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Functional investigations into the role of dopamine and serotonin in partial bilateral striatal 6-hydroxydopamine lesioned rats

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Abstract

In Parkinson's disease (PD), several neurotransmitter systems, such as the dopaminergic and serotonergic system, show signs of degeneration. This led to the suggestion that alterations in the serotonergic system play a role in the pathophysiology of PD. Partial bilateral dopaminergic lesions of the caudate putamen complex (CPu) of rats induced by 6-hydroxydopamine (6-OHDA) produce behavioral symptoms mimicking PD. In the present study, the role of serotonin and dopamine was investigated both behaviorally and neuroanatomically. In a reaction time task, motor initiation and motor performance were impaired in the lesioned animals compared to controls. The performance of rats treated with *d*-amphetamine or serotonergic ligands (DOI and ketanserin) in the reaction time task indicated that 5-HT and DA appear to be agonistically related in the CPu. The relation was the same in both control and 6-OHDA lesioned rats. 12 weeks after lesioning, motor initiation recovered, whereas motor performance did not. Parallel to the behavioral study, a second group of animals was lesioned and, at 3 days, 6 weeks and 12 weeks after lesioning, a subgroup was killed to obtain a qualitative indication of the degree of 6-OHDA lesion. Over the three time points, a substantial recovery of tyrosine hydroxylase staining in the CPu was visible. Taken together, since serotonergic ligands have the same effect as dopaminergic ligands on reaction time responding indicated that 5-HT and DA release are agonistically linked in control and 6-OHDA lesioned rats.

Keywords: Parkinson's disease; Behavior; Reaction time; Recovery; Caudate putamen

1. Introduction

The cardinal features of Parkinson's disease (PD) are all motor symptoms, namely, resting tremor, rigidity and bradykinesia. The main cause of PD symptoms lies in the degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc), leading to a reduction of DA in the caudate putamen complex (CPu). However, histological studies in both humans and animals have demonstrated loss of cells and pathological processes in a number of non-DAergic cell populations in PD, including noradrenergic neurons of the locus coeruleus and dorsal vagal nucleus, serotonergic neurons of the dorsal raphe nucleus (DR), cholinergic neurons within the substantia innominata and the pedunculo-pontine nucleus, and the occurrence of Lewy bodies in a range of cerebral areas (Braak et al., 2003; Jellinger, 1987, 1990; Mohr et al., 1995; Wadenberg, 1996). Damage to these systems may influence movement-related and non-movement-related aspects of PD, such as cognitive function and mood (Mohr et al., 1995; Cummings, 1992).

Approximately 25–40% of PD patients suffer from depressive disorder (Cummings, 1992; Leentjens, 2004). Dysfunction of the serotonergic neurotransmitter system is commonly associated with depression (Van Praag and De Haan, 1979). The first clues regarding serotonin (5-hydroxytryptamine, 5-HT) involvement in PD were based on postmortem neurochemical data (Ehringer and Hornykiewicz, 1960; Bernheimer et al., 1961; Hornykiewicz, 1963) and decreased levels of 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid

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(CSF) in brains of depressed PD patients (Johansson and Roos, 1967; Mayeux et al., 1984; Kostic et al., 1987; Kuhn et al., 1996). Initial results were based on data from postmortem research and thus generally based on patients in late stages of their disease. It is, however, also known that serotonin levels are found to be decreased in earlier stages of PD (Haapaniemi et al., 2001; Kerenyi et al., 2003). Serotonergic neurons in the DR and central 5-HT levels are reduced in the parkinsonian brain (Mohr et al., 1995; Chen et al., 1998), suggesting a link with depressive as well as non-depressive symptoms of PD. Serotonergic efferents from the DR have been found to innervate both the CPu and the SN (Steinbusch et al., 1981). It is, furthermore, known that PD patients may suffer from dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis resulting in abnormal cortisol and ACTH responses (Pfeiffer et al., 1986; Kostic et al., 1990; Rabey et al., 1990). Since the serotonergic axis influences the HPA system, the endocrine abnormalities found in PD patients might also be related to deficient functioning of the 5-HT system (Volpi et al., 1997). These findings have led to the suggestion that 5-HT may play a significant role in the pathophysiology of PD. Furthermore, more knowledge about the exact role of 5-HT in PD may lead to novel treatment strategies for PD, which at present are based on DA and acetylcholine (ACh).

The possible role of 5-HT in PD is described by two opposing hypotheses. The first hypothesis regards 5-HT dysfunction as the primary event in PD (Steinbusch and De Vente, 1997). In this hypothesis, 5-HT is agonistically linked to DA, i.e., a decrease in 5-HT leads to a decrease in DA. The second hypothesis regards 5-HT decrease as a secondary event in PD (Hornykiewicz, 1986; Mayeux, 1990; Jacobs and Fornal, 1993). Here it is assumed that 5-HT is antagonistically linked to DA, i.e., a decrease of 5-HT is a secondary compensatory mechanism for the initial DAergic degeneration and thus reduces the decrease in DA. Circumstantial evidence for this hypothesis can be found in the observation that SSRIs may worsen motor symptoms in PD (Leo, 1996).

