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Amelioration of behavioral deficits in a rat model of Huntington's disease by an excitotoxic lesion to the globus pallidus

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Abstract

Four groups of rats, sustaining a striatal quinolinic acid (QA) lesion, a pallidal QA lesion, a combined striatal + pallidal lesion, or sham operation, were tested in spontaneous and amphetamine-induced activity, spatial navigation in a water maze, position discrimination and reversal in a wet T maze, and food manipulation. The striatal lesion markedly impaired rats' performance on the motor and cognitive tasks. In contrast, rats sustaining a bilateral lesion to the GP in addition to the striatal lesion performed similarly to sham-operated rats on the motor and cognitive tasks, although they showed a transient decrease in activity levels. Given that a similar dysfunction of basal ganglia circuitry is thought to subserve the behavioral alterations seen in QA-lesioned rats and Huntington's disease (HD) patients, the present results raise the possibility that manipulations of the external segment of the globus pallidus (the primate analogue of the rat GP) could ameliorate some of HD symptoms.
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Keywords: Striatum; Globus pallidus; Quinolinic acid lesion; Basal ganglia-thalamocortical circuitry; Huntington's disease (HD); Rat

Introduction

Huntington's disease (HD), one of the CAG trinucleotide-repeat diseases (Huntington's Disease Collaborative Research Group, 1993), is a progressive neurodegenerative disorder of mid-life onset, characterized clinically by progressive involuntary choreiform movements, cognitive decline, and personality changes (Albin et al., 1989; Martin and Gusella, 1986; Penney and Young, 1986; Wilson and Garron, 1979). Brain imaging and neuropathological studies have revealed that in the early stages of the disease, the striatum is most severely affected, although other brain regions, including the cortex, may show some pathological changes (Albin and Tagle, 1995; Harris et al., 1996; Hayden et al., 1986; Penney and Young, 1986; Vonsattel and DiFiglia, 1998; Vonsattel et al., 1987). In the striatum, the early stages of the disease are marked by a selective loss of striatal neurons projecting to the external segment of the globus pallidus (GPe) and the substantia nigra pars reticulata, while neurons projecting to the internal segment of the globus pallidus are lost only in later stages (Albin and Tagle, 1995;

Albin et al., 1990a,b; Reiner et al., 1988; Sapp et al., 1995; Storey and Beal, 1993). Based on these findings, the leading model of HD, launched by (Penney and Young, 1986; Albin et al., 1989) and Albin and Penney (1989), views this disease as resulting from abnormal functioning of basal ganglia-thalamocortical circuitry. More specifically, loss of striatal innervation to GPe leads to overactivity of GPe, which results in turn in the disruption of basal ganglia output to the thalamus (Albin et al., 1989; Crossman, 1987; Penney and Young, 1986; for a detailed application of the model to HD, see Joel, 2001).

On the basis of the pathological mechanism suggested to underlie the symptomatology of HD, we suggested that lesion of the overactive GPe should ameliorate some of the symptoms of HD (Joel and Weiner, 1997). Using one of the leading rat models of HD, striatal quinolinic acid (QA) lesion, which has been shown to closely mimic the selective neural degeneration in the striatum of HD patients (Beal et al., 1986; Ellison et al., 1987; Figueredo-Cardenas et al., 1994; Roberts et al., 1993), we had previously shown that bilateral electrolytic lesion to the GP (the rat analogue of the primate GPe) ameliorated the deleterious effects of bilateral QA lesion to the striatum on post-surgery weight, activity level, and performance in a water maze task (Joel et al., 1998).

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The aim of the present study was 2-fold: to extend the assessment of the behavioral alterations induced by the striatal lesion, and to test whether these deficits can be ameliorated by an axon-sparing lesion to the GP. To this end, five groups of rats, (1) rats with bilateral QA lesion to the striatum, (2) rats with bilateral QA lesion to the striatum and bilateral QA lesion to the GP performed simultaneously with the striatal lesion, (3) rats with bilateral QA lesion to the striatum and bilateral QA lesion to the GP performed 1 month after the striatal lesion, (4) rats with bilateral QA lesion to the GP, and (5) sham-operated rats, were tested in several behavioral assays known to be sensitive to striatal damage: spontaneous and amphetamine-induced activity (Emerich and Sanberg, 1992; Sanberg et al., 1989), spatial navigation in a water maze (Block et al., 1993; Furtado and Mazurek, 1996; Joel et al., 1998; Whishaw et al., 1987), position discrimination and reversal in a T maze (Dunnett and Iversen, 1981; Pisa and Cyr, 1990; Reading et al., 1991), and food manipulation (Pisa, 1988).

Materials and methods

Subjects

Thirty-seven male Wistar rats (Tel Aviv University, Sackler Faculty of Medicine, Israel) approximately 4 months old, weighing 350–470 g, were housed four to a cage under reversed cycle lighting (lights on: 1900–0700 h) with food and water freely available, except for 2 days before and during the food manipulation task (see below). All experimental protocols were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University.

Surgery

Surgery was performed in two rounds. In the first round, sham ($n = 8$), striatal ($n = 9$), simultaneous combined striatal + pallidal ($n = 7$), and striatal lesion of the delayed combined striatal + pallidal ($n = 7$) were performed. The GP lesions in the delayed combined striatal + pallidal group and in the pallidal group ($n = 6$) were performed 30–40 days after the other three lesions (sham, striatal, and simultaneous combined striatal + pallidal). Rats received 3 mg/kg diazepam (i.p.), and 20 min later were anaesthetized with i.p. injection of avertin (1 ml/100 g). They were placed in a stereotaxic frame and an incision was made into the scalp to expose the skull. The vertical coordinates of bregma and lambda were measured to align them in same (level head) plane. Lesion coordinates were according to the atlas of Paxinos and Watson (1997). QA (Sigma Chemicals, Israel) was dissolved in 1 M NaOH and diluted with phosphate buffer to a final pH of 7.4 and the appropriate concentration. *Striatal lesion*: 31-gauge cannulae were vertically lowered into the brain through holes drilled in the skull at the following coordinates: 1.0 mm anterior to bregma, 2.5 mm lateral to the midline, and 4.3 mm ventral to dura. One microliter of QA in a concentration of 150 nmol/μl was infused at a constant rate over 3 min. The cannulae were left in place for additional 5 min, to reduce upward diffusion of the solution. *GP lesion*: Bilateral lesions were made by infusing 0.3 μl of QA in a concentration of 120 nmol/μl at the following coordinates: 0.5 mm posterior to bregma, 2.6 mm lateral to the midline, and 5.5 mm ventral to dura. *Simultaneous combined striatal and GP lesion*: Rats sustained a bilateral lesion to the striatum and a bilateral lesion to the pallidum as above. *Delayed combined striatal and GP lesion*: Rats sustained a bilateral lesion to the striatum and a bilateral lesion to the pallidum as above. Bregma was marked using a driller at the time of striatal surgery to be clearly seen at the time of the pallidal surgery. *Sham operation*: Rats underwent the same surgical procedure as striatal rats but 1 μl of vehicle was used instead of QA. Sterispon was used to cover the holes in the bone, the scalp incision was sutured by Michel clips, sulfonamide powder was sprinkled on the wound, and an additional dose of 3 mg/kg diazepam was given about 30 min following surgery to prevent seizures. Post-surgical maintenance and care followed closely the procedure described by Cromwell and Berridge (1996). Three rats (one from the pallidal group, one from the simultaneous combined striatal + pallidal group, and one from the delayed combined striatal + pallidal group) died following surgery.

