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Dopamine D3 receptors: a potential target to treat motivational deficits in Parkinson's disease

Mathieu Favier, Carole Carcenac, Marc Savasta, Sebastien Carnicella

Parkinson's disease (PD), which is traditionally viewed as a motor disorder involving the degeneration of dopaminergic (DA) neurons, has recently been identified as a quintessential neuropsychiatric condition. Indeed, a plethora of non-motor symptoms may occur in PD, including apathy. Apathy can be defined as a lack of motivation or a deficit of goal-directed behaviors and results in a pathological decrease of self-initiated voluntary behavior. Apathy in PD appears to fluctuate with the DA state of the patients, suggesting a critical role of DA neurotransmission in the pathophysiology of this neuropsychiatric syndrome. Using a lesion-based approach, we developed a rodent model which exhibit specific alteration in the preparatory component of motivational processes, reminiscent to apathy in PD. We found a selective decrease of DA D₃ receptors (D₃R) expression in the dorsal striatum of lesioned rats. Next, we showed that inhibition of D₃R neurotransmission in non-lesioned animals was sufficient to reproduce the motivational deficit observed in our model. Interestingly, we also found that pharmacologically targeting D₃R efficiently reversed the motivational deficit induced by the lesion. Our findings, among other recent data, suggest a critical role of D₃R in parkinsonian apathy and highlight this receptor as a promising target for treating motivational deficits.

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Beyond the motor symptoms of Parkinson's disease

Parkinson's disease (PD) is classically described as a motor disorder (hypokinesia, rigidity and resting tremor) consecutive to the degeneration of dopaminergic (DA) neurons from the substantia nigra pars compacta (SNc) [1]. Beyond classical motor symptoms, motivational and affective deficits are frequently observed in PD, dramatically impairing the quality of life of patients [2]. A wide variety of non-motor symptoms have been described, including sleep disturbance, cognitive impairments, psychosis, anxiety, depression, apathy, or impulsive/compulsive disorders [2-4]. Fatigue is also common among parkinsonian patients which induces a lack of energy and turns into difficulty initiating and supporting voluntary activity [5]. PD is usually not diagnosed until motor symptoms develop, although non-motor manifestations including depression, sleep problems and loss of smell, typically begin years earlier [6]. It is now recognized that this cluster of non-motor disorders negatively affects PD patients and their caregivers to the same extent as motor symptoms [3, 7], making their understanding a central element of therapeutic management.

Apathy as a pivotal symptom in PD

Among neuropsychiatric symptoms, apathy (generally defined as a motivational deficit), appears to be a typical psychiatric feature of PD [2, 7]. Apathy is the most common psychiatric disorder in the early stages of untreated PD and can therefore be considered as an hypodopaminergic syndrome, which also includes anxiety and depression [8, 9]. Apathy incidence in PD varies from 16.5 to 70 %, depending on the assessment scale used and the population studied [2, 10-12]. Most patients affected by PD can exhibit apathy in parallel with the progression of the disease [2, 13]. However, it is difficult to accurately establish the prevalence of apathy in PD, as signs of apathy may overlap with signs of other cognitive dysfunctions [14]. Finally, incidence can vary significantly depending on the non-motor fluctuations: during the off period, up to 75% of patients exhibited apathetic behavior [15, 16].

How to define apathy in PD?

A clear definition of apathy is difficult to find in the literature. Apathy is described as a frequent associated feature of PD, like anxiety and depression, two affective impairments that are prevalently associated with apathy in this neurodegenerative disorder [2, 3, 17]. Apathy may

therefore be regarded as a non-specific symptom, emerging from a general degradation of cognitive functions, with negligible implications for assessment or treatment. However, several pieces of evidence suggest that apathy is a true clinical construct [18, 19]. Robert Marin in the 1990s defined apathy in neurological disorders as a symptom or a distinct psychiatric syndrome [18, 20]. Marin first proposed diagnostic criteria for apathy syndrome based on the construct of deficits in goal-directed behaviors or a primary lack of motivation [21]. Marin structured the clinical expression of apathy around behavioral, cognitive, and emotional domains that were operationalized as follows:

1. Diminished goal-directed behavior, with a lack of effort, energy, initiative, and productivity.
2. Diminished goal-directed cognition, with decrease interests, lack of plans and goals, and lack of concern about one's personal problems.
3. Diminished emotional concomitants of goal-directed behavior, with a flattened effect and lack of emotional response to positive or negative events.

It was also stated that the symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Since Marin's work, several psychometric scales of apathy were developed, especially for PD [22-24]. All these scales rely on the behavioral and cognitive dimensions of apathy, but opinions differ on the inclusion of an emotional dimension. For Marin's scale, the term "goal-directed behavior" should be viewed as a "behavior directed toward a goal" and not as a "behavior directed by a goal", as the second has a strong theoretical connotation referring to a specific psychobiological process and putative functional sub-compartmentalization of the dorsal striatum [25, 26].

Levy and Dubois then proposed that the term motivation should not be used to defined apathy, pointing out that such definition would be a psychological interpretation of a behavioral change [27]. Instead, they defined apathy as a quantifiable behavioral syndrome, consisting of a quantitative reduction of self-generated voluntary and purposeful behaviors. This aspect of lack of self-generated action is pivotal, as apathetic patients are able to function properly and to perform daily activities if they receive repeated external stimulation [27, 28], indicating a clear deficit in their capacity to initiate and maintain behaviors toward a specific goal. Levy and Dubois also suggested that apathy should not be considered as a unique syndrome, but rather proposed multiple forms of apathetic states subscribing different dysfunctions of the cortico-striatal circuits involved in the chain of processes, from intention to action [27]. Thus apathy could be related to a) disruption of emotional and affective processing (the reduction of goal-directed behaviors is due to an inability to associate these signals with ongoing and forthcoming behaviors), b) alteration of cognitive processing (impairment of the cognitive functions needed to plan and carry out goal-directed behaviors) or c) an auto-activation deficit (difficulties in activating thoughts or

initiating the motor programs necessary to perform the behavior) [27]. Accordingly, in an elegant study using an implicit incentive task, Schmidt and colleagues reported that apathetic patients (in a PD and non-PD population) show a strong alteration in the motivational processes responsible for translating an expected reward into effort and action, but no change in the perception of reward value [29]. Specifically, apathetic patients were unable to modulate hand-grip force in order to obtain a monetary incentive, in function of its size, while their sensitivity to the relative value of the incentive was preserved [29]. Hence, these data and recent clinical observations reveal that apathetic state in PD may be specifically linked to the anticipatory component of anhedonia, which is a lack of association between pleasure and a specific action, without any change in consummatory responses reflecting the capacity of patients to experience pleasure when engaged in an enjoyable activity [30, 31].

