Mol Neurobiol (2016) 53:3891–3899
DOI 10.1007/s12035-015-9330-z

Temporal Dissociation of Striatum and Prefrontal Cortex Uncouples Anhedonia and Defense Behaviors Relevant to Depression in 6-OHDA-Lesioned Rats

Filipe C. Matheus[1] · Daniel Rial[1,2] · Joana I. Real[2] · Cristina Lemos[2] · Reinaldo N. Takahashi[1] · Leandro J. Bertoglio[1] · Rodrigo A. Cunha[2,3] · Rui D. Prediger[1]

Received: 2 February 2015 / Accepted: 1 July 2015 / Published online: 12 July 2015

Abstract The dorsolateral striatum (DLS) processes motor and non-motor functions and undergoes extensive dopaminergic degeneration in Parkinson's disease (PD). The nigrostriatal dopaminergic degeneration also affects other brain areas including the pre-frontal cortex (PFC), which has been associated with the appearance of anhedonia and depression at pre-motor phases of PD. Using behavioral, neurochemical, and electrophysiological approaches, we investigated the temporal dissociation between the role of the DLS and PFC in the appearance of anhedonia and defense behaviors relevant to depression in rats submitted to bilateral DLS lesions with 6-hydroxydopamine (6-OHDA; 10 μg/hemisphere). 6-OHDA induced partial dopaminergic nigrostriatal damage with no gross motor impairments. Anhedonic-like behaviors were observed in the splash and sucrose consumption tests only 7 days after 6-OHDA lesion. By contrast, defense behaviors relevant to depression evaluated in the forced swimming test and social withdrawal only emerged 21 days after 6-OHDA lesion when anhedonia was no longer present. These temporally dissociated behavioral alterations were coupled to temporal- and structure-dependent alterations in dopaminergic markers such as dopamine D_1 and D_2 receptors and dopamine transporter, leading to altered dopamine sensitivity in DLS and PFC circuits, evaluated electrophysiologically. These results provide the first demonstration of a dissociated involvement of the DLS and PFC in anhedonic-like and defense behaviors relevant to depression in 6-OHDA-lesioned rats, which was linked with temporal fluctuations in dopaminergic receptor density, leading to altered dopaminergic system sensitivity in these two brain structures. This sheds new light to the duality between depressive and anhedonic symptoms in PD.

Keywords Parkinson's disease · Depression · Anhedonia · Social isolation · Dorsolateral striatum · Pre-frontal cortex

Introduction

Parkinson's disease (PD) patients exhibit non-motor alterations several years before the onset of classical motor symptoms (i.e., rigidity, resting tremor, and bradykinesia) [1]. Mood disorders such as anhedonia and depression are insidious non-motor symptoms of PD and very detrimental to the quality of life of PD patients [2]. These pre-motor symptoms may fluctuate in intensity and also may be dissociated from each other over time during the progression of PD [3].

Depression has a heterogeneous symptomatology and responsiveness to treatment. Anhedonia, a decreased ability to experience pleasure, is a core symptom of this mental illness [4], and its occurrence seems to be variable in intensity and duration depending on the subtype of depression affecting the patients [5]. Social isolation is present in PD patients [6] and is also a symptom found in some cases of depression [4]. Importantly, non-motor symptoms of PD are underdiagnosed and

Electronic supplementary material The online version of this article (doi:10.1007/s12035-015-9330-z) contains supplementary material, which is available to authorized users.

✉ Rui D. Prediger
ruidsp@hotmail.com

[1] Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC 88049-900, Brazil

[2] Center for Neuroscience and Cell Biology (CNC), University of Coimbra, 3004-517 Coimbra, Portugal

[3] Faculty of Medicine, University of Coimbra, 3005-504 Coimbra, Portugal

consequently undertreated [3], and it is critical to improve the knowledge about the neurobiology of these symptoms in PD patients to offer adequate therapeutic strategies. For instance, many PD patients experience apathy independently of depression [7], warring for a better comprehension of the relative modifications of different brain structures involved in the modulation of emotional responses to clarify this duality between depression and anhedonia in PD.

Dopaminergic degeneration of dorsolateral striatum (DLS) is a cardinal feature of PD, but it also affects other brain structures such as the pre-frontal (PFC) [8]. Functional connectivity between cortical and striatal neuronal systems plays a pivotal role in sensory, emotion, and motor functions [9–11]. However, the neurobiology of anhedonia and depression in PD is still poorly understood, and the possible different participation of the DLS and PFC to process different non-motor symptoms of PD remains unknown.

In this study, through the use of behavioral, neurochemical, and electrophysiological approaches, we investigated the temporal and the structural dissociation between the role of DLS and PFC in the appearance of anhedonia and defense behaviors relevant to depression in rats submitted to bilateral 6-hydroxydopamine (6-OHDA) lesions in the DLS. Our findings provide the first evidence of a dissociative involvement of the DLS and PFC, respectively, in anhedonic and defense behaviors relevant to depression in 6-OHDA-lesioned rats. These behavioral modifications were accompanied by temporal fluctuations in the dopaminergic receptor density, leading to altered pre-synaptic control and dopaminergic sensitivity in the DLS and in the PFC.

Methods and Materials

Animals

Male Wistar rats weighing 300–350 g, aged 12–16 weeks at the time of testing, were used. All procedures were approved by the Institutional Ethical Committee for the care and use of laboratory animals of the Federal University of Santa Catarina (CEUA PP00830/2013) and the Ethical Committee of the Center for Neuroscience of Coimbra.

Drugs and Stereotaxic Surgery

The dose of 6-OHDA (Sigma-Aldrich, USA) was selected (Supplementary data Fig. S1 and Fig. S2) at a dose of 10 μg/hemisphere and was bilaterally injected into the DLS (AP, +0.2 mm; ML, ±3.5 mm; DV, −4.8 mm from bregma and dura). All animals were administered intra-peritoneally (i.p.) with desipramine (20 mg/kg) (Sigma-Aldrich, USA) 30 min before surgery, in order to protect noradrenergic terminals from 6-OHDA toxicity. The stereotaxic surgery was performed under ketamine (75 mg/kg)/xylazine (8 mg/kg) i.p. anesthesia. Sham-operated rats followed the same protocol except for the fact that vehicle was injected instead of 6-OHDA. Fluoxetine (Sigma-Aldrich, USA) and bupropion (Sigma-Aldrich, USA) were dissolved in saline solution, and the dose of these drugs was selected based on previous studies [12, 13]. The behavioral experiments were carried out 7 or 21 days after the surgery and either transcardially perfused or killed by decapitation after halothane anesthesia following behavioral tests.

