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Contributions of signaling by dopamine neurons in dorsal striatum to cognitive behaviors corresponding to those observed in Parkinson's disease

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Abstract

Although the cardinal features of Parkinson's disease (PD) are motor symptoms, PD also causes cognitive deficits including cognitive flexibility and working memory, which are strongly associated with prefrontal cortex (PFC) functions. Yet, early stage PD is not characterized by pathology in the PFC but by a loss of dopaminergic (DA) projections from the substantia nigra to the dorsal striatum. Moreover, the degree to which PD symptoms can be ascribed to the loss of DA alone or to the loss of DA neurons is unknown. We addressed these issues by comparing mouse models of either chronic DA depletion or loss of DA projections to the dorsal striatum. We achieved equal levels of striatal DA reduction in both models which ranged from mild (~ 25%) to moderate (~ 60%). Both models displayed DA concentration-dependent reductions of motor function as well as mild deficits of cognitive flexibility and working memory. Interestingly, whereas both motor function and cognitive flexibility were more severely impaired after mild ablation of DA neurons as compared to mild loss of DA alone, both models had equal deficits after moderate loss of DA. Our results confirm contributions of nigro-striatal dopamine signaling to cognitive behaviors that are affected in early stage PD. Furthermore, our findings suggest that the phenotype after ablation of DA neurons accrues from factors beyond the mere loss of DA.

Keywords

Dopamine-deficient mice; Neurodegeneration; Tyrosine hydroxylase; Viral inactivation; 6-hydroxydopamine; Executive function

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Introduction

A principal characteristic of PD is the degeneration of midbrain DA neurons in the substantia nigra pars compacta (SNpc) and the resulting loss of DA projections to the dorsal striatum, which contributes to the hallmark motor symptoms of the disease (Samii et al., 2004). However, there is substantial clinical evidence for psychiatric and cognitive symptoms in PD patients (Aarsland et al., 2009; Foltynie et al., 2004; Williams-Gray et al., 2007). Cognitive symptoms include deficits in visuospatial function, working memory, attention processes and cognitive flexibility (Giraudo et al., 1997; Lima et al., 2008; Owen et al., 1997; Sawamoto et al., 2008). Whereas these cognitive domains are thought to be regulated by processes involving primarily the PFC, cognitive impairment is already apparent at stages of PD when pathological changes are apparent in the SNpc but have not yet extended to the PFC (Aarsland et al., 2009; Braak et al., 2003; Williams-Gray et al., 2007). Data from rodents with neurotoxic lesions of nigro-striatal DA neurons indicate that the dorsal striatum contributes to visuospatial function and memory (Baunez and Robbins, 1999; Chudasama and Robbins, 2006; Da Cunha et al., 2002; De Leonibus et al., 2007; Mura and Feldon, 2003). This raises the possibility that DA projections from the SNpc to the dorsal striatum not only contribute to the well-known motor symptoms but also to cognitive impairment in PD. Although DA-related dysfunction in PD is traditionally attributed to the loss of DA signaling, the actual loss of SNpc DA neurons might affect processes beyond the mere loss of DA, including inflammatory responses, gliosis and loss of co-transmitter release from DA neurons. Yet, present models for PD do not distinguish the effects of DA depletion versus loss of DA neurons on motor or cognitive dysfunction.

Our lab has generated a genetic model, the conditional *Th* knock-out (KO) mouse that permits depletion of DA synthesis while leaving DA neurons otherwise intact (Jackson et al., 2012). *Th* alleles in these conditional KO mice can be inactivated in a region-specific manner through retrograde transport from the site of injection of a Cre recombinase-expressing virus, CAV2-Cre (Hnasko et al., 2006). By injecting either CAV2 Cre or the DA-specific neurotoxin 6-hydroxydopamine (6-OHDA) into the dorsal striatum we examined the consequences of disrupting DA synthesis versus loss of DA neurons to motor behaviors, visuospatial function, cognitive flexibility and working memory. Because both models of DA depletion allow for control of the loss of striatal dopamine, we can recapitulate in our mice the varying degrees of DA loss present at earlier and further advanced stages of PD, hence accounting for disease progression.

Materials and methods

Drugs

6-OHDA (Sigma) was dissolved in saline solution containing 0.2 % ascorbic acid to make a concentration of 3 mg/ml.

Animals

All experiments were approved by the Institutional Animal Care and Use Committee at the University of Washington. The conditional *Th* KO mice were generated as described

(Jackson et al., 2012). Mice were maintained on a C57Bl/6 genetic background and were housed under a 12-h, light-dark cycle in a temperature-controlled environment with food and water available *ad libitum* unless noted otherwise. CAV2-Cre virus was generated and titered as described (Kremer et al., 2000). The virus preparation had a titer of 3×10^{12} particles per ml. For *Th* inactivation, 0.75 μ l CAV2-Cre per site was bilaterally injected into the anterior (+0.9 mm anterior of Bregma) and posterior region (directly at Bregma) of the dorsal striatum (each $\pm$ 2.0 mm lateral to midline and 3 mm ventral from the skull surface) of 2- to 3-month-old homozygous (inactivation group) and heterozygous (sham control group) conditional *Th* KO mice. For DA neuron ablation, 2- to 3-month-old heterozygous conditional *Th* KO mice received injections of either 0.75 μ l 6-OHDA (ablation group) or ascorbic acid (sham control) into the previously described regions of the dorsal striatum. All surgeries were performed on anesthetized (Isoflurane) male and female mice and all animals were given 4 weeks of recovery after surgeries before behavior testing commenced. Male animals constituted 56.8% of all sham control, 56.3% of all *Th*-inactivated and 66.6% of all DA-neuron ablated mice. Female animals constituted 43.2% of all sham control, 43.7% of all *Th*-inactivated and 33.3% of all DA-neuron ablated mice.

Behavioral Studies

Behavioral experiments were performed in the order listed below with at least 7 days between motor tests and at least 14 days between water-escape tasks.

Motor coordination and grip strength were measured using the four-limb hang test (Perez and Palmiter, 2005). Mice were placed on a wire grid which was gently lowered and raised three times to prompt the animal to grip. The grid was then inverted and the latency to fall off was recorded and averaged over three trials with an intertrial interval (ITI) of 15 min. To avoid harm to the animals the fall from the grid was cushioned and animals were gently removed from the grid if they managed to hang on for more than 2 min.

Motor-skill learning and motor performance was assessed as the latency to fall from a rotating rotarod (Rotamex 4/8 system, Columbus Instruments) and was recorded over three consecutive days with 4 trials per day and an ITI of 10 min. On each trial, mice were placed on the rotarod, which began at 4 rpm and accelerated to 40 rpm over the course of 5 min.

