reflect the presence of drug-induced positive affect in rats (Simola and Brudzynski, 2018b), these results suggest that measuring the emission of 50-kHz USVs may be a relevant approach in preclinical studies that employ rat models of nigrostriatal dopaminergic denervation to investigate the affective/motivational properties of drugs used in the DRT of PD.

CRediT authorship contribution statement

Marcello Serra: Writing – original draft, Investigation, Formal analysis, Data curation. **Jacopo Marongiu:** Writing – original draft, Investigation, Formal analysis. **Nicola Simola:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization. **Giulia Costa:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support this study are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

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References

Ahrens, A.M., Ma, S.T., Maier, E.Y., Duvauchelle, C.L., Schallert, T., 2009. Repeated intravenous amphetamine exposure: rapid and persistent sensitization of 50-kHz ultrasonic trill calls in rats. Behav. Brain Res. 197, 205–209. https://doi.org/10.1016/j.bbr.2008.08.037.

Bastide, M.F., Meissner, W.G., Picconi, B., Fasano, S., Fernagut, P.O., Feyder, M., Francardo, V., Alcacer, C., Ding, Y., Brambilla, R., Fisone, G., Jon Stoessl, A., Bourdenx, M., Engeln, M., Navailles, S., De Deurwaerdère, P., Ko, W.K., Simola, N., Morelli, M., Groc, L., Rodriguez, M.C., Gurevich, E.V., Quik, M., Morari, M., Mellone, M., Gardoni, F., Tronci, E., Guehl, D., Tison, F., Crossman, A.R., Kang, U.J., Steece-Collier, K., Fox, S., Carta, M., Cenci, M.A., Bézard, E., 2015. Pathophysiology of L-dopa-induced motor and non-motor complications in Parkinson's disease. Prog. Neurobiol. 132, 96–168. https://doi.org/10.1016/j.pneurobio.2015.07.002.

Bing, G.Y., Filer, D., Miller, J.C., Stone, E.A., 1991. Noradrenergic activation of immediate early genes in rat cerebral cortex. Brain Res. Mol. Brain Res. 11, 43–46. https://doi.org/10.1016/0169-328x(91)90019-t.

Browning, J.R., Browning, D.A., Maxwell, A.O., Dong, Y., Jansen, H.T., Panksepp, J., Sorg, B.A., 2011. Positive affective vocalizations during cocaine and sucrose self-administration: a model for spontaneous drug desire in rats. Neuropharmacology 61, 268–275. https://doi.org/10.1016/j.neuropharm.2011.04.012.

Burgdorf, J., Panksepp, J., 2006. The neurobiology of positive emotions. Neurosci. Biobehav. Rev. 30, 173–187. https://doi.org/10.1016/j.neubiorev.2005.06.001.

Burgdorf, J., Knutson, B., Panksepp, J., Ikemoto, S., 2001. Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. Behav. Neurosci. 115, 940–944. https://doi.org/10.1037//0735-7044.115.4.940.

Burgdorf, J.S., Brudzynski, S.M., Moskal, J.R., 2020. Using rat ultrasonic vocalization to study the neurobiology of emotion: from basic science to the development of novel therapeutics for affective disorders. Curr. Opin. Neurobiol. 60, 192–200. https://doi.org/10.1016/j.conb.2019.12.008.

Campbell, J.C., Jeyamohan, S.B., De La Cruz, P., Chen, N., Shin, D., Pilitsis, J.G., 2014. Place conditioning to apomorphine in rat models of Parkinson's disease: differences by dose and side-effect expression. Behav. Brain Res. 275, 114–119. https://doi.org/10.1016/j.bbr.2014.09.002.

Costa, G., Morelli, M., Simola, N., 2015. Involvement of glutamate NMDA receptors in the acute, long-term, and conditioned effects of amphetamine on rat 50 kHz ultrasonic vocalizations. Int. J. Neuropsychopharmacol. 18, pyv057. https://doi.org/10.1093/ijnp/pyv057.

