ORIGINAL ARTICLE

Implication of dopamine D3 receptor activation in the reversion of Parkinson's disease-related motivational deficits

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In addition to the classical motor symptoms, motivational and affective deficits are core impairments of Parkinson's disease (PD). We recently demonstrated, by lesional approaches in rats, that degeneration of the substantia nigra pars compacta (SNc) dopaminergic (DA) neurons is likely to have a crucial role in the development of these neuropsychiatry symptoms. We have also shown that, as in clinical investigations, chronic treatment with levodopa or the DA D2/D3 receptor (D_2/D_3R) agonist ropinirole specifically reverses these PD-related motivational deficits. The roles of specific DA receptor subtypes in such reversal effects remain, however, unknown. We therefore investigated here the precise involvement of D_1, D_2 and D_3R in the reversal of the motivational and affective deficits related to SNc DA neuronal loss. Three weeks after bilateral and partial 6-hydroxydopamine (6-OHDA) SNc lesions, rats received 14 daily intraperitoneal administrations of the selective D1R agonist SKF-38393 (2.5 or 3.5 mg kg^{-1}), the selective D2R agonist sumanirole (0.1 or 0.15 mg kg^{-1}), or the preferring D3R gonist PD-128907 (0.1 or 0.15 mg kg^{-1}). Anxiety-, depressive-like and motivated behaviors were assessed in an elevated-plus maze, a forced-swim test, and an operant sucrose self-administration procedure, respectively. All DA agonists attenuated anxiety- and depressive-like behaviors. However, only PD-128907 reversed the motivational deficits induced by 6-OHDA SNc lesions. This effect was blocked by a selective D_3R (SB-277011A, 10 mg kg^{-1}), but not D_2R (L-741,626, 1.5 mg kg^{-1}), antagonist. These data provide strong evidence for the role of D3R in motivational processes and identify this receptor as a potentially valuable target for the treatment of PD-related neuropsychiatric symptoms.

INTRODUCTION

Parkinson's disease (PD) is a motor disorder resulting from the progressive degeneration of midbrain dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). However, in addition to the classical motor symptoms of the disease, a plethora of neuropsychiatric and cognitive impairments, including apathy, contribute significantly to patient's high morbidity, impairing critically their quality of life.[1] Apathy is observed in between 16.5 and 70% of PD patients, depending on the evaluation scale used and the population studied[2,3] and as such, is considered to be a major neuropsychiatric feature of the disease.[1] Apathy is classically defined as a lack of motivation, or a reduction in the 'cognitive and emotional concomitants of goal-directed behaviors'.[4] Apathetic patients indeed exhibit a global deficit in self-initiation and maintenance of voluntary and purposeful behavior, frequently accompanied by affective disorders, such as anxiety and depression,[1,2,5] resulting in low levels of activity and a loss of interest in sources of reinforcement.[6,7] A recent study revealed that apathy in PD and non PD patients is associated with an inability to translate expected reward into effort and action, with no change in the perception of reward value,[7] indicating that the core element of apathy resides within the motivational preparatory processes responsible for initiating voluntary actions. In addition, PD-related apathetic, anxiety and depressive symptoms have been shown to fluctuate with DA replacement therapy, occurring particularly in conditions in which DA medications are highly reduced, whereas these symptoms can be reversed by treatments with DA agonists,[1,2,5,6,8] suggesting a common underlying pathophysiological mechanism linked to DA neurotransmission in the basal ganglia.[5,8,9]

We have recently demonstrated that a part of these neuropsychiatric symptoms may stem directly from the loss of nigral DA neurons.[10] Specifically, we found that partial bilateral 6-hydroxydopamine (6-OHDA) lesions of SNc DA neurons in rats induced depressive- and anxiety-like behaviors and impairments of motivated behaviors, reminiscent of the apathetic symptoms observed in PD patients. Indeed, evaluation of behavior in several operant and nonoperant tasks revealed a profound deficit in the preparatory aspect of instrumental responses, with changes neither in locomotor activity nor in reward and Pavlovian processes. Moreover, we have shown, as in clinical investigations,[1,5,6] that this adverse phenotype was reversed by levodopa or the D2/D3R agonist ropinirole,[10] confirming the critical implication of DA.

DA acts. however, through multiple receptor subtypes with differential roles in motor, limbic and cognitive functions, depending on their neuronal and brain localizations in corticostriatal and limbic circuits.[11–14] As such, D1R, D2R and D3R have been shown to have important roles in regulating affective,[15–18] reward-related and motivational processes.[19–21] In the present

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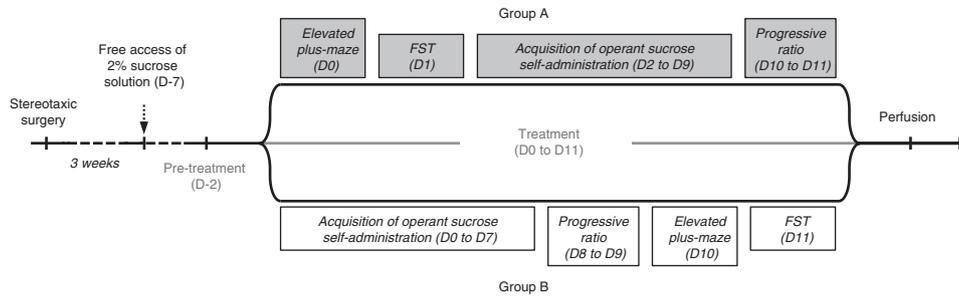[4]These authors contributed equally to this work.