Somatosensory function and front paw coordination were examined with the adhesive removal test (Gaugler et al., 2012). A circular adhesive label (12.7-mm diameter) was gently placed onto the forehead of the animal and the latency to remove it was recorded.

The Morris water maze was used to measure visuospatial learning, spatial reference memory and spatial working memory. For visuospatial learning and spatial reference memory, mice were trained to locate a hidden platform over a period of 4 days with four 90-s trials per day and an ITI of 5 min. On each trial, mice were released into the pool from a different location. For this procedure, the position of the platform was permanent for each mouse during all trials. All sessions were recorded with a camera and analyzed with Ethovision software (Noldus). The circular pool was 84 cm in diameter and filled with opaque water at 22 °C. No visual cues were present within the pool. External cues were provided through the wall decoration of the room. Visuospatial learning was measured as latency to reach the

hidden platform. Swim speed was also recorded. One day after the last training session, mice performed a 90-s probe trial in which no platform was present in the maze. Spatial reference memory was scored as the percentage of time spent in the quadrant of the pool where the platform was positioned during training. Spatial memory precision was measured during the probe trial as average proximity (distance) to the exact location where the platform was positioned during learning. Spatial working memory was assessed 3 days after the probe trial in the same room and maze using a delayed response paradigm with increasing ITIs (Wei et al., 2011). Mice were again trained to locate a hidden platform, but now there were only 2 trials per day and each day the position of the platform was changed to a new location in the pool; training extended over 16 days. On days 1–4 the ITI between the two trials was 60 s, on days 5–8 the ITI was 300 s, on days 9–12 the ITI was 900 s and on days 13–16 the ITI was back to 60 s. The difference between the latencies on the first and second trial on each day was calculated as a measure for retention of the daily changing platform position and averaged over each of the 4-day blocks of equal ITI length.

Cognitive flexibility was measured in a water-based, U-shaped maze (Darvas and Palmiter, 2011). Mice were released into a gray stem (45 cm) that ended in one black and one white choice arm (50 cm), which are bent back towards the stem so that the mouse could not see the escape platform which was always present at the end of one of the two arms (Gorski et al., 2003). The right-left orientations of the white and black arms of the maze were alternated daily in a non-repetitive, pseudo-random sequence so that either arm was equally located on both sides of the maze during the entire session. Each day, mice had 10 trials with an ITI of 3 to 5 min during which they were placed on a heating pad. The percentage of correct trials and latencies to reach the platform (escape latencies) were recorded. To make sure that all mice had an equal number of reinforced responses, mice stayed in the maze after wrong turns until a correct turn was made. During the first three days in the maze mice were trained to acquire a turn-based water escape strategy. For one half of the mice, the escape platform was always at the end of the left arm and for the other half it was in the right arm. Then the same mice were trained for 5 days during which the escape platform was now always at the end of the black arm for one half of the mice and at the end of the white arm for the other half (rule-shift to cue-based strategy). As a control procedure, an independent set of mice was trained for three days to solely acquire the cue-based escape strategy.

Immunohistochemistry, DA and DA-transporter measurement

All brains were collected 10–15 days after the last behavior test was finished. For immunohistochemistry, proteins were detected on 30- μ m brain sections by using the following primary antibodies: rabbit anti-TH (1:2000; Chemicon) and rat anti-DA-transporter (1:1000, Chemicon). Immunofluorescence was revealed by using CY2-(TH) and/or CY3-(DA transporter) labeled IgG secondary antibodies (1:200; Jackson ImmunoResearch). For determination of striatal DA content, punches (1 mm diameter, 2 mm thick) from brain regions of interest were collected, immediately frozen in liquid nitrogen and stored at -80°C until analysis. HPLC coupled with electrochemical detection was used to measure catecholamine content by the laboratory of Dr. Thomas J. Montine at the University of Washington. For DA transporter expression, tissue from the SNpc and

cerebellum was collected, immediately placed in liquid nitrogen and stored at -80°C until analysis. Samples were thawed into RIPA homogenization buffer (Sigma-Aldrich) containing Complete Mini protease inhibitors (Roche Applied Science), sonicated, and centrifuged at $1,000 \times g$ for 10 min. The supernatant was collected and assayed for protein concentration using a bicinchoninic assay (Thermo Scientific). DA-transporter expression was quantified by ELISA. Plates were coated with monoclonal rat anti DA-transporter antibody (1:500, Abcam), washed, unspecific sites blocked with 5% bovine serum albumin, washed and then incubated with 20 μg total protein. Then plates were washed, incubated with polyclonal rabbit anti DA-transporter antibody (1:20,000, Abcam), washed and incubated with a horse-radish peroxidase-conjugated, donkey anti-rabbit antibody (1:500, Jackson ImmunoResearch). After another wash samples were incubated with TMB solution (R & D Systems). The resulting color reaction was stopped by addition of 2N sulfuric acid and then absorption at 450 nm was measured.

Results

Expression of DA neuronal markers in the SNpc

We compared two different models of DA depletion. In one model, the DA neurons were ablated by injection of the neurotoxin, 6-OHDA into the dorsal striatum; in the other model, the essential enzyme for DA synthesis, tyrosine hydroxylase (TH), was inactivated in neurons that project to the dorsal striatum by injection of CAV2-Cre virus into the dorsal striatum of mice carrying two conditional alleles of *Th* (*Th^{lox/lox}*). The control mice for the viral experiments (sham controls) were heterozygous, conditional *Th* (*Th^{lox/+}*) mice that were also injected with CAV2-Cre; controls for the 6-OHDA injections received ascorbic acid vehicle injections. Because we did not observe any differences between control *Th^{lox/+}* mice that received either CAV2-Cre virus or ascorbic acid injections, we grouped them together for the presentation of our data.

To clarify the difference between our models (*Th* inactivation and 6-OHDA lesion) we compared expression of TH and DAT, which are expressed by most midbrain dopamine neurons, by immunohistochemistry. The sole purpose of this experiment was to demonstrate that Cre-mediated inactivation of *Th* results in loss of TH immuno-reactivity and leaves DAT expression unchanged and that 6-OHDA treatment results in loss of DA neurons with loss of both TH and DAT immuno-reactivity. Expression of TH was reduced in the SNpc of both *Th*-inactivated and 6-OHDA-treated mice and was barely affected in the ventral tegmental area (Figs. 1A-C). DAT expression was normal in the midbrain of *Th*-inactivated mice and reduced in the SNpc of 6-OHDA-treated mice (Figs. 1D-F). This confirms that in *Th*-inactivated mice only expression of TH was affected without loss of DA neurons and that in 6-OHDA-treated mice the number of DA neurons was reduced.