Costa, G., Serra, M., Pintori, N., Casu, M.A., Zanda, M.T., Murtas, D., De Luca, M.A., Simola, N., Fattore, L., 2019. The novel psychoactive substance methoxetamine induces persistent behavioral abnormalities and neurotoxicity in rats. Neuropharmacology 144, 219–232. https://doi.org/10.1016/j.neuropharm.2018.10.031.

Costa, G., Serra, M., Marongiu, J., Morelli, M., Simola, N., 2020. Influence of dopamine transmission in the medial prefrontal cortex and dorsal striatum on the emission of 50-kHz ultrasonic vocalizations in rats treated with amphetamine: effects on drug-stimulated and conditioned calls. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 97, 109797. https://doi.org/10.1016/j.pnpbp.2019.109797.

Czarna, M., Kuchniak, K., Chrapusta, S.J., Turzyńska, D., Płaźnik, A., Taracha, E., 2020. Diverging changes in rat striatal extracellular dopamine and DOPAC levels and in frequency-modulated 50-kHz ultrasonic vocalizations rate during repeated amphetamine treatment. Behav. Brain Res. 393, 112745 https://doi.org/10.1016/j.bbr.2020.112745.

Delpont, B., Lhommée, E., Klinger, H., Schmitt, E., Bichon, A., Fraix, V., Castrioto, A., Quesada, J.L., Pélissier, P., Kistner, A., Carnicella, S., Lüscher, C., Broussolle, E., Pollak, P., Thobois, S., Krack, P., 2017. Psychostimulant effect of dopaminergic treatment and addictions in Parkinson's disease. Mov. Disord. 32, 1566–1573. https://doi.org/10.1002/mds.27101.

Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M.A., Spina, L., Cadoni, C., Acquas, E., Carboni, E., Valentini, V., Lecca, D., 2004. Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 47 (Suppl. 1), 227–241. https://doi.org/10.1016/j.neuropharm.2004.06.032.

Eisinger, R.S., Ramirez-Zamora, A., Carbunaru, S., Ptak, B., Peng-Chen, Z., Okun, M.S., Gunduz, A., 2019. Medications, deep brain stimulation, and other factors influencing impulse control disorders in Parkinson's disease. Front. Neurol. 10, 86. https://doi.org/10.3389/fneur.2019.00086.

Everitt, B.J., Robbins, T.W., 2013. From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. Neurosci. Biobehav. Rev. 37, 1946–1954. https://doi.org/10.1016/j.neubiorev.2013.02.010.

Floris, G., Scheggi, S., Pes, R., Bortolato, M., 2022. The steroidogenic inhibitor finasteride reverses pramipexole-induced alterations in probability discounting. Brain Res. Bull. 181, 157–166. https://doi.org/10.1016/j.brainresbull.2022.01.020.

Frau, L., Morelli, M., Simola, N., 2013. Performance of movement in hemiparkinsonian rats influences the modifications induced by dopamine agonists in striatal efferent dynorphinergic neurons. Exp. Neurol. 247, 663–672. https://doi.org/10.1016/j.expneurol.2013.03.002.