Figure 1. Behavioral experiment schedules. Evaluation of the effects of different pharmacological treatments on the behavioral deficits induced by the 6-OHDA lesion. The behavioral studies began 3 weeks after surgery and tests were carried out in opposite orders for groups A and B. FST, forced-swim test; 6-OHDA, 6-hydroxydopamine.

study, we therefore set out to determine the respective contributions of these DA receptors in the reversal of the motivational and affective impairments induced by SNc DA neuronal loss. Using DA agonists selective for D_1R (SKF-38393), D_2R (sumanirole) or preferential for D3R (PD-128907),[22–26] we showed that all DA agonists corrected the affective impairments, but only the preferential D3R agonist reversed the motivational deficits induced by the SNc DA lesions, providing a potential specific target for the treatment of PD-related neuropsychiatric symptoms.

MATERIALS AND METHODS
See Supplementary Material for details regarding, animals, drugs and reagents, surgery, immunohistochemistry, DA denervation analyses and behavioral procedures, as well as data and statistical analysis.

Bilateral 6-OHDA lesions
A total of 6 µg of 6-OHDA dissolved in 2.3 µl of sterile 0.9% NaCl (6-OHDA conditions) or 2.3 µl of sterile 0.9% NaCl (sham conditions) were bilaterally and stereotaxically infused into the SNc as described in the Supplementary Material, at the following coordinates relative to bregma:[27] anterior-posterior, − 5.4 mm; medial-lateral, ± 1.8 mm and dorsal-ventral, − 8.1 mm. The posterior placement of the injectors is a determinant factor for sparing a part of DA ascending fibers, and therefore to lead to a partial (< 80%) DA denervation of the dorsal striatum that circumvent severe alterations of the motor function. Indeed, we previously shown that the SNc DA lesions with these parameters did not affect ambulatory behaviors, neither induced significant deficits in sensorimotor coordination or in stepping adjustement of the forepaws.[10]

Immunohistochemistry and DA denervation analyses
Immunohistochemical processing and analyses are described in the Supplementary Material. Coronal mesencephalic and striatal sections were processed for tyrosine hydroxylase (TH)- immunohistochemistry. Only TH-immunoreactivity (TH-IR) in the nucleus accumbens (NAc) and the dorsal striatum of sections localized around +1.2 mm anterior to bregma was quantified, as we previously observed that the dorsostriatal DA denervation at this level, is highly representative of the lesional extent and predictive of the motivational deficits.[10] We have ensured that pharmacological treatment did not modify TH-IR in sham-operated animals (no effect of treatment and no treatment × structure interaction were found: Fs < 1.26).

Experimental design
Three weeks after surgery and habituation to the 2% sucrose solution, rats were subjected to a sequence of behavioral tests, as summarized in Figure 1. Intraperitoneal administration of SKF-38393 (2.5 or 3.5 mg kg^{-1}), sumanirole (0.1 or 0.15 mg kg^{-1}), PD-128907 (0.1 or 0.15 mg kg^{-1}) or vehicle (0.9% NaCl, 1 ml kg^{-1}) began two days before the beginning of the behavioral sequence and were repeated daily for 14 days (Figure 1). Injections were carried out 30 min before the beginning of the behavioral sessions. Each of the selected doses has been shown to have

electrophysiological or neurochemical and behavioral effects through a specific action on the preferred DR subtype.[22,24–26,28–31]

Two subgroups of animals were constituted, the order of the tests being reversed in the second subgroup (see group A and B in Figure 1), to ensure that the effects of the lesion or of a pharmacological treatment did not change over the course of the study: half the animals were subjected first to the elevated-plus maze (EPM; day 0) and then the forced-swim test (FST; day 1), and operant sucrose self-administration (from day 2 to day 9) followed by a progressive ratio (PR) test (from day 10 to day 11), whereas the other half was subjected first to the self-administration procedure (from day 0 to day 7), followed by the PR test (from day 8 to day 9), the EPM (day 10) and the FST (day 11; Figure 1). No effect of order was found in any of the conditions tested (data not shown). In each experiment, all conditions (lesions and/or pharmacological treatments) were distributed among the different test chambers according to a Latin-square design.

This study was conducted in two separated experiments: a first one with the low dose of each agonists and a second with the higher doses. As the results obtained from sham-vehicle and 6-OHDA-vehicle rats were similar among the two experiments (data not shown), the data were pooled. The final number of subjects in each group for the sham and 6-OHDA condition, respectively, was as followed: n = 21 and 13 for vehicle, n = 12 and 8 for 2.5 mg kg^{-1} SKF-38393, n = 9 and 10 for 3.5 mg kg^{-1} SKF-38393, n = 9 and 8 for 0.10 mg kg^{-1} sumanirole, n = 11 and 9 for 0.15 mg kg^{-1} sumanirole, n = 13 and 12 for 0.10 mg kg^{-1} PD-128907 and n = 12 and 10 for 0.15 mg kg^{-1} PD-128907.