Behavioral Tests

Assessment of Motor Function

Spontaneous locomotor activity was assessed in the open field test and allowed to freely explore it during 15 min to measure, using the ANY-maze software (Stoelting, USA), the total distance travelled and the average speed as indicators of spontaneous locomotor activity, as described previously [14]. The balance and motor coordination of rats were tested in the accelerated rotarod (Insight, Ribeirão Preto, Brazil) with automatic increase speed of the cylinder rotation (phase 1–2, 16 rpm; phase 3–4, 20 rpm; phase 5–6, 25 rpm; phase 7–8, 28 rpm; phase 9–10, 37 rpm) to measure the latency to fall, as previously described [14]. The measurement of grip force was made using a computerized grip force meter (Grip Strength Meters, Columbus Instruments, Columbus, OH, USA), simultaneously evaluating the right and left limbs and expressing grip force as the difference in grams, as previously described [14].

Assessment of Emotional Parameters

The sucrose consumption test, frequently used to measure anhedonia [15], compared the consumption of water and of a 0.8 % sucrose solution during 48 h, as described previously [16]. Additionally, the splash test, which evaluates a form of motivational behavior considered to parallel some symptoms of anhedonia such as apathetic behavior [17], was carried out to measure the time spent grooming which was recorded for a period of 15 min after squirting a 10 % sucrose solution on the dorsal coat of the animals, as previously described [18]. Defense behaviors relevant to depression were evaluated in a forced swimming test, scoring the total duration of immobility and swimming during a 5-min test session in glass cylinders containing water, as previously described [14]. Social interaction was evaluated as previously described [19, 20] by measuring the amount of time spent by each rat in a pair sniffing, grooming, following, kicking, mounting, jumping on, wrestling/boxing, and crawling under/over the partner during a 10-min period of interaction. Anxiety-like behavior was evaluated using an elevated plus-maze by measuring during 5 min the

percent open arm entries (% open arm entries: open entries/total entries×100) and percent time on open arms (% open time), as previously described [14].

Immunohistochemistry

The density of tyrosine hydroxylase (TH) was evaluated in free-floating sections containing the DLS and substantia nigra (25 μm thick) stained with a rabbit anti-TH polyclonal antibody (1:1500, ab-112 Abcam, UK) followed by a goat anti-rabbit secondary antibody (1:2000; ab60317, Chromeo™ 546 Abcam, UK), and the fluorescent signals were detected using a fluorescent microscope (BioZero8000, Keyence Corp., Japan), as previously described [21].

Western Blotting

Striatum or PFC homogenates were separated by SDS-PAGE and, after electrotransfer, protein and molecular weight markers (BioRad, Mississauga, Canada) were revealed by Ponceau Red staining Then, membranes were incubated overnight at 4 °C with antibodies anti-D_1R (sc-14001; Santa Cruz Biotechnology, USA), anti-D_2R (sc-5303; Santa Cruz Biotechnology, USA), or anti-DAT (MAB369; Millipore, Germany) and, after washing, with horseradish peroxidase-conjugated anti-rabbit, anti-mouse, and anti-rat antibodies for 2 h. Blots were visualized using the PerkinElmer ECL system, as previously described [22].

Extracellular Electrophysiological Recordings

Electrophysiological recordings were carried out as previously described [23] by extracellularly recording population spikes (PSs) either in layer V of the medial prelimbic PFC upon stimulation of layers II/III in coronal slices (250–300 μm thick) containing the PFC or in the DLS upon stimulation of white matter above the DLS in slices (400 μm thick) containing the DLS. The intensity of stimulation was selected to yield 40–50 % of the maximum response, which was quantified by its population spike amplitude. Paired-pulse stimulation consisted of two stimuli delivered with an inter-stimuli interval of 50 or 250 ms for PFC slices or 20 or 160 ms for DLS slices. The paired-pulse ratio was calculated as the ratio of the second response to the first response. Dopamine (25, 50, 100 μM) was cumulatively added through the superfusion solution, and its effect estimated by changes of PS amplitude.

Experimental Protocol

Rats were first stereotaxically injected with 6-OHDA (or vehicle for controls) in the DLS (Supplementary data Fig. S3), and independent groups of animals were tested only once in the behavioral tasks after either 7 or 21 days: Motor function was first characterized using the rotarod, the open field, and the grip force tests; then, the rats were tested in the non-motor behavioral tasks such as sucrose preference and splash tests (anhedonia), forced swimming and social interaction (defense behaviors relevant to depression), and elevated plus-maze (anxiety-like behaviors). Some rats were then transcardially perfused, and their brains were processed for immunohistochemical quantification of tyrosine hydroxylase (TH) in the DLS and substantia nigra (SN). Other rats were killed and their brains were dissected for Western blotting quantification of D_1R, D_2R, and dopamine transporter in the striatum and PFC. A last set of rats were killed to carry out extracellular electrophysiological recordings in DLS and PFC slices. Finally, independent experiments were carried out following the same protocol, to test the impact of the antidepressant drugs fluoxetine or bupropion on the anhedonic-like and defense behaviors relevant to depression 7 and 21 days after 6-OHDA lesion.

Data Analysis and Statistics

Statistical analysis was performed by two-way analysis of variance (ANOVA) factorial followed by Newman-Keuls post hoc test, except the analysis of electrophysiological data that was carried out using Student's *t* tests. The values are expressed as mean±standard error of mean (s.e.m), and the significance level was $P<0.05$.

Results

Intra-DLS 6-OHDA Injection Induces Partial Loss of Dopaminergic Neurons in the Nigrostriatal Pathway with No Motor Impairments

The intra-striatal administration of 6-OHDA caused a retrograde lesion of the nigrostriatal dopaminergic pathway (Fig. 1). In fact, both at 7 and at 21 days after 6-OHDA injection, the number of TH-positive cells was reduced by 6-OHDA compared to control in both the DLS (7 days, 63.0±2.5 %, $F_{(2, 21)}=423.0$, $P<0.05$; 21 days, 65.6±1.8 %, $F_{(2, 21)}=399.5$, $P<0.05$) (Fig. 1a, b) and SN (7 days, 50.0±4.5 %, $F_{(2, 21)}=20.26$, $P<0.05$; 21 days, 68.0±4.4 %, $F_{(2, 21)}=20.26$, $P<0.05$) (Fig. 1c, d); thus, there was a progression over time of the lesion only in the SN (Fig. 1c), and the same extent of degeneration was observed in both hemispheres (left and right) in the DLS and SN at both time points after 6-OHDA administration (Fig. 1).