Histology

After the completion of behavioral testing, rats were overdosed with nembutal (60 mg/kg, i.p.) and perfused intracardially with a solution of 0.9% NaCl solution (Fluka, Buchs, Switzerland) at room temperature for 2 min (flow rate 35 ml/min), followed by 4% paraformaldehyde (Fluka) in 0.1 M phosphate buffer (PBS, pH 7.2) at 4°C at a flow rate of 35 ml/min for 15 min. The descending aorta was clamped and the animal's head was packed with ice during the entire perfusion. The brains were removed from their skulls and postfixed for 2 h in 4% paraformaldehyde in 0.1 M PBS and then placed in a solution of 30% sucrose (Fluka) in 0.1 M PBS, pH 7.4 for 2 days at 4°C under gentle agitation. Forty-micrometer frozen coronal sections were cut using a sliding microtome. For histological examination, every fifth section was mounted and stained with cresyl violet. Verification of placements of lesions used the atlas of Paxinos and Watson (1997). To estimate the lesion extent, digital images of the striatum of lesioned and control animals were obtained using the Image-Pro Plus image analysis system (version 4.01, Media Cybernetics, Silver Spring, MD, USA). For calibration purposes, images of a micrometer slide were also acquired at the same magnification as that used for the striatum images. Cross-sectional area of the striatum was then calculated using the Image-Pro program.

Apparatus and procedure

Weight

Each rat was weighted before surgery, and on days 2, 6, 10, and 30 post-surgery. The percentage of each post-surgery weight from the rat's pre-surgery weight was calculated.

Spontaneous and amphetamine-induced activity

Activity was measured in plastic chambers (46 × 57 × 37 cm), covered by 50 × 50 cm clear Perspex lids, located in a darkened and air-conditioned room. A Coulbourn Instruments infrared sensor unit was installed in the center of a front wall 22 cm from the side walls, and 12 cm above the grid floor, and protected by a wire fence measuring 10 × 13 × 6 cm to prevent animals' access. Blind areas of the sensor (the two corners of the triangles adjacent to the sensor, measuring 17 × 17 × 25) were blocked by two clear Perspex walls with dimensions of 25 × 57 cm. The movements detected by the sensor were transmitted through a Coulbourn Instruments eight-channel infrared motion interface to a Coulbourn Instruments infrared motion activity monitor controller/analyzer. Data recording was computer controlled.

For spontaneous activity measurement, rats were individually placed in the activity chambers and allowed 30 min of free exploration. The duration of movements performed by each animal was recorded in 6 min blocks. For amphetamine-induced activity measurement, each rat was allowed 30 min of free exploration as above, after which it was returned to its home cage, injected intraperitoneally with 1 mg/kg D-amphetamine (Sigma Chemical, Israel) dissolved in 1 ml saline, and replaced into the chamber for an additional 60 min. Pre- and post-amphetamine locomotor activity was recorded as above.

Spatial navigation in a water maze

The water maze task was conducted in a circular pool (diameter—137 cm, height—35 cm) in which a transparent plastic platform (15.5 × 15.5 cm) was located below the water, invisible to the rat. There were abundant extra-maze cues in the room kept constant throughout testing.

The task included three stages. On days 1–3 (acquisition), rats were given eight trials with the platform located in the center of the northeast quadrant, starting each trial at one of four starting locations (north, south, east, or west) in a pseudo-random order. If a rat found the platform, it was permitted to remain on it for 5 s. If it failed to find the platform for 60 s, it was taken out of the water and put on the platform for 5 s. Between trials, rats were kept in a holding cage for approximately 2 min. On day 4 (probe), rats were given four trials as in acquisition, and a 60-s probe trial, in which the platform was removed from the pool, was interposed between the 2nd and 3rd trials. On day 5 (reposition), rats were given four trials with the platform in the northeast quadrant, followed by four trials in which the platform was repositioned in the center of the southwest quadrant. All trials were

videotaped by a Sony camera mounted on the ceiling directly above the pool and analyzed by a computerized tracking system (HVS image, VP118 super tracker). The following measures were recorded: latency to find the platform on each
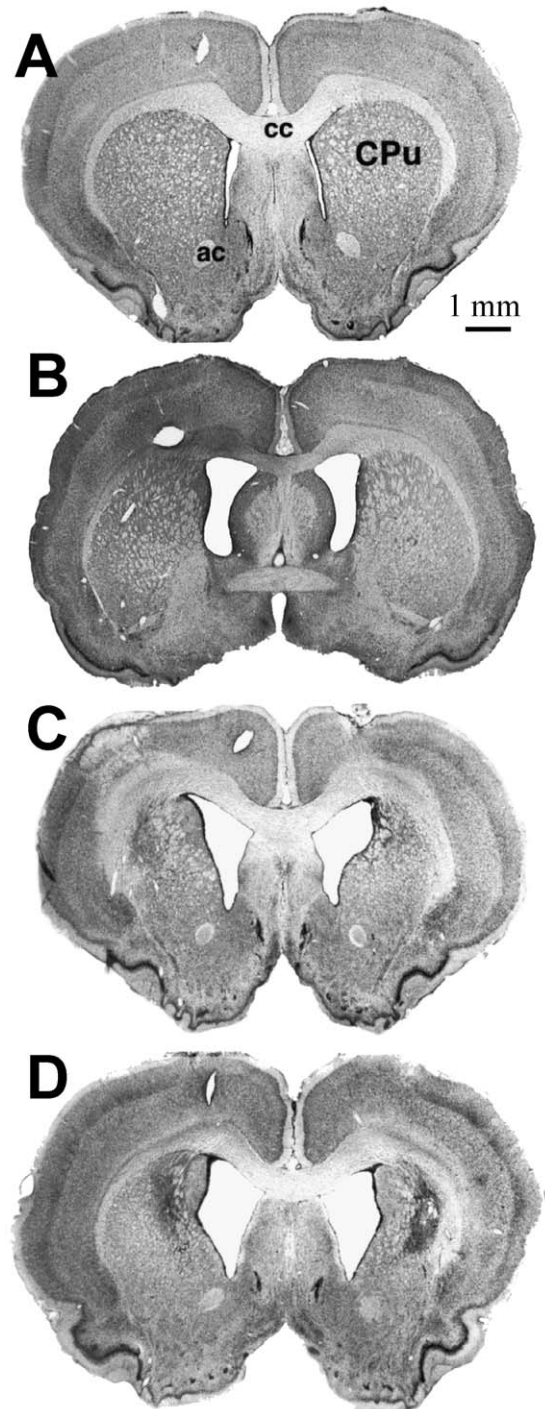

Plate 1. Representative photomicrographs of (A) the striatum of a sham-operated rat, (B) the pallidal lesion of a rat from the pallidal group, (C) the striatal lesion of a rat from the striatal group, and (D) the striatal lesion of a rat from the combined striatal + pallidal group. Abbreviations: ac, anterior commissure; cc, corpus callosum; CPu, caudate-putamen.

trial, time spent in each quadrant during the 60-s probe trial, and time spent in each of three concentric zones (center, platform, periphery) during the 60-s probe trial.