At the end of the second experiment, the self-administration procedure was continued or resumed (if after evaluation of affective-related behaviors) for 10 days, for the groups sham and 6-OHDA vehicle, 3.5 mg kg^{-1} SKF-38393 and 0.15 mg kg^{-1} PD-128907. During this period, SKF-38393 administration were discontinued and replaced after 2 days of withdrawal by the administration of the high-efficacy D_1R agonist SKF-82958[20,22] for 8 days. PD-128907 continued to be administered 30 min before each self-administration session. On days 3, 5 and 8, PD-128907 administration in sham and lesioned animals was preceded by a subcutaneous injection of the respective D_3R SB-277011A (10 mg kg^{-1}) or D_2R L-741626 (1.5 mg kg^{-1}) antagonist,[32] or vehicle (5% ethanol and 20% dimethyl sulfoxide in 0.9% NaCl, 1 ml kg^{-1}), in a within-subject design. These doses were chosen on the basis of previous reports,[25,32,33] and we had verified that they did not disrupt sucrose self-administration in sham-vehicle animals (no effect of treatment: F(2,14) = 0.39, data not shown). A second PR test was performed at the end of this 10-day period and then, locomotor activity of 6-OHDA 0.15 mg kg^{-1} PD-128907 animals was evaluated in an open area for two more days, in combination with SB-277011A or its vehicle (within-subject design).

RESULTS
6-OHDA-induced nigrostriatal DA lesions
Bilateral 6-OHDA infusion into the SNc led to a loss of DA neurons within this area, as revealed by TH immunolabeling (Figure 2a), and a less than 80% concomitant decrease in TH-IR in the dorsal striatum (Supplementary Table S1 and Figures 2b and c, significant effect of lesion: F(1,702) = 1066.01, P < 0.001), predominantly in the lateral portion (Figure 2b). By contrast, the mesolimbic pathway was relatively well-preserved (Figures 2a and b), with a less than 15% TH-IR loss in NAc (Supplementary Table S1 and
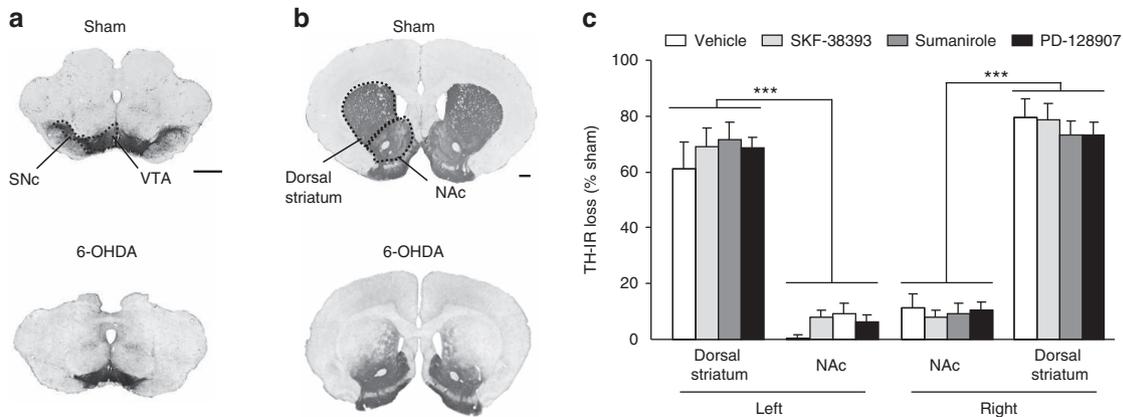
Figure 2. Bilateral partial lesions of the SNc result in a partial DA denervation of the dorsal striatum. (**a**, **b**) Photographs of coronal sections stained for TH at mesencephalic (**a**, anteriority − 5.2 mm from bregma) and striatal (**b**, anteriority +1.2 mm from bregma) levels, according to Paxinos and Watson (2005). (**c**) Quantifications of the loss of TH staining in the NAc and the dorsal striatum for each treatment, independently of the dose used, expressed as the percentage of the mean value obtained for sham-operated animals. Scale bar, 1 mm. DA, dopamine; NAc, nucleus accumbens; 6-OHDA, 6-hydroxydopamine; SNc, substantia nigra pars compacta; TH, tyrosine hydroxylase; VTA, ventral tegmental area. ***$P < 0.001$ ($n = 13$–22 per group).

Figure 2c, significant effect of structure and significant lesion × structure interaction: Fs > 240.86, Ps < 0.001. We previously showed that such mild degeneration of this pathway did not alter accumbal extracellular DA levels,[34] and did not provoke nor contribute to the behavioral phenotype induced by the 6-OHDA SNc lesions.[10] Moreover, analogous patterns of DA striatal denervation were observed for each treatment condition (Supplementary Table S1 and Figure 2c, no effect of treatment, structure × treatment interaction, lesion × treatment interaction, or lesion × treatment × structure interaction: Fs < 0.65).