To further demonstrate the difference between our models (*Th* inactivation and 6-OHDA lesion) we quantified DAT expression in the SNpc of an independent cohort of mice ($n = 4$) by ELISA. Because DAT is not expressed highly in the cerebellum, we also included the cerebellum of sham control mice in this analysis ("negative control"). Comparison of absorbances (i.e. DAT expression) in the SNpc by sham control, mice with *Th* inactivation, mice with ablation of DA neurons and in the cerebellum of sham

control mice (Fig S1) by one-way analysis of variance (ANOVA) revealed a significant effect of group ($F_{3, 12} = 26.49$, $p < 0.01$). Bonferroni's *post-hoc* comparisons using sham control SNpc as reference confirmed significant ($t = 2.81$, $p < 0.05$) reductions of DAT expression in mice with with ablation of DA neurons ($t = 3.70$, $p < 0.05$) and in the cerebellum of sham control mice ($t = 8.02$, $p < 0.01$). We therefore refer to 6-OHDA mice as DA neuron-ablated mice and conclude that DA neurons were not lost in *Th* inactivated mice.

Loss of nigro-striatal DA signaling

Estimates of striatal DA loss at the onset of PD vary between 20–70% (Lee et al., 2000; Morrish et al., 1998; Schwartz et al., 2004) and provide an average loss of ~ 60 % when first motor symptoms become apparent (Cheng et al., 2010). We therefore assigned DA depleted animals to either of the following groups: (1) mild DA depletion with > 70 % of sham level DA present in the dorsal striatum and (2) moderate DA depletion with < 70 % sham level DA in the dorsal striatum.

Mild DA depletion—Comparison of DA levels in the dorsal striatum of mice with mild DA depletion and sham controls (Fig. 2A) by ANOVA revealed a significant treatment effect ($F_{2, 77} = 5.62$, $p < 0.01$). Bonferroni's *post-hoc* comparison confirmed significant ($t = 2.81$, $p < 0.05$) reductions in mice with *Th* inactivation (mean: 85 % of sham control, range: 70–105 %) and in mice with ablation of DA neurons ($t = 2.81$, $p < 0.05$, mean 80 % of sham control, range: 70–103 %). Differences in DA levels between *Th*-inactivated and DA-neuron-ablated mice were not statistically significant ($t = 0.72$, $p > 0.05$). No significant changes of DA levels in the ventral striatum (Fig. 2A) were detected by ANOVA ($F_{2, 76} = 0.98$, $p > 0.05$). Analysis of DA metabolites and serotonin in the dorsal striatum by ANOVA showed no significant changes in levels of 3,4-dihydroxyphenylacetic acid (DOPAC, $F_{2, 94} = 1.26$, $p > 0.05$; Fig. 2B), the ratio of DOPAC to DA ($F_{2, 94} = 1.85$, $p > 0.05$; Fig. 2C), norepinephrine ($F_{2, 65} = 1.15$, $p > 0.05$; Fig. 2D) or serotonin ($F_{2, 83} = 1.81$, $p > 0.05$).

Moderate DA depletion—Comparison of DA levels in the dorsal striatum of mice with moderate DA depletion and sham controls (Fig. 2E) by ANOVA revealed a significant treatment effect ($F_{2, 67} = 50.47$, $p < 0.01$). Bonferroni's *post-hoc* comparison confirmed significant ($p < 0.01$) reductions in mice with *Th* inactivation (mean: 45 % of sham control, range: 7–69 %) and in mice with ablation of DA neurons (mean: 38 % of sham control, range: 12–62 %). Differences in dopamine levels between *Th* inactivated and DA neuron ablated mice were not statistically significant ($p > 0.05$). No significant changes of DA levels in the ventral striatum (Fig. 2E) were detected by ANOVA ($F_{2, 66} = 1.08$, $p > 0.05$). Analysis of DA metabolites and serotonin in the dorsal striatum by ANOVA showed significant changes in levels of DOPAC ($F_{2, 93} = 20.29$, $p < 0.01$; Fig. 2F), the ratio of DOPAC to DA ($F_{2, 94} = 7.87$, $p < 0.01$; Fig. 2G), but not of norepinephrine ($F_{2, 73} = 1.78$, $p > 0.05$; Fig. 2H) or serotonin ($F_{2, 73} = 1.86$, $p > 0.05$). Bonferroni's *post-hoc* comparison confirmed significant reductions of DOPAC in both mice with *Th* inactivation ($p < 0.01$) and ablation of DA neurons ($p < 0.01$) compared to sham levels and no significant differences in DOPAC levels between mice with *Th* inactivation and ablation of DA neurons ($p > 0.05$). Although comparison of the DOPAC to DA ratio by Bonferroni's *post-hoc* test did not detect significant changes relative to sham controls in mice with *Th* inactivation ($p > 0.05$), a

significant reduction of the ratio in mice with DA neuron ablation was detected when compared to both sham control ($p < 0.05$) and *Th*-inactivated mice ($p < 0.01$). In addition, we also performed an ANOVA of DA content in the dorsal striatum for all groups (sham, mild and moderate loss of DA) together and confirmed, consistent with our previous analysis, a significant effect of group ($F_{4, 111} = 41.32$, $p < 0.01$) ANOVA. Bonferroni's *post-hoc* test further confirmed that all *Th*-inactivated and DA neuron ablated groups had significantly less DA in the dorsal than sham controls (for mild groups: $p < 0.05$, for moderate groups: $p < 0.01$). More important, while differences between *Th*-inactivated and DA neuron ablated mice within mild and moderate groups were not significant ($p > 0.05$), all groups with moderate loss of DA had significantly lower DA contents than all mild groups ($p < 0.01$).

Taken together, we established groups of mice with mild and with moderate specific loss of DA in the dorsal striatum. Furthermore, analysis of DA metabolites in mice with moderate loss of striatal DA suggests that different neurochemical adaptations occur in mice with DA neuron ablation compared to those with *Th* inactivation.

Motor impairments

To establish the relationship between the degree of striatal DA dysfunction and motor impairment we employed tests that measure different aspects of motor functions: motor coordination/grip strength (four-limb hang test), motor skill (rotarod), and front paw coordination (adhesive removal).