Gerfen, C.R., 2003. D1 dopamine receptor supersensitivity in the dopamine-depleted striatum animal model of Parkinson's disease. Neuroscientist 9, 455–462. https://doi.org/10.1177/1073858403255839.
Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma Jr., F.J., Sibley, D.R., 1990. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250, 1429–1432. https://doi.org/10.1126/science.2147780.
Gerfen, C.R., Miyachi, S., Paletzki, R., Brown, P., 2002. D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J. Neurosci. 22, 5042–5054. https://doi.org/10.1523/JNEUROSCI.22-12-05042.2002.
Giovannoni, G., O'Sullivan, J.D., Turner, K., Manson, A.J., Lees, A.J., 2000. Hedonistic homeostatic dysregulation in patients with Parkinson's disease on dopamine replacement therapies. J. Neurol. Neurosurg. Psychiatry 68, 423–428. https://doi.org/10.1136/jnnp.68.4.423.
Godukhin, O.V., Zharikova, A.D., Budantsev, Ayu, 1984. Role of presynaptic dopamine receptors in regulation of the glutamatergic neurotransmission in rat neostriatum. Neuroscience 12, 377–383. https://doi.org/10.1016/0306-4522(84)90059-9.
Hoffmeister, J.D., Kelm-Nelson, C.A., Ciucci, M.R., 2022. Manipulation of vocal communication and anxiety through pharmacologic modulation of norepinephrine in the Pink1−/− rat model of Parkinson disease. Behav. Brain Res. 418, 113642 https://doi.org/10.1016/j.bbr.2021.113642.
Jiao, D., Liu, Y., Li, X., Liu, J., Zhao, M., 2015. The role of the GABA system in amphetamine-type stimulant use disorders. Front. Cell. Neurosci. 9, 162. https://doi.org/10.3389/fncel.2015.00162.
Jiménez-Urbieta, H., Gago, B., Quiroga-Varela, A., Rodríguez-Chinchilla, T., Merino-Galán, L., Oregi, A., Belloso-Iguerategui, A., Delgado-Alvarado, M., Navalpotro-Gómez, I., Marin, C., Fernagut, P.O., Rodríguez-Oroz, M.C., 2019. Pramipexole-induced impulsivity in mildparkinsonian rats: a model of impulse control disorders in Parkinson's disease. Neurobiol. Aging 75, 126–135. https://doi.org/10.1016/j.neurobiolaging.2018.11.021.
Kelly, M.J., Baig, F., Hu, M.T., Okai, D., 2020. Spectrum of impulse control behaviours in Parkinson's disease: pathophysiology and management. J. Neurol. Neurosurg. Psychiatry 91, 703–711. https://doi.org/10.1136/jnnp-2019-322453.
Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. Neuropsychopharmacology 35, 217–238. https://doi.org/10.1038/npp.2009.110.
Kuchniak, K., Wyszogrodzka, E., Chrapusta, S.J., Czarna, M., Michalak, M., Płaźnik, A., Krząścik, P., Mierzejewski, P., Taracha, E., 2019. Using anticipatory and drug-evoked appetitive ultrasonic vocalization for monitoring the rewarding effect ofamphetamine in a rat model of drug self-administration. Behav. Brain Res. 376, 112187 https://doi.org/10.1016/j.bbr.2019.112187.
Lenell, C., Broadfoot, C.K., Schaen-Heacock, N.E., Ciucci, M.R., 2021. Biological and acoustic sex differences in rat ultrasonic vocalization. Brain Sci. 11, 459. https://doi.org/10.3390/brainsci11040459.
Li, Y., Ge, S., Li, N., Chen, L., Zhang, S., Wang, J., Wu, H., Wang, X., Wang, X., 2016. NMDA and dopamine D1 receptors within NAc-shell regulate IEG proteins expression in reward circuit during cocaine memory reconsolidation. Neuroscience 315, 45–69. https://doi.org/10.1016/j.neuroscience.2015.11.063.
Ma, S.T., Maier, E.Y., Ahrens, A.M., Schallert, T., Duvauchelle, C.L., 2010. Repeated intravenous cocaine experience: development and escalation of pre-drug anticipatory 50-kHz ultrasonic vocalizations in rats. Behav. Brain Res. 212, 109–114. https://doi.org/10.1016/j.bbr.2010.04.001.
Magnard, R., Fouyssac, M., Vachez, Y.M., Cheng, Y., Dufourd, T., Carcenac, C., Boulet, S., Janak, P.H., Savasta, M., Belin, D., Carnicella, S., 2024. Pramipexole restores behavioral inhibition in highly impulsive rats through a paradoxical modulation of frontostriatal networks. Transl. Psychiatry 14, 86. https://doi.org/10.1038/s41398-024-02804-3.