DA receptors stimulation reverses the affect-related impairments induced by 6-OHDA SNc lesions

We first tested whether systemic administration of SKF-38393 (selective D1R agonist), sumanirole (selective D2R agonist) or PD-128907 (preferring D3R agonist), would correct the anxiety- and depression-like behaviors induced by the SNc DA lesions.[10]

All DA receptor agonists fully reversed the decrease in the time spent in the open arms induced by the SNc lesion in an EPM (Figure 3a, significant lesion × treatment interaction: F(6,143) = 5.49, P < 0.001), as well as the decrease in the time spent exploring the end of the open arms and in the number of nonprotected head dips (Figures 3b and c, significant lesion × treatment interaction: Fs = 2.77, Ps < 0.02), indicating a decrease in anxiety-like behaviors in lesioned-treated animals. Depending on the dose, SKF-38393- and sumanirole- even increased the time spent in the open arms of the EPM in lesioned rats compared with sham animals (Figure 3a), as well as nonprotected exploratory behaviors (Figures 3b and c). They also reduced the number of stretch attend postures toward the open arms (Figure 3d, significant lesion x treatment interaction: F(6,143) = 8.98, P < 0.001), a robust marker of risk assessment,[35] and tended to decrease the time spent in the central area (Figure 3e, significant effect of treatment: F(6,143) = 3.13, P < 0.01 and no lesion × treatment interaction: F(6,143) = 1.50), whereas PD-128907 normalized anxiety-related behaviors in lesioned-treated animals to control levels (Figures 3a–d). Conversely, sumanirole tends to reduce in sham animals exploration of the open arms (Figures 3a and b) and nonprotected head dipping (Figure 3c).

In addition, SKF-38393 and PD-128907 also reversed the depression-like behavior displayed in the FST (Figure 3f). Indeed, the method of contrasts (marginal lesion × treatment interaction: F(6,143) = 2.07, P = 0.06), found a significant increase in time spent immobile, a classical behavioral index of depression-like state,[36] in

vehicle-treated, but not in SKF-38393- or PD-128907-treated SNc-lesioned animals, when compared with their respective sham conditions. This effect was also observed in SNc-lesioned rats treated with sumanirole, but to a lesser extent as chronic administration of sumanirole tended to increase immobility time in sham animals (Figure 3f).

Only D3R agonist corrects the motivational deficits induced by 6-OHDA SNc lesions

We investigated whether sumanirole or PD-128907, as well as SKF-38393, could reverse the deficit in instrumental responses for sucrose self-administration induced by the SNc DA lesions, an impairment that was clearly linked to a motivational deficit and was notably reversed by the D2/D3R agonist ropinirole.[10]