Several animal models have been used for investigating PD. Unfortunately, there is no general agreement on what is the best method for modeling PD. In the present study, a partial bilateral striatal 6-hydroxydopamine (6-OHDA) lesion rat model was used. There are several arguments for using a bilateral model instead of the more widely used unilateral lesion model (Deumens et al., 2002; Orth and Tabrizi, 2003). Firstly, PD affects the human brain bilaterally, thus using a bilateral lesion resembles the human situation of PD more than using an unilateral model. Secondly, compensating mechanisms from the contralateral side are reduced (Van Oosten and Cools, 1999). Also, in rats with intrastriatal 6-OHDA lesions, it was demonstrated that bilateral 6-OHDA lesions are more suitable for testing behavioral motor paradigms than unilateral 6-OHDA lesions (Roedter et al., 2001). In the present study, the partial bilateral striatal 6-OHDA lesion was used to investigate the role of DA and 5-HT in an animal model of PD, using pharmacological challenge studies. Following the hypothesis of Mayeux (1990) described above, it was expected that DA and 5-HT would have antagonistic effects. In the light of this theory, stimulation of the DAergic system in 6-OHDA lesioned rats would have an opposite effect in the reaction time task as the 5-HTergic stimulation.

2. Materials and methods

2.1. Animals

The present experiments were approved by the animal ethical committee of Maastricht University (The Netherlands) and met governmental guidelines. For the behavioral part of the experiment 23 male Lewis rats (bred in our animal facility, Maastricht University, The Netherlands) being 2 months old and weighing 200–250 g at the beginning of the study were used. The rats were housed under standard conditions with free access to water but limited food access to reduce their weight by approximately 15% (Blokland and Honig, 1999). Animals were housed under a reversed 12 h light–dark cycle. A radio produced background noise. At the age of 3 months, rats were randomized over two groups according to pre-test scores achieved in the first month of the experiment on a reaction time task; this method created two groups who were equal in performance of the reaction time task: the experimental group ($n=14$) received twofold bilateral injections of 6-OHDA in the center of the CPu and the control group ($n=11$) received double bilateral injections of saline (sham condition) in the center of the CPu. Prior to surgery and 6–12 weeks post-lesion, several behavioral tests were performed. The second part of the experiment focused on the anatomical and immunohistochemical aspects of the 6-OHDA lesion. Here, 15 animals, identical to the ones used for the behavioral part, were used. Animals were divided into three groups, and in each group five animals received the abovementioned 6-OHDA lesion. The rats in these groups were killed at 3 days (group 1), 6 weeks (group 2) and 12 weeks (group 3) post-lesion. Table 1 provides an overview of the experimental setup.

Table 1
Schematic overview of the experimental setup

Project week	Experimental steps
−3	Training RT task
−2	Training RT task
−1	Training RT task
0	Surgery, ‘behavioral’ and ‘anatomical’ group
1	Recovery both groups, perfusion group 1 ‘anatomy’
2	Recovery both groups
3	Recovery both groups
4	Recovery both groups
5	Retraining RT task
6	Perfusion group 2 ‘anatomy’, testing *d*-amphetamine (0 and 0.1 mg/kg BW)
7	Testing *d*-amphetamine (0.3 and 1 mg/kg BW)
8	Testing DOI (0 and 0.3 mg/kg BW)
9	Testing DOI (1mg/kg BW)
10	No testing
11	Testing ketanserin (0 and 3 mg/kg BW)
12	Testing ketanserin (10 mg/kg BW), perfusion group 3 ‘anatomy’

2.2. Surgery

The animals, which were trained to perform a reaction time task, received stereotactic injections of 2 μl 6-OHDA (5 μg/μl dissolved in 0.9% saline and 0.2% ascorbic acid; Sigma, Zwijndrecht, The Netherlands) or the same injection amount of the vehicle solution (0.9% saline and 0.2% ascorbic acid) at four sites (two per hemisphere) in the CPu. One hour before the surgery, rats received desimipramine (20 mg/kg in 0.9% saline, injection volume 1 mg/kg i.p.). Desimipramine was given to protect noradrenergic neurons from possible damage by 6-OHDA and thus make the lesion more DA specific (Mason and Fibiger, 1979). The animals were anaesthetized using a combination of ketamine (50 mg/kg) and rompun (4 mg/kg) (i.p.) and subsequently placed in a stereotactic frame. The injections of 6-OHDA or vehicle were performed in the CPu at the following coordinates (Paxinos and Watson, 1996): AP: +0.7 and −0.4 mm, L: 2.8 and 3.4 mm, and DV: −5.0 and −5.0 mm (from bregma). Injection speed was 0.5 μl/min and the cannula was left in place for an additional 2 min before slowly retracting it. Post-operatively, buprenorfine (0.2 mg/kg) was administered subcutaneous as analgesic. The animals were put back in their home cages for recovery, and weighted and handled daily. The animals had free access to food and water after surgery. Food was pulverized and mixed with water into a porridge, and given inside the cages if rats had feeding problems. Water was consumed without problems. A number of animals died during the experiment as is further explained in Section 3.