Maier, E.Y., Abdalla, M., Ahrens, A.M., Schallert, T., Duvauchelle, C.L., 2012. The missing variable: ultrasonic vocalizations reveal hidden sensitization and tolerance-like effects during long-term cocaine administration. Psychopharmacology 219, 1141–1152. https://doi.org/10.1007/s00213-011-2445-7.
Marshall, J.F., Navarrete, R., Joyce, J.N., 1989. Decreased striatal D1 binding density following mesotelencephalic 6-hydroxydopamine injections: an autoradiographic analysis. Brain Res. 493, 247–257. https://doi.org/10.1016/0006-8993(89)91160-8.
Miller, D.W., Abercrombie, E.D., 1999. Role of high-affinity dopamine uptake and impulse activity in the appearance of extracellular dopamine in striatum after administration of exogenous L-DOPA: studies in intact and 6-hydroxydopamine-treated rats. J. Neurochem. 72, 1516–1522. https://doi.org/10.1046/j.1471-4159.1999.721516.x.
Mu, P., Fuchs, T., Saal, D.B., Sorg, B.A., Dong, Y., Panksepp, J., 2009. Repeated cocaine exposure induces sensitization of ultrasonic vocalization in rats. Neurosci. Lett. 453, 31–35. https://doi.org/10.1016/j.neulet.2009.02.007.
Mulvihill, K.G., Brudzynski, S.M., 2019. Effect of microinjections of dopamine into the nucleus accumbens shell on emission of 50 kHz USV: comparison with effects of d-amphetamine. Pharmacol. Biochem. Behav. 176, 23–32. https://doi.org/10.1016/j.pbb.2018.11.006.
Mulvihill, K.G., Brudzynski, S.M., 2020. Association of medial corticostriatal regions with amphetamine-induced emission of 50 kHz vocalizations as studied by Zif-268 expression in the rat brain. Brain Res. 1726, 146505 https://doi.org/10.1016/j.brainres.2019.146505.
Natusch, C., Schwarting, R.K., 2010. Using bedding in a test environment critically affects 50-kHz ultrasonic vocalizations in laboratory rats. Pharmacol. Biochem. Behav. 96, 251–259. https://doi.org/10.1016/j.pbb.2010.05.013.
Nevalainen, N., Af Bjerkén, S., Gerhardt, G.A., Strömberg, I., 2014. Serotonergic nerve fibers in L-DOPA-derived dopamine release and dyskinesia. Neuroscience 260, 73–86. https://doi.org/10.1016/j.neuroscience.2013.12.029.
Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, 4th ed. Academic Press, London.
Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J., 1979. A Stereotaxic Atlas of the Rat Brain. Plenum Press, New York.
Pereira, M., Andreatini, R., Schwarting, R.K., Brenes, J.C., 2014. Amphetamine-induced appetitive 50-kHz calls in rats: a marker of affect in mania? Psychopharmacology 231, 2567–2577. https://doi.org/10.1007/s00213-013-3413-1.
Picconi, B., Centonze, D., Rossi, S., Bernardi, G., Calabresi, P., 2004. Therapeutic doses of L-dopa reverse hypersensitivity of corticostriatal D2-dopamine receptors and glutamatergic overactivity in experimental parkinsonism. Brain 127, 1661–1669. https://doi.org/10.1093/brain/awh190.
Pinna, A., Ko, W.K., Costa, G., Tronci, E., Fidalgo, C., Simola, N., Li, Q., Tabrizi, M.A., Bezard, E., Carta, M., Morelli, M., 2016. Antidyskinetic effect of A2A and 5HT1A/1B receptor ligands in two animal models of Parkinson's disease. Mov. Disord. 31, 501–511. https://doi.org/10.1002/mds.26475.
Pintori, N., Castelli, M.P., Miliano, C., Simola, N., Fadda, P., Fattore, L., Scherma, M., Ennas, M.G., Mostallino, R., Flore, G., De Felice, M., Sagheddu, C., Pistis, M., Di Chiara, G., De Luca, M.A., 2021. Repeated exposure to JWH-018 induces adaptive changes in the mesolimbic and mesocortical dopaminergic pathways, glial cells alterations, and behavioural correlates. Br. J. Pharmacol. 178, 3476–3497. https://doi.org/10.1111/bph.15494.
Premoli, M., Pietropaolo, S., Wöhr, M., Simola, N., Bonini, S.A., 2023. Mouse and rat ultrasonic vocalizations in neuroscience and neuropharmacology: state of the art and future applications. Eur. J. Neurosci. 57, 2062–2096. https://doi.org/10.1111/ejn.15957.
Riddle, J.L., Rokosik, S.L., Napier, T.C., 2012. Pramipexole- and methamphetamine-induced reward-mediated behavior in a rodent model of Parkinson's disease and controls. Behav. Brain Res. 233, 15–23. https://doi.org/10.1016/j.bbr.2012.04.027.