Mild DA depletion—The latencies to fall from the hanging wire mesh did not detect any significant (ANOVA; $F_{2, 74} = 2.09$, $p > 0.05$) differences between sham control, *Th*-inactivated and DA-neuron-ablated mice (Fig. 3A). Although DA-depleted mice were impaired in the rotarod task, all animals showed increased performance during training (Fig. 3B). Two-way repeated measures ANOVA of rotarod performance revealed significant main effects of trial number ($F_{11, 748} = 42.27$, $p < 0.01$), group ($F_{2, 68} = 13.42$, $p < 0.01$), but not of trial number $\times$ group interaction ($F_{22, 748} = 0.99$, $p > 0.05$). Bonferroni's *post-hoc* comparison further showed that differences between sham control and DA-neuron-ablated mice on trials 5–10 ($p < 0.05$) and 12 ($p < 0.05$) were significant and that differences between *Th*-inactivated and DA-neuron-ablated mice were only significant on trial 11 ($p < 0.05$). The latencies to remove the adhesive labels failed to detect any significant differences between the groups (ANOVA; $F_{2, 56} = 0.65$, $p > 0.05$; Fig. 3C). We further analyzed the rotarod data by comparing the ratio of latency to fall on trial 12 vs. trial 1 as a measure of 'fold improvement', which may be analogous to motor learning. Sham control mice improved on average 229 % ($\pm$ SEM 9.25), *Th*-inactivated mice improved 234 % ($\pm$ SEM 15.19) and DA-neuron-ablated mice improved 273 % ($\pm$ SEM 28.08). ANOVA of fold improvement did not detect any significant differences (ANOVA; $F_{2, 68} = 1.78$, $p > 0.05$). We conclude that mild striatal DA depletion produced only moderate overall impairment of motor performance that was slightly more pronounced after loss of DA neurons than after loss of DA.

Moderate DA depletion—DA-depleted animals had reduced grip strength and motor coordination in the four-limb hang test. The latencies to fall revealed a significant main effect of group (ANOVA; $F_{2,65} = 7.07$, $p < 0.01$) and Bonferroni's *post-hoc* comparison confirmed that both *Th* inactivated ($p < 0.05$) and DA neuron-ablated mice ($p < 0.01$) had shorter latencies compared to sham controls (Fig. 3D). Impairment of rotarod performance by DA-depleted animals was severe, yet their learning of the motor skill was not abolished (Fig. 3E). Two-way, repeated-measures ANOVA of rotarod performance revealed significant main effects of trial number ($F_{11,759} = 46.32$, $p < 0.01$), group ($F_{2,759} = 14.74$, $p < 0.01$), but not of trial number $\times$ group interaction ($F_{22,759} = 1.41$, $p > 0.05$). Whereas Bonferroni's *post-hoc* comparison showed that differences between sham control and *Th*-inactivated mice were only significant on trials 5–9 and on trial 12 ($p < 0.05$), differences between sham and DA-neuron-ablated mice were significant on trial 2 ($p < 0.01$), trial 3 ($p < 0.05$) and trials 5–12 ($p < 0.01$). On trials 10 and 11 differences between *Th*-inactivated and DA-neuron-ablated mice were significant ($p < 0.05$). ANOVA of latencies in the adhesive removal test (Fig. 3F) narrowly failed to detect any significant differences between the groups ($F_{2,67} = 2.75$, $p = 0.07$). Again, we analyzed the rotarod data by comparing the ratio of latency to fall on trial 12 vs. trial 1 as a measure of 'fold improvement'. Sham control mice improved on average 229 % ($\pm$ SEM 9.25), *Th*-inactivated mice improved 265 % ($\pm$ SEM 16.96) and DA neuron-ablated mice improved 256 % ($\pm$ SEM 24.82). ANOVA of fold improvement did not detect any significant differences (ANOVA; $F_{2,69} = 1.69$, $p > 0.05$).

Taken together, we infer that moderate striatal DA depletion produced clear overall motor impairment which was, in the case of motor skill performance, more aggravated after loss of DA neurons than after loss of DA alone. In addition, motor skill performance deficits were observed after mild loss of DA neurons but not that much after mild *Th* inactivation, suggesting involvement of processes beyond loss of TH activity.

Spatial reference memory and visuospatial learning

To ascertain if there is an effect of DA depletion visuospatial function and spatial memory in the two mouse models we tested them in a Morris water maze (MWM).

Mild DA depletion—Visuospatial learning was intact in DA depleted animals. Two-way, repeated-measures ANOVA of escape latencies (Fig. 4A), swim speed (Fig. 4B) and path length (Fig. 4C) in the MWM showed that all animals improved water escape over time as indicated by significant main effects of trial number for escape latencies ($F_{3,183} = 54.41$, $p < 0.01$) and path length ($F_{3,183} = 67.38$, $p < 0.01$), but not for swim-speed ($F_{3,183} = 2.51$, $p > 0.05$). There were no significant effects of group on escape latencies ($F_{2,183} = 0.01$, $p > 0.05$), path length ($F_{2,183} = 0.21$, $p > 0.05$) or swim-speed ($F_{2,183} = 0.51$, $p > 0.05$). We did not observe animals consistently swimming close to the walls (thigmotaxis) and locating the platform by circling process alone. On the probe trial, all animals displayed a clear preference for the quadrant where the platform had been located (Fig. 4D). ANOVA of the time spent in the quadrants of the maze showed significant effects of quadrant location for sham control ($F_{3,135} = 50.61$, $p < 0.01$), *Th* inactivated ($F_{3,87} = 20.74$, $p < 0.01$) and DA neuron ablated mice ($F_{3,31} = 5.22$, $p < 0.01$). Bonferroni's *post-hoc* comparison confirmed that all groups preferred the reinforced quadrant over all other quadrants ($p < 0.01$).

However, analysis of average proximity to the exact platform location (Fig. 4E) by ANOVA showed significant effects of DA depletion ($F_{2, 35} = 7.13$, $p < 0.01$). Bonferroni's *post-hoc* comparison confirmed that both *Th*-inactivated ($p < 0.01$) and DA-neuron-ablated mice ($p < 0.05$) maintained higher average distances from the platform position than sham control mice.

