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Interplay Between Inhibitory Control and Behavioural Flexibility: Impact of Dorsomedial Striatal Dopamine Denervation in Mice

Juliette Lhost, Simon More, Isabelle Watabe, Didier Louber, Abdel-Mouttalib Ouagazzal, Martine Liberge and Marianne Amalric*

Aix Marseille Univ, CNRS, Marseille, France

Abstract—In Parkinson's disease, nigrostriatal dopamine (DA) degeneration is commonly associated with motor symptomatology. However, non-motor symptoms affecting cognitive function, such as behavioural flexibility and inhibitory control may also appear early in the disease. Here we addressed the role of DA innervation of the dorsomedial striatum (DMS) in mediating these functions in 6-hydroxydopamine (6-OHDA)-lesioned mice using instrumental conditioning in various tasks. Behavioural flexibility was studied in a simple reversal task (nose-poke discrimination) or in reversal of a two-step sequence of actions (central followed by lateral nose-poke). Our results show that mild DA lesions of the DMS induces behavioural flexibility deficits in the sequential reversal learning only. In the first sessions following reversal of contingency, lesioned mice enhanced perseverative sequence of actions to the initial rewarded side then produced premature responses directly to the correct side omitting the central response, thus disrupting the two-step sequence of actions. These deficits may be linked to increased impulsivity as 6-OHDA-lesioned mice were unable to inhibit a previously learned motor response in a cued response inhibition task assessing proactive inhibitory control. Our findings show that partial DA denervation restricted to DMS impairs behavioural flexibility and proactive response inhibition in mice. Such striatal DA lesion may thus represent a valuable animal model for exploring deficits in executive control documented in early stage of Parkinson's disease.

Key words: Parkinson's disease, non-motor symptoms, behavioural flexibility, inhibitory control, dopamine, dorsomedial striatum.

INTRODUCTION

The loss of nigrostriatal dopaminergic (DA) neurons plays a crucial role in the physiopathology of Parkinson's disease (PD) (Kish et al., 1988; Del Tredici and Braak, 2012; Politis and Niccolini, 2015). This neuronal loss results in the occurrence of motor deficits including akinesia, bradykinesia, muscle rigidity and tremor at rest when the level of DA depletion in the striatum reaches 50–80% (Dauer and Przedborski, 2003). In addition, patients with PD may also exhibit cognitive deficits early in the disease, when DA neuronal loss is presumably less important, impairing attentional processes, inhibitory control and behavioural flexibility (Cools et al., 2001; Chaudhuri and Schapira, 2009; Chaudhuri and Odin, 2010; Voon and Dalley, 2011; Jahanshahi et al., 2015). Flexibility, a key component of executive function, requires the ability to adapt quickly to environmental changes. Inflexibility can result from two distinct deficits: an inability to inhibit an old strategy no longer relevant (perseverative deficit) and/or an inability to implement a new strategy adapted to the changing environment (regressive deficit). Inflexibility is also linked to an overall deficit of inhibitory control (Izquierdo and Jentsch, 2012; Bari and Robbins, 2013). Behavioural flexibility is often assessed in reversal learning tasks in PD patients or in animal models of PD (O'Neill and Brown, 2007; Buelow et al., 2015; Grospe et al., 2018) and critically relies on frontal cortex-basal ganglia circuits. Preclinical studies, in rodent or non-human primate models of PD, found that behavioural flexibility is impaired following DA depletion of the dorsal striatum (O'Neill and Brown, 2007; Haik et al., 2008; Clarke et al., 2008; Tait et al., 2017). However, to what extent these flexibility deficits, observed in PD patients at an early stage, result from an impairment of proactive inhibitory control remains an open question.

A low baseline of dopamine level in early PD may in some instance be associated with impulsive behaviour (Aarts et al., 2012), although impulse control disorders are usually observed in 15–17% of PD patients after long-term treatment with dopaminergic receptor agonists (Evans et al., 2005; Weintraub et al., 2010; Voon and

*Corresponding author. Address: Aix-Marseille University, CNRS, UMR7291, Laboratoire de Neurosciences Cognitives (LNC), Case C, 3, place Victor Hugo, 13331 Marseille cedex 03, France.
E-mail address: marianne.amalric@univ-amu.fr (M. Amalric).

Abbreviations: 6-OHDA, 6-hydroxydopamine; DA, dopamine; DLS, dorsolateral striatum; DMS, dorsomedial striatum; ITI, intertrial interval; PD, Parkinson's disease; SNc, substantia nigra pars compacta; TH, tyrosine hydroxylase.

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Dalley, 2011; Garcia-Ruiz et al., 2014). Previous studies conducted in animals highlight the involvement of the mesolimbic dopaminergic system (Robbins, 2002; Dalley et al., 2007) and dopamine signalling in the dorsal striatum in impulse control disorders (Agnoli et al., 2013) but do not allow to conclude on the involvement of medial or lateral part of the striatum in these effects. We therefore investigated the effect of a restricted DA denervation of the dorsomedial striatum on behavioural flexibility in mice trained in operant reversal learning tasks. We then addressed the question whether the flexibility deficits produced by such lesions could be related to a global impairment of inhibitory control when mice are tested in the cued response inhibition task. This task assesses aspects of proactive inhibition control related to preparation and initiation of motor action by measuring the ability of the animals to withhold a pre-learned motor response during a signaled response inhibition period. To rule out gross locomotor impairments produced by striatal DA denervation, we also assessed locomotor and exploratory activity of lesioned animals.

EXPERIMENTAL PROCEDURES

Animals

Mice were housed in groups of 4–5, with food and water ad libitum in a temperature-controlled room (24 °C) on a 12:12 h dark–light cycle (lights on at 07:00). All procedures were approved by the French National Ethical Committee (authorization no. 00196.01) and in accordance with the recommendations of the EEC (2010/63/UE) for care and use of laboratory animals. In line with our previous work (Ztaou et al., 2018), we used $Rosa^{eNpHR/+}$::$ChAT^{cre/+}$ male and female mice, with a Bl6/C57J background, genetically modified to express halorhodopsin in cholinergic neurons, to first assess the effects of DMS DA lesion on operant behaviour then of striatal cholinergic interneurons inhibition by optogenetics (study not presented here).

Stereotaxic surgery

Mice were deeply anaesthetized with intraperitoneal (i.p.) injections of ketamine and xylazine (100 and 10 mg/kg, respectively) in a volume of 10 ml/kg and mounted on a stereotaxic apparatus (David Kopf Instruments, California, USA). An analgesic (Buprenorphine, Vetergesic®, 0.05 mg/kg) was administered subcutaneously at least 30 min before surgery.

Apparatus

Behavioural tasks were conducted in four operant chambers housed in a sound-attenuating cubicle, equipped with a fan to provide ventilation and mask extraneous noise (25 × 25 × 20 cm, Med associates, St Albans, Vermont, USA). The front wall of the chamber was fitted with nose-poke holes (two or three according to the task), positioned 2.5 cm above the grid floor and equally spaced. Individual LEDs positioned in each nose-poke hole were used to illuminate each location according to the task parameters. An incandescent houselight and a central food magazine, by which food reinforcement (sucrose pellet, 20 mg, TestDiet, Saint Louis, Missouri, USA) was delivered by a pellet dispenser, were located in the back wall. Entries into the magazine and nose-pokes were detected by infrared beams crossing each opening horizontally.

Experimental design

Three cohorts of naïve mice were tested in the different operant behavioural tasks.

The first cohort of mice (n = 24) was tested for the effects of striatal DMS DA lesions on acquisition and reversal learning of a *single nose-poke discrimination* task. They first received 6-OHDA lesion (sham n = 10; 5 females, 5 males and 6-OHDA n = 14; 8 females, 6 males) and were then tested for the acquisition of the task. As no impact of DA lesion was observed, they were then tested for reversal of the task contingencies. The second cohort of mice (n = 28) was first trained to acquire a *sequence of two consecutive responses* and then received 6-OHDA lesion (sham n = 14; 3 females, 11 males and 6-OHDA n = 14; 6 females, 8 males). They were then tested for reversal learning of the learned sequence of responses after recovery of baseline performance. The third cohort of mice (n = 32) was first trained to perform a *cued response inhibition* task and then received 6-OHDA lesion (sham n = 12; 7 females, 5 males and 6-OHDA n = 20; 7 females, 13 males). They were then tested in response inhibition task followed by an extinction task. Subjects of cohorts 2 and 3 were further tested in locomotor activity cages at different postoperative days: 21 (sham n = 12; 6-OHDA n = 21) and 35 (sham n = 8; 6-OHDA n = 7) to assess potential motor deficits produced by 6-OHDA lesions.

Operant behavioural procedures

Food restriction. Three to five days prior to the onset of training in operant tasks, mice received restricted access to food in their home cages. This food regimen was sufficient to achieve a 10–15% decline of their free-feeding weight at the onset of testing and to motivate animals to perform the appetitive operant tasks. Standard laboratory chow amount, provided daily after testing, was adjusted for each mouse to maintain the subjects at 85–90% of their free-feeding body weight.