Position discrimination and reversal task in a wet T maze

The task was conducted in a T maze (width: 15.5 cm, height of walls above the water: 11 cm, length of stem: 70 cm, length of cross piece: 121 cm) located in the circular pool described above. Two guillotine doors were located 1 cm from the beginning of each of the arms. A hidden transparent plastic platform (15.5 × 15.5 cm) was located 1 cm below the water at the end of one of the arms.

The task included two stages. On day 1 (position discrimination), rats were trained to swim to the platform which was consistently located in one of the arms (left and right sides counterbalanced within groups), until they reached a criterion of five consecutive correct trials. At the start of each trial, the rat was placed in the starting box, facing the wall opposite the cross piece, and allowed to swim and choose between the two arms. Once it had entered an arm, the guillotine door blocking that arm was lowered preventing the rat from retracing. If the rat chose the correct arm, it was allowed to remain on the platform for 5 s after which it was removed from the maze to a holding cage for a 10-s inter-trial

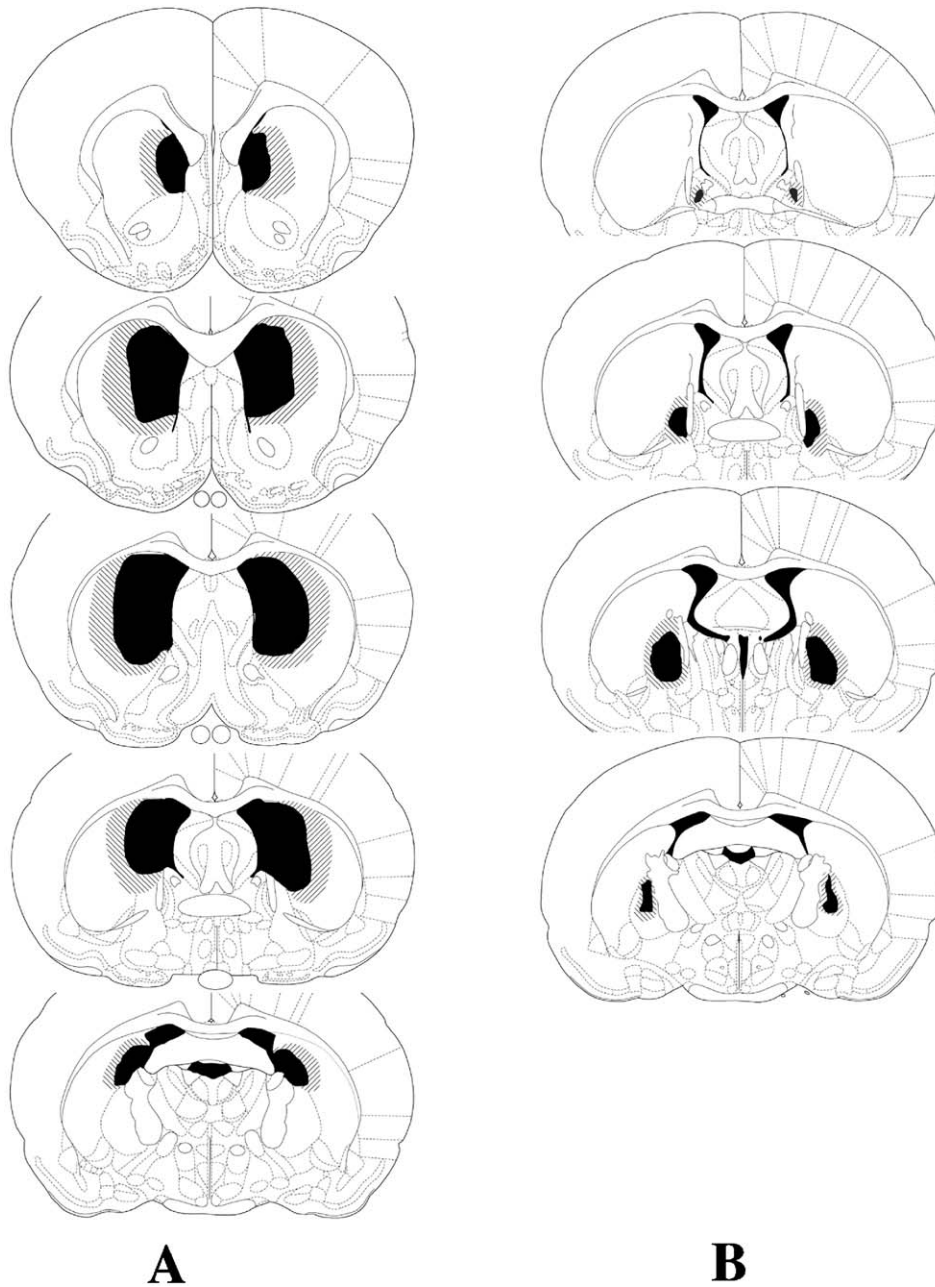**A**   **B**

Plate 2. Reconstruction of the striatal (A) and pallidal (B) lesions in successive brain sections representing the minimal (black) and the maximal (hatched) extent of the damage.

interval. If the wrong arm was chosen, the rat was confined to the arm for approximately 5 s and then removed from the maze to a holding cage for the duration of the inter-trial interval. On days 2–5 (position discrimination and reversal), each rat was first retrained until criterion on the position discrimination on which it was trained last on the previous day (i.e., on day 2, rats were retrained on the initial discrimination, whereas on days 3–5, they were retrained on the reversal of the previous day) and then trained until criterion on the reversal of this discrimination, that is, with the platform located in the opposite arm. Other than that, training continued exactly as on day 1. The arm chosen and the time to reach the platform on each trial were recorded. The mean number of trials to reach criterion on the five discriminations (days 1–5), the mean number of trials to reach criterion on the four reversals (days 2–5), and the mean time to reach the platform in the five criterion trials of the initial discrimination were calculated.

Food manipulation

Rats were put on a 23-h food restriction schedule. On the following day (habituation), rats were placed in a 38 × 21 cm Plexiglas observation box for 10 min. On the next day (test), rats were placed in the observation box, and 5 min later, several Purina rat food pellets (approximately 2 g each) were introduced into the box. Rats were given 10 min to consume the food pellet. Rats' ability to manipulate the food pellet was rated independently by two observers, according to the scale of Kolb and Holmes (1983): 0, the animal was unable to manipulate the food pellet with the forepaws; 1, the animal held the food pellet against the floor with its forepaws when it ate; 2, the animal ate the food pellet from the floor and sometimes held the food pellet in its forepaws; 3, the animal picked the food pellet up in its forepaws and partially

ate it but dropped the food pellet before it was all consumed; and 4, the animal sat up on its hind paws and held the food pellet in its front paws until it was finished. Ratings given by the two observers (inter-judge reliability: 0.98) were recorded, and the average of the two ratings was used as the animal's score.