Overall, these studies indicate that at least some forms of apathy in PD are related to dysfunctions of preparatory, but not consummatory, subcomponent of motivated behaviors.

Apathy and its relation with the dopaminergic state of PD patient

Apathetic symptoms as well as mood disorders such as depression or anxiety, are often reported even before the onset of motor symptoms, or early in the disease, in untreated PD patients [2, 8, 32, 33]. Apathy incidence and severity are also typically rising in patients undergoing surgical treatment of PD (deep brain stimulation of the subthalamic nucleus, STN-DBS) [13, 34], particularly in cases of strong reduction of DA medication [17]. At the opposite, apathetic symptoms can be treated at different stages of the disease using the DA precursor levodopa or DA D_2/D_3 receptor (D_2/D_3R) agonists, such as ropinirole or pramipexole [7, 17, 35-39]. These clinical observations therefore indicate that, at least some forms of apathy in PD depend on the DA state of the patients, suggesting an important role of DA neurotransmission in the pathophysiology of this non-motor symptom [17, 39, 40]. In line, functional imaging studies in humans have reported an association between PD-related apathy, as well as anxiety and depression, and the extent of DA denervation in several brain regions, including the ventral and dorsal striatum and the prefrontal cortex [17, 41, 42].

Thereby, the resurgence of apathy observed during STN-DBS might be the consequence of a DA withdrawal-like syndrome, secondary to the reduction of pharmacotherapies [17]. However, as previously described, several forms of apathy resulting from different neurobiological dysfunctions exist, and at least two types of apathy can be observed in PD: (1) a fluctuating apathetic state directly related to a hypodopaminergic state resulting from the DA neuronal loss, and that can be revealed by the decrease in DA medication associated with STN-DBS, and (2) a more protracted apathy, resistant to DA medication and observed in the chronic stage after surgery [13, 43, 44]. Some researchers have proposed that postoperative apathy may be directly

linked to STN-DBS [13, 39, 43]. In two longitudinal studies, Verin and coworkers found no association between the increase of apathy following STN-DBS surgery and the reduction in the DA medication dose, arguing against the aforementioned hypothesis [43, 45, 46]. In Drapier's study, the stimulation electrodes were placed more ventrally in the STN of apathetic than non-apathetic DBS patients [43], suggesting that apathy could be due to the diffusion of electric current to the limbic sub-compartment of the STN [45]. In addition, other neurotransmitters than DA may be involved in the pathogenesis of apathetic symptoms, including the well-described degeneration of noradrenergic and serotonergic systems in PD patients [47].

The pathogenesis of apathy in PD remains difficult to decipher. For instance, there are no clear mechanisms of action of DBS on the basal ganglia loops that could account for a direct effect on mood and motivation [40, 48]. Moreover, it is almost impossible to clearly distinguish the role of the DA denervation and the effect of DBS since it has been reported that STN-DBS also directly impacts DA function [49, 50]. Nevertheless, exploring this question with preclinical approaches makes it possible to separately examine potentially interacting factors. In the laboratory, we recently showed that chronic STN-DBS in rats diminishes reward seeking and basal activity in both control animals and in a model of PD-neuropsychiatric symptoms, without any DA medication [51]. Thus, our study provides evidence that STN-DBS by itself could promote loss of motivation reminiscent to apathy in PD. Of note, we also demonstrated that pramipexole was effective to reverse the STN-DBS motivational deficit, arguing for a mechanism involving D_2/D_3 receptors, in line with our previous observation of a decreased expression of these receptors under acute STN-DBS [50].

Neurobiological mechanisms underlying apathy: a pivotal role of the dopaminergic system

Most studies have linked apathy, and its related affective disorders, to the DA denervation of the mesocorticolimbic system occurring in PD patients [40, 52]. Clinically, estimation of DA neuronal loss within the VTA in post-mortem studies varied from 30 to 60% [53], leading to a ~50% DA denervation of the ventral head of the caudate nucleus of PD patients in the late stages of the disease [54]. Interestingly, Torack and Morris [55] found a partial DA loss in the mesolimbic system exclusively in depressed, but not in non-depressed PD patients. Functional imaging studies also suggest that apathy, anxiety, and depression in PD are associated with a DA hypofunction within the ventral striatum [17, 42]. Using complete mesolimbic lesions in animals models, studies have shown a reduction of motivated behaviours [56, 57], consistently with the pivotal role of DA mesolimbic system in motivational processes [58, 59].

On the other hand, several data suggest that the partial loss of DA neurons in the VTA is not severe enough, especially in the early stages of the disease, to induce strong neuropsychiatric symptoms such as apathy [27]. Instead, parkinsonian apathy could arise directly from the loss of DA neurons in the SNc, the main nucleus initially affected in the disease. In fact, beyond its role in motor functions, the DA nigrostriatal system is actually strongly implicated in the control of motivated behaviors [25, 26, 60-62]. Strikingly, depression and anxiety symptoms have been also found specifically associated with a greater DA denervation affecting the putamen of PD patients [41].

How to disentangle potential implication the mesocorticolimbic and nigrostriatal dopaminergic systems?

In the laboratory, we developed a lesion-based model using stereotaxic bilateral injections of the neurotoxin 6-hydroxydopamine (6-OHDA) into discrete areas of the rat SNc or VTA, to selectively induce degeneration of the DA nigrostriatal and/or mesocorticolimbic systems, respectively. SNc and VTA 6-OHDA lesions resulted in distinct, non-overlapping, complementary patterns of DA denervation (**Figure 1**) and DA loss throughout striatal territories [63, 64]. Importantly, infusion of the neurotoxin can lead to a 40–60 % tyrosine hydroxylase immunoreactivity (TH-IR) loss in the nucleus accumbens (NAc), reproducing the partial denervation of the ventral striatum observed in PD [54]. Our study was aimed at determining whether such limited DA loss would impact motivational processes.

The SNc lesions also led to a partial DA denervation in the dorsal striatum (**Figure 1**). The loss of TH-IR was predominant in the lateral striatal portion, but remained below 80 % [63], leading to a 70 % decrease in basal extracellular DA levels in the dorsal striatum, with no changes in the NAc [64]. This partial denervation was crucial for preventing the severe alterations of the motor functions that usually occur for denervation around or above 80 % [65, 66]. This approach allowed us to study specifically the role of the nigrostriatal DA system in motivational and affective processes, in the absence of a potential bias related to major locomotor impairments. Using an exhaustive battery of tests, we consistently found that our partial DA SNc lesion did not induce significant motor alterations [63, 64].