Magazine training. All mice first underwent magazine training for 2 to 3 sessions. This consisted in 30 sucrose pellets delivery in the magazine under a random time schedule (30–90 s). Magazine entries were recorded and used as an indicator that mice had learned the location of sucrose pellet delivery.

Reversal learning: Single nose-poke discrimination. Acquisition phase: After magazine training, mice were trained to respond in one of the two illuminated nose poke apertures to be reinforced. At the beginning of the session, a green cue-light was turned on for 5 s

in each of the two nose-poke apertures, equally placed left, and right side of the front wall. Only a response in a rewarded side (left or right hole counterbalanced in the group) triggered sucrose pellet delivery and counted as a *correct response*. Nose poking into the other aperture was counted as *incorrect response* and had no consequences. No response during the 5 s-cue ON period was counted as an *omission* and followed by a 5 s-intertrial interval (ITI) during which no reinforcement was available. The house light was turned ON throughout the session, except during reward delivery. Animals were tested in 15 min-daily sessions and trained until they achieved a criterion of $\geq$70% correct choices for three consecutive sessions. Mice that did not reach a level of 10 trials/session were excluded from the experiment (sham n = 1, 6-OHDA n = 3). Each session ended after 15 min.

Reversal learning phase: After reaching the performance criterion on the initial acquisition phase, mice were tested for reversal of the reinforcement contingencies. Hence, a correct response required a nose-poke in the opposite hole to the one previously reinforced in the initial acquisition stage. All mice completed the reversal phase.

Behavioural measures: Correct responses were calculated as the percentage of responses out of the total number of trials [100 $\times$ (number of correct responses/number of trials)]. The percentage of omissions [100 $\times$ (number of omissions/number of trials)] and total number of responses (i.e. nose-poke) and completed trials were also measured.

Reversal learning: sequence of two consecutive responses. Mice underwent food restriction as mentioned before, then followed different training before surgery.

Central nose-poke: The front wall of the chamber was fitted with three nose-poke apertures (one central and two adjacent left and right). After magazine training, animals were trained to nose-poke in the central illuminated (yellow visual cue) aperture to obtain a sucrose pellet. Mice were training daily for a 30-min session. Repeated nose-pokes in the central hole or nose-poke in any other aperture earned a time-out with house-light ON for 8 s during which no response was allowed. When mice earned a minimum of 30 pellets, they were trained in the next procedure.

Acquisition of the sequence: This consisted of 30-min training session per day in which mice were trained to perform a sequence of two nose-poke responses to be rewarded. Each trial started by the central nose-poke aperture illuminated as before. Nose-poke in the central aperture turned off the light and triggered illumination of a green visual cue in the two flanking apertures. A sequence of responses in the central hole then in *any* flanking aperture turned off the green light cue and triggered food pellet delivery. Five seconds after collection of the pellet in the magazine, the central hole was illuminated initiating a new trial. In the subsequent training phase, only a response into the left *or* right aperture was rewarded. For each mice, the reinforced sequence (central-left or central-right response) was selected based on its side preference developed in the previous training phase. The preference for response in the left or right side was overall equally distributed among subjects. There was no ITI in the training session, each trial was initiated by animal poking into the central aperture and ended after a response in a lateral nose-poke, triggering cue-lights extinction. Training was carried daily until mice learned the two-step sequence of actions by earning a minimum of 30 pellets per session. Mice then received stereotaxic lesion surgery. After a two-week recovery period, mice were submitted first to a reversal learning of the initial sequence of responses until they reached a stable baseline of 70% correct responses for four consecutive sessions. Mice with no side preference (below 60% of responses in one side) or unable to learn the task (less than 10 trials/session) were excluded from final analysis (n = 3 and n = 6 for sham and 6-OHDA group respectively). Repetitive nose-pokes in the central aperture or direct nose-pokes in any lateral aperture with no central responses first (termed premature responses) were not rewarded and followed by a time-out with house-light ON for 10 s.

Two-step sequential reversal learning: When mice reached stable baseline performance (70% correct responses) for four consecutive sessions, they were submitted to a second reversal learning that corresponds to the initial reinforced sequence of responses (central-left or central-right response). Correct new sequences of responses were rewarded by a food pellet. Incorrect sequence of responses (i.e. perseverative responses on the initial rewarded side or in the central hole) earned a time-out with all lights extinguished for 3 s. Premature response in either side, omitting the central nose-poke, was followed by a time-out (house-light ON for 10 s).

Behavioural measures. The percentage of correct sequences were calculated out of the total number of trials [100 $\times$ (number of correct sequences/number of trials)]. Perseverative responses on the previously rewarded sequence of responses were calculated as a percentage [100 $\times$ (number of incorrect sequences/number of trials)]. Perseverative responses to the incorrect sequences were further subdivided as *perseverative error*, if the incorrect sequence occurred *before* three consecutive correct sequences were made, and *regressive error*, if it occurred *after* three consecutive correct sequences. Premature responses (i.e. direct response to a lateral nose-poke aperture) were calculated as a percentage of premature responses to the novel rewarded side (termed% premature correct) or to the previously reinforced side (termed % premature incorrect).

Cued response inhibition task. In the cued response inhibition task, each chamber was equipped in the front wall with a central cue-light, flanked by two lateral nose-poke response holes (on the right or left side). In this test, adapted from Olmstead et al. (2009) and Simon

et al. (2013), animals must inhibit a response (nose-poke in a lit hole) during a waiting phase, indicated by the illumination of a central visual cue for a variable duration (pseudorandomly selected length of 2, 4, 6, 8 and 10 s). This test allows to measure the animal's ability to inhibit a response that has not yet been initiated (proactive motor inhibition). Mice were trained, in 30-min daily sessions, for three consecutive phases.

In *phase* 1 and *phase* 2, mice were submitted to a simple appetitive instrumental learning task. A trial was initiated by illumination of both left and right nose-poke apertures (green LED cue-light) while the house-light was turned off. A single nose-poke into the rewarded side (correct response) elicited sucrose food pellet delivery, cue-light extinction and house-light turned ON until mice collected the pellet. Assignment of the rewarded hole was counterbalanced among the animals. Nose-pokes into the incorrect aperture had no consequence. Training progressed until mice obtained 25 rewards by session and reached a minimum performance of 80% correct responses in three sessions. In Phase 2, illumination of nose-poke apertures was reduced to 5 s and trials started after a fixed inter-trial interval (ITI) of 30 s. Only responses in the illuminated hole within 5 s were rewarded. Training progressed to phase 3 when animals performed a minimum of 80% of reinforced nose-pokes and obtained a minimum of 20 rewards for 3 consecutive sessions. In *phase* 3 (i.e. cued response inhibition test), a 15-s ITI was followed by a response inhibitory period (waiting phase) during which the central cue-light was turned ON for variable durations (pseudo-randomly generated length of 2, 4, 6, 8 and 10 s). Nose-poke into the rewarded port during this waiting phase were recorded as premature responses (failure to appropriately withhold an action), earned a timeout period of ITI with house light OFF and extinction of light-cues. When the inhibitory period was completed, the two nose-pokes were illuminated for 5 s and a response in the rewarded side was reinforced. If the animal did not respond within the 5-s response period, it was counted as an omission and resulted into ITI, house light and light-cue OFF. Phase 3 testing continued until animals obtained a minimum of 30 rewards for 3 consecutive sessions. When mice reached a stable baseline level of 60% correct response, they underwent stereotaxic 6-OHDA surgery. After a two-week recovery period, mice were tested for 11 consecutive sessions.

Extinction task. In order to evaluate the animal's ability to inhibit a response previously rewarded but no longer reinforced, mice were tested for an extinction phase. Experimental parameters were identical to the phase 3 of the cued response inhibition task, except that a correct response was no longer rewarded.

Behavioural measures. Different parameters were measured to evaluate the performance: percentage correct responses [100 × (number of correct responses/total number of trials)], percentage omissions [100 × (number of omissions/number of trials)], percentage premature responses [100 × (number of premature responses/number of trials)], total number of nose-pokes during ITI, correct response latency (time in s between the onset of the stimulus and a nose-poke in the correct hole), and latency to collect reward (time in s between a correct response and a poke into the food magazine). In addition, the percentage of correct and premature responses was plotted as a function of the inhibitory cue durations.

Locomotor activity. Testing was carried in a rack of eight actimetry cages (20 × 11 × 17 cm, Imetronic, Bordeaux, France). The rack was enclosed in a dimly lit sound-attenuating cubicle. Each cage was fitted with two frames containing infrared detectors positioned at 2 and 8 cm from floor. Interruptions of photocell beams located in the lower and upper frames provided automated measures of locomotor activity and rearing behaviour, respectively. The session lasted 60 min photocell beam interruptions were recorded every 5-min bins.