2.3. Drugs

Several drugs were tested in a reaction time task on the following behavioral parameters: reaction time, motor time and ratio premature responses. The drugs tested were the partial DA agonist *d*-amphetamine (Sigma, Steinheim, Germany tested at doses 0, 0.3 and 1 mg/kg body weight (BW)), the 5-$HT_{2a/2c}$ receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane hydrochloride (*R*-(−)-DOI, DOI; Sugimoto and Yamada, 2000) (Sigma; tested at doses 0, 0.3 and 1 mg/kg BW), and the 5-$HT_{2a/2c}$ receptor antagonist ketanserin (Jorgenson et al., 1999) (Tocris Cookson Ltd., Avonmouth, UK; tested at doses 1, 3 and 10 mg/kg BW). All drugs were dissolved in 0.9% NaCl and injected i.p. 30 min before test trials started. Injections of 0.9% NaCl were also used as a control condition (as depicted in Figs. 2–5). Although there are substances with higher specificity for the DAergic system, *d*-amphetamine was used in order to make comparisons possible with earlier experiments of our group (Blokland, 1998; Blokland et al., 2005). Order and time frame of drug testing are provided in Table 1.

2.4. Behavioral tests

2.4.1. Reaction time task

The reaction time task used in the present experiment was identical to the task used in earlier experiments of our group. Animals are trained (by means of operant conditioning) to respond to a high or low tone (cue) by pressing one of two levers in a Skinner box in order to obtain a food reward. For an overview, see Blokland and Honig (1999) and Blokland (1998). This task provides information about several aspects of reaction time responding of rats. The task was always performed at the same time of day.

The following parameters were used to evaluate the responding of the rats.

Reaction time (RT): the main latency between the onset of the tone and the release of the hinged food tray panel (after pushing it away). It is generally accepted that response latencies shorter than 100 ms may not reflect true RT (Blokland and Honig, 1999). These latencies were counted as premature responses. Response latencies longer than 1500 ms were not considered to be a task-related RT. The RT performance was evaluated in two ways: the mean reaction time of all responses and the mean RT per hold duration was examined.

Motor time (MT): the mean latency between the release of the hinged food tray panel and the lever press. It was assumed that MT of more than 2 s do not reflect true MT (Blokland and Honig, 1999).

Number of trials: the total number of trials the rats completed in a session of maximum 30 min.

Premature responses (PR): the total number of times that the rat released the hinged panel before the hold duration had elapsed. In the present study, we always used the ratio of PR (i.e., the PR divided by the total number of completed trials, with a maximum of 60). When the total number of completed trials was not 60, this is mentioned in the text. Furthermore, a PR caused the trial to start over again.

2.5. Immunohistochemistry

2.5.1. Perfusion and sectioning

At three subsequent post-lesion time points (3 days, 6 weeks and 12 weeks), a group of animals was deeply anesthetized and perfused transcardially with Somogyi fixative consisting of 4% depolymerized parformaldehyde, 0.05% glutaraldehyde and 15% picric acid in 0.1 M phosphate buffer (pH 7.4). The brains were removed and post-fixed for 2 h at 4 °C in the same fixative. After 2 h, the brains were immersed in 15% sucrose in 0.1 M phosphate buffer (pH 7.4) for 24 h at 4 °C. Subsequently, the brains were frozen with carbogen snow. Coronal free floating sections of 35 μm thickness were cut using a cryostat (Leica, Rijswijk, The Netherlands) at the level of the CPu. The CPu sections were stained for TH and mounted on gelatin-coated slides.

2.5.2. TH immunohistochemistry

The sections were incubated overnight at 4 °C with a primary mouse anti-TH antibody (diluted 1:100, generous gift from Dr. C. Cuello, Montreal, Canada). After three washing steps (Tris-buffered saline (TBS), 0.1 M; TBS containing 0.3% Triton X-100 (TBS-T); TBS), the sections were incubated with biotinylated donkey anti-mouse antibody (Jackson Immunoresearch Laboratories, USA) for 60 min at room temperature. After washing, the sections were incubated with ABC-kit

(Vector Laboratories, USA) for 2 h at room temperature. Afterwards, the sections were incubated with DAB to visualize the horse radish peroxidase (HRP) reaction product. After washing with TBS, the sections were mounted on gelatin-coated slides, dehydrated and coverslipped for microscopy.

2.5.3. Imaging

Sections of the CPu in between the two injection sites showing the highest damage due to the 6-OHDA lesion were visualized with an Olympus BX 51 microscope equipped with a Hitachi HV-C20A camera (Hitachi Kokusai Electronics, Tokyo, Japan) connected to a computer which generated one single montage of multiple, adjacent images with the Virtual Slice application in the StereoInvestigator software program (Micro-Brightfield Inc., Burlington, VT, USA). Final figures were constructed using Corel Draw (Version 11.0, Corel, Ottawa, Canada). Only minor adjustments of contrast and brightness were made, which in no case altered the appearance of the original materials.

2.6. Statistics

The data obtained from the reaction time task, concerning RT, MT and PR over time or over increasing doses of drugs were evaluated with a two-factorial (time/dose and group) analysis of variance (ANOVA) with repeated measures over time or concentration. *t*-Statistics were performed to analyze whether the 6-OHDA lesioned group and the control group differed from each other at every time point or dose. As mentioned above, three different parameters were recorded in the reaction time task: RT, MT and PR. Also, the mean number of completed trials was analyzed in every session. In all sessions except the one after administering the highest dose of DOI, the rats completed the maximum of 60 trials. To get an indication of the performance over time, the vehicle injections at post-lesion weeks 6, 7, 8, 9, 11 and 12 were analyzed. The baseline values did not differ from the pre-lesion data (data not shown). In order to enhance reliability, the performance at weeks 6 and 7, 8 and 9, and 11 and 12 were averaged. All calculations were carried out using SPSS Version 10 (SPSS Inc., Chicago, USA). $p<0.05$ was considered statistically significant.