Bilateral and partial dopaminergic lesion of the nigrostriatal projection specifically affects motivated and affective-related behaviors

Because it is suggested that apathy in PD is related to preparatory, but not consummatory, behavioral deficits, we used various non-operant and operant tasks to distinguish between the potential effects of the lesions on these two sub-components of motivated behaviors.

We showed that bilateral and partial DA lesions of the SNc, but not of the VTA, dramatically impaired operant responding for obtaining a sucrose solution (**Figure 2**). The absence of effect of the partial DA mesocorticolimbic lesion on motivation was confirmed under a progressive ratio schedule of reinforcement [63]. This result is in line with other data showing that complete lesions of the mesocorticolimbic system are necessary to decrease motivated behaviors [56, 57]. In addition, we found a robust negative correlation between operant performances and the loss of TH-IR within the dorsal striatum (**Figure 2**), strongly supporting the implication of the DA nigrostriatal system in motivational processes.

The reduced behavioral response of SNc-lesioned animals could not be attributed to an impairment in instrumental learning since their capacities to discriminate between a reinforced and non-reinforced lever were preserved [63]. Moreover, this behavioral deficit was observed after the full acquisition of the instrumental task, confirming that a learning impairment did not account for this effect [64]. Furthermore, the reduced behavioral response of SNc-lesioned rats could not be attributed to a decrease in the sensitivity to the rewarding properties of sucrose since rodents demonstrated a clear preference for the sucrose solution in a two-bottle choice procedure (**Figure 2**). This indicates that partial DA SNc lesions do not affect hedonic, consummatory processes, but rather selectively alter preparatory behaviors. This selective effect of the SNc DA lesion on preparatory behaviors, was also confirmed in two other operant tasks, namely the runway task and cue-light self-administration. In a runway task (evaluating the progressive reduction in the latency to reach a palatable food at the end of a straight alley), acquisition was similar for all groups but SNc DA lesions exhibited decreased asymptotic performance compared to controls (**Figure 2**). This indicates that motivation to reach the goal but not learning was affected by the DA SNc lesion. Finally, cue-light acted as a robust reinforcer in both VTA-lesioned and controls animals, but not in SNc-lesioned rats (**Figure 2**).

In contrast, neither VTA nor SNc lesions impaired preference for a novel environment in a non-instrumental novelty place preference procedure (**Figure 2**). The marked motivational deficit observed in animals with partial DA SNc lesions was only present when an instrumental preparatory action was required.

As aforementioned, apathy in PD is frequently associated with anxiety and depression. We then also investigated affective-related behaviors. We found that SNc-, but not VTA-, lesioned rats displayed reduced social interaction, anxiety-related behaviors in an elevated plus-maze, and a dark/light avoidance test, as well as a depression-related behavior, as reflected by an increase in the time spent immobile in a forced-swim test [63].

Pharmacological reversion of the behavioral deficits of SNc-lesioned rats highlights a critical involvement of dopamine D₃ receptor

The various functions of DA are mediated by different DA receptor subtypes and depend on their neuronal and brain localizations [67]. Moreover, expression and function of these DA receptors can be strongly affected by DA denervation, with potential pathophysiological implications in PD [68, 69]. To further examine the role of DA in our rodent model of parkinsonian apathy, we tested whether sub-chronic systemic administration of different pharmacological DA agents classically used in PD could correct the behavioral impairments induced by partial DA SNc lesions.

Anxiety- and depressive-like symptoms displayed by SNc-lesioned rats were fully reversed by L-Dopa and the D₂/D₃R agonist ropinirole, as indicated by a reversal of the reduction in time spent in the open arms of the elevated plus-maze and immobility in the forced swim-test (**Figure 3**) [63]. In addition, ropinirole was the only pharmacological agent that significantly improved instrumental performances of SNc-lesioned rats under a fixed- or a progressive-ratio of reinforcement, in an operant sucrose self-administration procedure (**Figure 3** and [63]). The efficacy of D₂/D₃R agonists in reversing the motivational deficits induced by the SNc lesions was confirmed with the use of pramipexole in the same operant procedure [64]. Notably, discontinuation of pramipexole treatment induces the resurgence of motivational deficits [64], thereby mimicking the re-emergence, or worsening, of apathetic symptoms when DA medication is reduced or withdrawn in PD patients [17].

The beneficial effects of D₂/D₃R agonists on the motivational deficits induced by DA SNc lesions are likely to be mediated specifically by the D₃R subtype. Indeed, while subchronic administration of a D₁R (SKF-38393), D₂R (Sumanriole), or D₃R agonist (PD-128907) fully reversed the anxiety- and depression-related behaviors induced by the SNc lesions (**Figure 3** and [70]), only the D₃R preferential agonist reversed the deleterious impact of the SNc lesion on operant sucrose self-administration (**Figure 3**). The absence of effect of SKF-38393 and Sumanriole was particularly striking, as both agonists dose-dependently improved the instrumental performances of sham animals (**Figure 3**), indicating that the lack of effect was most likely not due to insufficient dosage. Moreover, the effect of PD-128907 in SNc-lesioned animals was selectively blocked by the D₃R antagonist SB-277011A, but not the D₂R antagonist L-741,626 [70], confirming the D₃R-mediated action of the agonist.

Taken together, these data strongly suggest a pivotal role of D₃R in motivational processes. This is consistent with earlier evidence that this receptor contributes to the control of

affective and motivated behaviors and mediates the therapeutic effect of DA medication on the neuropsychiatric symptoms in PD [71].

Autoradiographic analysis reveals down-regulation of D₃R in the dorsal striatum of SNc-lesioned rats

Using our rodent model reminiscent of parkinsonian apathy, we performed a semi-quantitative autoradiographic analysis to evaluate the changes of D₁, D₂ and D₃ receptors (D₁R, D₂R and D₃R) expression induced by SNc lesion (**Figure 4**) [64]. We found a selective decrease in D₃R levels within the dorsal striatum of SNc-lesioned rats. Neither D₁ nor D₂ receptors expression was modified by the nigrostriatal lesion. In physiological conditions, D₃R expression is high in the NAc shell, intermediate in the NAc core and lower, but detectable, in the dorsal striatum [72, 73]. Due to this low expression, the study of the possible modification of D₃R expression in the dorsal striatum following PD-related DA lesions remains challenging and has given conflicting results. For instance, previous studies reported a decrease or no change in dorsostriatal D₃R expression in DA-depleted rats, depending on the method of detection and analysis [74-76]. In this study, using an adapted protocol to detect changes of expression in brain areas where D₃R expression is low [77, 78], we demonstrated that D₃R is specifically downregulated in the DLS after bilateral SNc 6 OHDA-lesion (**Figure 4**).