However, the extent of 6-OHDA-induced dopaminergic injury was not sufficient to impair motor function, as assessed in different behavioral tests, namely, in the open field (Fig. 1e, f), rotarod (Fig. 1g), and grip force tests (Fig. 1h).

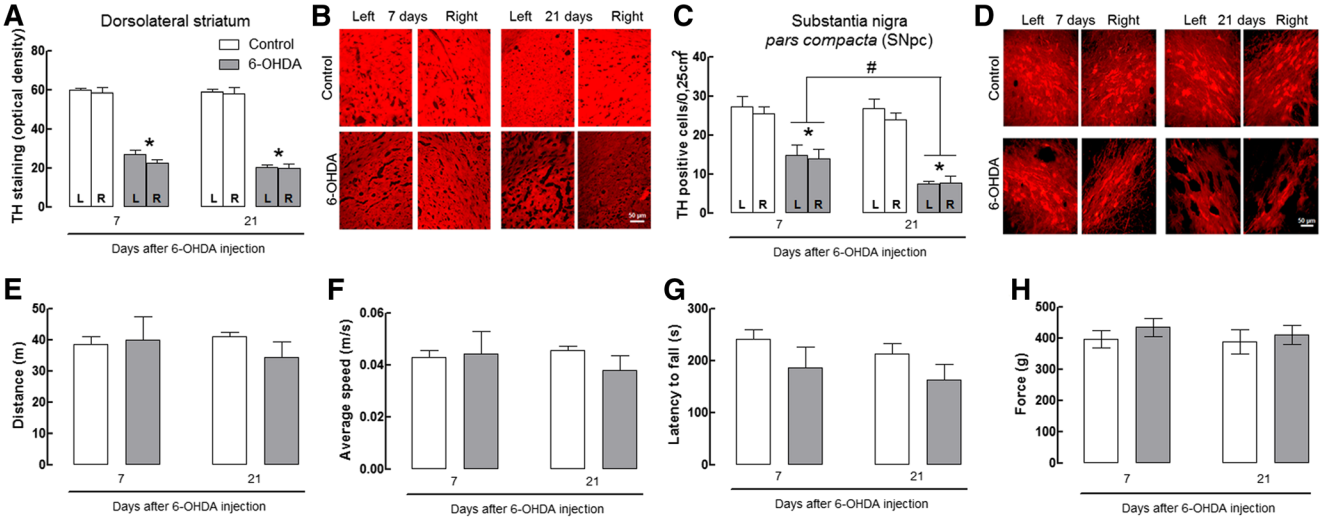

Fig. 1 6-Hydroxydopamine (6-OHDA) decreased the optical density of tyrosine hydroxylase (TH) in the dorsolateral striatum (DLS) and the number of TH-positive cells in the substantia nigra (SN) to a similar extent in both hemispheres left (L) and right (R) with no motor impairments. **a** Immunohistochemical quantification of TH staining in the DLS. **b** Representative coronal sections of the DLS. **c** Immunohistochemical quantification of TH-positive cells in the SN. **d** Representative coronal sections of the SN. **e** Total distance travelled during 15 min in the open field apparatus. **f** Average speed in the open field. **g** Latency to fall in the accelerated rotarod test. **h** Grip force test to evaluate the strength of the forelimbs. (*$P<0.05$ vs. control group; $^{\#}P<0.05$ vs. 6-OHDA group 7 days after injection, two-way ANOVA followed by the Newman-Keuls post hoc tests; $N=5–10$ rats/group)

Decoupling Anhedonia and Defense Behaviors Relevant to Depression in 6-OHDA-Lesioned Rats

When compared to the control group, rats lesioned with 6-OHDA displayed a decreased sucrose consumption ($F_{(1, 33)}=7.35$, $P<0.05$) (Fig. 2a) and a decreased time of grooming ($F_{(1, 33)}=8.57$, $P<0.05$) (Fig. 2c) only 7 days after 6-OHDA injection (but not after 21 days), which is indicative of an anhedonic-like behavior. Conversely, in the forced swimming test, 6-OHDA-lesioned rats displayed an increased immobility time ($F_{(1, 33)}=12.93$, $P<0.05$) (Fig. 2d) and a decreased swimming time ($F_{(1, 33)}=13.22$, $P<0.05$) only at 21 days (but not after 7 days) after 6-OHDA injection (Fig. 2e). At this later time point after 6-OHDA injection (21 days), the lesioned rats also exhibited a significant reduction of social interaction behaviors (sniffing, grooming, following, kicking, mounting, jumping on, wrestling/boxing, and crawling under/over the partner) (Fig. 2f), which was not observed 7 days after 6-OHDA injection. On the other hand, 6-OHDA-lesioned rats did not exhibit a modified anxiety-like behavior in the elevated plus-maze at neither 7 nor 21 days after 6-OHDA injection (Supplementary data Fig. S4).

The 6-OHDA-induced anhedonia observed only at 7 days was prevented by a treatment with bupropion (10 mg/kg, i.p., during 7 days) in both the sucrose preference task ($F_{(1, 27)}=7.73$, $P<0.05$) (Fig. 3a) and in the splash test ($F_{(1, 26)}=62.52$, $P<0.05$) (Fig. 3c). The treatment with fluoxetine (10 mg/kg, i.p., during 7 days) prevented the impairments induced by 6-OHDA only in splash test ($F_{(1, 28)}=46.39$, $P<0.05$) (Fig. 3d) but not in sucrose preference task ($F_{(1, 27)}=1.14$, $P>0.05$) (Fig. 3b). Regarding the 6-OHDA-induced defense behaviors relevant to depression observed only at 21 days, a chronic i.p. treatment during 21 days with both fluoxetine (10 mg/kg) or bupropion (10 mg/kg) prevented these alterations in immobility time (bupropion, $F_{(1, 28)}=18.05$, $P<0.05$; fluoxetine, $F_{(1, 27)}=19.85$, $P<0.05$) (Fig. 3e, f). Likewise, the 6-OHDA-induced decreased investigation time in the social interaction test observed only at 21 days was also prevented by a chronic i.p. treatment during 21 days with both fluoxetine (10 mg/kg; $F_{(1, 26)}=33.21$, $P<0.05$) or bupropion (10 mg/kg; $F_{(1, 28)}=8.41$, $P<0.05$) (Fig. 3g, h).