Experimental design

Rats were weighted before surgery, and again on days 2, 6, 10, and 30 post-surgery. Spontaneous activity was assessed on days 2 and 10 after surgery. For each rat, both measurements were taken at the appropriate time relative to the rat's surgery day. Therefore, rats from the sham, striatal, and simultaneous combined striatal + pallidal groups were run on the same days, and rats from the pallidal and delayed combined striatal + pallidal groups were run on the same days.

Testing in the water maze task commenced 2 weeks after the performance of the pallidal lesion in the pallidal and in the delayed combined striatal + pallidal groups (i.e., 7–8 weeks after surgery for the sham, striatal, and simultaneous combined striatal + pallidal groups). Eleven days after the termination of the water maze task, training in the position discrimination and reversal task began. Eight to 14 days following the termination of the latter task, rats were tested in the food manipulation task. Amphetamine-induced activity was assessed after the completion of the food manipulation task. In all four tests, rats from all the lesion conditions were run on the same days.

Statistical analysis

The behavioral data were analyzed using analyses of variance (ANOVA) with a main factor of lesion and repeated measurements factors appropriate to the design of each
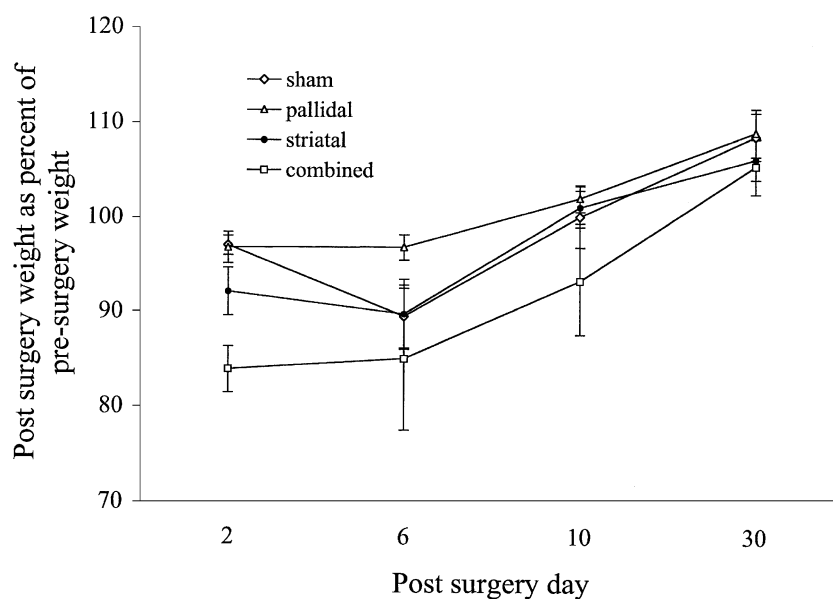

Fig. 1. Means and standard errors of the post-surgery weight (calculated as percentage of pre-surgery weight) of the sham, striatal, pallidal, and combined striatal + pallidal groups, on days 2, 6, 10, and 30 post-surgery.

experiment (Weight, days; Spontaneous and amphetamine-induced activity, sessions and blocks; Water maze, days and blocks; quadrants; zones; location and blocks; Position discrimination and reversal, reversal; Food manipulation, no repeated measurements factor). In all experiments, post hoc least significant difference (LSD) comparisons were used to unravel the source of the significant effect of lesion or of its interactions with other factors.

Results

Anatomical

In five out of six rats with a delayed combined striatal + pallidal lesion, a very extensive striatal damage prevented verification of the placement of the pallidal lesion. This group was therefore not included in further analyses. Representative photomicrographs at mid-rostral level through the striatum of a rat from the sham, pallidal, striatal, and combined striatal + pallidal groups are presented in Plate 1. Reconstruction of the striatal and pallidal lesions in successive brain sections representing the minimal (black) and the maximal (hatched) extent of the damage is presented in Plate 2, columns A and B, respectively. As can be seen, a substantial shrinkage of the striatum and an enlargement of the lateral ventricles were observed in rats with a striatal or a combined striatal + pallidal lesion. Compared with the sham group, the striatal area in striatal, combined striatal + pallidal, and pallidal groups was on the average reduced to 57%, 62%, and 95%, respectively. An ANOVA with a main factor of lesion and a repeated measurements factor of hemisphere
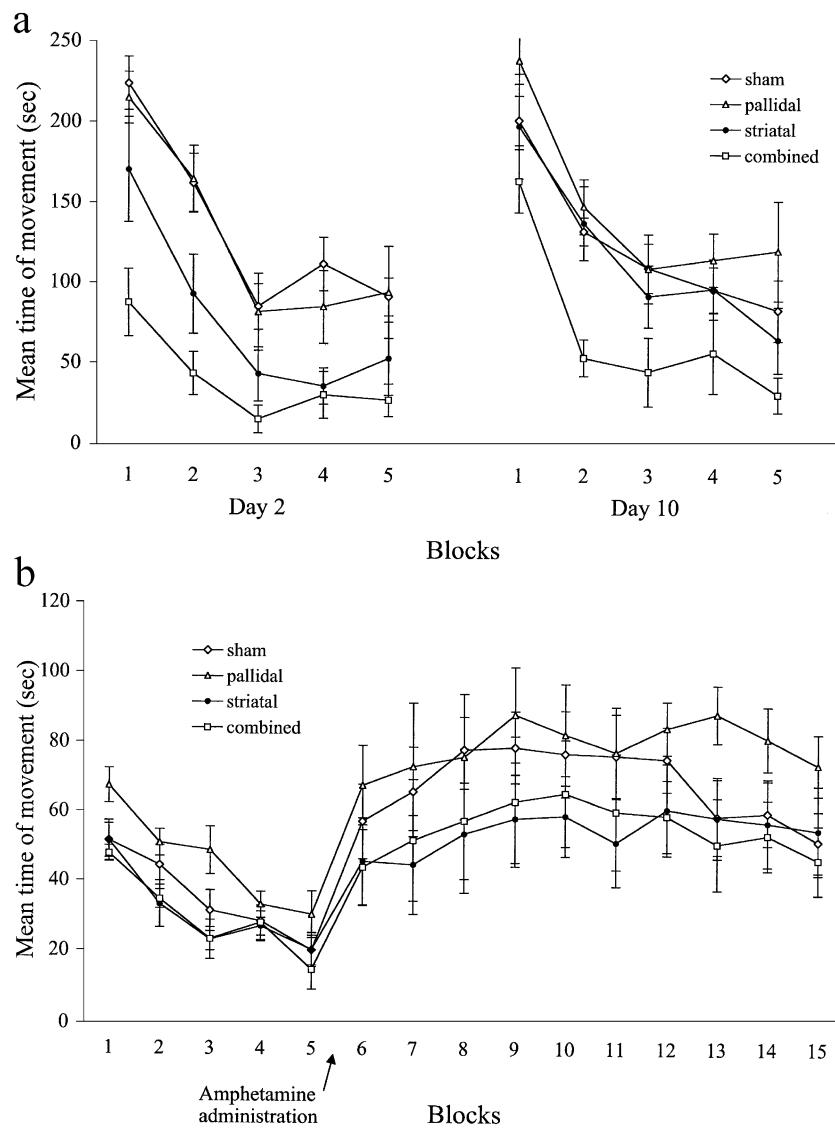