Due to a technical problem, data of six control animals concerning the ketanserin measures was, unfortunately, lost. Therefore, the data analysis of ketanserin was performed with the five remaining control animals.

3. Results

3.1. Survival and body weights

After surgery, all animals were fed inside the cages, because of problems with eating from the food rack. Weight loss during the first week post-lesion was about 14% for the 6-OHDA lesioned animals, while the control group only lost 1% of their body weight in the first week after surgery. After 4 weeks, the control animals weighed about 19% more than their pre-operative weights, while the lesioned animals only weighed 9% more than their pre-operative weights. During this period, the animals received food ad libitum, and were not tested behaviorally. After these 4 weeks, the animals were food restricted again before behavioral testing. During the course of the present experiment, the lesioned animals remained lighter than the control animals (time: $F_{2,46}=58.19$, $p<0.001$) (Fig. 1).

3.2. Behavioral studies

3.2.1. Baseline performance

The different substances were tested at different time points during the experiment, as summarized in Table 1. *d*-Amphetamine was tested at weeks 6 and 7. DOI was tested in weeks 8 and 9, and ketanserin was tested in weeks 11 and 12. In order to obtain an impression of the baseline performance of both groups over the time course of the experiment, the performance of the animals (after the injection of saline) on several measures over time are shown in Fig. 2.

3.2.1.1. Reaction time. The mean RT of 6-OHDA lesioned animals was significantly higher than that of control animals at 6/7 weeks post-lesion ($t=3.22$, $p<0.01$) (Fig. 2A). This effect was not present anymore at 8 and 9 as well as at 11 and 12 weeks post-lesion (Fig. 2A). All RTs were also analyzed per hold duration. With increasing hold duration, all rats reacted faster. Initially, 6-OHDA lesioned rats were slower; this effect was the same at each hold duration (data not shown). Therefore, only the mean RT is always presented.

3.2.1.2. Motor time. The mean MT of the 6-OHDA lesioned animals was higher than that of the control group at all time points (group: $F_{1,17}=14.36$, $p<0.01$) (Fig. 2B). Neither control nor 6-OHDA lesioned animals showed alterations in mean MT over time (time: $F_{2,34}=0.66$, n.s.); therefore, the difference between the two groups remained the same (group by time: $F_{2,34}=1.35$, n.s.) (Fig. 2B).

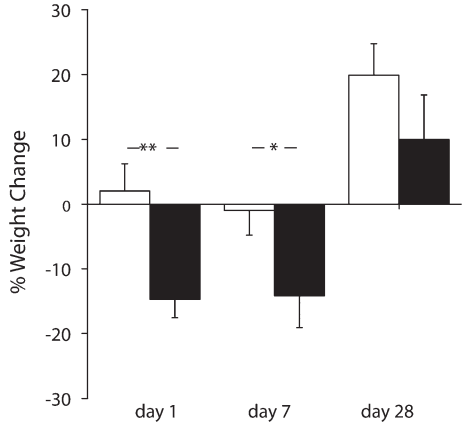

Fig. 1. Mean and S.E.M. of the body weight of sham-operated control rats (open bars) and 6-OHDA lesioned rats (closed bars) at three different time points after operation (or sham-operation, respectively). Data are expressed as percentage of the animals' pre-operative weight: *$p<0.05$, **$p<0.001$.

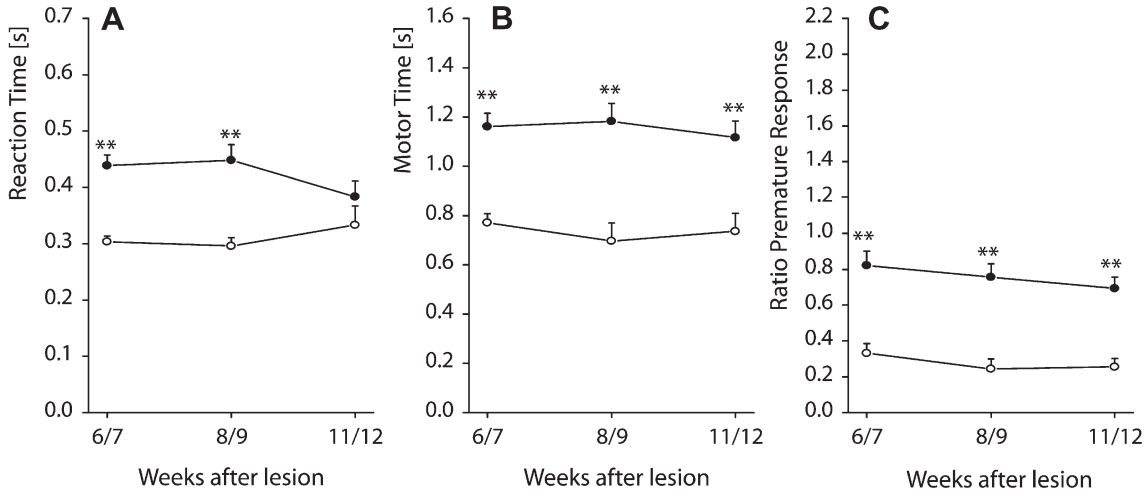

Fig. 2. Mean and S.E.M. of baseline performance (A, reaction time; B, motor time; C, ratio premature responses) of sham-operated control rats (open dots) and 6-OHDA lesioned rats (closed dots) as a function of the time interval after operation (or sham-operation, respectively). Data from respectively weeks 6 and 7, weeks 8 and 9, and weeks 11 and 12 were pooled. *$p<0.05$, **$p<0.01$ (differences between sham-operated control rats and 6-OHDA lesioned rats).