In the striatum, D₃R can mediate the action of DA as a post-synaptic receptor or exert an inhibitory effect on DA release as a putative pre-synaptic receptor [67]. However, D₃R autoradiographic binding and mRNA levels are highly colocalized in the striatum, thereby suggesting that these localizations primarily correspond to dendrites or soma of striatal neurons rather than to long axon terminals from distant neurons [79] and the decreased D₃R expression in the present study is associated with a phenotype related to a loss of DA function (observed in SNc-lesioned rats), reproduced by the pharmacological blockade of dorsostriatal D₃R. Thus, our data suggest that the loss of D₃R was due, at least in part, to changes of expression in post-synaptic neurons. The implication of the D₃R subtype in PD-related non-motor deficits has rarely been investigated in animals [80]. Because modulation of the D₃R function influences motivated behaviors [81, 82], we hypothesized that downregulation of this receptor within the dorsal striatum may participate in the motivational deficits induced by the DA SNc lesion.

Intracerebral infusions of a D₃R antagonist confirm the causal implication of dorsostriatal D₃R in the motivational deficits of SNc-lesioned rats

In order to test the hypothesis, we decided to functionally reproduce the decrease in expression of D₃R in control rats. We found that pharmacological blocking of the D₃R within the dorsal striatum, but not in the NAc, in non-lesioned rats mimicked the behavioral deficits induced by partial and bilateral SNc DA lesions, suggesting that the motivational deficits observed in SNc-lesioned rats are causally related to the functional downregulation of dorsostriatal D₃R. Indeed, our data show that selective blockade of D₃R, with the antagonist SB-277011A, within the dorsal striatum in non-lesioned rats impaired operant sucrose self-administration, and specifically the maintenance of the operant response, without affecting the rewarding properties of the reinforcer, as observed in lesioned rats (**Figure 5**). In addition, we confirmed that the behavioral effects of SB-277011A were not mediated by D₂R, as infusion of a selective D₂R receptor antagonist, L-741,626 [83], in the dorsal striatum, had no effect on the operant performances of the animals [64].

Although both blockade of D₃R in the dorsal striatum and SNc DA lesions impaired the maintenance of operant behaviors, the effect on operant performance of the latter [63, 64] was stronger than of the former. Moreover, the curves showing the pattern of operant activity across time appear similar, but not identical. In addition to the decrease of D₃R expression revealed by autoradiography in the present study, SNc-lesioned rats also exhibited a dramatic reduction (~70%) of extracellular DA levels in the dorsal striatum [64]. These cumulative DA dysfunctions in 6-OHDA rats may account for these differences, indicating that pharmacological blockade of dorsostriatal D₃R may reproduce only a part of the deficits induced by the SNc lesion.

Taken together, these data clearly emphasize a critical, while probably non-exclusive, implication of dorsostriatal D₃R in the pathophysiology of motivational deficits in PD patients related to apathy.

Distinct functional roles of D₃R in the dorsal striatum and nucleus accumbens

With the notable exception of experimental studies on drugs of abuse and L-Dopa-induced dyskinesia [73-75], the role of dorsostriatal D₃R in motivational processes remains poorly investigated because of its low basal level of expression within this area.

We show that infusion of the selective D₃R antagonist in the dorsal striatum specifically alters the maintenance, but not the initiation of operant sucrose self-administration (**Figure 5**). This effect is highly reminiscent of the “extinction-like” effect observed after systemic [58] or dorsostriatal, but not accumbal, intracerebral infusions of broad-spectrum DA antagonists at moderate doses [84]. However, and contrary to the initial postulate of Wise [85], a decrease in the

hedonic/rewarding effects of sucrose cannot account for this result. Indeed, the effect of the D₃R antagonist on maintenance of operant behaviors was observed even in absence of the reward, and neither consummatory behaviors nor preference for the sucrose solution in a two-bottle choice procedure were affected by the dorsostriatal infusion of the D₃R antagonist. Therefore, our data clearly demonstrate that blocking or reducing D₃R transmission within the dorsal striatum induces a specific deficit in goal-oriented behavior.

According to the concept of incentive salience developed by Berridge and collaborators [86], DA signaling is necessary for transforming the “neutral” perception of a conditioned stimulus, or a goal object at a distance into an attractive incentive capable of eliciting appetitive or instrumental behaviors towards it (wanting). During motivational tasks, incentive salience assignment to reward-related stimuli and actions are maintained or strengthened by the presentation of the reinforcer (i.e, a correct prediction has been made). It is proposed that DA mediates this “reboosting” effect so that the reinforcer and associated cues remains “wanted” at later occasions [86, 87]. Based on this data, we hypothesize that blockade of dorsostriatal D₃R may induce a specific impairment of “reboosting” processes, accounting for the early termination in instrumental activity reported in the present study. Consistently, Howe et al. showed that prolonged tonic DA signals, or “ramps”, detected by fast-scan cyclic voltammetry in the dorsal striatum could provide the motivational drive needed to maintain instrumental behavior [88]. Besides, the dorsal striatum and especially its lateral part have also been shown to mediate the properties of reward-related stimuli to stimulate operant responding [89]. Our findings suggest that, despite a limited level of expression, dorsostriatal D₃R may have a critical functional implication in these DA-mediated processes.

In contrast to the dorsal striatum, infusion of a D₃R antagonist into the NAc did not affect the maintenance of instrumental behaviors, but rather induced a discrete decrease in the rate of responses for sucrose (**Figure 6**). The effects related to D₃R blockade in the NAc are consistent with the literature since in operant tasks with low ratio requirement, as in the FR1 procedure used here, only slowing in the rate of responding and increased tendency to pause are observed after blockade of DA receptors or DA depletion in the NAc [90, 91]. The differential implication of accumbal and dorsostriatal D₃R in instrumental behaviors and motivational processes deserves further investigation, notably by relying on cue-driven reward-seeking procedures [92, 93].