Histology, immunohistochemistry and microscopy

Animals were deeply anesthetized with pentobarbital (100 mg/kg) and then transcardially perfused with an ice-cold solution of paraformaldehyde 4% in PBS. After dissection, brains were post-fixed overnight in the same fixative at 4 °C, cryoprotected in 30% sucrose dissolved in 1X PBS for an additional 36 h at 4 °C and frozen. Coronal cryostat sections (40 μm) covering the antero-posterior extent of the substantia nigra *pars compacta* (SNc) were used for labeling. Sections were incubated overnight at 4 °C with mouse anti-tyrosine hydroxylase antibody (TH) (1/1000, Millipore, MAB318). They were then incubated in Alexa Fluor 594 goat anti-mouse (1/500, Invitrogen, A11005). Immunostaining was done on free-floating sections, which were then mounted onto SuperFrost Plus glass slides (VWR) and coverslipped with Roti®-Mount FluorCare mounting medium (Carl Roth). Six to eighteen images of TH-immunostained alternated slices from each animal were then acquired on a fluorescence microscope (Leitz Aristoplan light microscope) equipped with a Nikon high resolution digital camera (756 × 581 pixels; Nikon, Tokyo, Japan) with a 10x objective, interfaced to a PC computer and Image software (Lucia, Nikon). The number of TH-positive DAergic neurons in SNc was quantified in sections taken from −2.80 to −3.28 mm from bregma. Slices were chosen to cover the entire rostral to caudal extension of the SNc. TH-positive cells in the SNc were counted manually in four regions of interest (ROIs, 1100 × 1100 μm) per hemisphere, using the cell counter plugin of FIJI software (ImageJ, National Institutes of Health). ROIs were determined to cover the whole medio-lateral extension of the structure based on the stereotaxic mouse atlas (Paxinos and Franklin, 2001). Cells number was calculated as the mean number/ROI.

We also examined qualitatively DA depletion extent in the dorsal striatum in a few subjects. Coronal sections (40 μm) covering the anteroposterior extent of the

dorsal striatum were incubated overnight at 4 °C with mouse anti-tyrosine hydroxylase antibody (TH) (1/1000, Millipore, MAB318). Thereafter, they were incubated with a biotinylated secondary antibody (goat anti-mouse, 1/200; Jackson Immunoresearch; 115-065-003) for 1 hour and then in a solution containing 0.01% DAB (3,3-diaminobenzidine) and 3% H_2O_2 diluted in PBS 1× for 3 min. Sections were finally mounted on slides as described before. Images were acquired using a bright field microscope (Nikon Leica DMLB) at 10× magnification.

Statistical analyses

Histological data on TH+ neurons were presented as median and first and third quartiles for sham and lesion group. Box-and-whisker plots show median, first/third quartile, and maximum/minimum values. Statistical evaluations were performed using a non-parametric when appropriate. Two-way repeated measures ANOVA evaluated the loss of DA neurons in the SNc at different anteriority levels with groups (sham/lesion) as the independent between-factor and anteriority level as the within-factor. Behavioural data, presented as mean ± standard error of the mean (SEM), from the different operant tasks were recorded using Med-PC software. Two-way repeated measures ANOVA with groups (sham/lesion) as the independent between-factor and time as the within-factor (training sessions or time-bins for locomotion) was used in the different tests. When significant, multiple comparisons (Sidak post-hoc analyses) were used to evaluate differences between groups at different time points. Student's *t*-test compared performance of two independent groups. $P < 0.05$ was considered as significant for all analyses. Latencies to collect reward exceeded 80 s after surgery in three subjects (sham $n = 1$; 6-OHDA $n = 2$) compared to the rest of the group (mean latency = 1 s). Data on this parameter for the three subjects were excluded in the pre- and post-operative sessions. Statistical analyses were performed using Prism6 (GraphPad Software Inc., La Jolla, USA).

RESULTS

Histology

Fig. 1A illustrates representative sections of the dorsal striatum showing tyrosine hydroxylase (TH) immunohistochemical staining (DAB staining) of sham and 6-OHDA lesioned mice. The loss of TH-positive fibers was circumscribed to the dorsomedial part of the striatum (DMS) extending from +0.74 to −0.10 mm relative to bregma, according to Paxinos and Franklin's atlas (2001). Fig. 1B illustrates the extension of lesion size in the striatum (shaded gray of largest to smallest size) in animals from the different cohorts. The lesions did not extend to the ventral striatum and DA innervation in dorsolateral striatum (DLS) was mostly spared (Fig. 1B). Analysis of TH-positive cells (TH immunofluorescent staining) in the SNc, from −2.92 to −3.40 relative to bregma, revealed a relatively partial DA neuronal loss (average 30%), as illustrated in Fig. 1C. TH-positive cells were still present in the lateral part of the SNc and in the ventral tegmental area (VTA). Quantification of TH-positive cells in regions of interest (ROI) at four anteriority levels of the SNc (Fig. 1C) showed a similar DA neuronal loss at the different anteriority levels [ANOVA main group effect ($F_{1,153} = 77.51$, $p < 0.0001$), no effect of anteriority ($F_{3,153} = 0.146$, $p = 0.93$), nor group × anteriority interaction, post-hoc multiple comparison Sidak test $p < 0.001$ at each anteriority level]. Post-hoc test revealed a significant decrease of TH-positive cells in 6-OHDA lesioned mice relative to controls when collapsed over anteriority levels (*t*-test, $t = 6.502$, df49, $p < 0.0001$, box-and-whisker plots, Fig. 1D).

Effect of DMS DA denervation on single nose-poke reversal learning

Partial DA depletion of the DMS did not alter acquisition of a single nose-poke discrimination. Sham and lesioned mice similarly and progressively improved their performance across the 10th first sessions (Fig. 2A). Overall, the two groups needed 15 sessions to reach the criterion of 70% correct responses (sham, 15 ± 1.46; 6-OHDA, 14.18 ± 1.27; Student's *t*-test, $p = 0.67$, Fig. 2A inset). Mean number of trials at the first session did not differ between sham and 6-OHDA groups (45.11 ± 0.75 vs 44.09 ± 0.99). At the last session of acquisition, shams reached 48.78 ± 1.40 trials and lesioned mice 48.64 ± 1.27 trials (Table 1). As the percentage of correct responses increased over sessions, percentage of omissions gradually decreased (Fig. 2A). Two-way repeated measures ANOVA revealed a significant main effect of sessions on % correct responses and omissions ($F_{9,162} = 29.09$ and 26.84, $p < 0.0001$, respectively) but no main effect of lesion ($F_{1,18} = 0.17$ and 0.26, $p = 0.6$), nor a training session × lesion interaction ($F_{9,162} = 0.5$ and 0.76, $p = 0.6$, respectively). Mice were then submitted to reversal learning to assess the impact of DMS DA denervation on behavioural flexibility. Both sham and lesioned mice significantly decreased the percentage of correct responses in the first two sessions following reversal of task contingency compared with the last acquisition session (paired t test, in sham and 6-OHDA groups, $t = 10.8$, df8 and $t = 20$ df10, respectively $p < 0.0001$) to the same extent (no group effect $F_{1,18} = 0.63$, $p = 0.43$) (Fig. 2B). The two groups recovered their pre-reversal performances after a few training sessions and no significant difference was detected between groups ($F_{1,18} = 0.82$, $p = 0.4$, Fig. 2B). The percentage of omissions was not modified by the rule inversion whatever the group ($F_{1,18} = 0.13$, $p = 0.7$, Fig. 2B). Similarly, the total number of trials completed in the first reversal session was comparable between sham and 6-OHDA groups and tended to remain stable with the progression of the training (Table 1).