Fig. 2. Means and standard errors of the duration of movement, in 6 min blocks, of the sham, pallidal, striatal, and combined striatal + pallidal groups, during (a) 30 min spontaneous activity sessions on days 2 and 10 post-surgery, and (b) 30 min of spontaneous activity and 60 min of amphetamine-induced activity on day 50 post-surgery.

yielded only a significant main effect of lesion, $F(3,20) = 16.317$, $P < 0.0001$. Post hoc comparisons revealed that the striatal area of the striatal and combined striatal + pallidal groups was significantly smaller than that of the sham and pallidal groups (all P's < 0.01). In addition, the morphology of the lesioned striatum was somewhat disrupted, showing accumulation of glia cells in the lesioned area. The striatum displayed a spongy-like appearance, with cavitation in the lesioned area. The lesion was confined to the dorsal striatum involving mainly its medial parts. In some cases, a thinning of the corpus callosum and a gliosis in the ventrolateral nucleus of the thalamus were also observed. The ventral striatum was spared in the majority of cases. The striatal lesion in most animals extended from 1.7 mm anterior to bregma to 1.5 mm posterior to bregma (A–P), from 1.5 to 4.0 mm lateral to the midline (M–L), and from 3.3 to 7.4 mm ventral from dura (D–V). Maximal damage was between 1.4 to -0.8 mm A–P, 1.9 to 3.9 mm M–L, and 3.6 to 6.7 mm D–V. The pallidal lesion in most animals extended from -0.26 to -1.6 mm A–P, from 1.5 to 4.5 mm M–L, and from 4.8 to 7.7 mm D–V. Maximal damage was between -0.3 and -1.3 mm A–P, 1.9 and 3.9 mm M–L, and 5 and 7 mm D–V.

Following histological examination, three rats were excluded from the final analysis: a rat with a striatal lesion and a rat with a combined striatal + pallidal lesion in whom the striatal lesion was evident only unilaterally, and one sham rat with very enlarged ventricles. Data of one rat with a combined striatal + pallidal lesion in the water maze task were lost due to computer failure. Thus, the final analysis included seven, five, eight, and five (four in the water maze task) rats in the sham, pallidal, striatal, and simultaneous combined striatal + pallidal groups, respectively.

Behavioral

Post-surgery weight

Mean post-surgery weight, calculated as percentage of pre-surgery weight, of the sham, striatal, pallidal, and combined striatal + pallidal groups, on days 2, 6, 10, and 30 post-surgery, is depicted in Fig. 1. As can be seen, all the groups have gained weight over the period assessed (repeated measurements factor of days, $F(3,63) = 45.79$, $P < 0.0001$). Although the combined striatal + pallidal group had a lower post-surgery weight on day 2, this difference was not significant and disappeared by day 6.

Spontaneous and amphetamine-induced activity

Fig. 2a presents the mean duration of movement, in 6 min blocks, of the sham, pallidal, striatal, and combined striatal + pallidal groups, during 30 min spontaneous activity sessions on days 2 and 10 post-surgery. As can be seen, spontaneous activity of all groups declined similarly within each session (repeated measurements factors of blocks, $F(4,84) = 126.04$, $P < 0.0001$) but the combined striatal + pallidal group exhibited the lowest levels of activity (main effect of lesion, $F(3,84) = 5.31$, $P < 0.01$). Post hoc comparisons confirmed that the mean activity (over both sessions) of the combined striatal + pallidal group was significantly lower than that of the sham and pallidal groups (all P's < 0.005).

Fig. 2b presents the mean duration of movement, in 6 min blocks, of the sham, pallidal, striatal, and combined striatal + pallidal groups, during the 30 min of spontaneous activity and the 60 min of amphetamine-induced activity on day 50 post-surgery. There were no differences in the spontaneous activity level of the four groups, and amphetamine administration led to a similar increase in the activity of all groups
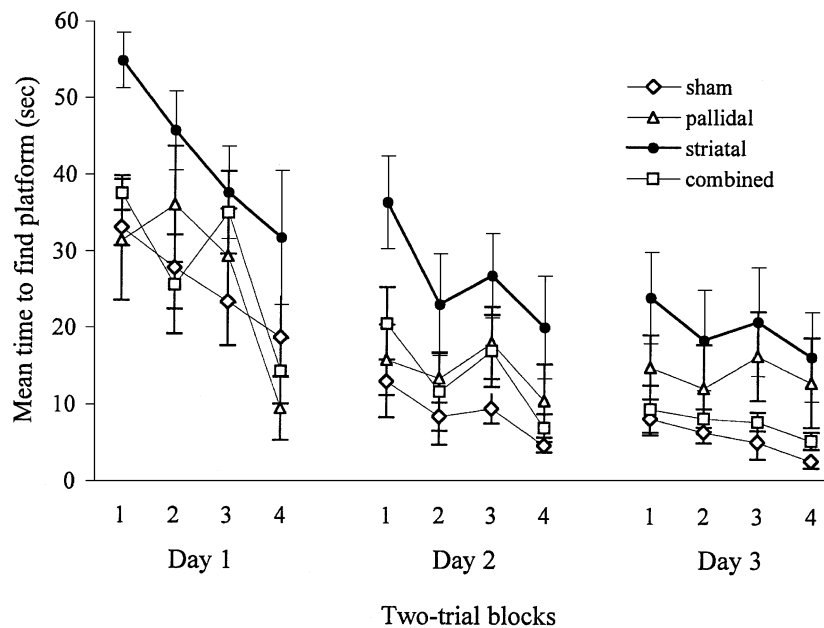

Fig. 3. Means and standard errors of the latency, in two-trial blocks, of the sham, pallidal, striatal, and combined striatal + pallidal groups to find the platform in the three acquisition days of the water maze task.

(repeated measurements factors of sessions, $F(2,168) = 21.9$, $P < 0.0001$, and blocks, $F(4,168) = 7.33$, $P < 0.0001$).

Spatial navigation in a water maze

Fig. 3 presents the mean latency, in two-trial blocks, of the sham, pallidal, striatal, and combined striatal + pallidal groups to find the platform in the three acquisition days of the water maze task. As can be seen, the latency to find the platform decreased with training in all groups (repeated measurements factors of days, $F(2,120) = 42.37$, $P < 0.0001$, and blocks, $F(3,120) = 19.06$, $P < 0.0001$). However, the striatal group was slower to find the platform throughout training, whereas rats sustaining a combined striatal + pallidal lesion performed similarly to sham rats

(main effect of lesion, $F(3,120) = 5.05$, $P < 0.01$). Post hoc comparisons confirmed that the striatal group needed a significantly longer time (over the three acquisition days) to find the platform than the sham, pallidal, and combined striatal + pallidal groups (all P's < 0.05).