Conclusion

Our work, along with other lesion-based studies [94], selective optogenetic [95-99] and chemogenetic (Goutaudier and Carnicella, unpublished data) modulation of SNc DA neurons,

reinforces the role of the nigrostriatal DA system, and specifically the dorsolateral striatum, in motivational processes. The precise role of nigrostriatal DA neurotransmission remains however to be elucidated. A cohort of recent studies suggests that dorsostriatal DA may be more involved in an alerting aspect (response to sensory events that reflect surprise) and instrumental reinforcement, i.e. signaling movement, while that ventrostriatal DA contributes to reward prediction error and reward identity prediction, i.e. signaling reward, as different parts of an actor/critic model [61, 88, 97-99]. Alternatively, the variety of DA signals within the striatum may be interpreted as a modulation of resource allocation decisions. In the dorsolateral striatum the resource would be movement (decide to move or not to move), in the dorsomedial striatum the resources could be cognitive processes (attention and working memory) and in the NAc the resource might be time (engage in a task means that other beneficial ways of spending time must be foregone) [100]. In addition, our findings emphasize a critical role of dorsostriatal D₃R neurotransmission in the pathophysiology of PD-related neuropsychiatric disorders. Consistently with recent clinical and preclinical data suggesting that stimulation of D₃R may have important beneficial effects in apathy and depression in PD [38, 50, 70, 101-104], our findings clearly designated D₃R as a promising target for the treatment of apathy and other affective disorders in PD patients.

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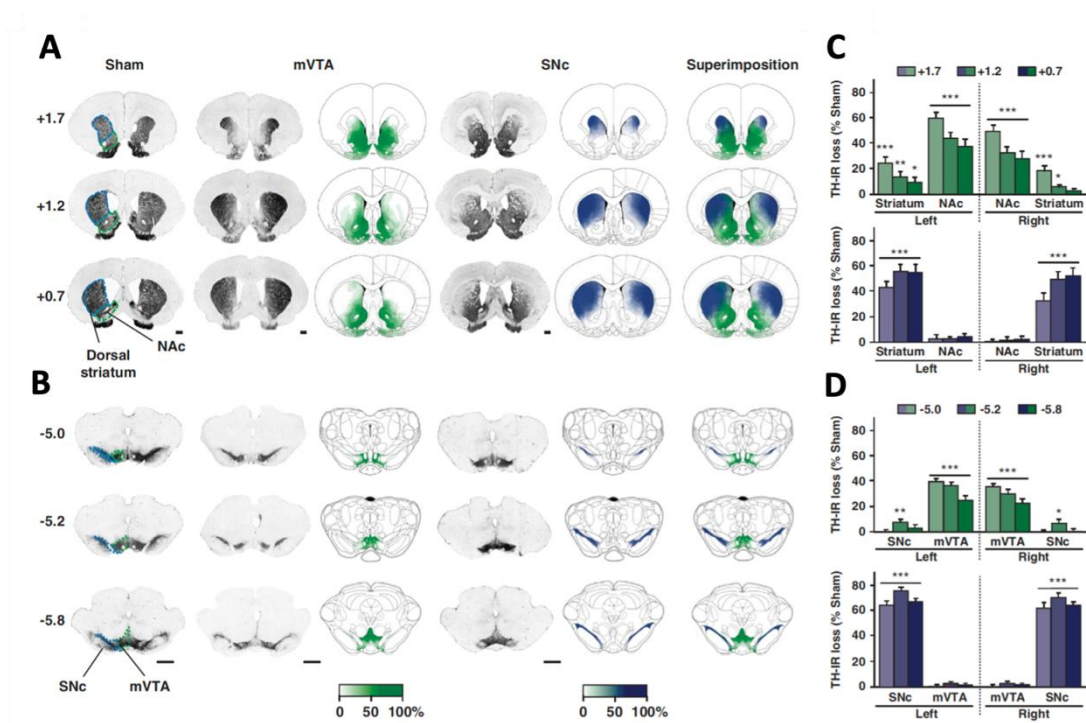


Figure 1. Bilateral partial lesions of the mVTA or SNc result in distinct, non-overlapping, complementary patterns of dopaminergic (DA) depletion throughout striatal territories.

(A, B) Representative photomicrographs of coronal sections stained for tyrosine hydroxylase (TH) in striatal (1.7 to 0.7mm anterior to bregma) (A) and mesencephalic (-5 to -5.8mm anterior to bregma) (B) regions according to the stereotaxic atlas of Paxinos and Watson. Bar: 1 mm. The intensity of the gradient of color (white to green or white to blue) in schematic sections corresponds to the measured DA-lesioned area in the different brain structures studied for each lesion performed. mVTA lesion is shown in green and SNc lesion in blue. The highest intensity of green or blue color (100%) indicates that all animals had lesions in the corresponding area, whereas the lowest color intensity (white, 0%) corresponds to a non-lesioned or denervated area. (C, D) Quantification of the loss of TH staining in the different mesencephalic (C) and striatal (D) structures, expressed as percentage of the mean value obtained for sham-operated animals. Two-way ANOVAs revealed significant interactions between the lesion and the brain region considered ($F_s > 9.49$, $P_s < 0.001$). $n = 22-28$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. IR, immunoreactivity; mVTA, medial ventral tegmental area; NAc, nucleus accumbens; SNc, substantia nigra pars compacta. From Drui et al., 2014.