Analysis of operant performances session by session revealed that only PD-128907 reversed the deleterious impact of the SNc lesion on sucrose self-administration under a continuous schedule of reinforcement (Figure 4, vehicle, SKF-38393 and sumanirole treatments: significant effect of lesion and session × lesion interaction: Fs > 5.26, Ps < 0.001; 0.1 mg kg^{-1} PD-128907 treatment: significant effect of lesion: F(1,168) = 7.68, P = 0.01, but no session × lesion interaction: F(7,168) = 0.81; 0.15 mg kg^{-1} PD-128907 treatment: no significant effect of lesion and interaction; Fs < 0.22), progressively increasing the number of sucrose deliveries in lesioned animals, to eventually reach the level of reinforcers earned by sham animals (see also Figure 5a, significant lesion × treatment interaction: F(6,143) = 8.08, P < 0.001). The absence of positive effects of the other treatments in lesioned animals was particularly striking, as the D1R and D2R agonist dose-dependently improved the instrumental performances of the sham animals. Similar results were observed with the potent full D1R agonist SKF-82958[22] (Supplementary Figure S1). Importantly, PD-128907 administration in 6-OHDA rats selectively increased the number of presses on the active lever over the number of presses on the inactive lever (effect of lever: Fs > 14.95, P < 0.004 and significant session × lever interaction: Fs > 5.64, P < 0.001; data not shown), indicating that PD-128907 did not induce a general increase in operant activity, but selectively toward the reinforced lever. This reversal effect of PD-128907 (0.15 mg kg^{-1}) in SNc-lesioned animals was selectively blocked by the D$_3$R antagonist SB-277011A, but not the D$_2$R antagonist L-741626 (Figure 5b, significant lesion × treatment interaction: F(2,38) = 9.15, P < 0.001). This effect was not due to a disruptive action of SB-277011A in
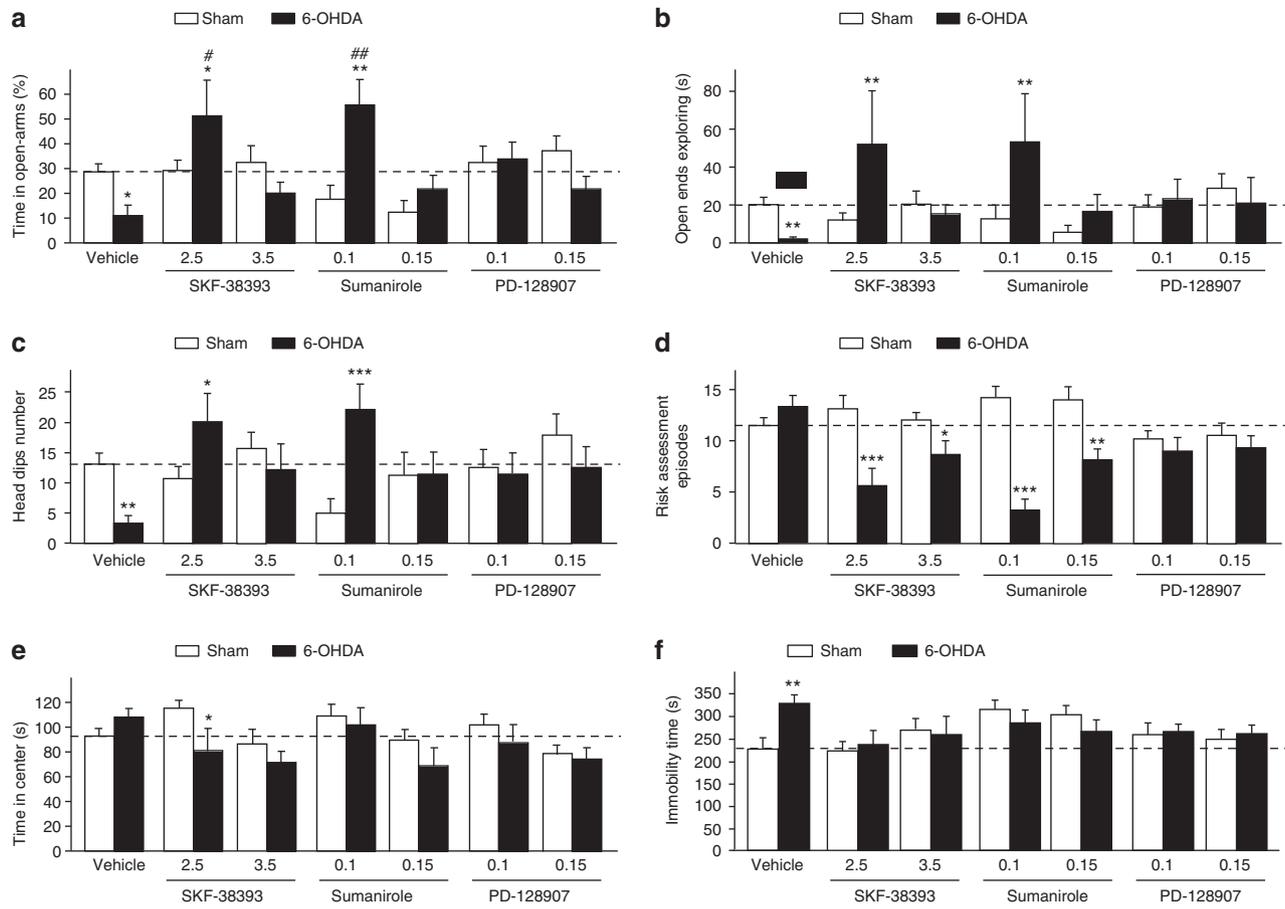
Figure 3. Effects of dopaminergic agonists on affective-related deficits induced by 6-OHDA SNc lesions. Evaluation of the effects of subchronic administration of SKF-38193 (2.5 and 3.5 mg kg^{-1}), sumanirole (0.1 and 0.15 mg kg^{-1}) and PD-128907 (0.1 and 0.15 mg kg^{-1}) in the elevated-plus maze (**a–e**) and the forced-swim test (**f**), in sham-operated (white histograms) and 6-OHDA SNc-lesioned (black histograms) animals. Indexes of anxiety-related behaviors evaluated here were the percentage of time spent in the open arms (**a**), the time spent specifically at the end of the open arms (**b**), the number of head dips when animals are exploring the open arms (**c**), the number of stretched attend episodes toward the open arms (**d**) and the time spent in the central area (**e**). Overall, DA agonists reversed the anxiety-like behaviors induced by 6-OHDA SNc lesions, as well as the increase in the time spent immobile in the forced-swim test. Dot lines represented the mean of the behavioral performances of vehicle-treated sham animals. *$P < 0.05$; **$P < 0.01$, ***$P < 0.01$ sham-operated versus lesioned within the same treatment and #$P < 0.05$, ##$P < 0.01$ between the treatments for sham-operated and lesioned conditions, respectively ($n = 8–21$ per group). 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; SNc, substantia nigra pars compacta.

lesioned animals, as locomotor behavior was not affected by the antagonist (Supplementary Figure S1).

Moreover, when the workload required to obtain the reward increased exponentially under a PR reinforcement schedule, an index of motivation,[37] only PD-128907 significantly increased the performance of lesioned animals, leading to a partial or a full reversion of the deficit, depending of the dose administered (Figure 5c, significant lesion × treatment interaction: F (6,143) = 5.08, $P < 0.001$). Ten more days of PD-128907 (0.15 mg kg^{-1}) administration, when responding for sucrose is stable, asymptotic and instrumental strategies become presumably exploitative,[38] led to a similar reversal of PR performance deficits (Figure 5d, marginal lesion × treatment interaction: F(1,30) = 3.67, $P = 0.06$).