6-OHDA Induces Time-Dependent Changes of Dopamine Receptors (D_1R and D_2R) and Transporter (DAT) in the Striatum and PFC

The observed anhedonic and defense behaviors relevant to depression caused by 6-OHDA administration were accompanied by temporal fluctuations of the density of dopaminergic markers. Thus, 6-OHDA increased the density of D_1R ($F_{(1, 18)}=8.99$, $P<0.05$) (Fig. 4a) and D_2R ($F_{(1, 18)}=3.68$, $P<0.05$) (Fig. 4b) in the striatum after 7 days, which returned to control levels at 21 days after 6-OHDA administration (Fig. 4a, b). By contrast, 6-OHDA did not modify the density of either D_1R or D_2R in the PFC either after 7 days or after 21 days (Fig. 4d, e). Finally, we report that 6-OHDA decreased DAT density both in the striatum ($F_{(1, 18)}=10.58$, $P<0.05$) (Fig. 4c) as well as in the PFC ($F_{(1, 17)}=13.65$, $P<0.05$) (Fig. 4f) only at 21 days.

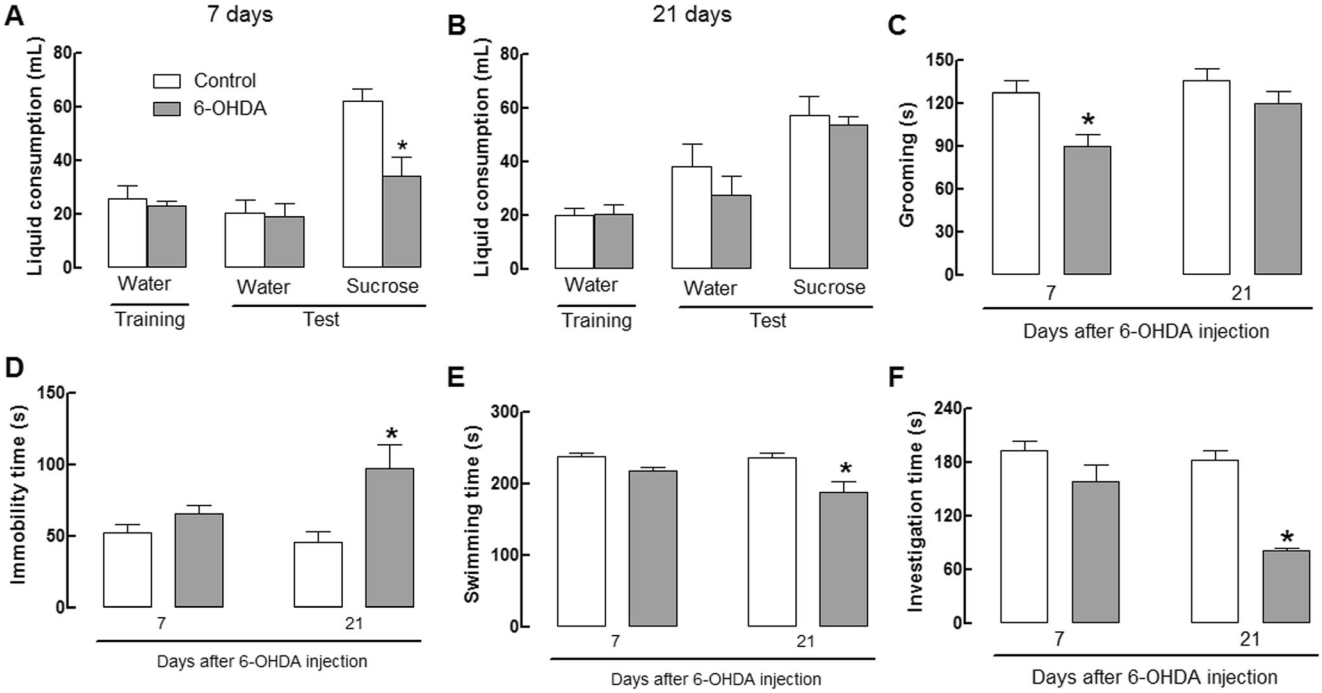

Fig. 2 Temporal dissociation between anhedonic-like and defense behaviors in 6-hydroxydopamine (6-OHDA)-lesioned rats. 6-OHDA decreased sucrose preference when compared to control group 7 days after 6-OHDA injection, (**a**) and this anhedonic-like behavior was not present 21 days after the 6-OHDA injection (**b**). The same pattern of response was observed in the splash test, confirming that anhedonic-like behavior was present only after 7 days (**c**). 6-OHDA increased the immobility time (**d**) and decreased the swimming time (**e**) in the forced swimming test only 21 days after 6-OHDA injection. In the social interaction test, 6-OHDA increased the social isolation when compared to control group only 21 days after 6-OHDA injection (**f**). (*$P<0.05$ vs. control group, two-way ANOVA followed by the Newman-Keuls post hoc tests; $N=8$–10 rats/group)