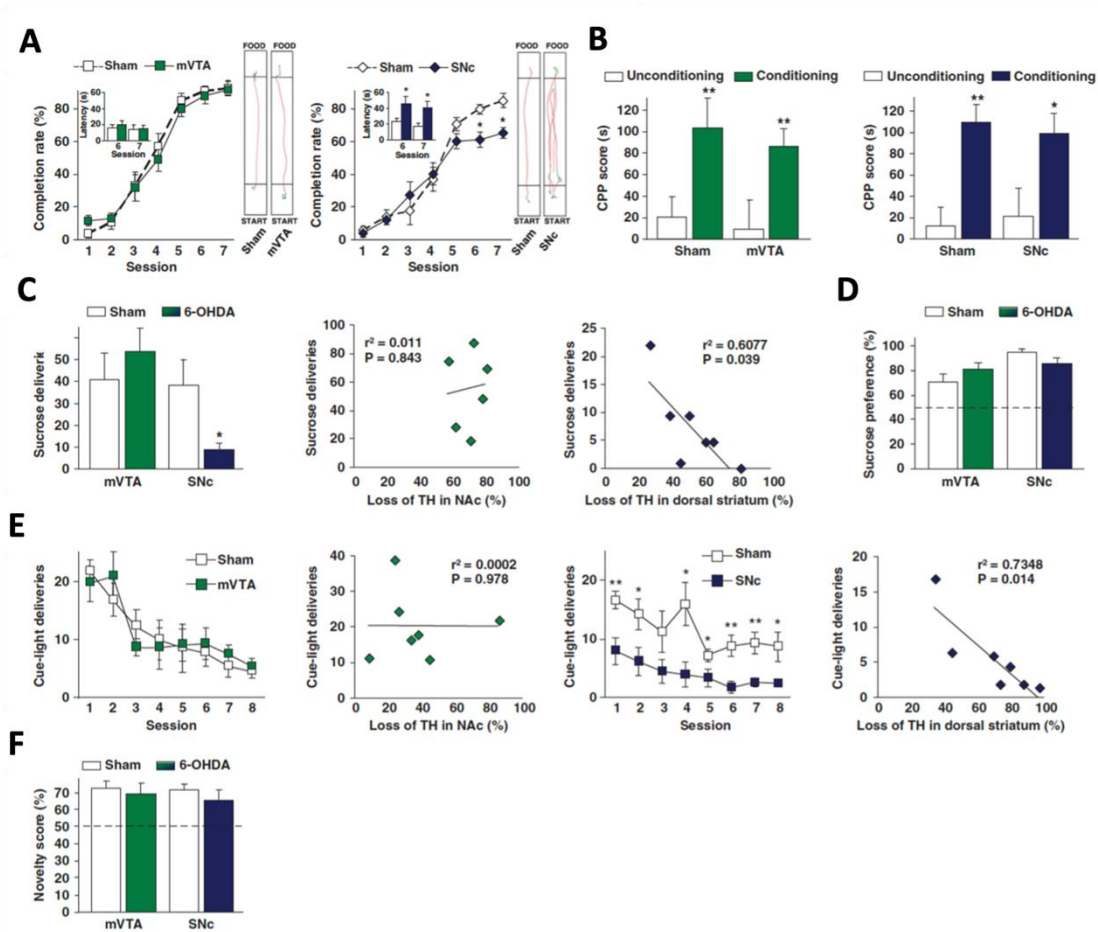


Figure 2. Bilateral partial 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra pars compacta (SNc), but not of the medial ventral tegmental area (mVTA), impair motivated behaviors.

(A, B) SNc (lesion x session interaction: $F_{6,108} = 2.65$, $P < 0.02$, $n = 19-22$) but not mVTA (lesion x session interaction: $F_{6,66} = 0.84$, $P = 0.55$, $n = 12-15$) lesions increased the latency to reach a palatable food in a runway paradigm at the asymptotic level (A), with no incidence on conditioning place preference (CPP) for the same reward (B, effect of conditioning: $F_s > 11.61$, $P_s < 0.001$, no effect of lesion: $F_s < 0.50$, $P_s > 40.48$ and no interaction: $F_s < 0.16$, $P_s > 0.69$, $n = 10-17$). (C, D) SNc (* $P < 0.05$) but not mVTA ($P=0.41$) lesions decreased operant sucrose self-administration (c, representation of the mean of the three last fixed ratio 1 sessions (first graph) and linear regressions between sucrose deliveries and the loss of tyrosine hydroxylase (TH) in the nucleus accumbens (NAc) (second graph) and the dorsal striatum (third graph) for mVTA and SNc lesions, respectively, $n=6-9$), while having no significant effect on sucrose preference in a two-bottle choice procedure (D, $P_s > 0.08$, $n=12-19$). (E) SNc but not mVTA lesions (effect of lesion: $F_{1,98} = 14.1$, $P < 0.01$ and $F_{1,91} \cdot 0.01$, $P = 0.94$, respectively, $n = 7-8$) reduced self-activation of a cue-light during an operant procedure. Linear regressions between cue-light deliveries and the loss of TH in the NAc and the dorsal striatum for mVTA and SNc lesions, respectively. (F) No effect of the 6-OHDA lesions were found on the preference for a novel environment ($P_s > 0.73$), $n = 6-8$. * $P < 0.05$, ** $P < 0.01$, sham-operated vs lesioned. From Drui et al., 2014.