Robbins, T.W., Everitt, B.J., 2002. Limbic-striatal memory systems and drug addiction. Neurobiol. Learn. Mem. 78 (3), 625–636. https://doi.org/10.1006/nlme.2002.4103.
Rokosik, S.L., Napier, T.C., 2012. Pramipexole-induced increased probabilistic discounting: comparison between a rodent model of Parkinson's disease and controls. Neuropsychopharmacology 37, 1397–1408. https://doi.org/10.1038/npp.2011.325.
Schallert, T., Fleming, S.M., Leasure, J.L., Tillerson, J.L., Bland, S.T., 2000. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 39, 777–787. https://doi.org/10.1016/s0028-3908(00)00005-8.
Schwarting, R.K.W., 2023. Behavioral analysis in laboratory rats: challenges and usefulness of 50-kHz ultrasonic vocalizations. Neurosci. Biobehav. Rev. 152, 105260 https://doi.org/10.1016/j.neubiorev.2023.105260.
Seeman, P., 2009. Schizophrenia model of elevated D2(high) receptors: haloperidol reverses the amphetamine-induced elevation in dopamine D2(high). Schizophr. Res. 109, 191–192. https://doi.org/10.1016/j.schres.2008.12.024.
Serra, M., Costa, G., Onaivi, E., Simola, N., 2024. Divergent acute and enduring changes in 50-kHz ultrasonic vocalizations in rats repeatedly treated with amphetamine and dopaminergic antagonists: new insights on the role of dopamine in calling behavior. Int. J. Neuropsychopharmacol. 27, pyae001 https://doi.org/10.1093/ijnp/pyae001.
Shimoju, R., Shibata, H., 2021. Simultaneous antagonism of dopamine D1/D2/D3 receptor in the NAc reduces 50-kHz ultrasonic calls in response to rhythmic tactile stroking. Behav. Brain Res. 405, 113211 https://doi.org/10.1016/j.bbr.2021.113211.
Simola, N., Brudzynski, S.M., 2018a. In: Brudzynski, S.M. (Ed.), Repertoire and biological function of ultrasonic vocalizations in adolescent and adult rats. In Handbook of Ultrasonic Vocalization: A Window into the Emotional Brain. Elsevier Academic Press, Cambridge, MA, USA, pp. 177–186.
Simola, N., Brudzynski, S.M., 2018b. Rat 50-kHz ultrasonic vocalizations as a tool in studying neurochemical mechanisms that regulate positive emotional states. J. Neurosci. Methods 310, 33–44. https://doi.org/10.1016/j.jneumeth.2018.06.018.
Simola, N., Costa, G., 2018. Emission of categorized 50-kHz ultrasonic vocalizations in rats repeatedly treated with amphetamine or apomorphine: possible relevance to drug-induced modifications in the emotional state. Behav. Brain Res. 347, 88–98. https://doi.org/10.1016/j.bbr.2018.02.041.
Simola, N., Granon, S., 2019. Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. Neuropharmacology 159, 107420. https://doi.org/10.1016/j.neuropharm.2018.11.008.
Simola, N., Morelli, M., 2015. Repeated amphetamine administration and long-term effects on 50-kHz ultrasonic vocalizations: possible relevance to the motivational and dopamine-stimulating properties of the drug. Eur. Neuropsychopharmacol. 25, 343–355. https://doi.org/10.1016/j.euroneuro.2015.01.010.
Simola, N., Morelli, M., Carta, A.R., 2007. The 6-hydroxydopamine model of Parkinson's disease. Neurotox. Res. 11, 151–167. https://doi.org/10.1007/bf03033565.
Simola, N., Pinna, A., Fenu, S., 2010. Pharmacological therapy of Parkinson's disease: current options and new avenues. Recent Pat. CNS Drug Discov. 5 (3), 221–238. https://doi.org/10.2174/157488910793362421.
Simola, N., Fenu, S., Costa, G., Pinna, A., Plumitallo, A., Morelli, M., 2012. Pharmacological characterization of 50-kHz ultrasonic vocalizations in rats: comparison of the effects of different psychoactive drugs and relevance in drug-induced reward. Neuropharmacology 63, 224–234. https://doi.org/10.1016/j.neuropharm.2012.03.013.
Simola, N., Frau, L., Plumitallo, A., Morelli, M., 2014. Direct and long-lasting effects elicited by repeated drug administration on 50-kHz ultrasonic vocalizations are regulated differently: implications for the study of the affective properties of drugs of