Figs. 4a and b present the mean percentage of time spent by the sham, pallidal, striatal, and combined striatal + pallidal groups in different pool quadrants and in different pool zones, respectively, during the 60-s probe trial. Rats in all groups showed a similar preference to the northeast quadrant, in which the platform was located during the three acquisition days (Fig. 4a; repeated measurements factor of quadrants, $F(3,60) = 38.58$, $P < 0.0001$). Although rats in all groups spent most of the 60 s in the pool periphery, and least in the
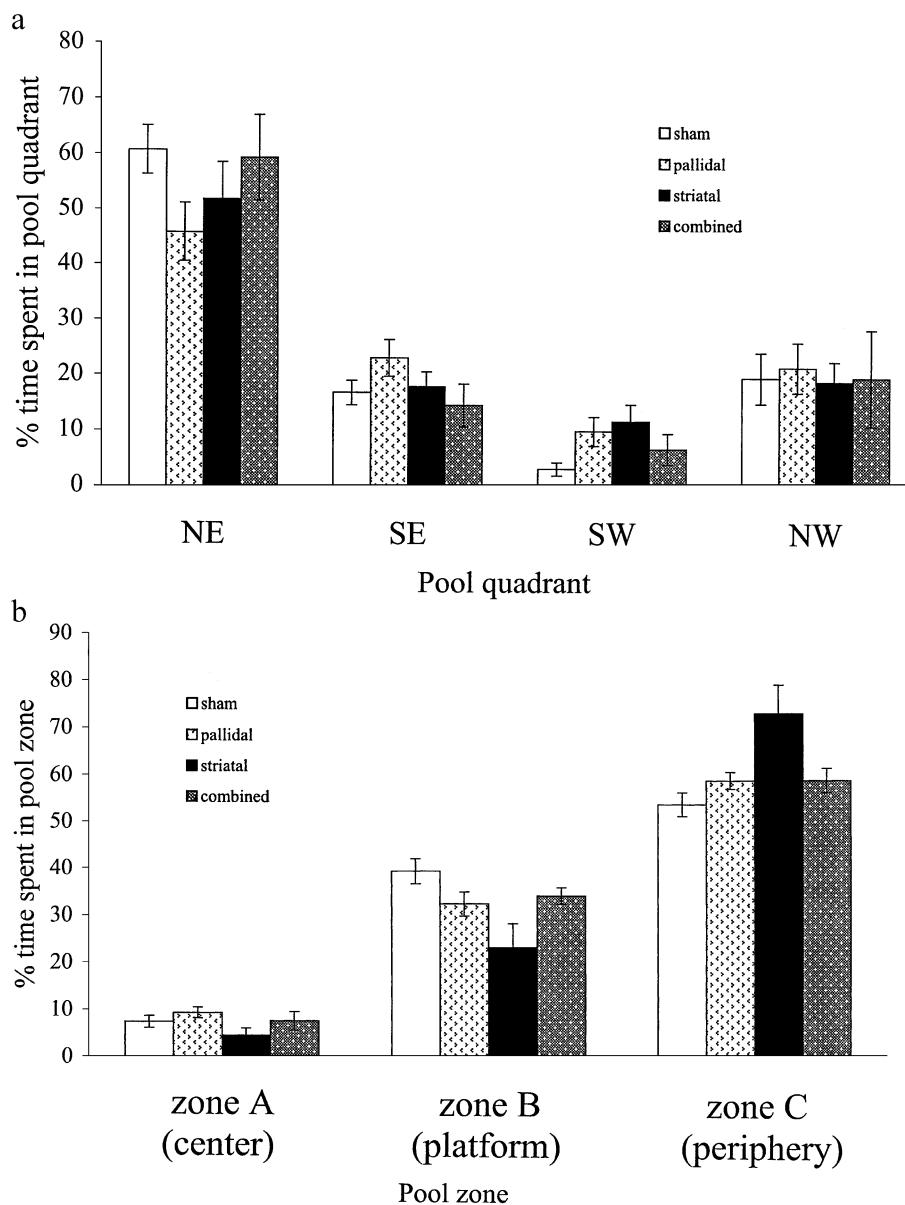

Fig. 4. Means and standard errors of the percentage of time spent by the sham, pallidal, striatal, and combined striatal + pallidal groups in (a) different pool quadrants and (b) different pool zones during the 60-s probe trial (day 4).
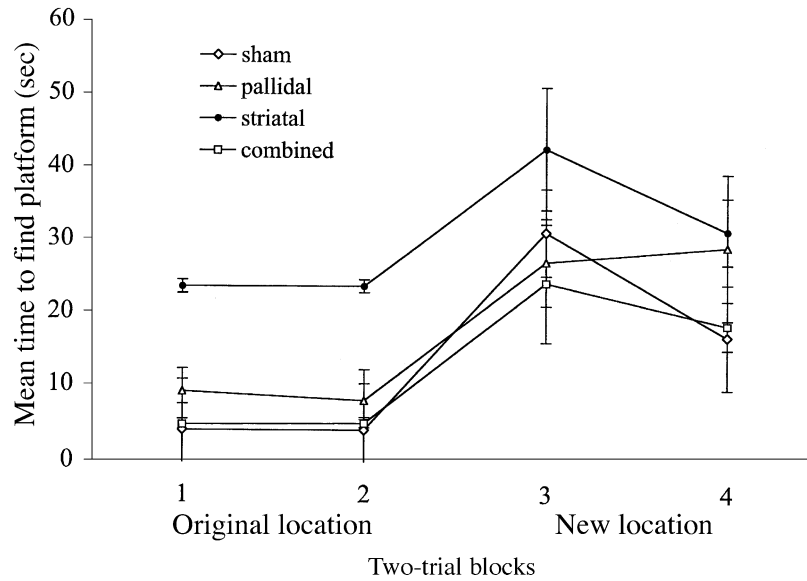
Fig. 5. Means and standard errors of the latency, in two-trial blocks, to find the platform in the four trials before and following reposition of the platform in the southwest quadrant, of the sham, pallidal, striatal, and combined striatal + pallidal groups (day 5).

pool center (Fig. 4b; repeated measurements factor of zone, $F(2,40) = 135.06$, $P < 0.0001$), the striatal group spent significantly more time in the periphery and less time in the platform concentric zone than the sham group, whereas the preferences of the combined striatal + pallidal group were similar to those of the sham group (Fig. 4b; significant lesion $\times$ zone interaction, $F(6,40) = 3.71$, $P < 0.005$). Post hoc comparisons confirmed a significant difference between the striatal and the sham groups in the platform zone, and significant differences between the striatal group and the other three groups in the pool periphery (all P's < 0.05).