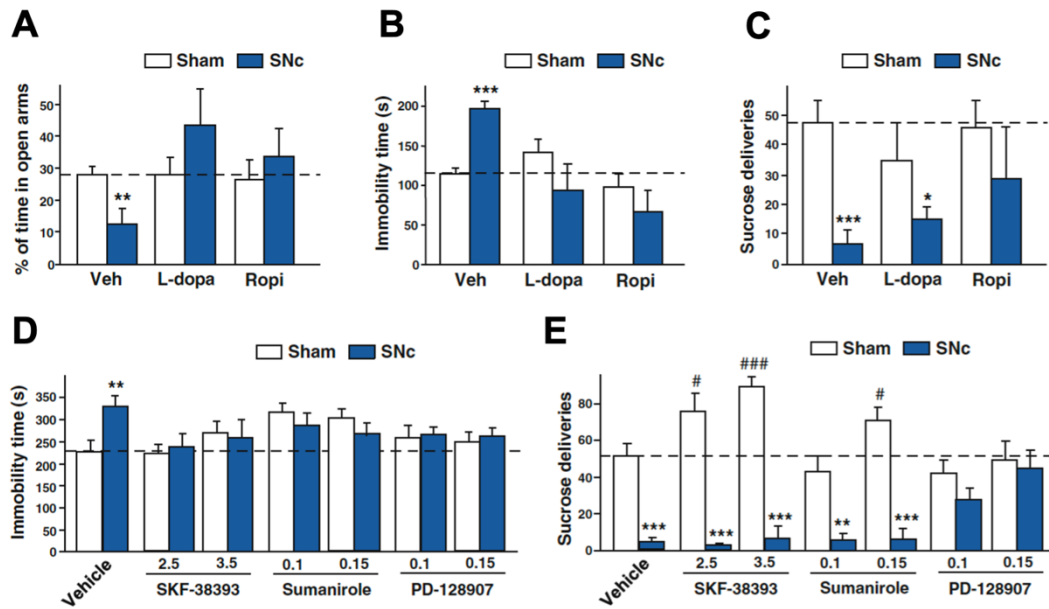


Figure 3. Pharmacological reversion suggests a pivotal role of D_3R in motivational deficits of SNc-lesioned rats. (A-E) Effects of i.p. sub-chronic administration of L-Dopa (12.5 mg/kg), Ropinirole (Ropi, 1 mg/kg) or vehicle (veh) were evaluated in an elevated plus-maze (A), a forced-swim test (B) and in an operant sucrose self-administration procedure (C), $n = 6-11$. (D-E) Effects of i.p. sub-chronic administration of SKF-38393 (2.5 and 3.5 mg/kg), Sumanitrole (0.1 and 0.15 mg/kg), PD-128907 (0.1 and 0.15 mg/kg), or vehicle were evaluated in a forced-swim test (D) and in an operant sucrose self-administration procedure (E), $n = 8-21$. Data are expressed as mean \pm SEM. Dotted lines represent the mean of the behavioral performances of vehicle-treated sham animals. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, sham-operated versus lesioned within the same treatment and # $p < 0.05$, ### $p < 0.001$ between the treatments for sham-operated and lesioned conditions respectively. SNc substantia nigra pars compacta, TH tyrosine hydroxylase, i.p. intraperitoneal. Adapted from Drui et al., 2014 and Carnicella et al. 2014.

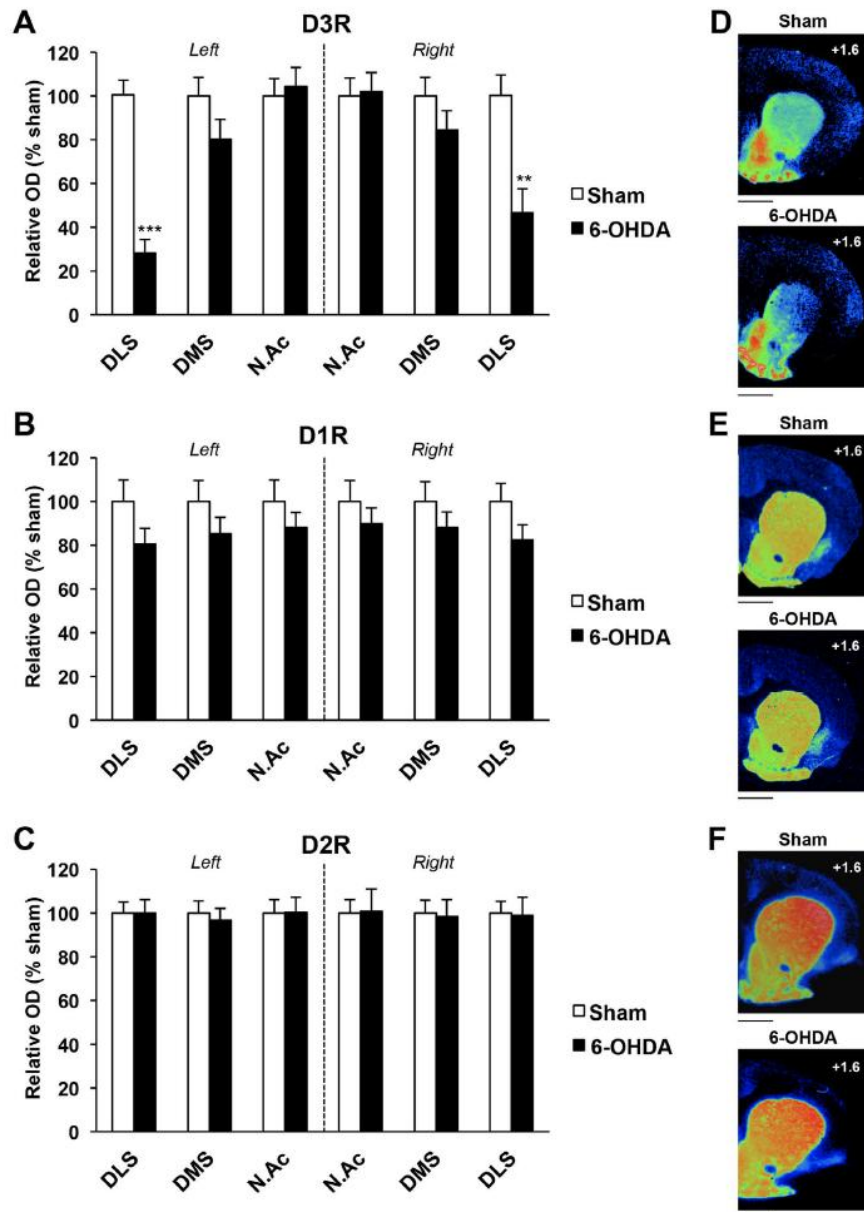


Figure 4. Bilateral 6-OHDA SNc lesion induces a selective decrease of D₃R expression in dorsolateral striatum. (A-C), Mean \pm SEM optical density (expressed as arbitrary units) of D₃R, D₁R and D₂R receptor binding density at striatal level, as measured by semi-quantitative autoradiography in sham and 6-OHDA lesioned rats. SNc lesions induced a marked decrease of D₃R binding, specifically within the dorsolateral striatum. Two-way ANOVAs and post-hoc analyses with the method of contrasts were used. **p < 0.01, ***p < 0.001, sham (n = 8) vs 6-OHDA (n = 7). DLS: dorsolateral striatum; DMS: dorsomedial striatum; NAc: nucleus accumbens. (D-F) Photographs of autoradiograms obtained at striatal level for sham and 6-OHDA-lesioned rats, colorized using Autoradio V4.03 Software. D, D₃R; E, D₁R; F, D₂R. AP levels are indicated from bregma. Scale: 1mm. AP: Anteroposterior. **From Favier et al., 2017.**