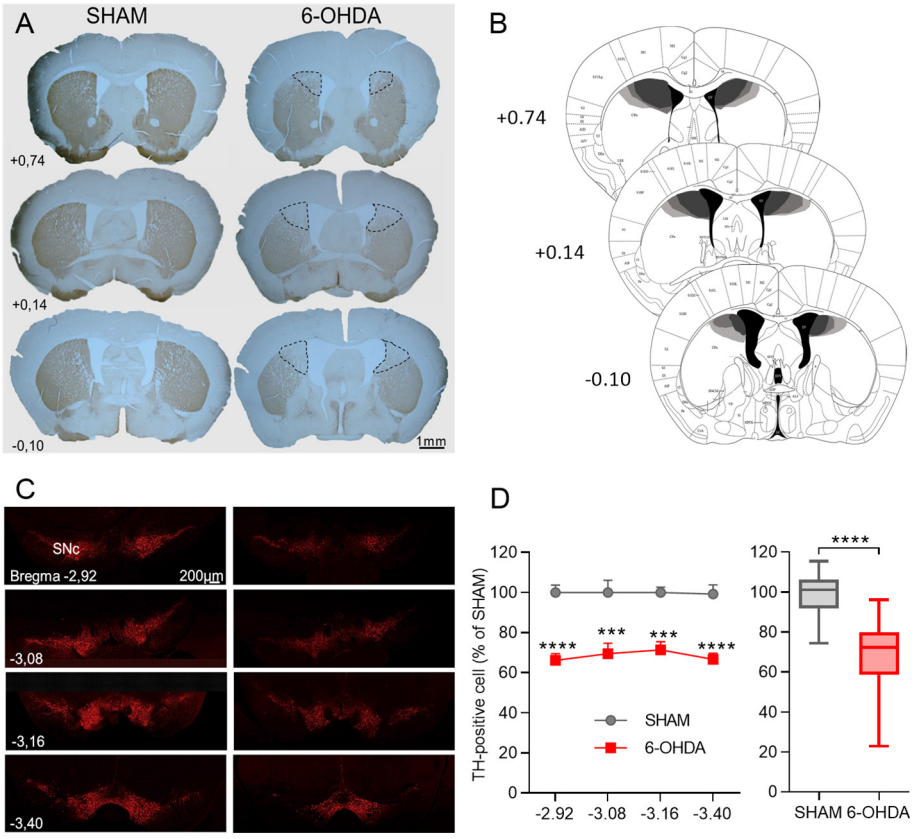

Fig. 1. Bilateral 6-hydroxydopamine (6-OHDA)-induced dopaminergic lesions of the striatum. **(A)** Loss of tyrosine hydroxylase (TH) immunoreactivity in the dorsal striatum at three anteriority levels: +0.74, +0.14, −0.10 mm (Paxinos and Franklin's atlas, 2001) in coronal sections of representative control (SHAM, left) or bilateral 6-OHDA-lesioned mice (6-OHDA, right). Dotted lines delineate the extension of TH immunoreactivity in the dorsomedial striatum. Scale bar: 1 mm. **(B)** The extension of 6-OHDA lesions from the largest (light gray), intermediate (dark gray) to the smallest (black) size is illustrated in coronal sections at the same anteriority levels. **(C)** Coronal immunofluorescence images showing TH-positive cells in the substantia nigra pars compacta (SNc) of representative control (SHAM, left) and lesioned mice (6-OHDA, right). Scale bar: 200 µm. **(D)** Quantification of TH-positive cells in the SNc of control (SHAM $n = 15$) and lesioned (6-OHDA $n = 37$) mice taken from the three experimental cohorts. Left: There was a significant decrease of TH immunoreactive cells (expressed as mean ± SEM percentage of sham) in 6-OHDA lesioned compared with control mice at four anteriority levels within SNc (−2.92, −3.08, −3.16, −3.40 mm related to bregma). Sidak post-hoc analysis ***$p < 0.001$, ****$p < 0.0001$, 6-OHDA *vs* sham group following significant two-way ANOVA. Right: box-and-whisker plots representing median values, first/third quartile, and maximum/minimum values collapsed over the four anteriority levels. Post-hoc *t*-test ****$p < 0.0001$.

Effect of DMS DA denervation on two-step sequential reversal learning

We next asked whether the impact of DMS DA denervation on behavioural flexibility depends on the difficulty of the reversal learning. A novel cohort of sham and lesioned mice was therefore tested in a sequential reversal learning task in which a sequence of actions, consisting of a central nose-poke followed by a response in a lateral aperture (left or right to the central one), was rewarded if performed in the opposite side to the initial training. When the two groups of mice reached stable performance (70% correct response) over four consecutive sessions, they were submitted to a reversal learning to evaluate their behavioural flexibility skills (Fig. 3A). There was no difference between sham and 6-OHDA groups in the number of trials executed at first session (54.18 ± 8.81 vs 54.75 ± 6.06, respectively) or at the last session before reversal (53.91 ± 4.88 vs 57.50 ± 4.96; Table 1).

After reversal, the percentage of correct responses was markedly decreased in both sham and lesion groups (Fig. 3A). Whereas sham animals rapidly recovered their baseline performance by session five, lesioned animals attained criterion level by the 7th session (Fig. 3A). Analysis of the correct sequence ratio over sessions revealed a main effect of lesion ($F_{1,17} = 11.29$, $p < 0.005$), of training sessions ($F_{9,153} = 62.76$, $p < 0.0001$) and a significant interaction between the two factors ($F_{9,153} = 67.85$, $p < 0.0001$). Significant difference between sham and lesion group was found on sessions 2 to 5 (Sidak post-hoc test $p < 0.05$). Incorrect sequence previously rewarded (i.e. perseveration) showed the inverse pattern (Fig. 3B) with a significant difference between sham and lesioned mice found on the first two sessions after reversal [(Sidak post-hoc test; $p < 0.05$ after significant ANOVA (main effect of group ($F_{1,17} = 8.39$, $p < 0.01$), sessions ($F_{9,153} = 83.99$, $p < 0.0001$) and interaction between the two factors ($F_{9,153} = 4.67$, $p < 0.0001$)]. A detailed analysis of these incorrect sequence of responses was added to see whether impairment in behavioural flexibility was due to enhanced perseverative responding or to a deficit in acquiring and maintaining a new strategy. When mice continued to perform the previously reinforced sequence until three consecutive correct responses were made, it was counted as a *perseverative error,* and an incorrect sequence that occurred afterwards was counted as *a regressive error*. As shown in Fig. 3C, D, the two groups of mice made selectively more perseverative rather than regressive errors. Analysis of these errors identified a significant main effect of group ($F_{1,17} = 7.55$, $p < 0.05$), group × session interaction ($F_{9,153} = 2.04$, $p < 0.05$) and a main effect of sessions ($F_{9,153} = 39.01$, $p < 0.0001$). Lesioned mice significantly increased perseverative errors during the first two sessions after reversal (post-hoc Sidak test, $p < 0.05$). In addition to this type of errors, lesioned mice displayed a higher premature responding after reversal (Fig. 3E). They increased premature responding directly to the novel side (Fig. 3E) but not

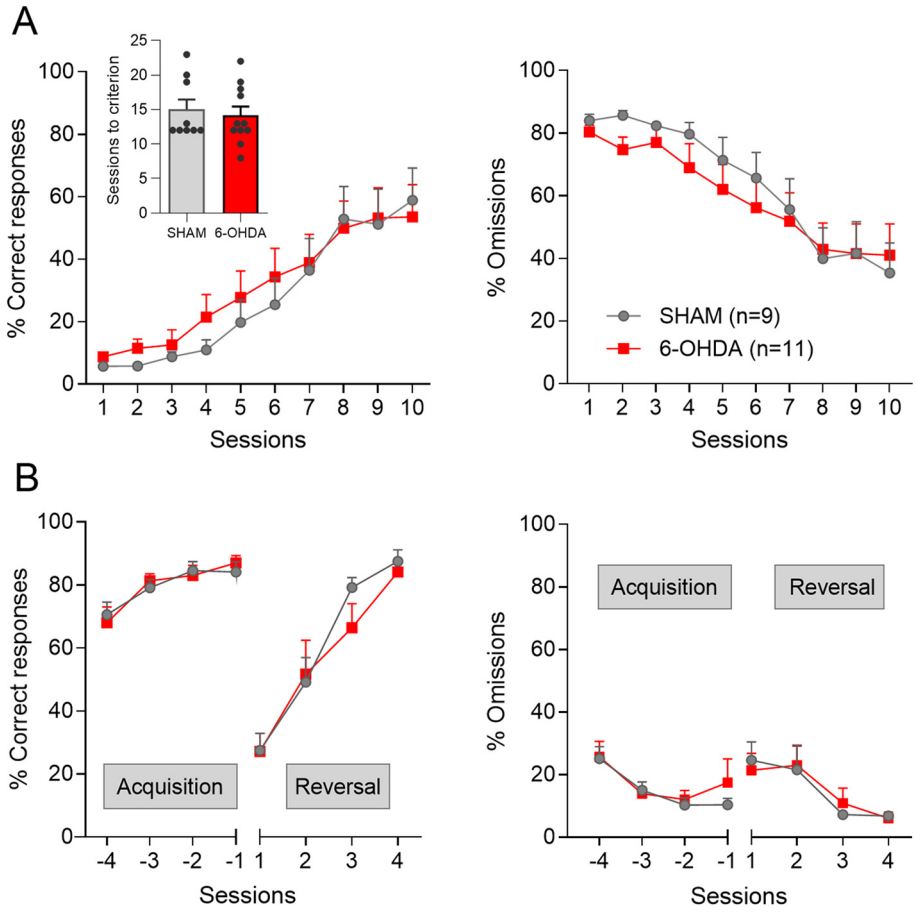

Fig. 2. Dorsomedial striatal dopamine (DA) denervation does not modify acquisition nor reversal learning of a single nose-poke discrimination. **(A)** Percentage of correct responses and omissions (mean ± SEM) of the ten first sessions of learning phase acquisition in SHAM (n = 9) and 6-OHDA lesioned (n = 11) mice. Inset: Mean number of sessions (±SEM) to criterion. **(B)** Percentage of correct responses and omissions (mean ± SEM) in the last four sessions of acquisition phase and in the four sessions after reversal of contingencies.

the previously rewarded side (Fig. 3F). This indicates that they could discriminate the novel reinforced side but they were unable to implement the correct motor response sequence. Analysis of these responses across sessions showed a significant main effect of group ($F_{1,17}$ = 5.7, p < 0.05), sessions ($F_{9,153}$ = 8.44, p < 0.0001) and group × sessions interaction ($F_{9,153}$ = 3.19, p < 0.001). There was no significant difference in the first two sessions after rule inversion (whatever the group or side of responses), nevertheless at the 3rd session, lesioned mice made higher premature responses than sham-treated mice (Sidak post-hoc test, p < 0.01). This effect progressively declined to baseline level at session 6. There was no change in the total number of responses (nose-pokes) by session before or after rule inversion (data not shown) suggesting that motivation was not affected by partial DA depletion ($F_{1,17}$ = 0.16, p > 0.05). The number of trials by session increased after reversal, compared to the pre-reversal session, and remained stable over time in both groups (Table 1).