DISCUSSION

By using DA agonists with different affinities for D1R, D2R and D3R, we show here a differential implication of these receptors in the reversal of the affective and motivational impairments induced by a DA denervation of the nigrostriatal system in rats. D1R, D2R and D3R agonists reversed the anxiety- and depressive-like behaviors induced by partial and bilateral 6-OHDA SNc lesions.

The most important finding was that only the pharmacological stimulation of D3R reversed the instrumental and motivational deficits of lesioned animals, thereby suggesting a critical role for this receptor in the reversal of processes that are directly related to the pathophysiology of PD.

SKF-38393 is a well-characterized agonist of D1R, with a much higher selectivity for this receptor than for D2R and D3R,[22] contrasting with sumanirole and PD-128907 that exhibit a very low affinity for D1R.[24,26] Sumanirole also has a 250-fold selectivity for D2 over D3 receptors,[26] but the affinity of PD-128907 for D$_3$R is only 15 to 30 times higher than that for D2R in rats.[23] Thus, sumanirole is unlikely to interact efficiently with D3R at relevant pharmacological concentrations, but PD-128907, depending on the dose used, may act as a D3R or a D2/D3R agonist.[25,26,30] Pharmacological and genetic studies have provided strong evidence that two very characteristic effects of DA agonists, hypothermia and yawning, are mediated by D2R and D3R respectively.[39–41] On the basis of these findings, Collins and colleagues[39–41] elegantly demonstrated that systemic administration of PD-128907 in Sprague–Dawley rats, the strain used here, induced robust yawning with no effects on body temperature for doses ranging from 0.01 to 0.1 mg kg^{-1}, thereby indicating a functional in vivo selective activation of D3R over D2R within this
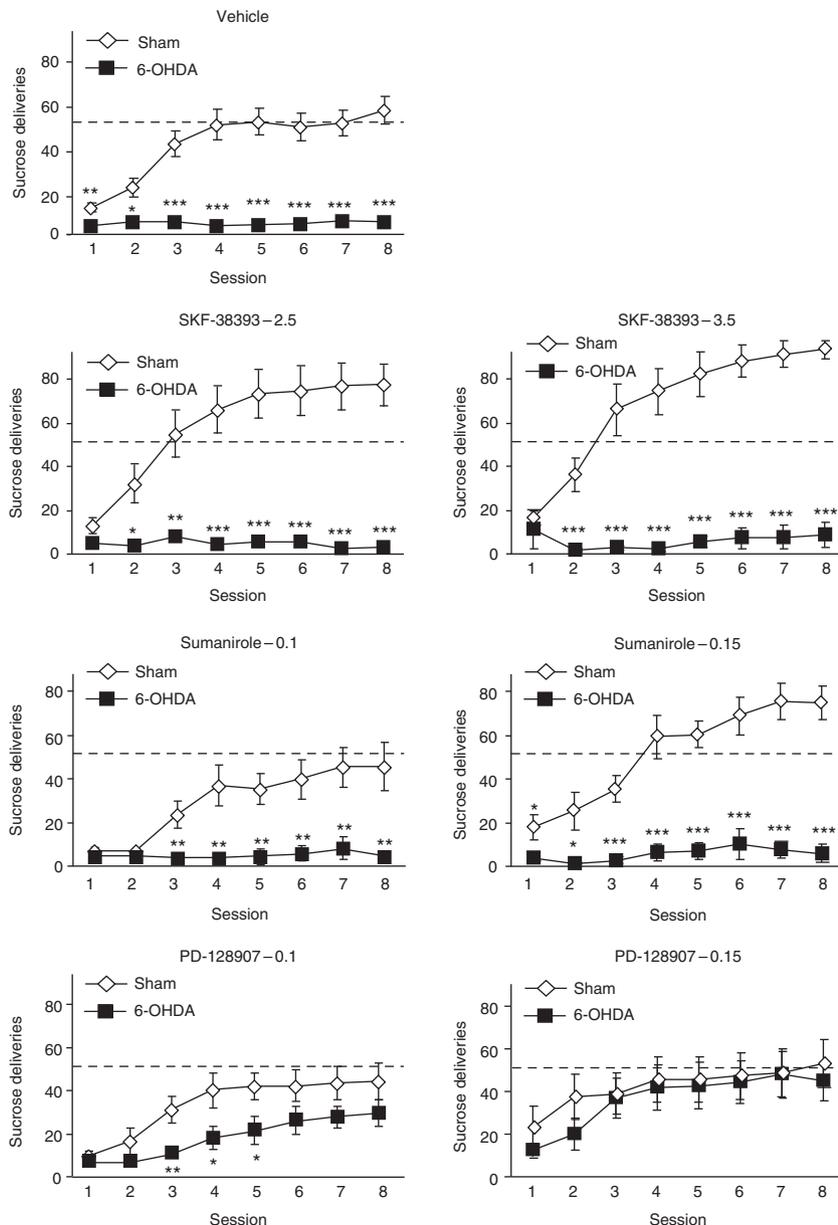
Figure 4. Only the preferential D_3R agonist, PD-128907, reverses the instrumental deficit induced by 6-OHDA SNc lesions. Evolution of operant sucrose (2% in water) self-administration performances, under a fixed ratio 1 (FR1) schedule of reinforcement, in sham-operated (white chips) and lesioned (black chips) animals, during the eight instrumental sessions. Subchronic administration of PD-128907 (0.1 and 0.15 mg kg^{-1}), but not of SKF-38393 (2.5 and 3.5 mg kg^{-1}) or sumanirole (0.1 and 0.15 mg kg^{-1}), improved the instrumental performances of 6-OHDA-lesioned rats. Dot lines represented the mean of the instrumental performances of the three last FR1 sessions of vehicle-treated sham animals. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, sham-operated versus lesioned within the same treatment ($n = 8$–21 per group). 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; SNc, substantia nigra pars compacta.