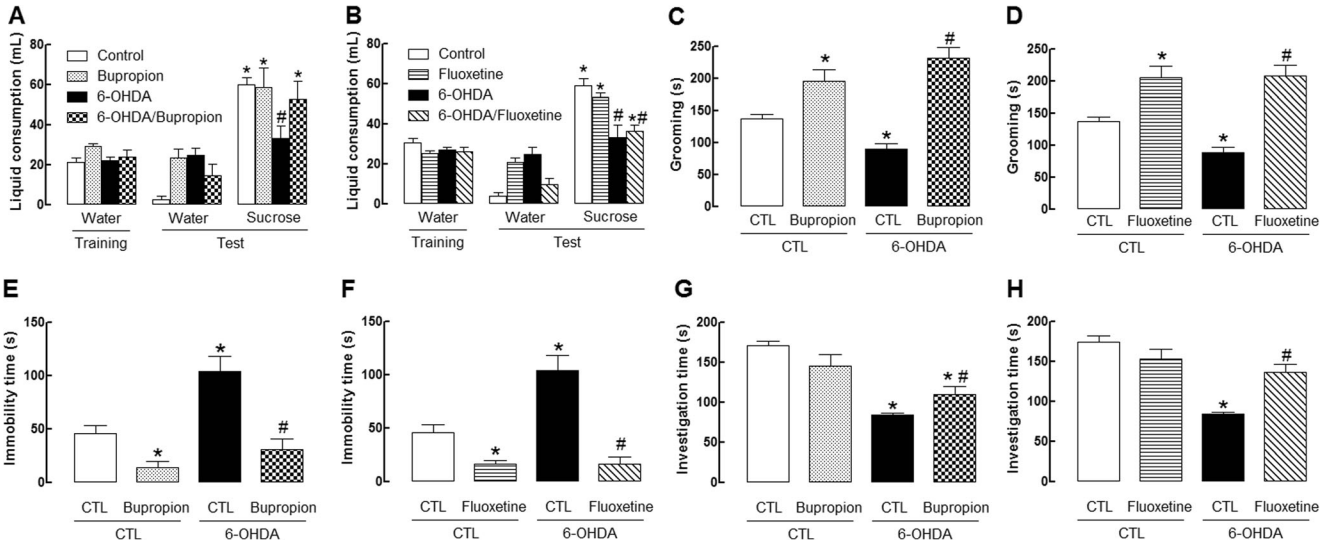

Fig. 3 The effects of bupropion (10 mg/kg, i.p.) and fluoxetine (10 mg/kg, i.p.) on the anhedonic-like and defense behaviors of 6-hydroxydopamine (6-OHDA)-lesioned rats. Treatment with bupropion (**a**), but not with fluoxetine (**b**), during 7 days reversed the anhedonic-like behaviors induced by 6-OHDA lesion. Both bupropion (**c**) and fluoxetine (**d**) treatments during 7 days reversed the 6-OHDA-induced reduction of grooming time in splash test. Chronic treatments during 21 days with bupropion or fluoxetine reversed the helpless behavior in the forced swimming test (**e**, **f**) and social isolation (**g**, **h**) induced by 6-OHDA. (Sucrose preference test, *$P<0.05$ vs. when compared sucrose consumption with water consumption in the test phase by each group; $^{\#}P<0.05$ vs. sucrose consumption of control group, two-way ANOVA. Splash, forced swimming, and social interaction tests, *$P<0.05$ vs. control group; $^{\#}P<0.05$ vs. 6-OHDA group, two-way ANOVA followed by the Newman-Keuls post hoc tests; $N=8$–10 rats/group)

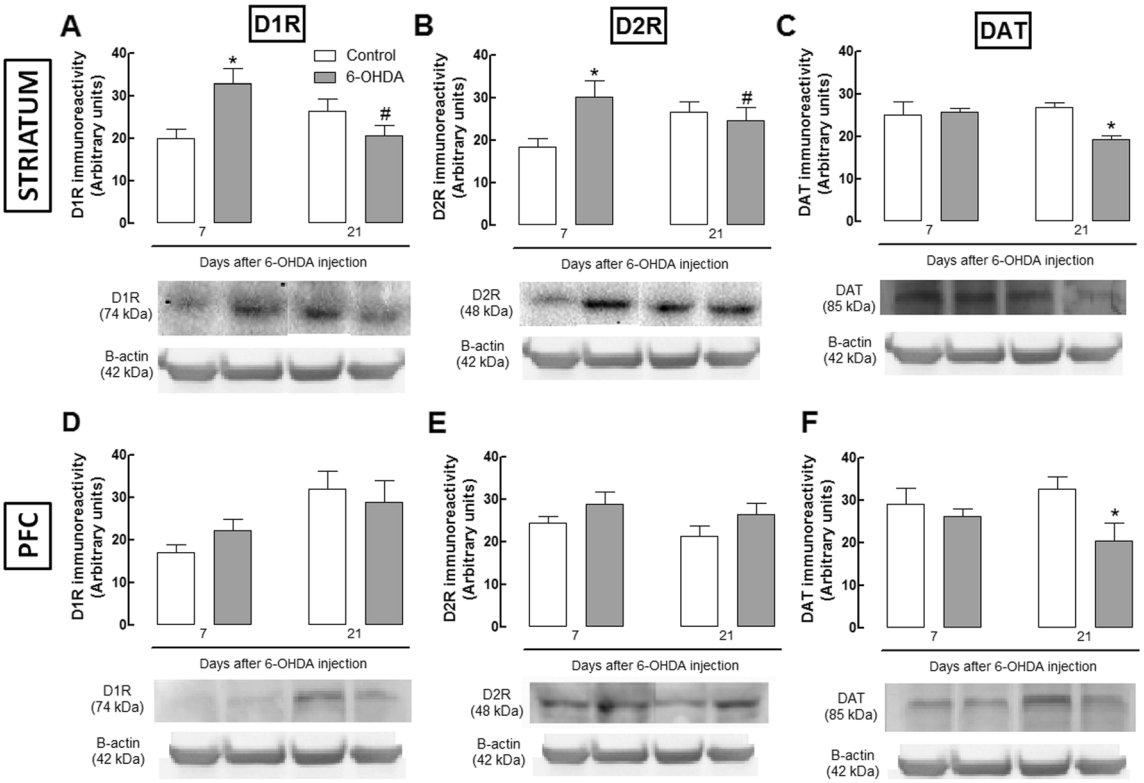

Fig. 4 6-Hydroxydopamine (6-OHDA) differently altered the density of dopamine receptors (D_1R and D_2R) and dopamine transporter (DAT) with time (7 and 21 days after 6-OHDA injection) in the striatum and pre-frontal cortex (PFC). Western blot quantification of D_1R (**a**), D_2R (**b**), and DAT (**c**) in striatum; D_1R (**d**), D_2R (**e**), and DAT (**f**) in PFC. (*$P<0.05$ vs. control group, $^{\#}P<0.05$ vs. 6-OHDA group 7 days after injection, two-way ANOVA followed by the Newman-Keuls post hoc tests; $N=5$ rats/group)

6-OHDA Induces Temporal Dissociation of Dopamine Sensitivity in the DLS and PFC

Electrophysiological recordings in the DLS or in PFC revealed that the 6-OHDA administration did not alter the stimulus-sensitivity curve at either 7 or 21 days after lesion (Supplementary data Fig. S5). Paired-pulse stimulation showed a significant decrease of paired-pulse facilitation in the DLS only at 7 (but not 21) days after 6-OHDA lesion (Fig. 5a), indicative of an early striatal pre-synaptic disruption, whereas paired-pulse stimulation was not modified in the PFC either 7 or 21 days after 6-OHDA administration (Fig. 5e).

Since 6-OHDA induces different time-dependent changes of dopamine receptors (D_1R and D_2R) and DAT in the striatum and PFC, we next investigated possible 6-OHDA-induced changes of dopamine sensitivity on synaptic transmission in DLS and PFC slices. In contrast to slices from control rats, dopamine increased synaptic transmission in the DLS at concentrations of 25 μM (75±31 % over baseline, $n=4$, $P<0.05$) and 50 μM (64±35 % over baseline, $n=4$, $P<0.05$) at 7 days (Fig. 5b) but not at 21 days (Fig. 5c) after 6-OHDA lesion. By contrast, in the PFC, there was no alterations of dopamine sensitivity at 7 days (Fig. 5f), but we observed a decreased dopamine sensitivity selectively at 21 days after the 6-OHDA lesion (21±6 % inhibition by 25 μM dopamine in 6-OHDA-lesioned vs. 62±5 % inhibition in control, $n=4$, $P<0.05$; 35±5 % inhibition by 50 μM dopamine in 6-OHDA-challenged vs. 67±7 % inhibition in control, $n=4$, $P<0.05$ (Fig. 5g).