Moderate DA depletion—DA-depleted mice also had no deficit in visuospatial learning. Two-way, repeated-measures ANOVA of escape latencies (Fig. 4F), swim speed (Fig. 4G) and path length (Fig. 4H) in the MWM showed that all animals had improved water escape over time as indicated by significant main effects of trial number for escape latencies ($F_{3, 183} = 97.76$, $p < 0.01$) and path length ($F_{3, 183} = 103.40$, $p < 0.01$), but not for swim-speed ($F_{3, 183} = 1.48$, $p > 0.05$). There were no significant effects of group on escape latencies ($F_{2, 183} = 0.86$, $p > 0.05$), path length ($F_{2, 183} = 0.84$, $p > 0.05$) or swim-speed ($F_{2, 183} = 0.85$, $p > 0.05$). We did not observe animals swimming consistently close to the walls (thigmotaxis) and locating the platform by circling process alone. On the probe trial, all animals displayed a clear preference for the quadrant where the platform had been located (Fig. 4I). ANOVA of the time spent in the quadrants of the maze showed significant effects of quadrant location for sham control ($F_{3, 135} = 50.61$, $p < 0.01$), *Th*-inactivated ($F_{3, 59} = 16.07$, $p < 0.01$) and DA-neuron-ablated mice ($F_{3, 31} = 10.66$, $p < 0.01$). Bonferroni's *post-hoc* comparison confirmed that all groups preferred the reinforced quadrant over all other quadrants ($p < 0.01$). Analysis of average proximity to the exact platform location (Fig. 4J) by ANOVA showed significant effects of DA depletion ($F_{2, 36} = 8.98$, $p < 0.01$). Bonferroni's *post-hoc* comparison confirmed that both *Th*-inactivated ($p < 0.01$) and DA-neuron-ablated mice ($p < 0.05$) maintained higher average distances from the platform position than sham control mice.

We conclude from these data that although even a moderate loss of striatal dopamine did not result in impaired visuospatial learning, both mild and moderate striatal DA depletion resulted in slightly impaired spatial memory.

Spatial working memory

Spatial working memory was assessed in sham-control, *Th*-inactivated and DA-neuron-ablated mice that previously underwent the MWM testing for visuospatial function and memory. This was done to make sure that all animals were familiar with the maze and water escape and because intact visuospatial function is an essential prerequisite for MWM-based examination of spatial working memory. In the working memory test, the mice are given two trials separated by a variable interval. Enhanced performance on the second trial relative to the first trial is a measure of working memory, which decays within a few minutes. Performance was measured with ITIs of 60, 300 and 900 s and then the mice were tested with a 60-s ITI again.

Mild DA depletion—Retention of the location of platform position during the second trial was intact in all animals with only a 60-s ITI as indicated by the 10- to 20-s shorter latency (Fig. 5A). The delta-scores were significantly higher than zero for sham-control ($p < 0.01$), *Th*-inactivated ($p < 0.01$) and DA-neuron-ablated mice ($p < 0.01$). With the 300-s ITI delay

condition (Fig. 5A), the delta-scores were only significantly elevated from zero in sham-control mice ($p < 0.01$). Delta-scores with the 900-s ITI delay condition the delta scores were not significantly different from zero for any of the groups (Fig. 5A). In addition, we also performed a two-way repeated-measures ANOVA analysis of the delta-scores and confirmed only a significant effect of ITI delay condition ($F_{3, 138} = 8.12$, $p < 0.01$), but not of group ($F_{2, 46} = 1.75$, $p > 0.05$) or ITI delay condition $\times$ group interaction ($F_{6, 138} = 1.76$, $p > 0.05$).

Moderate DA depletion—With the 60-s ITI delay condition, sham-control ($p < 0.01$), *Th*-inactivated ($p < 0.05$) and DA-neuron-ablated ($p < 0.01$) mice had delta-scores significantly higher than zero, suggesting that retention of the platform position during the second trial was intact in all animals with the short delay (Fig. 5B). With the 300-s ITI delay condition (Fig. 5B), delta-scores were only significantly elevated from zero in sham-control mice ($p < 0.01$) but not in the other groups. Delta-scores with the 900-s ITI delay condition (Fig. 5B) were not significantly different from zero any of the groups.). In addition, we also performed a two-way, repeated-measures ANOVA analysis of the delta scores which confirmed significant effects of ITI delay condition ($F_{3, 156} = 9.31$, $p < 0.01$) and ITI delay condition $\times$ group interaction ($F_{6, 156} = 3.33$, $p < 0.01$), but not of group ($F_{2, 52} = 1.96$, $p > 0.05$). Bonferroni's *post-hoc* comparison confirmed significant differences for the 300-s ITI delay condition between sham control and DA-neuron-ablated mice ($p < 0.05$).

Taken together, we conclude that mild DA depletion had inconclusive effects on our measure of working memory. We noted however a tendency for reduced performance by mildly DA-depleted mice. Moderate DA depletion caused by DA neuron ablation resulted in impaired working memory, which was indistinguishable between the two groups of mice. However, all animals were capable of learning the cognitive flexibility task eventually. We can rule out the possibility that prolonged testing caused impairments in performance, because all groups of mice showed recovery of their delta scores when conditions were shifted back to a 60-s ITI delay for the last training sessions (Figs. 5A-B). However, the performance of the moderate DA neuron-ablated group during the second 60-s ITI trial was significantly impaired compared to the first trial ($p < 0.05$).

Cognitive flexibility and cue-dependent learning

The effect of DA depletion produced in *Th*-inactivated and DA-neuron-ablated mice on cognitive flexibility was tested using a water-escape, U-maze with one black arm and one white arm. After training the mice to use a turn-based strategy (independent of arm color), they had to learn a new escape strategy in which they had to use the color cues.

Mild DA depletion—Learning of the turn-based escape strategy in the first phase of the strategy-shifting task was intact in all DA-depleted animals (Fig. 6A). Two-way, repeated-measures ANOVA analysis of the percentage of correct trials per day revealed significant effects of the number of training days ($F_{2, 84} = 104.0$, $p < 0.01$), but not of group ($F_{2, 42} = 2.34$, $p > 0.05$) or training day $\times$ group interaction ($F_{4, 84} = 0.56$, $p > 0.05$). Analysis of the correct trials during the subsequent shift to a cue-based escape strategy with two-way, repeated-measures ANOVA showed significant main effects of number of training days

($F_{4,168} = 149.4$, $p < 0.01$), group ($F_{2, 42} = 4.35$, $p < 0.05$) and training day $\times$ group interaction ($F_{8,168} = 2.61$, $p < 0.01$) (Fig. 6B). Whereas Bonferroni's *post-hoc* comparison failed to show significant differences between sham-control and *Th*-inactivated mice on any individual training day, it confirmed significant differences between sham and DA-neuron-ablated mice for training days 2 ($p < 0.01$) and 3 ($p < 0.05$) of the strategy-shifting task. Another cohort of DA-depleted animals was trained to acquire only a cue-based escape strategy (Fig. 6C). Two-way, repeated-measures ANOVA analysis of the percentage of correct trials per day revealed only significant effects of the number of training days ($F_{2, 42} = 40.76$, $p < 0.01$), but not of group ($F_{2, 21} = 1.11$, $p > 0.05$) or training day $\times$ group interaction ($F_{4, 42} = 0.15$, $p > 0.05$). Based on these analyses we conclude that deficits observed in strategy-shifting were not due to an inherent impairment of cue-based learning.