Effect of DMS DA depletion on proactive inhibitory control

To verify whether the high premature response displayed by lesioned mice in sequential reversal learning may in part be a manifestation of enhanced motor impulsivity, we studied the effects of DMS DA lesion on inhibitory control. Another group of animals was therefore tested in a cued response inhibition task that requires the subjects to withhold a prepotent response during a waiting period of variable duration. When mice reached a minimum of 60% correct responses for four consecutive sessions, they were submitted to 6-OHDA or sham-lesions and re-tested in the cued response inhibition task after a recovery period. As illustrated in Fig. 4A, the percentage of correct responses of sham and lesioned mice decreased in the first sessions following surgery. Sham mice rapidly recovered their preoperative baseline level

Table 1. Number of trials in the first and last session before and after reversal of rule contingencies in the reversal learning tasks. Sessions in the single nose-poke discrimination task lasted 15 min and 30 min in the task with a sequence of two consecutive responses

	SHAM		6-OHDA	
	First session	*Last session*	*First session*	*Last session*
Single nose-poke discrimination				
Number of trials before reversal	45.11 ± 0.75	48.78 ± 1.40	44.09 ± 0.99	48.64 ± 1.27
Number of trials after reversal	63.22 ± 1.20	58.11 ± 2.41	68.32 ± 2.27	56.73 ± 1.18
Two-step sequential reversal learning				
Number of trials before reversal	54.18 ± 8.81	53.91 ± 4.88	54.75 ± 6.06	57.50 ± 4.96
Number of trials after reversal	77.45 ± 9.17	65.18 ± 4.32	92.00 ± 8.84	62.50 ± 5.06

Measures are expressed as mean trials ± SEM by session.
6-OHDA = 6-hydroxydopamine.

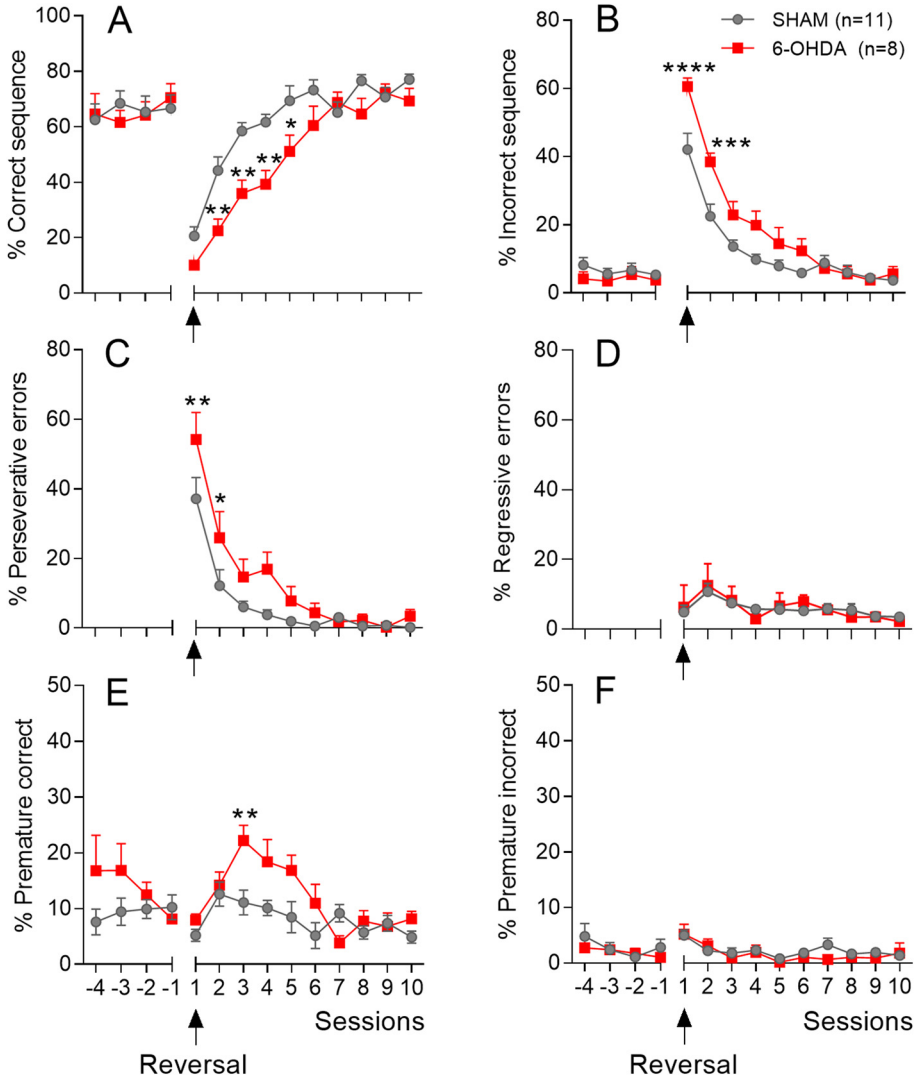

Fig. 3. Dorsomedial striatal DA denervation disrupts reversal learning when a two-step sequence is required. Performance of SHAM (n = 11) and 6-OHDA lesioned (n = 8) mice before and after reversal phase of the two-step sequential task. Mice were initially trained to perform a sequence of responses: central nose-poke followed by a lateral response (left or right nose-poke to the central hole). They were then tested in the reversal phase where the correct sequence had to be performed in the opposite side to initial training. Mean percentage (±SEM) of responses for the last four baseline sessions preceding reversal and for the following ten sessions. **(A, B)** Percentage of correct **(A)** and incorrect **(B)** sequence of responses before and after reversal. **(C, D)** Incorrect sequence of responses were further subdivided as percentage of perseverative errors **(C)** if it occurred *before* three consecutive correct sequences and of regressive errors **(D)** if it occurred *after* three consecutive correct sequences. **(E–D)** Percentage of premature correct and premature incorrect responses (mean ± SEM). Direct responses to the lateral aperture opposite to the previously rewarded hole, with no central nose-poke, were termed premature correct **(E)**, while premature responses to the previously reinforced side was termed premature incorrect **(F)**. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 post-hoc Sidak test after significant two-way ANOVA.

of correct responses by the 5th postoperative session while lesioned mice remained at a low level across testing sessions. Analysis of correct response ratios revealed a significant main effect of lesion ($F_{1,29}$ = 6.02, p < 0.05), a main session effect ($F_{10,290}$ = 12.44, p < 0.0001) and a group × sessions interaction ($F_{10,290}$ = 4.72, p < 0.0001). Post-hoc Sidak multiple comparison test indicated that scores of 6-OHDA mice significantly differed from sham at sessions 7, 10 and 11 (p < 0.05, Fig. 4A).

Performances were similarly lower in the first postoperative sessions (1–4) for the two groups (ANOVA shows no main group effect in all parameters measured, NS). Latencies to respond (Fig. 4B) or collect food reward (Fig. 4F) and omissions (Fig. 4E) were massively increased in the two groups. This was associated with low percentage of premature responses and ITI responses (Fig. 4C, D). These effects were transient in sham mice. In contrast, DMS DA denervation impaired the execution of the cued inhibition task for the remaining testing sessions 5 to 11. Lesioned mice took longer to initiate their response [main group effect ($F_{1,29}$ = 5.24, p = 0.03) and no session effect, NS; Fig. 4B)]. They also produced higher percentage of premature responses than sham mice which showed a reduction of these errors over time [Fig. 4C; significant main effect of lesion ($F_{1,29}$ = 6.51, p = 0.01)]. This was associated with increased number of responses during ITI across sessions compared with sham treated mice (Fig. 4D) [main group effect ($F_{1,29}$ = 3.96, p = 0.05) and no group × session interaction, NS]. Omissions remained at a high level in the lesion group across sessions (main group effect $F_{1,29}$ = 5.94, p = 0.02) that tended to progressively decrease over time (main session effect, $F_{6,174}$ = 2.12, p = 0.05) (Fig. 4E). Lesioned mice took slightly longer to collect reward than sham mice ($F_{1,26}$ = 10.64, p = 0.03; Fig. 4F).