Discussion

The present study demonstrates that a mild bilateral dopaminergic lesion of the rat DLS, which was insufficient to trigger motor deficits, mimicked some core non-motor symptoms of PD such as anhedonia and defense behaviors relevant to depression, as occurs in the prodrome phase of PD. Most importantly, we observed a temporal dissociation between anhedonia and defense behaviors relevant to depression that was coupled with a parallel temporal dissociation of neurochemical and electrophysiological alterations in the dorsolateral striatum and in the pre-frontal cortex. This indicates a dissociated neurobiological basis for the depressive and anhedonic symptoms in early PD.

The present model of 6-OHDA administration together with desipramine triggered a stable decrease of TH immunoreactivity in the striatum but an evolving dopaminergic lesion in the substantia nigra (40 % at 7 days and 68 % at 21 days), as occurs in PD patients [1]. Importantly, this observed extent of dopaminergic degeneration was not sufficient to impair motor

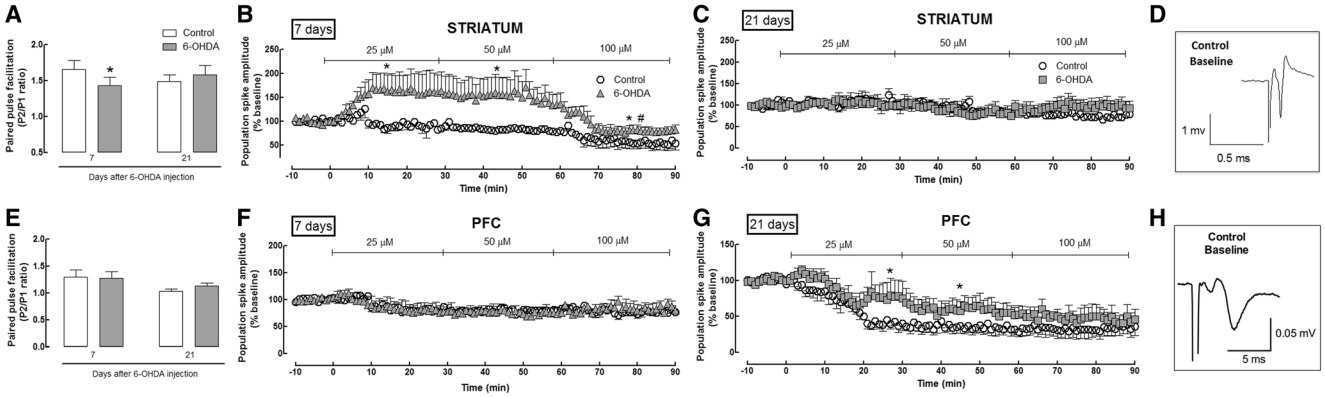

Fig. 5 6-Hydroxydopamine (6-OHDA) induces a temporal and structural dissociation of dopamine sensitivity in excitatory synapses of the dorsolateral striatum (DLS) and in the medial prefrontal cortex (PFC). In population spikes recorded in the DLS (**a**), the paired-pulse facilitation (ratio of the second vs. first pulse, P2/P1 ratio, with an inter-pulse interval of 20 ms) was decreased at 7 but not at 21 days after injection of 6-OHDA (**b**). Dopamine (25 and 50 µM) increased synaptic transmission in the DLS, when compared to control group at 7 days after the injection of 6-OHDA (**c**) but was devoid of effects at 21 days after 6-OHDA injection (**d**). In population spikes recorded in layer 5 of the PFC (**e**), the paired-pulse facilitation (ratio of the second vs. first pulse, P2/P1 ratio, with an inter-pulse interval of 50 ms) was not altered either at 7 but not at 21 days after 6-OHDA injection (**f**). Dopamine sensitivity was not affected 7 days after 6-OHDA injection (**g**), but there was a decreased sensitivity (25 and 50 µM) to inhibit synaptic transmission 7 days after the injection of 6-OHDA when compared to control group at (**h**). (*P<0.05 vs. control group, Student's t test; N=4 rats/group)

function, as gauged by the lack of modification of the performance in the open field (horizontal activity), rotarod (balance and coordination), and grip force (strength of the forelimbs) tests. However, this mild dopaminergic damage triggered mood-related behavioral alterations, such as anhedonia, defense behaviors, and impaired social interaction, as also observed in previous studies [24–26]; thus, this 6-OHDA-based model is an experimental model well-suited to evaluate behaviors related to symptoms occurring in the PD prodrome [3, 27, 28], without affecting motor functions that could confound the interpretation of emotional parameters associated to defense behaviors relevant to depression and anhedonia. Furthermore, we now confirmed that fluoxetine and the atypical antidepressant drug bupropion prevented the appearance of these behavioral alterations, further validating the pertinence of these alterations to the PD-related depressive status [29, 30]. This is particularly relevant since PD patients exhibit a wide range of non-motor symptoms such as anhedonia, depression, and anxiety that affect their quality of life and represent a major unmet therapeutic need [2, 3, 6].

A main finding of the present study was the temporal dissociation of the appearance of these different behavioral alterations: thus, anhedonic-like behaviors, evaluated in sucrose preference and in splash tests, were observed at early (7 days) but not at later (21 days) time points after 6-OHDA injection; by contrast, defense behaviors relevant to depression were present only at later periods (21 days) after 6-OHDA injection. Interestingly, parkinsonian patients also exhibit this dissociation of symptoms of anhedonia and depression [31, 32]. Since 6-OHDA triggers functional alterations in the striatum [33, 34] as well as in the PFC [35, 36] and these two interconnected brain regions [37, 38] are involved in the control of motivation, reward, and defense behaviors relevant to depression [reviewed in [39–42], we explored neurochemical and electrophysiological alterations in these two brain regions at 7 and at 21 days after 6-OHDA administration to gain a mechanistic insight on this temporal dissociation between anhedonia and defense behaviors relevant to depression. We observed that the appearance of anhedonia at 7 days was selectively associated with an increased density of both D_1 and D_2 receptors in the striatum, together with a decreased short-term plasticity and increased dopaminergic sensitivity in corticostriatal synapses. This is in notable agreement with the involvement in operant motivation of aberrant dopaminergic signaling in the striatum [43–46], suggesting that abnormal dopaminergic control of striatal function might be selectively associated with the hedonic alterations observed 7 days after 6-OHDA administration. This is re-enforced by the observation that there was a parallel disappearance of anhedonic behavior as well as striatal dopaminergic alterations at 21 days after 6-OHDA administration. By contrast, at 21 days after 6-OHDA administration, we observed the emergence of defense behaviors relevant to depression that paralleled the appearance of changes now in the PFC, typified by a reduction of the density of DAT and a decreased inhibition by dopamine of excitatory transmission in the PFC. This is in agreement with the involvement of the PFC in the control of emotional responses [reviewed in [39–42] and in the appearance of non-motor symptoms in PD [27, 28]. Also, PFC dysfunction underlies major depressive disorders [40, 47, 48] with preserved or disrupted hedonic function [49] and is also involved in the emergence of social phobia and their comorbidities [50]. This tentative association of PFC modifications with helpless and social impaired behaviors is re-enforced by the observation