range of doses. In line with these results, it has been shown in D3R knockout mice, that PD-128907 must be administered at doses higher than 0.1 mg kg^{-1} to induce a significant D2 autoreceptors-mediated reduction of basal striatal DA extracellular levels.[30] Consistently, Zapata et al.[31] have shown in D_3R knockout mice that low doses of PD-128907, ranging from 0.03 to 0.3 mg kg^{-1}, reduce basal striatal DA extracellular levels through a selective activation of D3R. By using these selective agonists, together with a D3R preferring over D2R agonist at low doses, that selectively affect D3R but not D2R-mediated behaviors, we were able to accurately dissociate the effects of D1R, D2R and D3R activation on the behavioral deficits induced by a lesion of the DA nigrostriatal system.

We previously showed that anxiety- and depression-like behaviors induced by a DA SNc lesion can be reversed by levodopa and ropinirole.[10] SKF-38393, sumanirole and PD-128907 fully abolished the lesion-induced increase in anxiety observed in the EPM and exhibited beneficial effects in the FST, indicating a broad implication of DA receptors in the reversal of such affective impairments. It notably suggests that activation of both D2R and D3R may account for the reversal effects of ropinirole, a D2/D3R agonist, but that activation of D1R may, in addition, contribute to the effects of levodopa, which has, by its presynaptic mechanisms, no selective action on DA receptors. It has long been suggested that the DA function is involved in the physiopathology of depression and anxiety and as such, is considered as a potential target for the treatment of these disorders.[42–44] However, its
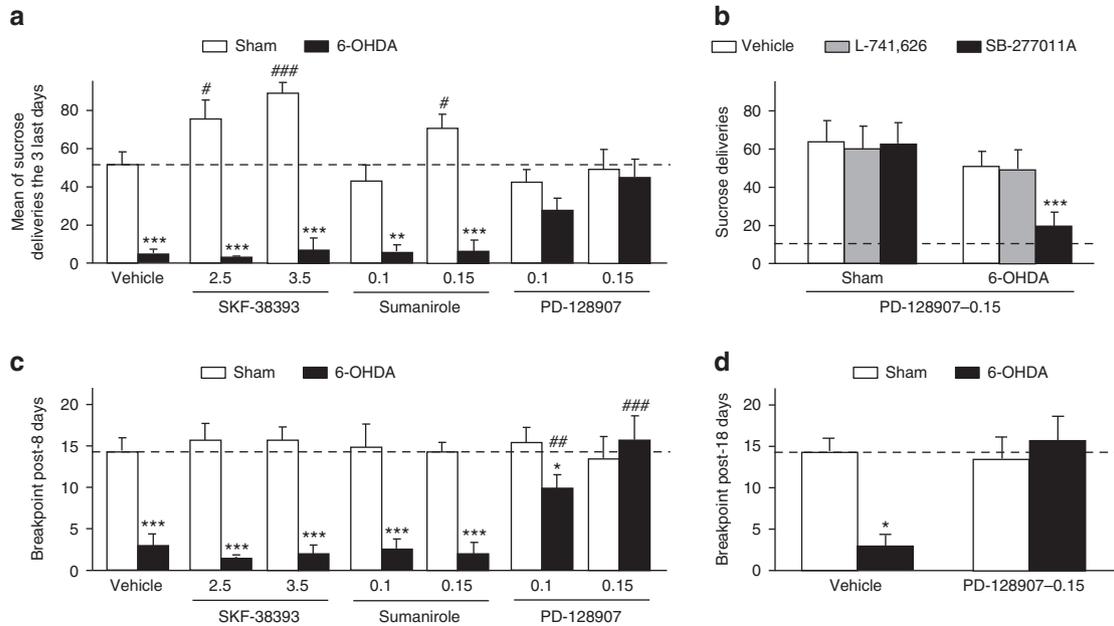
Figure 5. Reversion of the motivational deficits induced by 6-OHDA SNc lesions by the D3R agonist PD-128907 was blocked by the D$_3$R antagonist SB-277011A, but not by the D$_2$R antagonist L-741626. (**a**) Representation of the mean number of sucrose deliveries of the three last fixed ratio 1 (FR1) sessions in sham-operated (white histograms) and lesioned (black histograms) animals. Subchronic administration of PD-128907 (0.1 and 0.15 mg kg^{-1}), but not of SKF-38393 (2.5 and 3.5 mg kg^{-1}) or sumanirole (0.1 and 0.15 mg kg^{-1}), improved instrumental performances of the 6-OHDA-lesioned rats. (**b**) SB-277011A (10 mg kg^{-1}) but not L-741626 (1.5 mg kg^{-1}) blocked specifically this reversal effect of PD-128907 (0.15 mg kg^{-1}) in lesioned rats. (**c**) PD-128907, but not SKF-38393 or sumanirole enhanced the breakpoint (that is, last ratio completed) of 6-OHDA-lesioned rats after 8 days of sucrose self-administration. (**d**) A similar effect of PD-128907 (0.15 mg kg^{-1}) was observed after 18 days of sucrose self-administration. Dot lines represented the mean of the instrumental performances of the three last FR1 sessions of vehicle-treated sham animals (**a**), the mean of the number of sucrose deliveries of vehicle-treated 6-OHDA-lesioned animals during antagonists tests (**b**) or the mean of the breakpoint of vehicle-treated 6-OHDA-lesioned animals (**c–d**). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, sham-operated versus lesioned within the same treatment and #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ between the treatments for sham-operated and lesioned conditions, respectively ($n = 8$–21 per group). 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; SNc, substantia nigra pars compacta.