Analysis of performance of sham and lesioned mice as a function of waiting phase duration at the end of the training period (i.e., preoperative session S-1, Fig. 5) revealed that the percentage of correct responses declined as a function of increasing duration of the inhibitory period while an opposite pattern was found on premature responses for the two groups (significant effect of waiting phase duration $F_{4,116}$ = 10.82, and 30.30, respectively; p = 0.0001). This pattern disappeared on the first post-operative session, when the correct responses ratio was reduced in both groups, whatever the waiting phase duration. By

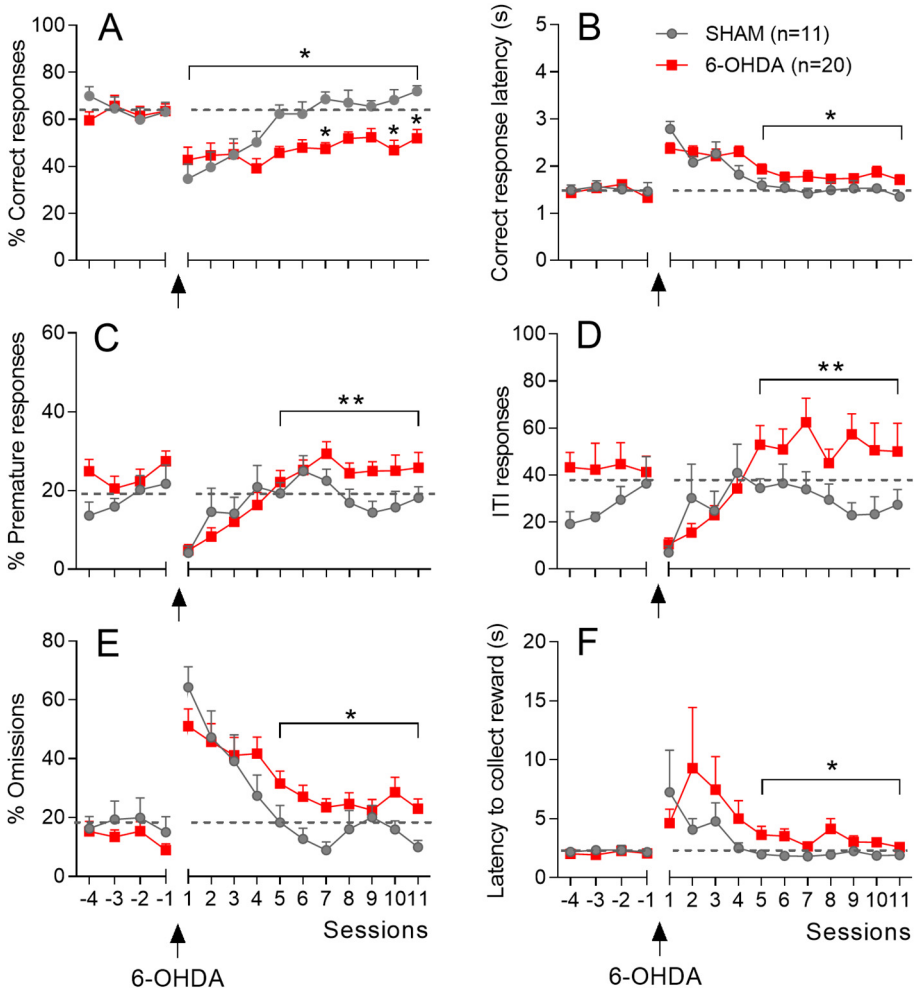

Fig. 4. Dorsomedial striatal DA denervation disrupts performance in the cued-response inhibition task. Performance of SHAM (n = 11) and 6-OHDA lesioned (n = 20) mice in the cued-response inhibition task before (sessions −4 to 1) and after (sessions 1 to 11) sham or DMS DA lesions (6-OHDA). Compared to controls, lesioned mice could not withhold a pre-learned nose-poke response during variable and randomly generated delays (2–10 s) signalled by a cue-light. **(A)** Mean percentage (±SEM) of correct responses. **(B)** Mean (±SEM) correct response latency (s). **(C)** Mean percentage (±SEM) of premature responses during cued-response inhibition waiting phase. **(D)** Mean number (±SEM) of responses during intertrial intervals (ITI). **(E)** Mean percentage (±SEM) of omissions (no response for 5 s after response inhibition). **(F)** Mean (±SEM) latency to collect reward (s). *p < 0.05, **p < 0.01, ***p < 0.001; post-hoc Sidak test following significant two-way ANOVA.

session 5, lesioned mice displayed a decreased correct responses ratio as a function of waiting phase duration that significantly differed from sham treated mice at 10 s interval (p < 0.05 Sidak test after significant main effect of waiting phase duration [($F_{4,116}$ = 6.42, p < 0.0001) and group effect ($F_{1,29}$ = 12.29, p < 0.01), Fig. 5]. In both groups, premature responding increased as a function of the length of inhibitory cue duration ($F_{4,116}$ = 29.74, p < 0.0001) but this did not differ between groups (Fig. 5).

These effects were enhanced across testing sessions in the lesion group (Fig. 6). There was a significant main effect of group on correct response ratio (main group effect $F_{1,29}$ = 25.71, 5.62, 10.01, 2.67, 10.01, 7.99 on sessions 7, 8, 9, 10, 11, respectively; all p < 0.05, except on session 9). Lesioned mice increased premature responding at each delay reflecting enhanced motor impulsivity with the prolongation of the waiting period (main effect of waiting phase duration $F_{4,116}$ = 52.08, 92.36, 20.11, 21.72, 27.51 on sessions 7, 8, 9, 10, 11, respectively; all p < 0.0001). This effect was significantly different from sham group at the longest cue duration (post-hoc Sidak test, p < 0.01 and p < 0.05 in sessions 9 and 10). The rate of omissions significantly differed between lesion and control groups ($F_{1,150}$ = 7.63, p < 0.01) and this was independent of the variable delay duration ($F_{4,150}$ = 1.30, p > 0.05, not shown).

Extinction phase

The ability of lesioned mice to extinguish a learned response when the reward is not any more associated with a correct response was further tested in a subgroup of sham (n = 5) and lesioned mice (n = 9) after completion of the cued response inhibition task. 6-OHDA and control mice showed a progressive decrease of responding over the 6 sessions of extinction ($F_{1,12}$ = 1.64, p > 0.05) indicating that DMS DA denervation did not impair the stimulus-reward association over time (data not shown).

Effect of DMS DA depletion on locomotor and exploratory behaviour

Spontaneous locomotor and exploratory activity were not modified either in sham or lesioned mice, when tested at 21 and 35 days after surgery (Table 2). Overall, DMS DA denervation did not produce any major motor impairment on spontaneous locomotion or rearing behaviour as measured by horizontal and vertical photocell beam interruptions for the 60-min test duration (p > 0.05, N.S. post-hoc Student's test between 6-OHDA and sham groups).

DISCUSSION

A growing body of evidence suggests that the striatum contributes to the regulation of flexibility (Owen et al., 1992; Cools et al., 2001; Ragozzino, 2007; Clarke et al., 2008; Floresco et al., 2009; Castañé Anna et al., 2010). Whereas preclinical and human studies support the involvement of the limbic cortico-ventral striatal circuits in mediating flexibility and inhibitory control (Fineberg et al., 2014; Vaghi et al., 2017), less is known about the