abuse. Int. J. Neuropsychopharmacol. 17, 429–441. https://doi.org/10.1017/S1461145713001235.

Simola, N., Costa, G., Morelli, M., 2016. Activation of adenosine a_{2a} receptors suppresses the emission of pro-social and drug-stimulated 50-kHz ultrasonic vocalizations in rats: possible relevance to reward and motivation. Psychopharmacology 233, 507–519. https://doi.org/10.1007/s00213-015-4130-8.

Simola, N., Serra, M., Marongiu, J., Costa, G., Morelli, M., 2021. Increased emissions of 50-kHz ultrasonic vocalizations in hemiparkinsonian rats repeatedly treated with dopaminomimetic drugs: a potential preclinical model for studying the affective properties of dopamine replacement therapy in Parkinson's disease. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 108, 110184. https://doi.org/10.1016/j.pnpbp.2020.110184.

Stamos, J.P., Ma, S., Pawlak, A.P., Engelhard, N., Horvitz, J.C., West, M.O., 2022. Reward versus motoric activations in nucleus accumbens core of rats during Pavlovian conditioning. Eur. J. Neurosci. 56 (1), 3570–3590. https://doi.org/10.1111/ejn.15680.

Sulzer, D., Sonders, M.S., Poulsen, N.W., Galli, A., 2005. Mechanisms of neurotransmitter release by amphetamines: a review. Prog. Neurobiol. 75, 406–433. https://doi.org/10.1016/j.pneurobio.2005.04.003.

Tassan Mazzocco, M., Serra, M., Maspero, M., Coliva, A., Presotto, L., Casu, M.A., Morelli, M., Moresco, R.M., Belloli, S., Pinna, A., 2024. Positive relation between dopamine neuron degeneration and metabolic connectivity disruption in the MPTP plus probenecid mouse model of Parkinson's disease. Exp. Neurol. 374, 114704 https://doi.org/10.1016/j.expneurol.2024.114704.

Tassin, J.P., Simon, H., Hervé, D., Blanc, G., Le Moal, M., Glowinski, J., Bockaert, J., 1982. Non-dopaminergic fibres may regulate dopamine-sensitive adenylate cyclase in the prefrontal cortex and nucleus accumbens. Nature 295, 696–698. https://doi.org/10.1038/295696a0.

Terenzi, D., Rumiati, R.I., Catalan, M., Antonutti, L., Furlanis, G., Garlasco, P., Polverino, P., Bertolotti, C., Manganotti, P., Aiello, M., 2018. Reward sensitivity in Parkinson's patients with binge eating. Parkinsonism Relat. Disord. 51, 79–84. https://doi.org/10.1016/j.parkreldis.2018.03.007.

Thompson, B., Leonard, K.C., Brudzynski, S.M., 2006. Amphetamine-induced 50 kHz calls from rat nucleus accumbens: a quantitative mapping study and acoustic analysis. Behav. Brain Res. 168, 64–73. https://doi.org/10.1016/j.bbr.2005.10.012.