Fig. 5 presents the mean latency, in two-trial blocks, to find the platform in the four trials before and following reposition of the platform in the southwest quadrant, of the sham, pallidal, striatal, and combined striatal + pallidal groups. As can be seen, repositioning of the platform in a new location led to an increased latency to find the platform in all groups (significant repeated measurements factor of location (original, new), $F(1,20) = 22.75$, $P < 0.0001$). In addition, rats sustaining a striatal, but not a combined striatal + pallidal, lesion were slower to find the platform both before and following platform repositioning (main
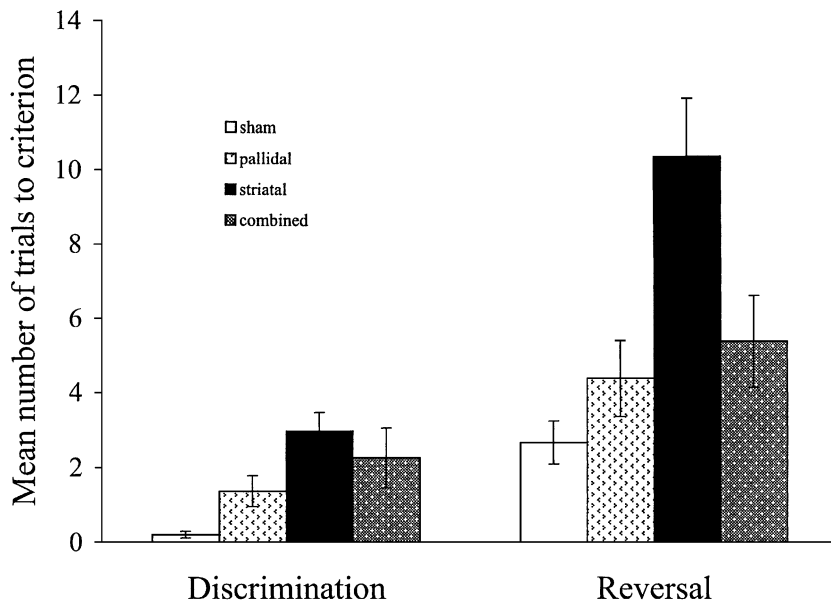

Fig. 6. Means and standard errors of the number of trials to reach criterion of the sham, striatal, pallidal, and combined striatal + pallidal groups, on the five position discriminations (days 1–5) and on the four reversals (days 2–5).

effect of lesion, $F(3,20) = 4.44$, $P < 0.05$). Post hoc comparisons confirmed that the striatal group had a higher mean latency (over the four blocks) to find the platform than the sham, the pallidal, and the combined striatal + pallidal groups (all P's < 0.05).

Position discrimination and reversal task

There were no differences between the groups on the mean time to reach the platform in the five criterion trials of the initial discrimination ($F < 1$). Fig. 6 presents the mean number of trials to reach criterion of the sham, striatal, pallidal, and combined striatal + pallidal groups, on the five position discriminations (days 1–5) and on the four reversals (days 2–5). As can be seen, striatal rats needed more trials to reach criterion on both the discriminations and the reversals compared with the sham and pallidal rats, whereas rats with a combined striatal + pallidal lesion were only slightly impaired compared to the sham group (significant effects of lesion, $F(3,21) = 9.91$, $P < 0.0005$; reversal, $F(1,21) = 63.29$, $P < 0.0001$; significant lesion × reversal interaction, $F(3,21) = 6.46$, $P < 0.005$). Post hoc comparisons within each condition (discriminations, reversals) revealed that the striatal group needed more trials to criterion than the sham group on the discriminations and the reversals (P's < 0.0001), and that rats sustaining a combined striatal + pallidal lesion performed better than striatal rats on the reversals ($P < 0.0001$), although they needed more trials to criterion than the sham group at this stage ($P < 0.05$).

Food manipulation

Fig. 7 presents the food manipulation scores of the sham, pallidal, striatal, and combined striatal + pallidal groups. As can be seen, striatal and pallidal rats had lower scores compared to the sham and combined striatal + pallidal groups. Pallidal rats attempted to gnaw on the pellet without picking it up as normal rats do; rather, they held the pellet on the cage floor with their forepaws. Striatal rats picked up the pellet in their forepaws but dropped it before it was all consumed. In contrast to striatal rats, all rats in the combined
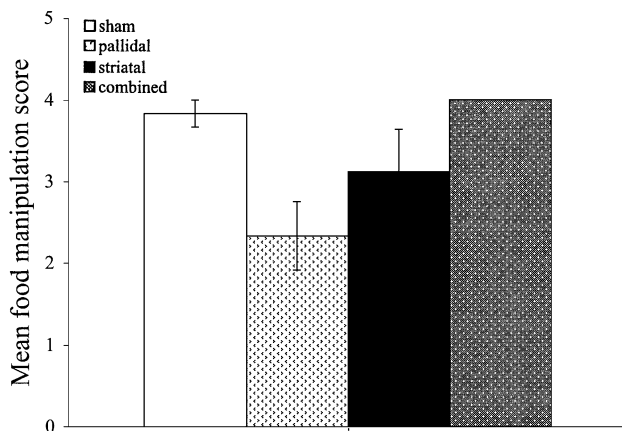
Fig. 7. Means and standard errors of the food manipulation scores of the sham, pallidal, striatal, and combined striatal + pallidal groups.

striatal + pallidal group ate the entire pellet while sitting up and holding the pellet by their forepaws (main effect of lesion, ($F(3,21) = 3.72$, $P < 0.05$). Post hoc comparisons revealed that the pallidal group had a lower score than the sham and combined striatal + pallidal groups (P's < 0.01).

Discussion

Consistent with previous reports in the literature, we found that a QA lesion to the striatum impaired spatial navigation in a water maze (Block et al., 1993; Devan et al., 1996; Furtado and Mazurek, 1996; Joel et al., 1998; Whishaw et al., 1987), the acquisition and reversal of a position discrimination (Dunnett and Iversen, 1981; Mitchell and Hall, 1988; Mitchell et al., 1985; Pisa and Cyr, 1990; Reading et al., 1991; but see Oliveira et al., 1997), and the ability to manipulate food (Pisa, 1988). The striatal lesion was without an effect on activity level, either spontaneous or amphetamine induced, in agreement with some reports (Antoniou and Kafetzopoulos, 1992; Furtado and Mazurek, 1996; Hauber and Schmidt, 1993) but not others (Sanberg et al., 1989). The striatal lesion did not lead to weight loss, in contrast to previous reports (Berridge and Cromwell, 1990; Emerich and Sanberg, 1992; Pisa et al., 1980; Sanberg et al., 1989) including ours (Joel et al., 1998). This is likely a result of our improvement of the post-surgical maintenance and care since our first study (Joel et al., 1998).

The novel finding of the present study is that rats sustaining an excitotoxic lesion to the GP in addition to the striatal lesion, performed better than striatal-lesioned rats. In fact, with the exception of a mild deficit in reversal and a transient decrease in activity level, these rats performed similarly to sham rats. The reason for the lower activity in the combined striatal + pallidal lesion group is not clear, because these rats were not slower that sham rats in the water maze and T-maze tasks.