3.2.1.3. Premature responses. The mean proportion of PR was significantly higher in the 6-OHDA lesioned group than in the control group (group: $F_{1,17}=20.38$, $p<0.01$) (Fig. 2C). The ratio of the PR changed neither for the control nor for the 6-OHDA lesioned group over time (time: $F_{2,34}=1.56$, n.s.). The difference between both groups remained the same (group by time: $F_{2,34}=0.20$, n.s.) (Fig. 2C).

3.2.2. d-Amphetamine

Effects of the partial DA agonist *d*-amphetamine were tested at increasing doses (0, 0.3 and 1 mg/kg BW) at 6 and 7 weeks post-lesion.

3.2.2.1. Reaction time. The mean RT in 6-OHDA lesioned and control animals differed significantly (group: $F_{1,21}=10.19$, $p<0.01$) (Fig. 3A). There was an overall reduction of RT at increasing doses of *d*-amphetamine (dose: $F_{2,42}=19.68$, $p<0.001$). However, the effect was more pronounced in the 6-OHDA lesioned group when compared with the control group (dose by group: $F_{2,42}=3.69$, $p<0.05$). *t*-Tests revealed that the performance of the groups significantly differed after treatment with 0 mg/kg BW and 0.3 mg/kg BW (both *t* values>2.64, $p<0.05$), but not 1.0 mg/kg BW ($t_{23}=1.82$, n.s.) (Fig. 3A).

3.2.2.2. Motor time. The mean MT of the 6-OHDA lesioned animals was higher than that of the controls (group: $F_{1,21}=28.62$, $p<0.001$) (Fig. 3B). Increasing doses of *d*-amphetamine did not change MT in both groups (dose: $F_{2,42}=2.47$, n.s.). *t*-Tests showed that the groups differed significantly over all doses (all *t* values>4.16, $p<0.001$). In both control and 6-OHDA lesioned rats, administration of *d*-amphetamine had no effect (group by dose: $F_{2,42}=0.73$, n.s.) (Fig. 3B).

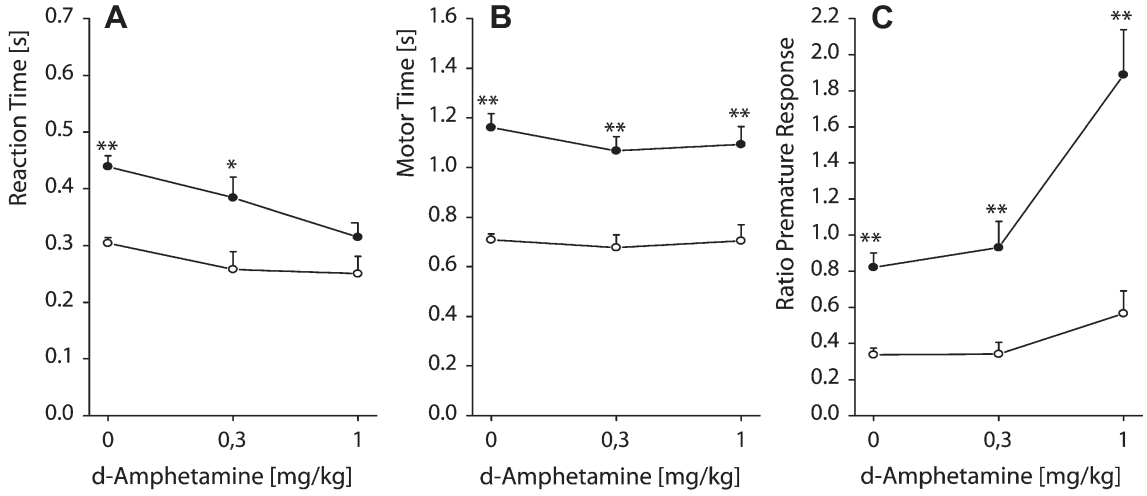

Fig. 3. Mean and S.E.M. of reaction time (A), motor time (B) and ratio premature responses (C) of sham-operated control rats (open dots) and 6-OHDA lesioned rats (closed dots) as a function of the dose of *d*-amphetamine at 6 and 7 weeks after operation (or sham-operation, respectively). *$p<0.05$, **$p<0.01$ (differences between sham-operated control rats and 6-OHDA lesioned rats).