Todorova, A., Samuel, M., Brown, R.G., Chaudhuri, K.R., 2015. Infusion therapies and development of impulse control disorders in advanced Parkinson disease: clinical experience after 3 years' follow-up. Clin. Neuropharmacol. 38, 132–134. https://doi.org/10.1097/WNF.0000000000000091.

Voon, V., Napier, T.C., Frank, M.J., Sgambato-Faure, V., Grace, A.A., Rodriguez-Oroz, M., Obeso, J., Bezard, E., Fernagut, P.O., 2017. Impulse control disorders and levodopa-induced dyskinesias in Parkinson's disease: an update. Lancet Neurol. 16, 238–250. https://doi.org/10.1016/S1474-4422(17)30004-2.

Wang, J.Q., 1998. Regulation of immediate early gene c-fos and zif/268 mRNA expression in rat striatum by metabotropic glutamate receptor. Brain Res. Mol. Brain Res. 57, 46–53. https://doi.org/10.1016/s0169-328x(98)00060-6.

Wang, J.Q., McGinty, J.F., 1995. Differential effects of D1 and D2 dopamine receptor antagonists on acute amphetamine- or methamphetamine-induced up-regulation of zif/268 mRNA expression in rat forebrain. J. Neurochem. 65, 2706–2715. https://doi.org/10.1046/j.1471-4159.1995.65062706.x.

Weintraub, D., David, A.S., Evans, A.H., Grant, J.E., Stacy, M., 2015. Clinical spectrum of impulse control disorders in Parkinson's disease. Mov. Disord. 30, 121–127. https://doi.org/10.1002/mds.26016.

Williams, S.N., Undieh, A.S., 2016. Dopamine-sensitive signaling mediators modulate psychostimulant-induced ultrasonic vocalization behavior in rats. Behav. Brain Res. 296, 1–6. https://doi.org/10.1016/j.bbr.2015.08.008.

Wöhr, M., Rippberger, H., Schwarting, R.K., van Gaalen, M.M., 2015. Critical involvement of 5-HT2C receptor function in amphetamine-induced 50-kHz ultrasonic vocalizations in rats. Psychopharmacology 232, 1817–1829. https://doi.org/10.1007/s00213-014-3814-9.

Wright, J.M., Gourdon, J.C., Clarke, P.B., 2010. Identification of multiple call categories within the rich repertoire of adult rat 50-kHz ultrasonic vocalizations: effects of amphetamine and social context. Psychopharmacology 211, 1–13. https://doi.org/10.1007/s00213-010-1859-y.

Wright, J.M., Dobosiewicz, M.R., Clarke, P.B., 2012. α- and β-adrenergic receptors differentially modulate the emission of spontaneous and amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats. Neuropsychopharmacology 37, 808–821. https://doi.org/10.1038/npp.2011.258.

Wright, J.M., Dobosiewicz, M.R., Clarke, P.B., 2013. The role of dopaminergic transmission through D1-like and D2-like receptors in amphetamine-induced rat ultrasonic vocalizations. Psychopharmacology 225, 853–868. https://doi.org/10.1007/s00213-012-2871-1.

Yano, M., Beverley, J.A., Steiner, H., 2006. Inhibition of methylphenidate-induced gene expression in the striatum by local blockade of D1 dopamine receptors: interhemispheric effects. Neuroscience 140, 699–709. https://doi.org/10.1016/j.neuroscience.2006.02.017.

Zengin-Toktas, Y., Authier, N., Denizot, H., Chassain, C., Hafidi, A., Llorca, P.M., Durif, F., 2013. Motivational properties of D2 and D3 dopamine receptors agonists and cocaine, but not with D1 dopamine receptors agonist and L-dopa, in bilateral 6-OHDA-lesioned rat. Neuropharmacology 70, 74–82. https://doi.org/10.1016/j.neuropharm.2012.12.011.