that there was a lack of neurochemical and electrophysiological modifications at 7 days after 6-OHDA administration, which paralleled the lack of modification of defense behaviors relevant to depression. These observations lead us to propose that the behavioral dissociation between anhedonia and defense behaviors relevant to depression could be related to time-dependent alternations in these two different brain regions, namely, the striatum and PFC, respectively. This is line with the complex impact [51, 52] of the dopaminergic system in the control of anhedonia and other defense behaviors relevant to depression [reviewed in [53, 54] and with the different levels of dopaminergic denervation causing different changes in dopaminergic signaling in different brain regions [55]. This is also in notable agreement with some clinical studies suggesting a different involvement of different brain areas in the relationship of anhedonia and depression in PD patients [32, 56].

Overall, these results provide pioneering evidence of a temporal and structural dissociation of the role of DLS and PFC related to different non-motor behavioral impairments in rats submitted to 6-OHDA. These behavioral modifications are accompanied by different temporal fluctuations in the striatum and PFC of the dopaminergic receptor density, leading to altered pre-synaptic control and dopaminergic system sensitivity. This sheds new light on the neurobiological basis underlying the dissociation of anhedonia and defense behaviors relevant to depression in early PD, although the exact pathophysiological mechanisms underlying these conditions still need to be clarified.

Acknowledgments This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-FCT), Programa de Apoio aos Núcleos de Excelência (PRONEX - Project NENASC), Fundação de Apoio à Pesquisa do Estado de Santa Catarina (FAPESC), Ciência sem Fronteiras, DARPA (09-68-ESR-FP-010), NARSAD, QREN (09-68-ESR-FP-010), and Santa Casa da Misericórdia. F.C.M and D.R received scholarships from CNPq. R.N.T. and R.D.P. are supported by research fellowship from CNPq.

Conflict of interest All authors reported no biomedical financial interests or potential conflicts of interest.

References

1. Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K (2004) Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 318:121–134
2. Martinez-Martin P, Rodriguez-Blazquez C, Kurtis MM, Chaudhuri KR, NMSS Validation Group (2011) The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. Mov Disord 3:399–406
3. Chaudhuri KR, Healy DG, Schapira AH, National Institute for Clinical Excellence (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol 5:235–245
4. American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (5th ed.). Washington, DC
5. Benazzi F (2006) Various forms of depression. Dialogues Clin Neurosci 8:151–161
6. Schrag A (2006) Quality of life and depression in Parkinson's disease. J Neurol Sci 248:151–157
7. Martínez-Horta S, Riba J, de Bobadilla RF, Pagonabarraga J, Pascual-Sedano B, Antonijoan RM et al (2014) Apathy in Parkinson's disease: neurophysiological evidence of impaired incentive processing. J Neurosci 17:5918–5926
8. Kehagia AA, Barker RA, Robbins TW (2010) Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease. Lancet Neurol 12:1200–1213
9. Rieger JW, Schoenfeld MA, Heinze HJ, Bodis-Wollner I (2008) Different spatial organizations of saccade related BOLD activation in parietal and striate cortex. Brain Res 1233:89–97
10. Syed EC, Sharott A, Moll CK, Engel AK, Kral A (2011) Effect of sensory stimulation in rat barrel cortex, dorsolateral striatum and on corticostriatal functional connectivity. Eur J Neurosci 3:461–470
11. Tops M, Koole SL, IJzerman H, Buisman-Pijlman FT (2014) Why social attachment and oxytocin protect against addiction and stress: insights from the dynamics between ventral and dorsal corticostriatal systems. Pharmacol Biochem Behav 119:39–48
12. Cryan JF, Lucki I (2000) Antidepressant-like behavioral effects mediated by 5-hydroxytryptamine(2C) receptors. J Pharmacol Exp Ther 295:1120–1126
13. Lopez-Rubalcava C, Lucki I (2000) Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. Neuropsychopharmacology 22:191–199
14. Rial D, Castro AA, Machado N, Garção P, Gonçalves FQ, Silva HB et al (2014) Behavioral phenotyping of parkin-deficient mice: looking for early preclinical features of Parkinson's disease. PLoS One 9:e114216
15. Craft TK, DeVries AC (2006) Role of IL-1 in poststroke depressive-like behavior in mice. Biol Psychiatry 60:812–818
16. Slattery DA, Markou A, Cryan JF (2007) Evaluation of reward processes in an animal model of depression. Psychopharmacology 190:555–568
17. Willner P (2005) Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52:90–110
18. Machado DG, Cunha MP, Neis VB, Balen GO, Colla AR, Grando J et al (2012) Rosmarinus officinalis L. hydroalcoholic extract, similar to fluoxetine, reverses depressive-like behavior without altering learning deficit in olfactory bulbectomized mice. J Ethnopharmacol 143:158–169
19. Koros E, Rosenbrock H, Birk G, Weiss C, Sams-Dodd F (2007) The selective mGlu5 receptor antagonist MTEP, similar to NMDA receptor antagonists, induces social isolation in rats. Neuropsychopharmacology 32:562–576
20. O'Shea M, McGregor IS, Mallet PE (2006) Repeated cannabinoid exposure during perinatal, adolescent or early adult ages produces similar long-lasting deficits in object recognition and reduced social interaction in rats. J Psychopharmacol 20:611–621
21. Matheus FC, Aguiar AS Jr, Castro AA, Villarinho JG, Ferreira J, Figueiredo CP et al (2012) Neuroprotective effects of agmatine in mice infused with a single intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Behav Brain Res 235:263–272
22. Pandolfo P, Machado NJ, Köfalvi A, Takahashi RN, Cunha RA (2013) Caffeine regulates frontocorticostriatal dopamine transporter density and improves attention and cognitive deficits in an animal model of attention deficit hyperactivity disorder. Eur Neuropsychopharmacol 23:317–328
23. Costenla AR, Diógenes MJ, Canas PM, Rodrigues RJ, Nogueira C, Maroco J et al (2011) Enhanced role of adenosine A_{2A} receptors in