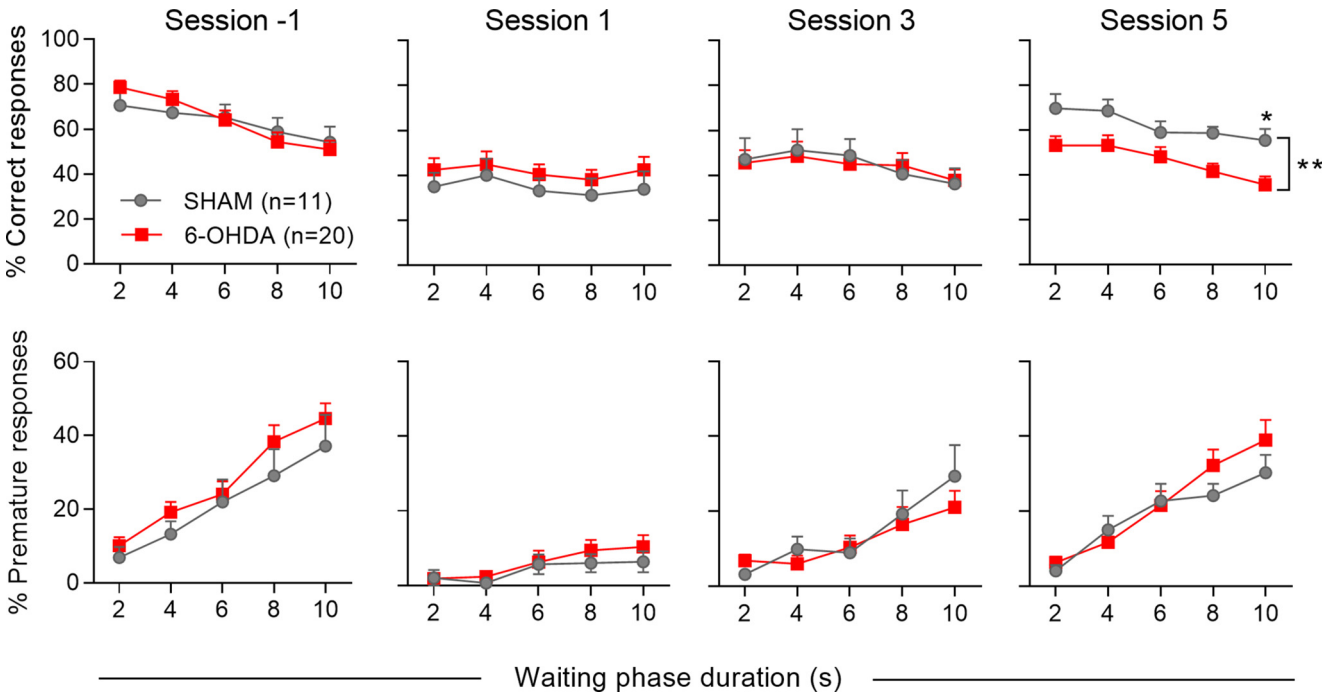

Fig. 5. Performance of mice as a function of the waiting phase duration before and in the first sessions following 6-OHDA lesion. Performance of SHAM ($n = 11$) and 6-OHDA lesioned ($n = 20$) mice as a function of the inhibitory cue duration in a preoperative session (S-1) and in three post-operative sessions 1, 3 and 5. In S-1, the mean (±SEM) percentage of correct responses declined as delays duration increased (upper graphs) while the percentage of premature responses increased. This pattern of responses, disrupted after surgery in the two groups at sessions 1 and 3, was recovered at session 5 with a lower ratio of correct responses in lesioned mice compared with sham. $^{**}p < 0.01$ Sidak test after significant two-way ANOVA.

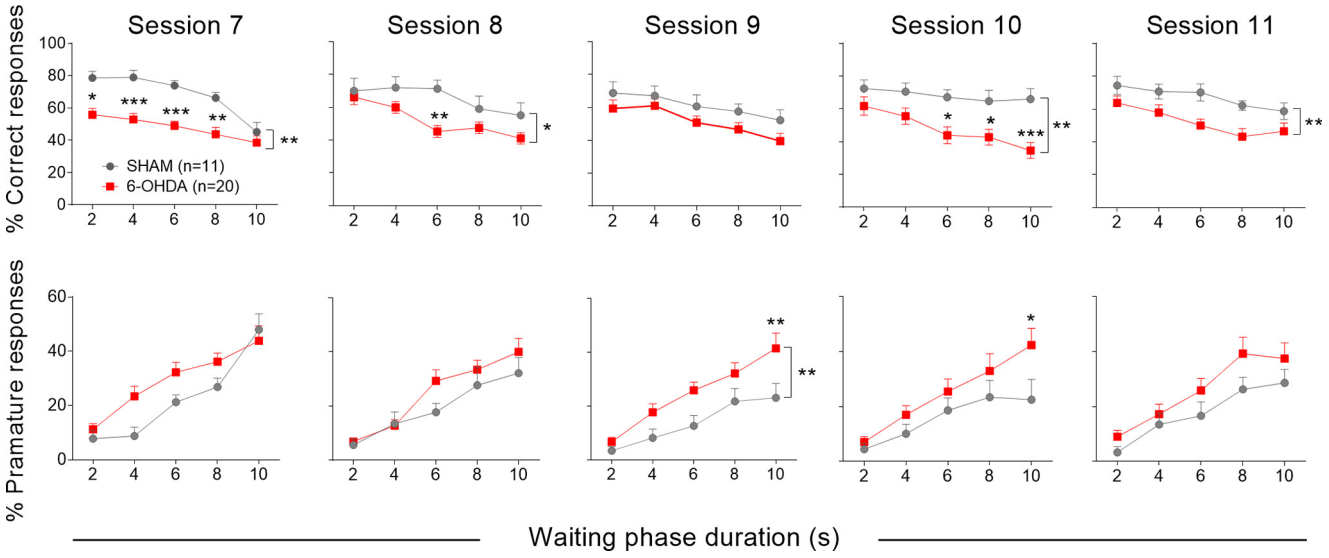

Fig. 6. Impairment of lesioned mice performance as a function of the waiting phase duration in sessions 7–11. Decreased mean (±SEM) percentage of correct responses over inhibitory cue duration in the 6-OHDA lesion group compared to SHAM for the following sessions (S 7–11, upper graphs). Increased mean (±SEM) percentage of premature responses group as a function of delays duration in the 6-OHDA lesion group compared to SHAM (lower graphs). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; post-hoc Sidak test following significant two-way ANOVA.

consequences on these processes of DA denervation restricted to the medial dorsal striatum (DMS). Here we show that DA denervation of the DMS impairs behavioural flexibility in a reversal learning instrumental task, only if a sequential set of two actions is required. Lesioned mice were unable to rapidly shift their pattern of response under changing task contingencies and showed an increase in perseverative responses in the first sessions

after reversal. DA lesion then disrupted the completion of the two-action sequences as shown by the increase of premature responses in the new reinforced side. This suggests that mice could learn the new reinforcement contingencies but omitted the first action (i.e. central nose-poke). We characterized further this impulsive behaviour in a cued response inhibition task and showed that lesioned mice also exhibited difficulty to withhold a previously learned appetitive conditioned response during variable waiting phase duration. These findings emphasize the critical role of DA in the DMS in behavioural flexibility and proactive inhibitory control.

Early cognitive deficits in Parkinson's disease include executive dysfunction which may impair decision making, problem solving and attentional shifting. A prominent deficit observed in these patients is their difficulties to adapt strategies or rules to the environmental changes (Owen et al., 1992; Muslimovic et al., 2005; Peterson et al., 2009). These flexibility deficits are in part reproduced here after selective DA depletion of the DMS in instrumental reversal learning tasks. Previous studies in rats points to a critical role of the DMS in spatial reversal learning (O'Neill and Brown, 2007; Haik et al., 2008; Tait et al., 2017). While DA depletion of dorsolateral striatum (DLS) does not impair acquisition nor reversal learning of a spatial discrimination (Castañé Anna et al., 2010; Braun et al., 2015) but impairs an instrumental habit response (Faure et al., 2010). This is in line with the classical view of instrumental learning which posits that habit learning is mediated by the DLS (Yin and Knowlton, 2006) while goal-directed learning is mediated by the DMS (Yin et al., 2005). Although this view has been recently challenged by a recent study showing that DMS and DLS act in an opposite manner in the acquisition of an automatic five-step sequential nose-poke task. DMS lesioned animals are able to enhance acquisition of the automatised action sequences (i.e. habit-like response) while DLS lesions impair it (Turner et al., 2021). Consistent with the opposite functions of DMS and DLS in learning ability, DMS lesions impaired acquisition of a spatial T-maze task that requires flexible behaviour over time (instead of an automatic response) while DLS lesions enhanced it (Moussa et al., 2011). In the present study, 6-OHDA-induced DA denervation of the DMS dramatically impaired reversal learning of a two-step sequence of actions but had no effect in a single nose-poke reversal learning. 6-OHDA lesions did not impair either acquisition or reversal learning of a single nose-poke response. Both lesioned and control groups required the same number of sessions to reach criterion and were similarly impaired after reversal. This suggests that the cognitive load involved in this version of the task is not high enough to be disrupted by mild striatal DA depletion. In contrast, lesioned mice took longer than sham mice to reach criterion in the reversal learning phase of the two-step sequence of actions. In the first two sessions following reversal, lesioned mice displayed an increased number of perseverative errors suggesting that animals were deficient in inhibiting the previous strategy that is no longer relevant. This is consistent with previous studies reporting increased errors during reversal learning after either DA denervation of the DMS (O'Neill and Brown, 2007), quinolinic acid lesions to DMS (Castañé Anna et al., 2010; Braun and Hauber, 2011; Lindgren et al., 2013) or pharmacological manipulation of DMS function (Tzavos et al., 2004; Ragozzino, 2007; McCool et al., 2008; Grospe et al., 2018). DMS lesioned rats, when tested in the cross maze, committed regressive in addition to perseverative errors suggesting that this area could be implicated to maintain a novel strategy once selected (Parikh et al., 2016; Grospe et al., 2018). Differences in the extent of DMS DA depletion or testing procedures (probabilistic reversal learning) may explain the lack of regressive errors observed in the present study.