Moderate DA depletion—Acquisition of the turn-based escape strategy in the first phase of the strategy-shifting task was intact in all DA-depleted animals. Two-way, repeated-measures ANOVA of the percentage of correct trials per day revealed significant effects of the number of training days ($F_{2, 80} = 68.03$, $p < 0.01$), but not of group ($F_{2, 40} = 1.77$, $p > 0.05$) or training day $\times$ group interaction ($F_{4, 80} = 0.13$, $p > 0.05$) (Fig. 6D). Two-way, repeated-measures ANOVA analysis of the correct trials during the subsequent shift to a cue-based escape strategy showed significant main effects of number of training days ($F_{4,160} = 88.76$, $p < 0.01$), group ($F_{2, 40} = 6.85$, $p < 0.01$) and training day $\times$ group interaction ($F_{8,160} = 2.48$, $p < 0.05$; Fig. 6E). Bonferroni's *post-hoc* comparison confirmed significant differences between sham control and *Th*-inactivated mice on day 2 ($p < 0.05$) and between sham and DA neuron-ablated mice on day 4 ($p < 0.01$). Another cohort of DA depleted animals was trained to acquire only a cue-based escape strategy. Two-way, repeated-measures ANOVA analysis of the percentage of correct trials per day (Fig. 6F) revealed only significant effects of the number of training days ($F_{2, 44} = 41.68$, $p < 0.01$), but not of group ($F_{2, 22} = 0.45$, $p > 0.05$) or training day $\times$ group interaction ($F_{4, 44} = 0.07$, $p > 0.05$). Based on these analyses we conclude that deficits observed in the strategy-shifting phase were not due to an inherent impairment of cue-based learning.

Taken together, we conclude that mild DA depletion following DA neuron ablation slightly impaired cognitive flexibility and that moderate DA depletion caused by either *Th* inactivation or DA neuron ablation also resulted in slightly deficient cognitive flexibility, which was indistinguishable between the two groups of mice. However, all animals were eventually capable of learning the cognitive flexibility task.

Correlations between DA in the dorsal striatum and memory performance and cognitive flexibility

To further elaborate on the findings related to our tests of cognitive behaviors we calculated correlation coefficients (Spearman's r) between levels of DA depletion and performance in our tests of spatial reference memory, working memory and cognitive flexibility. For spatial reference memory (Fig. 7A), we could not confirm a significant correlation coefficient for the percentage time spent in the target quadrant ($r = 0.04$, $p > 0.05$) or for the average proximity to the exact platform location ($r = -0.21$, $p > 0.05$). For spatial working memory (Fig. 7B), we confirmed a significant positive correlation between

striatal DA and delta scores only for the 300-s ITI condition ($r = 0.44$, $p < 0.01$), but not for the 60-s ($r = -0.08$, $p > 0.05$), the 900-s ($r = 0.15$, $p > 0.5$) or the Re-60-s ITI ($r = 0.22$, $p > 0.05$) conditions. For cognitive flexibility (Fig. 7C), we confirmed positive correlations between striatal DA and percentage of correct trials on rule-shift training days 3 ($r = 0.27$, $p < 0.05$) and 4 ($r = 0.39$, $p < 0.01$), but not on day 1 ($r = 0.03$, $p > 0.05$), day 2 ($r = 0.16$, $p > 0.05$) or day 5 ($r = 0.19$, $p > 0.05$) after the rule shift. Taken together, the correlation analyses confirm a quantitative relationship between DA in the dorsal striatum and performance in working memory and cognitive flexibility tasks.

Gender differences in behavioral test results

We did not observe differences related to gender in most of our behavior tests. The notable exception is the hanging wire test. Here male animals in all tested groups had shorter latencies to fall from the grid than females. Still, we could not detect any significant differences between sham control and *Th*-inactivated or DA-neuron ablated male mice with mild DA depletion. For moderate DA depletion, however, ANOVA of latencies to fall by male mice revealed a significant effect of group on latency ($F_{2, 33} = 7.38$, $p < 0.01$), yet only DA-neuron ablated male mice had shorter latencies than sham, control mice ($t = 3.81$, $p < 0.01$). Interestingly, DA-neuron ablated male mice also had shorter latencies to fall than *Th*-inactivated male mice ($t = 2.68$, $p < 0.05$). We did not detect any significant differences of latencies to fall between female mice of any of the groups ($p > 0.05$).

Discussion

In this study we investigated whether the loss of DA neurons has a more severe effect on cognitive behaviors than just the loss of DA synthesis. We developed a genetic approach (*Th* gene inactivation) to cause permanent loss of striatal DA while leaving DA neurons intact and compared that approach with 6-OHDA-mediated ablation of DA neurons (Ungerstedt, 1971). Both techniques targeted the same striatal projection area of nigro-striatal DA neurons and produced partial loss of DA in the dorsal striatum. The depletion of DA in our models was within the range of DA loss observed in PD at the time when motor symptoms first become apparent and cognitive impairment is already observed in some PD patients (Lee et al., 2000; Morrish et al., 1998; Morrish et al., 1995; Schwartz et al., 2004; Tissingh et al., 1998; Williams-Gray et al., 2007). Hence it is important that we achieved DA depletions corresponding to this time point (moderate loss of DA), but also smaller DA depletions (mild loss of DA) which might correspond to prodromal PD. Because cognitive impairment in PD has been reported for spatial memory (Giraud et al., 1997; Owen et al., 1993), spatial working memory (Owen et al., 1997) and cognitive flexibility (Lima et al., 2008), we used procedures that measure similar cognitive behaviors in mice along with tests for motor ability.

Ablation of DA neurons is known to trigger a well-described increased DA turnover in non-affected DA neurons (Zigmond et al., 1990). Our observation that for moderate DA depletion only DA neuron loss resulted in an elevated DOPAC/DA ratio is surprising and indicates that compensatory increase in DA turnover might be triggered by processes related to the loss of DA neurons rather than the loss of DA alone.

Consistent with the classical motor symptoms of PD patients (Samii et al., 2004) and motor deficits observed in animal models of DA neuron loss (Meredith and Kang, 2006), we observed that motor deficits become more severe when more DA was depleted. However, contrary to reports from studies using SNpc lesion of DA neurons in rats (Da Cunha et al., 2002; Mura and Feldon, 2003), we did not observe deficits in visuospatial function or spatial memory with either mild or moderate loss of DA, in agreement with our previous results in mice with 80 % depletion of DA (Darvas and Palmiter, 2010). The effects observed in more severe lesioning models may be due to loss of co-transmitters and/or the responses to neuronal destruction (Palmiter, 2008; Willis and Kennedy, 2004).