precise role has just begun to be clarified (for example, as seen in Tye et al.[45]). Notably, the specific role of the different DA receptor subtypes remains to be determined. Consistent with our data, loss of D1R in transgenic mice has been shown to promote anxiety-related behaviors,[18] and the antidepressant-like effects of DA agents in the FST are at least partly mediated by D1R,[16] identifying this receptor as a potentially interesting target for the treatment of affective disorders. The relationships between D2R and D3R and mood are less clear. Indeed, although D2/D3R agonists, such as pramipexole and ropinirole, have been shown to exhibit antidepressant and anxiolytic activities in preclinical models and clinical studies,[1,10,46,47] conflicting results have been yielded concerning the relative contribution of D2R and D3R,[15,17,47,48] probably due to the pre- and postsynaptic localization of these receptors[15,16,49] that frequently leads to dual, opposite functions. Our data suggest that, at least under pathophysiological conditions, associated with a loss of or a decrease in presynaptic function and a complex postsynaptic redistribution of D2R and D3R following DA denervation and treatments,[13,50,51] these receptors may be of particular interest for the treatment of affective-related disorders.

Such receptors modifications may also account for the strong anxiolytic properties that SKF-38393 and sumanirole acquired following DA denervation. Indeed, depending on the dose, the D1R and D2R agonist not only induced a dramatic increase in the time spent in the open arms of the EPM, but also strongly influences ethological factors related to disinhibition and risk-taking behaviors,[34,52] by increasing head dippings and exploration in nonprotected areas and reducing risk assessments. Although it deserves further investigation, these data may provide useful

information for understanding the iatrogenic mechanisms of DA medications that lead to the development of impulse control behaviors in PD.[53]

In addition to reversing affective impairments, PD-128907 was the only agonist which improved the instrumental deficits in lesioned rats, a beneficial effect blocked by a D3R, but not a D2R antagonist, thereby suggesting a specific implication of the D3R in the reversion of motivational impairments related to nigrostriatal DA denervation. It is also unlikely that the lack of effect of the D1R and D2R agonists resulted from the administration of too low doses. Indeed, the doses of SKF-38393, SKF-82958 and sumanirole used here have been shown to be behaviorally effective in previous studies,[26,28,29,54] as well as in the present work. It is particularly striking for SKF-38393 that, despite its lack of effect on 6-OHDA-lesioned animals, significantly increased operant performance under a continuous ratio in controls, consistent with the function of D1R in conditioned reinforcement.[20,55] Interestingly, this effect does not appear to be strictly motivational, as SKF-38393 did not lead to a higher breakpoint under a progressive ratio procedure. Taken with the results above, this differential implication of DA receptors suggests that the development of affective and motivational deficits following DA SNc lesioning may be underlain by dysfunctions of different neurobiological substrates. For instance, our previous study clearly associated the motivational impairments with striatal DA denervation, but mood alterations may result from a loss of DA signals in extrastriatal regions, such as the amygdala which also receives DA inputs from the SNc.[10]

Our data further suggest that D$_3$R is crucial for mediating the beneficial effects of DA agonists on the behavioral alterations

induced by nigrostriatal DA denervation. D_3R is thought to significantly contribute to the control of affective and motivated behaviors and as such, has been identified as a potential target for the treatment of neuropsychiatric symptoms in several disorders,[19] including PD.[56,57] However, the relationship between D_3R and motivation has been investigated mostly in the framework of drug addiction[21] and behavioral evidences of a wider involvement of this receptor in affective and motivational functions are still lacking. Our data strongly suggest a pivotal role of D3R in the control of mood and motivation and in behavioral dysfunctions related to the pathophysiology of PD. Overall, these data identify D3R as an interesting pharmacological target for the treatment of apathy and affective disorders, such as anxiety and depression in PD.

In conclusion, this study provides new insights into the specific roles of DA receptors in motivational and affective functions, highlighting the critical role of D3R in the beneficial effects of DA agonists on the nonmotor behavioral alterations associated with nigral DA neuronal loss. Our data therefore support the use of D_2/D_3R agonists as effective treatments for PD-related neuropsychiatric symptoms and put forward D3R as a promising therapeutic target.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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