3.2.2.3. Premature responses. The mean proportion PR was higher for the 6-OHDA lesioned animals than for controls (group: $F_{1,21}=24.21$, $p<0.001$) (Fig. 3C). Increasing doses of *d*-amphetamine increased the PR (dose: $F_{2,42}=16.12$, $p<0.001$). This effect was more pronounced in the 6-OHDA lesioned animals than in the control animals (dose by group: $F_{2,42}=6.70$, $p<0.01$). *t*-Tests showed that there was a significant difference between both groups at all doses (all *t* values >3.29, $p<0.01$) (Fig. 3C).

3.2.3. DOI

Effects of the 5-$HT_{2a/2c}$ receptor agonist DOI were tested at increasing doses (0, 0.3 and 1 mg/kg BW) at weeks 8 and 9 post-lesion.

3.2.3.1. Reaction time. Overall, there was a significant difference between the RT of the 6-OHDA lesioned rats and the control rats (group: $F_{1,19}=8.64$, $p<0.01$) (Fig. 4A). Increasing doses of DOI appeared to decrease the RT in the lesioned animals, while RT in the control group remained at approximately the same level regardless the dose. This was supported by an interaction effect (dose by group: $F_{2,38}=3.36$, $p<0.05$). On the other hand, no main effect of dose was found (dose: $F_{2,38}=0.49$, n.s.). *t*-Tests showed that 6-OHDA lesioned animals were slower than controls at the doses of 0 and 0.3 mg/kg (both t values >3.74, $p<0.001$), but the performance at a dose of 1.0 mg/kg was not different ($t_{19}=1.19$, n.s.) (Fig 4A).

3.2.3.2. Motor time. The mean MT of the 6-OHDA lesioned animals was higher than that of the control animals (group: $F_{1,19}=25.97$, $p<0.001$) (Fig. 4B). Increasing doses of DOI increased MT in the rats (dose: $F_{2,38}=20.83$, $p<0.001$). The effect of DOI was similar in the control and lesion group (dose by group: $F_{2,38}=1.44$, n.s.). *t*-Tests showed a significant difference between both groups over all doses (all *t* values 3.90, $p<0.01$) (Fig. 4B).

3.2.3.3. Premature responses. The mean ratio PR was higher for the 6-OHDA lesioned animals than for the controls (group: $F_{1,19}=35.19$, $p<0.001$) (Fig. 4C). Increasing doses of the drug increased the mean ratio of PR (dose: $F_{2,38}=12.22$, $p<0.001$). However, DOI did not affect both groups differentially (dose by group: $F_{2,38}=1.54$, n.s.). *t*-Tests showed that the groups significantly differed over all doses (all *t* values >2.92, $p<0.01$) (Fig. 4C).

3.2.3.4. Number of trials. Only with the highest dose of the drug DOI (1 mg/kg), the number of completed trials was affected. Data of three animals from the lesion group had to be excluded (in the highest dose condition) due to the insufficient number of completed trials. After injection of the highest dose, both groups showed a reduction of the number of trials completed (52.82 and 46.73 trials for the control and lesion group, respectively, when compared with the maximum of 60 trials which were completed in all other test conditions) (dose: $F_{2,40}=12.16$, $p<0.001$), but there was no group difference (group: $F_{1,20}=1.09$, n.s.).

3.2.4. Ketanserin

The effects of the 5-$HT_{2a/2c}$ receptor antagonist ketanserin were tested at increasing doses (0, 1, 3 and 10 mg/kg BW) in weeks 11 and 12 after lesioning.

3.2.4.1. Reaction time. The RT did not differ between the 6-OHDA lesioned animals and the controls (group: $F_{1,14}=1.01$, n.s.) (Fig. 5A). Increasing doses of ketanserin worsened RT (dose: $F_{3,42}=13.43$, $p<0.01$). Ketanserin had the same effect in both groups (dose by group: $F_{3,42}=0.17$, n.s.).

3.2.4.2. Motor time. The overall MT of the 6-OHDA lesioned animals were higher than those of the controls (group: $F_{1,14}=9.26$, $p<0.01$) (Fig. 5B). Ketanserin increased the MT in a dose dependent manner (dose: $F_{3,42}=37.08$, $p<0.01$) and was similar in both groups (dose by group: $F_{3,42}=0.87$, n.s.). *t*-