Besides enhancing perseverative errors, DMS DA denervation produced a marked increase in premature responses for correct choice. Lesioned mice tended to omit the central hole and directly respond in the newly reinforced lateral nose-poke, thus reflecting their inability to implement a correct motor response sequence. This unexpected pattern of premature responses was not seen in the first two sessions, during which the rate of perseverative errors was particularly high, suggesting that it does not reflect a non-specific increase in responding as consequence of low success rate (less reinforced trials) but a specific behavioural deficit that emerges independently as mice learn the new contingency. This incapacity to acquire a two-step sequential reversal learning task after DMS DA lesions may be due to disruption of initiation and execution of chunked motor sequence (Yin, 2014). Basal ganglia circuits are involved in organizing motor and cognitive actions into chunks (Graybiel, 1998; Yin, 2014). Although the sequential reversal learning paradigm used here was not specifically designed to study the role of DA in the DMS in the acquisition and reversal of an automatised

Table 2. Effect of dorsomedial striatal dopamine denervation on motor activity

	SHAM		6-OHDA	
	Postoperative day 21	Postoperative day 35	Postoperative day 21	Postoperative day 35
Locomotor activity	1586.25 ± 86.71	1292.75 ± 84.70	1347.96 ± 113.19	1200.43 ± 78.24
Rearing behavior	150.00 ± 24.01 (n = 12)	174.38 ± 23.22 (n = 8)	130.00 ± 19.10 (n = 21)	112.57 ± 17.21 (n = 7)

Locomotion and rearing behavior were measured in 8 photocell activity cages equipped with infrared detectors positioned at 2 and 8 cm from floor. Interruptions of photocell beams located in the lower and upper frames provided automated measures of locomotor activity and rearing behavior, respectively. Measures are expressed as mean ± SEM of photocell beams interruptions for the 60-min testing period. There was no significant difference of locomotor activity or rearing behavior between sham and 6-OHDA-lesioned mice whatever the day of testing (Unpaired t-test day 21 t_{31} = 1.83, p = 0.08 and day 35 t_{13} = 0.79, p = 0.44 for locomotor activity; day 21 t_{31} = 0.9, p = 0.37 and day 35 t_{13} = 2.08, p = 0.06 for rearing behavior).
6-OHDA = 6-hydroxydopamine.

action sequences as the subjects were not forced to respond rapidly (no time limit to respond) nor to learn a complex set of action sequences, it might be postulated that lesioned mice failed to reverse the sequence-based strategy previously acquired to favour the response associated with the most proximal outcome (reward-associated side). This is supported by the absence of premature responses to the non-rewarded side. DAergic lesions in the striatum have severe effects on sequential learning when they are performed before training phase compared with lesions that are set thereafter, as in the present study. The specific increase in premature responses for correct choice may also reflect increased impulsive behaviour caused by DMS DA lesion as evidenced by the findings from the cued response inhibition task. Indeed, when trained to withhold a prepotent nose-poke response during variable and randomly generated delays, lesioned mice displayed a high rate of premature responding as a function of the length of the waiting period and a high rate of nose-poking during intertrial intervals (ITI) than sham-treated mice, behavioural impairments indicative of enhanced motor impulsivity.

In the cued response inhibition task, lesioned mice displayed a range of behavioural deficits that contribute to their poor performances. In the first post-operative testing sessions, both sham and lesioned mice had similarly a poor performance compared to their pre-surgery level, which was mainly due to reduced responding rate (high number of omitted trials). Such decline in performances may be attributed to the interruption of the training over the post-surgery recovery period because a gradual increase in response rate was observed with the progression of retraining. Interestingly, a clear dissociation between the two groups emerged from the 5th session, when sham mice recovered their pre-surgery level. Lesioned mice showed increased premature responses and a higher rate of nose-poking during the ITI, thus indicating a deficit in inhibitory control processes. They also had higher omission scores compared to sham mice. It is unlikely that such deficit may be due to motor impairment or reduced motivation because lesioned mice displayed greater responding during the waiting phase and the ITI. Furthermore, in both single nose-poke discrimination and two-step sequential reversal learning tasks lesioned mice showed high responding rates (Table 1). Finally, when tested in actimetry cages, they display normal locomotor activity level and rearing behaviour compared to sham mice (Table 2). Accurate responding in the cued response inhibition task depends on mice ability to adequately detect and attend to relevant visual cues (offset of the inhibitory cue and illumination of nose-poke aperture). Hence, the increase in omissions exhibited by lesioned mice may therefore reflect reduced visuospatial attentional processing. Overall, the pattern of deficit displayed by lesioned mice points to impairments in various cognitive domains necessary for task performance. Motor impulsivity is often associated with enhanced activity in the mesocorticolimbic dopaminergic system (Dalley et al., 2007, 2011; Pattij et al., 2007; Robbins, 2002). Stimulation of DA neuronal activity with D-amphetamine or methylphenidate disrupts response inhibition in the same task (Hayton et al., 2012; Simon and Moghaddam, 2017) and produces impulsive responses in a stop-signal task (Bari and Robbins, 2013). Partial DMS DA depletion also produces impulsivity though to a lesser degree. Lower nigrostriatal DAergic activity may result in enhanced mesolimbic DA activation associated with increased motor impulsivity because of a loss of balance between the two systems (Cools et al., 2003). It has been suggested that a low baseline dopamine state could contribute to impulse behaviour in early PD (Aarts et al., 2012) and the frequency of impulse control disorders induced by DA medication might be related to underlying predisposing factors (impulsivity, novelty seeking, and so on) in *de novo* patients (Antonini et al., 2011), which could be determined early in the disease progression.

Interestingly in addition to these multiple cognitive deficits, partial striatal DA depletion induced motor initiation impairment as lesioned mice also increased response latency to initiate nose-pokes after the waiting phase and produced high number of omissions (responses over 5 s). It might be suggested that the restricted DA denervation of the DMS could alter both inhibitory control implementation (as reflected by the higher number of premature and ITI responses) and the removal of this inhibitory control, as lesioned mice require more time to initiate a correct response after the waiting period (Favre et al., 2013). In the same line, dorsal striatal excitotoxic lesions increase the time required to initiate a response following a ‘‘Go” signal, in a stop signal reaction time task, in which rats had to hold their response in the stop condition (Eagle and Robbins, 2003). The activation of a conflicting impulsive response (when instructed to stop) can indeed interfere with the speed of the goal-directed response (Wylie et al., 2010). Contrasting results in the literature of Parkinson’s disease have been found on the loss of inhibitory control of PD patients. A recent study demonstrated that PD patients at early stages show selective impairment in reactive but not proactive inhibition (Di Caprio et al., 2020). It should be noted, however, that this was demonstrated in a stop-signal task, which is more commonly used to evidence reactive inhibition deficit. In contrast, another study in PD patients showed that slowness in movement initiation is associated with proactive inhibitory network dysfunction in PD (Criaud et al., 2016) consistent with our results in rodent model of early PD.

Blunted motivation to perform operant tasks here may also account for the deficits exhibited by striatal DA-depleted mice. This is unlikely, however, as lesioned animals performed the same number of trials by session as controls in the two tasks. Furthermore, in extinction condition, both control and lesioned mice progressively stopped performing the inhibition task when the reward was no longer provided. A general motor disability produced by the mild striatal DA lesions, here, is unlikely as there was no effect on overall response rate in the two operant tasks in the lesion group, and no change of locomotor and exploratory activity in photocell cages. These findings, consistent with earlier studies

reporting little or no major motor impairment with similar levels of DA depletion in rodents (Amalric et al., 1995; Branchi et al., 2008; Haik et al., 2008; Tadaiesky et al., 2008; Chen et al., 2014; Ztaou et al., 2018), argue upon a selective disruption of the cognitive processes involved in the two behavioural tasks.

Overall, we showed that bilateral DA denervation of the dorsomedial striatum of mice impairs flexible behaviour and induces proactive inhibition deficits. Flexibility disorders observed in PD patients may thus directly result from their inability to restrain an action that is not appropriate. These findings suggest that impulsivity observed in PD patients could result from nigrostriatal DA depletion early in the disease and not only from DA treatments as commonly observed. These early cognitive impairments could ultimately be used as a screening device to identify patients with PD prior to the onset of motor symptoms.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Juliette Lhost: Investigation, Methodology, Formal analysis, Writing - original draft. **Simon More:** Software. **Isabelle Watabe:** Investigation. **Didier Louber:** Methodology. **Abdel-Mouttalib Ouagazzal:** Conceptualization, Writing - review & editing, Project administration. **Martine Liberge:** Conceptualization, Investigation, Writing - review & editing, Visualization, Supervision. **Marianne Amalric:** Conceptualization, Writing - review & editing, Visualization, Supervision, Funding acquisition.

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Data are available via request to the authors.

None of the authors has a conflict of interest.

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