In the water maze task, striatal rats had prolonged latency to find the platform compared to sham rats. The impaired performance of the striatal group was unlikely a result of a motor or a motivational deficit because striatal rats swam as fast as sham-operated rats. Likewise, analysis of rats' behavior in the probe and reposition trials revealed that striatal rats were not impaired in their ability to use spatial information, because their preference for the platform quadrant in the probe trial and the increase in the latency to find the platform after it was repositioned, were comparable to those of sham rats. Striatal rats' behavior in the probe trial, namely, swimming close to the pool's side walls (thigmotaxis), does suggest that their impairment in the water maze resulted from the use of an inappropriate strategy, or from the interference of an incompatible behavioral tendency. A similar behavioral pattern in the water maze task following a striatal lesion had been described previously, and had led to a similar conclusion (Block et al., 1993; Devan et al., 1996; Whishaw et al., 1987). In contrast to striatal rats, rats sustaining a combined

striatal + pallidal lesion were indistinguishable from sham rats on all the behavioral measures in the water maze task. Reversal of the striatal lesion-induced deficit by the pallidal lesion is unlikely to reflect a simple additive effect of the two lesions, because pallidal lesion by itself had no effect on rats' performance in the water maze task. Rather, the fact that rats with a combined striatal + pallidal lesion distributed their swim time between the three concentric zones of the pool as did sham rats suggests that the beneficial effect of the pallidal lesion was obtained because rats with a combined striatal + pallidal lesion were able to use the appropriate spatial strategy, as opposed to the tendency to swim close to the pool's side walls that was induced by the striatal lesion.

In the T-maze task, striatal lesioned rats were impaired in the discriminations and in the reversals, whereas rats sustaining in addition a lesion to the GP performed significantly better than striatal rats, and were only mildly impaired compared to the sham rats. Similar to the water maze task, the impaired performance induced by the striatal lesion and the ameliorating effect of the pallidal lesion did not seem to result from lesion effects on motor or motivational aspects of the task, because the sham, striatal, pallidal, and combined striatal + pallidal groups had similar latencies to reach the platform. Also in this test, the ameliorating effect of the pallidal lesion in the combined striatal + pallidal group was not a result of a combined effect of the two lesions, because pallidal lesion in itself had no effect on this task.

In the food manipulation test, a few of the striatal lesioned rats were impaired to some degree, whereas all the rats sustaining a combined striatal + pallidal lesion performed perfectly. This result is of particular interest because this is the only behavioral assay, in which the pallidal lesion by itself caused a marked impairment in performance, thus providing the strongest evidence that the ameliorating effect of the pallidal lesion was not due to an additive effect.

Taken together, the present findings demonstrate that a pallidal lesion can ameliorate motor and cognitive deficits induced by a lesion to the striatum. Since an axon-sparing lesion of the GP was used, the ameliorating effect is due to damage to cell bodies of the GP rather than to fibers of passage. However, it could be argued that because we did not include a group of striatal lesioned rats with a sham operation of the GP, the beneficial effect of the pallidal lesion was a result of a non-specific damage caused by the insertion of the cannula into the GP rather than of QA-induced neuronal death in the GP. This possibility is unlikely, however, because we have recently shown that whereas intra-pallidal infusion of the GABA$_A$ agonist muscimol reversed striatal QA lesion-induced behavioral deficit in the position discrimination and reversal task used here, intra-pallidal infusion of vehicle was without an effect (Joel et al., in press).

The present finding that damage to the GP can reverse the behavioral deficits resulting from damage to the striatum suggests that striatal lesion-induced behavioral deficits reflect disrupted functioning of basal ganglia circuitry rather than dysfunction of the damaged striatum only, consistent with the central assumption of the anatomo-functional models of basal ganglia circuitry, that complex behavioral pathologies resulting from damage to one of the circuit's components reflect dysfunction of the circuit as a whole (e.g., Albin et al., 1989; Alexander and Crutcher, 1990; Alexander et al., 1986, 1990; DeLong and Georgopoulos, 1981; Groenewegen and Berendse, 1994; Joel and Weiner, 1994, 1997; Parent, 1990). To date, most of the support for this assumption has come from the demonstration that in Parkinson's disease patients and in parkinsonian non-human primates, a lesion to the internal segment of the GP or to the subthalamic nucleus alleviates symptoms resulting from degeneration of dopaminergic neurons (e.g., Bergman et al., 1990; Marsden and Obeso, 1994). The present findings further reinforce this notion, and provide the first demonstration that an excitotoxic lesion to the pallidal component can ameliorate behavioral deficits resulting from a lesion to the striatal component of the basal ganglia. Our results also strengthen the validity of the QA rat model of HD by showing that the behavioral consequences of the striatal QA lesion, which are believed to model HD symptoms (e.g., Furtado and Mazurek, 1996; Sanberg et al., 1989), are likely to reflect lesion-induced dysfunction in basal ganglia circuitry, as is postulated for the clinical condition (Albin et al., 1989; Alexander et al., 1990; Brandt, 1986; Crossman, 1987; Cummings, 1993; Fedio et al., 1979; Joel, 2001; Joel and Weiner, 1997; Penney and Young, 1986; Wilson and Garron, 1979).

As detailed in the introduction, the suggestion that GP lesion can ameliorate the deleterious effects of striatal lesion was based on the leading model of HD, according to which overactivity of the projections of GPe (GP in rats) to the subthalamic nucleus is believed to play a major role in the pathological mechanism underlying HD. Based on this model, the present results may be interpreted as reflecting disruption of GP projections to the subthalamic nucleus. However, other alterations in basal ganglia circuitry which could result from a pallidal lesion, could have contributed to the observed ameliorating effect. Specifically, the pallidal lesion could have disrupted pallidal projections to the entopeduncular nucleus (the rat analogue of the primate internal segment of GP), substantia nigra, and striatum (Gerfen and Wilson, 1996; Haber et al., 1985; Staines and Fibiger, 1984), as well as to the reticular nucleus of the thalamus (Carter and Fibiger, 1978), and disruption of each of these projections could have affected the functioning of basal ganglia-thalamocortical circuitry. In addition, since in rats many of the striato-entopeduncular and striato-nigral projections are collaterals of striato-pallidal projections (Kawaguchi et al., 1990), disruption of the latter could have led to disruption of the former, again leading to alterations in the functioning of basal ganglia circuitry.

Although the mechanism/s by which the pallidal lesion ameliorated the behavioral effects of the striatal lesion remain/s to be elucidated, given that a similar dysfunction

of basal ganglia circuitry is thought to subserve the behavioral alterations seen in QA-lesioned rats and HD patients, the present results raise the possibility that manipulations of the GPe could ameliorate some of HD symptoms, similarly to the ameliorating effects of manipulations of the internal segment of the GP in Parkinson's disease (Marsden and Obeso, 1994). In this context, it is important to note that we have recently shown that striatal QA lesion-induced behavioral deficit in the position discrimination and reversal task used here was effectively reversed by a GP lesion performed about 1 month following the striatal lesion. Furthermore, a temporary unilateral GP inactivation (by intra-pallidal infusion of the GABA$_A$ agonist muscimol) performed about 1 month following the striatal lesion was also successful in reversing striatal deficit (Joel et al., in press). These findings are particularly encouraging because they suggest that GPe manipulations, including a relatively safe and limited manipulation, applied on the background of a pre-existing striatal damage, could be beneficial in the treatment of HD. The present finding that a pallidal lesion can ameliorate motor and cognitive deficits induced by a striatal lesion further suggests that manipulations of the GPe may have a positive effect on both motor and cognitive symptoms in HD, which in the early stages of the disease are thought to reflect basal ganglia dysfunction.

Acknowledgment

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