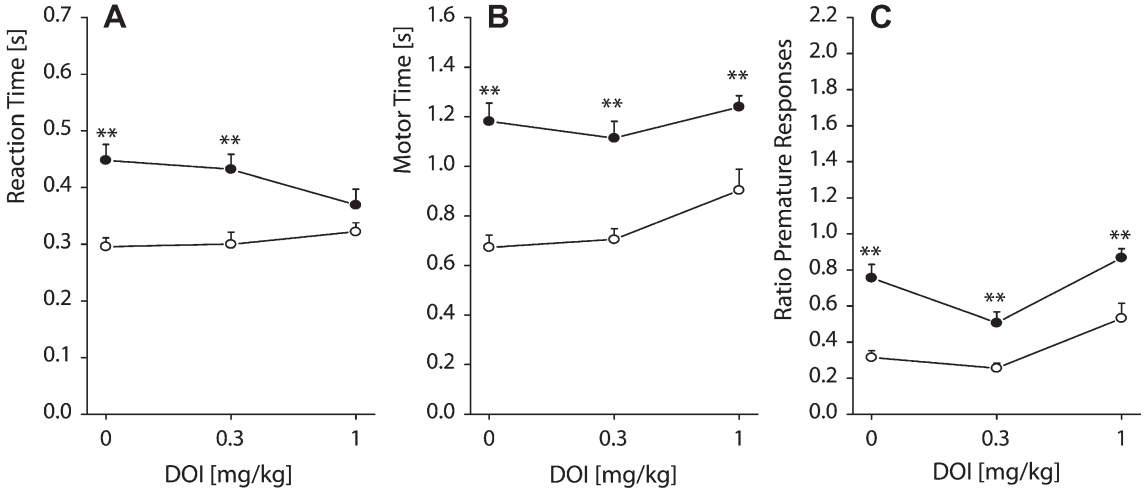

Fig. 4. Mean and S.E.M. of reaction time (A), motor time (B) and ratio premature responses (C) of sham-operated control rats (open dots) and 6-OHDA lesioned rats (closed dots) as a function of the dose of DOI at 8 and 9 weeks after operation (or sham-operation, respectively).*$p<0.05$, **$p<0.01$ (differences between sham-operated control rats and 6-OHDA lesioned rats).

Tests showed that the groups significantly differed at all doses (all t values >2.52, $p<0.05$) (Fig. 5B).

3.2.4.3. Premature responses. The proportion of PR was higher in the group of 6-OHDA lesioned animals than in the group of control animals (group: $F_{1,15}=9.16$, $p<0.01$) (Fig. 5C). Increasing doses of ketanserin did not affect PR (dose: $F_{3,45}=0.60$, n.s.). In addition, no interaction effect was observed (dose by group: $F_{3,45}=0.25$, n.s.). t-Tests showed that the groups significantly differed over the following doses 0, 1 and 10 mg/kg (all t values >2.41, $p<0.05$). However, after injection of 3 mg/kg, the groups did not differ due to large variations in the 6-OHDA lesioned group ($t_{16}=1.54$, n.s.) (Fig. 5C).

3.2.5. Recovery of TH immunohistochemistry over time

Injection of 6-OHDA introduced a significant reduction of TH immunopositive tissue in the CPu (Fig. 6A). Over time, however, a clear recovery of the lesioned CPu was observed (Fig. 6B,C). This recovery is visible as a gradual decrease in the difference in staining intensity between unlesioned and lesioned parts of the CPu with increasing post-lesion time. The recovery depicted in Fig. 6 was representative for the total group of lesioned animals.

4. Discussion

The results of the present study indicate that partial bilaterally 6-OHDA lesioned rats exhibit a wide range of behavioral and neurochemical deficits that resemble those observed in patients suffering from mild forms of PD. Impairments were found in motor initiation, motor performance and response inhibition (RT, MT and PR, respectively) in a reaction time task. The improvement in RT in the course of the experiment suggested that the 6-OHDA lesioned rats recovered from the lesion. Previously, it has been shown that striatal DA levels need to be decreased by more than 95% to impair RT (Smith et al., 2002). Over the course of the present experiment, a clear recovery of the 6-OHDA lesion was observed, as indicated by TH staining, signifying that the lesion was not permanent. The observed recovery in TH staining was paralleled by a recovery in RT, but not in MT and PR. Based on reaction time responding after treatment with *d*-amphetamine, DOI and ketanserin, it can be argued that the 5-HT_2 system is very likely to be agonistically related to the DAergic system.

4.1. Behavioral deficit

The partial bilateral 6-OHDA lesion induced in the terminal site of the nigrostriatal pathway (i.e., CPu) caused impairments in all behavioral parameters investigated. Increased RT and MT in 6-OHDA lesioned animals in our reaction time task reflect impairments in motor initiation and motor performance, respectively. In a simple reaction time paradigm, it has recently been found that RT increases after a bilateral 6-OHDA medial forebrain bundle (MFB) lesion, associated with a decrease of DA in the CPu of 95% (Smith et al., 2002). In the present study, the RT of 6-OHDA lesioned rats recovered to normal levels during the post-lesion period at around 11 weeks post-lesion. Thus, it may be assumed that, after lesioning, the striatal damage in the present experiment was about 95%, at least in the first 2 months post-lesion.

Compensatory mechanisms, or perhaps regeneration of the damaged structures, might be able to counteract the neurochemical loss produced by the lesion and consequently eliminate behavioral deficits (Bezard and Gross, 1998). Results from other studies also showed recovery of the 6-OHDA model, indicating that the observed recovery in the present experiment is not unique (Courtiere et al., 2005; Eslamboli et al., 2003).

MT and PR of the 6-OHDA lesioned rats were increased and remained at the same level throughout the post-lesion weeks. The increased number of PR in 6-OHDA lesioned rats corroborates findings in studies employing a simple reaction time task (Amalric et al., 1995; Baunez et al., 1995). This