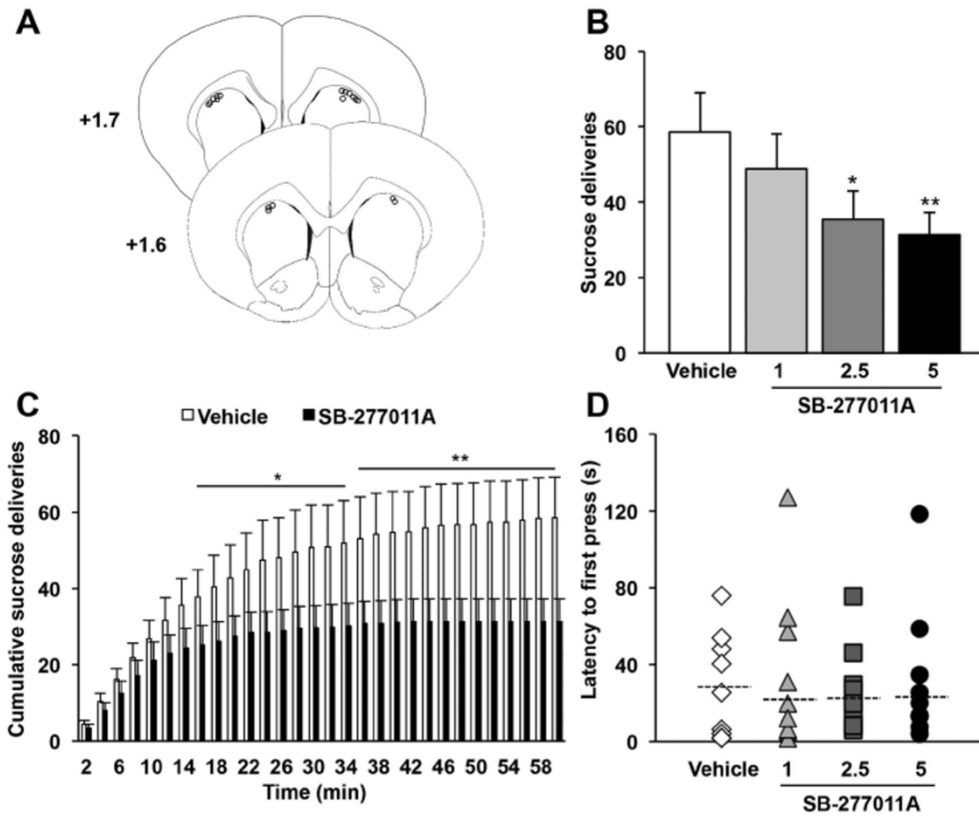


Figure 5. Intracerebral

infusion into the dorsal striatum of a selective D₃R antagonist decreases operant sucrose self-administration.

(A) Diagrams showing the locations of each individual guide cannula for the animals included in behavioral experiments (n = 9). The circles represent the tips of the microinjectors, visualized on coronal sections counterstained with cresyl violet. AP levels are indicated in mm from bregma. (B) Mean ± SEM number of sucrose deliveries over 60-minute sessions. Dorsostriatal infusion of the D₃R antagonist SB-277011A dose-dependently decreased instrumental performance for sucrose self-administration. One-way repeated ANOVA and post-hoc analyses with Student-Newman-Keuls test were used. *p < 0.05, **p < 0.01, vehicle vs SB-277011A (n = 9 per group). (C) Mean ± SEM number of cumulative sucrose deliveries within 2-minute bins over 60 minutes sessions. SB-277011A administration (5 μg μl⁻¹) specifically affected maintaining the instrumental response while no effect was seen during the first part of the procedure. Two-way repeated ANOVA and post-hoc analyses with the method of contrasts were used. *p < 0.05, **p < 0.01, vehicle vs SB-277011A (n = 9 per group). (D) Median latencies for the first active lever press (dotted bars) and individual values. SB-277011A administration did not modify the latency for the first active lever press (Friedman test). Each microinjection experiment with vehicle vs SB-277011A was conducted by using a counterbalanced within-subject design. AP: Anteroposterior. From Favier et al., 2017.

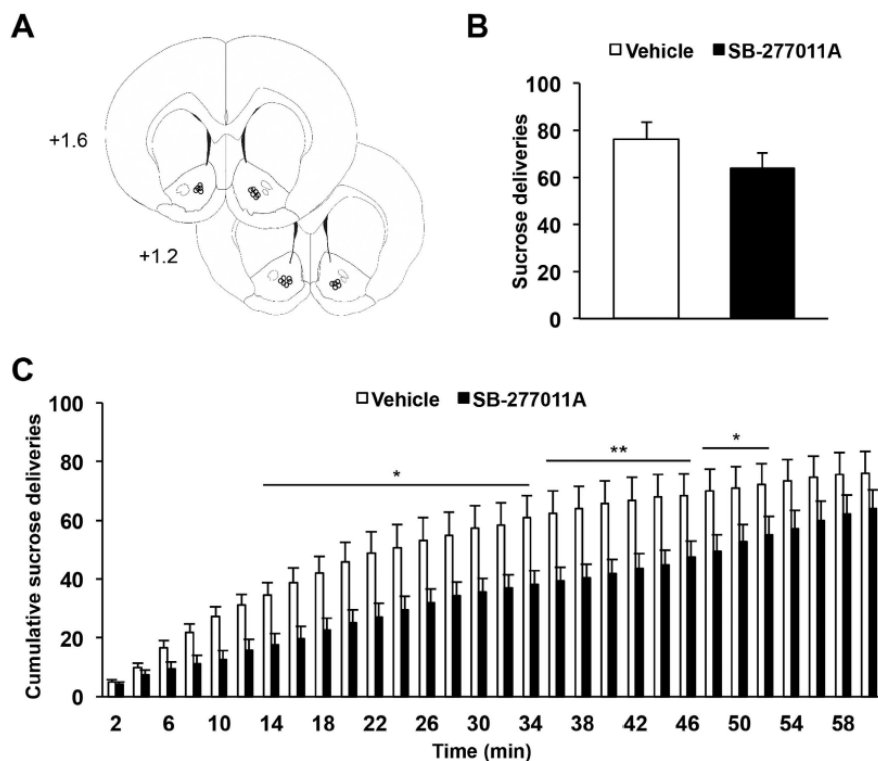


Figure 6. Intracerebral infusion of a selective D₃R antagonist into the nucleus accumbens does not reduce operant sucrose self-administration.

(A) Diagrams showing the locations of each individual guide cannula of animals included in behavioral experiments (n = 11). The circles represent the tips of the microinjectors, visualized on coronal sections counterstained with cresyl violet. AP levels are indicated in mm from bregma. (B) Mean \pm SEM number of sucrose deliveries over 60 minutes sessions. (C) Mean \pm SEM number of cumulative sucrose deliveries within 2 minutes bins over 60 minutes operant self-administration sessions. Intra-accumbal infusion of SB-277011A (5 μ g, μ l-1) did not modify instrumental performance for sucrose self-administration over the entire duration of the test, but instrumental performance of the animals was temporarily decreased in the middle period of the test. Two-way repeated ANOVA and post-hoc analyses with the method of contrasts (cumulative number of sucrose deliveries) or t-test (total number of sucrose deliveries) were used. *p < 0.05, **p < 0.01, vehicle vs SB-277011A (n = 11 per group). Each microinjection experiment was conducted by using a counterbalanced within-subject design. AP: Anteroposterior. **From Favier et al., 2017.**