Interestingly, moderate striatal DA depletion resulted in deficits in spatial working memory. Our data as well as studies on mice transiently over-expressing D2 receptors (Kellendonk et al., 2006) or lacking adenosine A_{2A} receptors (Wei et al., 2011) implicate a role for striatal circuitry in mediating aspects of working memory. Our data are also in agreement with both PET studies in healthy subjects that have shown a correlation between striatal D2 receptor occupancy together with impaired working memory after treatment with a D2 receptor antagonist (Mehta et al., 2008) and with imaging studies of patients with early-stage PD that had impaired spatial working memory together with impaired nigro-striatal function (Sawamoto et al., 2008). Our studies isolate the contributions of nigro-striatal DA signaling from other processes related to altered D2R-signalling and PD-related changes in other brain circuits (Braak and Del Tredici, 2008) and hence implicate a role of striatal DA signaling for working memory.

Working memory and cognitive flexibility are thought to be elements of cognitive control processes, termed executive function, which control how the brain optimizes given cognitive resources to address currently relevant tasks (Mansouri et al., 2009). Traditionally, executive function has been seen primarily as a domain of the prefrontal cortex (Birrell and Brown, 2000; Brown and Bowman, 2002; Buckner, 2004; Simpson et al., 2010; Stefani et al., 2003), but several reports have also implicated striatal circuits in mediating cognitive flexibility in animal models (Floresco et al., 2006a; Ragozzino et al., 2009; Ragozzino et al., 2002; Wang et al., 2013). Our finding of mildly impaired cognitive flexibility after striatal DA depletion agrees with these previous studies and, more importantly, by demonstrating isolated contributions of nigro-striatal DA our data illustrate how one neural circuit that is affected in early-stage PD can cause impaired cognitive flexibility observed in patients (Leverenz et al., 2009; Lima et al., 2008). We observed an effect of striatal DA depletion on the ability to shift from a turn-based to a cue-based strategy, which has been shown to be also dependent on DA signaling in the prefrontal cortex (Floresco et al., 2006b; Ragozzino, 2002). Another aspect of cognitive flexibility, reversal learning, has been shown to depend on serotonergic signaling in the prefrontal cortex, suggesting that different aspects of executive function depend on different loci and transmitter systems in the PFC (Clarke et al., 2004; Clarke et al., 2005). An interesting future direction would be to investigate potential differences between contributions of striatal DA to reversal learning vs. turn-cue strategy shifting.

Our results clearly show an involvement of mild to moderate changes of nigro-striatal DA signaling in mediating two aspects of executive function in mice, and hence implicate one neuroanatomical substrate for mild cognitive impairment in early-stage PD that may be

corrected by pharmacological intervention. Yet, although drugs acting on DA receptors have been well established for the treatment of PD motor symptoms (Antonini et al., 2009; Perez-Lloret and Rascol, 2010; Schapira and Olanow, 2008), little has been shown to support their capability to improve cognition. PD is a complex degenerative disease that affects several cortical and sub-cortical neurotransmitter systems, all of which might contribute to the range and severity of disease symptoms. There is no experimental animal model that reflects this complexity. Our approach serves to estimate the isolated contributions of DA neurons to some of the motor and cognitive symptoms of the disease.

Another important finding is the observation of several differences between mice with *Th* gene inactivation and mice with loss of DA neurons. We noticed that while motor deficits already emerged in mice when only little striatal DA was lost due to neuron ablation, the same amount of DA depletion due to loss of TH did not show much motor impairment. Similarly, under conditions of mild DA depletion, deficits in cognitive flexibility were more apparent in mice with DA neuron ablation than loss of TH only. This difference could be due to loss of other signaling molecules made by DA neurons and/or to gliosis and inflammatory responses to neuronal death. Although the observed differences were small, our finding is important for the treatment of PD symptoms because non-DA drug targets that impact striatal circuitry could provide treatment alternatives or additions to the traditional DA replacement strategy in PD which is often complicated by unwanted side effects of dopaminergic drugs (Kalia et al., 2012; Perez-Lloret and Rascol, 2010). In fact, much of the current effort to establish novel therapeutic approaches for PD involves non-DA drugs (Ahlskog, 2007; Meissner et al., 2011) and our animal models would provide a suitable preclinical test of the efficacy of these drugs.

As PD progresses, more DA neurons degenerate and more striatal DA is lost (Fearnley and Lees, 1991), hence warranting investigation of more severe loss of striatal DA. An important limitation of all animal models of PD is that only certain aspects of PD are modeled and the disease is more complicated than loss of DA neurons (Langston, 2002; Langston, 2006). At the time SNpc DA neurons are affected in PD, many other brain regions also show signs of neurodegeneration, some of which project directly to midbrain DA neurons or to other components of the basal-ganglia circuitry of which DA neurons are merely one part (Braak and Del Tredici, 2008; Del Tredici and Braak, 2012; Langston, 2006). Consequently, the inputs that modulate the activity of DA neurons, as well as other neurons in the basal ganglia, are likely to be dysfunctional. An interesting future approach would be to combine manipulations to both SNpc DA neurons and other affected areas and assess the consequences on motor and cognitive behavior. Now that we have data to support the idea that other factors, beyond the loss of DA, contribute to the DA neuron ablation phenotype, the next step is to identify these factors. The challenge will be to discriminate between secondary processes of DA neuron degeneration like inflammation and gliosis and the loss of other signaling molecules (e.g. brain-derived neurotrophic factor, cholecystokinin, glutamate, serotonin and GABA) that are known to be released by DA neurons (Seutin, 2005; Tritsch et al., 2012).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Striatal dopamine depletion results in cognitive deficits resembling early onset PD.
- Loss of dopamine signaling in striatum, rather than prefrontal cortex, causes cognitive decline.
- Motor and cognitive impairment becomes more severe with increased loss of striatal dopamine.
- Loss of dopaminergic neurons causes greater behavioral defects than the loss of dopamine alone.

Fig. 1.

Expression of TH and DAT in the midbrain. Expression of TH and DAT was visualized in coronal midbrain sections of sham control, *Th*-inactivated and DA neuron ablated mice by immunostaining for TH (red) and DAT (green). A–C: TH expression pattern in sections through the SNpc and VTA of sham control (A), *Th*-inactivated (B) and DA neuron-ablated mice (C). Expression of TH is similarly reduced in the SNpc of *Th*-inactivated and DA neuron-ablated mice. D–F: DAT expression pattern in sections through SNpc and VTA of sham control (D), *Th*-inactivated (E) and DA neuron-ablated mice (F). Expression of DAT is not altered in *Th*-inactivated mice and clearly reduced in the SNpc of DA neuron-ablated mice.

Fig. 2.