the modulation of LTP in the rat hippocampus upon ageing. Eur J Neurosci 34:12–21
24. Tadaiesky MT, Dombrowski PA, Figueiredo CP, Cargnin-Ferreira E, Da Cunha C, Takahashi RN (2008) Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. Neuroscience 156:830–840
25. Santiago RM, Barbieiro J, Lima MM, Dombrowski PA, Andreatini R, Vital MA (2010) Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. Prog Neuropsychopharmacol Biol Psychiatry 34: 1104–1114
26. Rampersaud N, Harkavyi A, Giordano G, Lever R, Whitton J, Whitton PS (2012) Exendin-4 reverses biochemical and behavioral deficits in a pre-motor 5 rodent model of Parkinson's disease with combined noradrenergic and serotonergic 6 lesions. Neuropeptides 46:183–193
27. Ferrer I, Martinez A, Blanco R, Dalfó E, Carmona M (2011) Neuropathology of sporadic Parkinson disease before the appearance of parkinsonism: preclinical Parkinson disease. J Neural Transm 118:821–839
28. Schapira AH, Tolosa E (2013) Molecular and clinical prodrome of Parkinson disease: implications for treatment. Nat Rev Neurol 6: 309–317
29. Connolly B, Fox SH (2014) Treatment of cognitive, psychiatric, and affective disorders associated with Parkinson's disease. Neurotherapeutics 11:78–91
30. Lemke MR (2008) Depressive symptoms in Parkinson's disease. Eur J Neurol 15(Suppl 1):21–25
31. Loas G, Krystkowiak P, Godefroy O (2012) Anhedonia in Parkinson's disease: an overview. J Neuropsychiatry Clin Neurosci 24:444–451
32. Starkstein S, Dragovic M, Jorge R, Brockman S, Merello M, Robinson RG et al (2011) Diagnostic criteria for depression in Parkinson's disease: a study of symptom patterns using latent class analysis. Mov Disord 26:2239–2245
33. Agrawal AK, Husain R, Raghubir R, Kumar A, Seth PK (1995) Neurobehavioral, neurochemical and electrophysiological studies in 6-hydroxydopamine lesioned and neural transplanted rats. Int J Dev Neurosci 13:105–111
34. Deumens R, Blokland A, Prickaerts J (2002) Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. Exp Neurol 175:303–317
35. Gui ZH, Zhang QJ, Liu J, Zhang L, Ali U, Hou C et al (2011) Unilateral lesion ofthe nigrostriatal pathway decreases the response of fast-spiking interneurons in the medialprefrontal cortex to 5-HT1A receptor agonist and expression of the receptor in parvalbumin-positive neurons in the rat. Neurochem Int 59:618–627
36. Zhang QJ, Li LB, Niu XL, Liu J, Gui ZH, Feng JJ et al (2011) The pyramidal neurons in the medial prefrontal cortex show decreased response to 5-hydroxytryptamine-3 receptor stimulation in a rodent model of Parkinson's disease. Brain Res 1384:69–79
37. Ernst M, Fudge JL (2009) A developmental neurobiological model of motivated behavior: anatomy, connectivity and ontogeny of the triadic nodes. Neurosci Biobehav Rev 33:367–382
38. Morgane PJ, Galler JR, Mokler DJ (2005) A review of systems and networks of the limbic forebrain/limbic midbrain. Prog Neurobiol 75:143–160
39. Krishnan V, Nestler EJ (2010) Linking molecules to mood: new insight into the biology of depression. Am J Psychiatry 167: 1305–1320
40. Price JL, Drevets WC (2012) Neural circuits underlying the pathophysiology of mood disorders. Trends Cogn Sci 16:61–71
41. Riga D, Matos MR, Glas A, Smit AB, Spijker S, Van den Oever MC (2014) Optogenetic dissection of medial prefrontal cortex circuitry. Front Syst Neurosci 8:230
42. Volman SF, Lammel S, Margolis EB, Kim Y, Richard JM, Roitman MF et al (2013) New insights into the specificity and plasticity of reward and aversion encoding in the mesolimbic system. J Neurosci 33:17569–71576
43. Drew MR, Simpson EH, Kellendonk C, Herzberg WG, Lipatova O, Fairhurst S et al (2007) Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. J Neurosci 27:7731–7739
44. Der-Avakian A, Markou A (2012) The neurobiology of anhedonia and other reward-related deficits. Trends Neurosci 35:68–77
45. Marchand WR (2012) Self-referential thinking, suicide, and function of the cortical midline structures and striatum in mood disorders: possible implications for treatment studies of mindfulness-based interventions for bipolar depression. Depress Res Treat 2012:246725
46. Shepherd GM (2013) Corticostriatal connectivity and its role in disease. Nat Rev Neurosci 14:278–291
47. Johnstone T, van Reekum CM, Urry HL, Kalin NH, Davidson RJ (2007) Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. J Neurosci 27:8877–8884
48. Duman RS, Aghajanian GK (2012) Synaptic dysfunction in depression: potential therapeutic targets. Science 338:68–72
49. Downar J, Geraci J, Salomons TV, Dunlop K, Wheeler S, McAndrews MP et al (2014) Anhedonia and reward-circuit connectivity distinguish non-responders from responders to dorsomedial prefrontal repetitive transcranial magnetic stimulation in major depression. Biol Psychiatry 76:176–185
50. Hamilton JP, Chen MC, Waugh CE, Joormann J, Gotlib IH (2014) Distinctive and common neural underpinnings of major depression, social anxiety, and their comorbidity. Soc Cogn Affect Neurosci (in press)
51. Cools R, D'Esposito M (2011) Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biol Psychiatry 69:e113–e125
52. Floresco SB (2013) Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions. Front Neurosci 7:62
53. Grace AA, Floresco SB, Goto Y, Lodge DJ (2007) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 30:220–227
54. Yadid G, Friedman A (2008) Dynamics of the dopaminergic system as a key component to the understanding of depression. Prog Brain Res 172:265–286
55. Dreyer JK (2014) Three mechanisms by which striatal denervation causes breakdown of dopamine signaling. J Neurosci 37:12444–12456
56. Lemke MR, Brecht HM, Koester J, Kraus PH, Reichmann H (2005) Anhedonia, depression, and motor functioning in Parkinson's disease during treatment with pramipexole. J Neuropsychiatry Clin Neurosci 17:214–220