Tissue content of catecholamines in the striatum. A–D: Shows data for sham control (N = 29), and *Th*-inactivated (N = 35) and DA neuron-ablated mice (N = 13) with mild depletion of DA (> 70 % of sham level) in the dorsal striatum. A: DA content in the dorsal and ventral striatum. B: DOPAC levels in the dorsal striatum. C: Ratio of DOPAC/DA in the dorsal striatum. D: Norepinephrine levels in the dorsal striatum. E–H: Shows data for sham control (N = 29), and *Th*-inactivated (N = 21) and DA neuron-ablated mice (N = 17) with moderate depletion of DA (< 70 % of sham level) in the dorsal striatum. E: DA content in the dorsal and ventral striatum. F: DOPAC levels in the dorsal striatum. G: Ratio of DOPAC/DA in the dorsal striatum. H: Norepinephrine levels in the dorsal striatum. Significant effects are marked with an asterisk (★ ★p < 0.01, ★ p < 0.05) or with a diamond (p < 0.05, comparison between *Th*-inactivated and DA-neuron-ablated mice). All data are shown as means ± SEM.

Fig. 3.

Motor functions. A–C: Show data for sham control and mice with mild depletion of DA in the dorsal striatum for *Th*-inactivated and DA neuron-ablated mice. (A) Latency to fall in the hanging-wire/four-limb hang test by sham control (N = 33), *Th*-inactivated (N = 32) and DA neuron ablated mice (N = 10). (B) Latency to fall in an accelerated rotarod test over a 12-trial testing period by sham control (N = 33), *Th*-inactivated (N = 25) and DA neuron-ablated mice (N = 13). (C) Latency to remove a circular adhesive placed on the animal's forehead by sham control (N = 40), *Th*-inactivated (N = 9) and DA neuron-ablated mice (N = 10). D–F: Show data for mice with moderate depletion of DA in the dorsal striatum. (D) Latency to fall in the hanging-wire/four-limb hang test by sham control (N = 33), *Th*-inactivated (N = 18) and DA neuron-ablated mice (N = 15). (E) Latency to fall in an accelerated rotarod test over a 12-trial testing period by sham control (N = 33), *Th*-inactivated (N = 21) and DA neuron-ablated mice (N = 15). (F) Latency to remove a circular adhesive placed on the animal's forehead by sham control (N = 40), *Th*-inactivated (N = 13) and DA neuron-ablated mice (N = 15). Significant effects are marked with an asterisk (★ ★ p < 0.01, ★ p < 0.05). All data are shown as means ± SEM.

Fig. 4.

Visuospatial function and spatial reference memory. Data were obtained using the Morris water maze procedure with a 4-day training procedure and 4 trials per day. A–E: Show data for sham control (N = 34), and *Th*-inactivated (N = 22) and DA neuron-ablated mice (N = 8) with mild depletion of DA in the dorsal striatum. (A) Latency to climb onto the hidden platform during training. (B) Swim speed in the maze during training. (C) Path length traveled during training sessions. (D) Time spent searching in the quadrants of the Morris water maze after 4 days of training. (E) Average proximity to the exact platform position after 4 days of training. F–J: Show data for sham control (N = 34), and *Th*-inactivated (N = 15) and DA neuron ablated mice (N = 15) with moderate depletion of DA in the dorsal striatum. (F) Latency to climb onto the hidden platform during training. (G) Swim speed in the maze during training. (H) Path length traveled during training sessions. (I) Time spent searching

in the quadrants of the Morris water maze after 4 days of training. (J) Average proximity to the exact platform position after 4 days of training. Significant effects are marked with an asterisk (★ ★ $p < 0.01$, ★ $p < 0.05$). All data are shown as means $\pm$ SEM.

Fig. 5.

Spatial working memory. Working memory was recorded using a modified Morris water maze procedure. Animals received 4 blocks of a 4-day training procedure with 2 trials per day and the position of the platform was changed every day. The time between trial pairs was constant within each block and varied between blocks (60 s, 300 s, 900 s and back to 60 s). Working memory was calculated for each block as average difference between escape latencies on the trial pairs (Trial 1 – Trial2). A positive Δ -score that was significantly higher than zero was interpreted as intact working memory. A: Δ -scores by sham control (N = 29), and *Th*-inactivated (N = 8) and DA neuron-ablated mice (N = 12) with mild depletion of DA in the dorsal striatum. B: Δ -scores by sham control (N = 29), and *Th*-inactivated (N = 10) and DA neuron-ablated mice (N = 15) with moderate depletion of DA (< 70 % of sham level) in the dorsal striatum. Significant effects are marked with an asterisk (★ $p < 0.05$). All data are shown as means $\pm$ SEM.

Fig. 6.

Cognitive flexibility and cue-dependent learning. Animals were first trained to acquire a turn-based water-escape strategy and then had to learn a new cue-based water-escape strategy. A-B: Show cognitive flexibility and cue-dependent learning data for sham control ($N = 18$), and *Th*-inactivated ($N = 15$) and DA neuron-ablated mice ($N = 12$) with mild depletion of DA in the dorsal striatum. Percentage of correct trials during 3-day training of turn-based water escape (A) and percentage of correct trials during 5-days of training a cue-based water-escape condition by animals that previously learned turn-based water-escape (B). C: Percentage of correct trials during 3-day training of cue-based water-escape by animals that were not previously trained to learn turn-based water-escape (sham $N = 14$, mild DA depletion: *Th*-inactivated $N = 5$ and DA neuron-ablated $N = 5$). D-E: Shows data for sham control ($N = 18$), and *Th*-inactivated ($N = 10$) and DA neuron-ablated mice ($N = 15$) with moderate depletion of DA in the dorsal striatum. Percentage of correct trials during 3-day training of turn-based water escape (D) and percentage of correct trials during 5-days of training a cue-based water-escape condition by animals that previously learned turn-based water-escape (E). F: Percentage of correct trials during 3-day training of cue-based, water-escape by animals that were not previously trained to learn turn-based water-escape (sham $N = 14$, moderate DA depletion: *Th*-inactivated $N = 5$ and DA neuron-ablated $N = 6$). All data are shown as means $\pm$ SEM.

Fig. 7.

Correlation analysis of striatal DA and cognitive behaviors. Correlation coefficients (Spearman's r) were calculated for levels of striatal DA and performance on the following cognitive tests. A: Spatial reference memory (measures of quadrant preference and average proximity to exact platform position). B: Spatial working memory (d' -scores for all ITI conditions: 60-s, 300-s, 900-s and Re-60-s). C: Cognitive flexibility (percentage of correct trials on 5 days following rule shift). Significant correlations are marked with an asterisk (★ ★ $p < 0.01$, ★ $p < 0.05$).