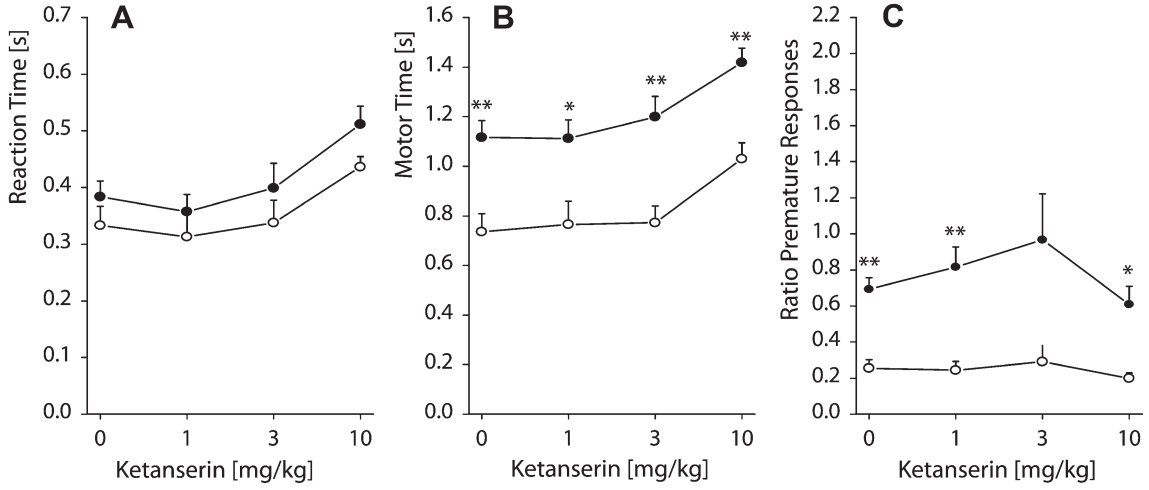

Fig. 5. Mean and S.E.M. of reaction time (A), motor time (B) and ratio premature responses (C) of sham-operated control rats (open dots) and 6-OHDA lesioned rats (closed dots) as a function of the dose of ketanserin at 11 and 12 weeks after operation (or sham-operation, respectively). *$p<0.05$, **$p<0.01$ (differences between sham-operated control rats and 6-OHDA lesioned rats).

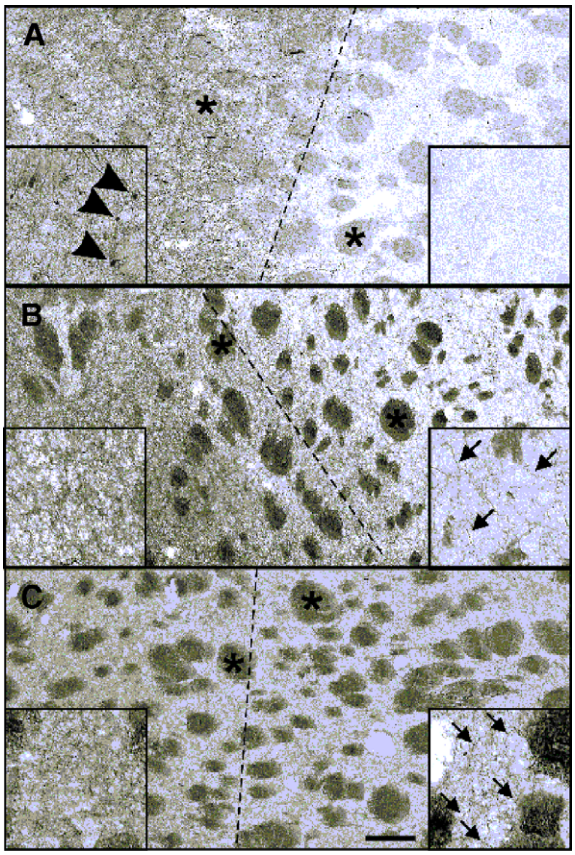

Fig. 6. Representative photomicrographs of the CPu at 3 days (A), 6 weeks (B) and 12 weeks (C) post-lesion, showing immunohistochemical labeling for TH. The dashed lines indicate the boundaries between unlesioned parts (on the left) and lesioned parts (on the right) of the CPu. Note the gradual decrease in the difference in staining intensity between unlesioned and lesioned parts of the CPu with increasing time post lesion. Direct comparisons of TH labeling intensity between the investigated time points were complicated by increasing unspecific staining of myelin (asterisks). Insets show TH positive fibers at high magnification in unlesioned parts (on the left) and lesioned parts (on the right) of the CPu. Note the aberrant varicosities in TH positive fibers within the unlesioned part of the CPu close to the lesioned part (arrowheads) as well as the complete absence of TH positive fibers within the lesioned part at 3 days (A), as well as the gradual recovery of TH positive fibers within the lesioned part of the CPu with increasing time post lesion (arrows in B and C). Scale bar=100 μm and 30 μm for the insets.

increase might be explained by a higher level of motor activation or preparation which makes the animals reacting faster than required (Baunez et al., 1995). The dissociation in recovery between RT and MT/PR indicates that different pathways and brain subregions are responsible for the different components of the reaction time task.

4.2. Drug effects

I.p. injections of the partial DA agonist *d*-amphetamine at 6 and 7 weeks post-lesion improved RT of 6-OHDA lesioned rats. As expected, increasing doses of the drug resulted in faster RT in 6-OHDA lesioned animals. However, in contrast to our expectations (cf. Blokland, 1998), control rats did not react faster or better to the different parameters of the reaction time task at increasing doses of *d*-amphetamine. Most likely, control animals already reacted at an optimal level and the effects of (further increased) *d*-amphetamine were undetectable because of a ceiling effect.

MT remained unaffected by *d*-amphetamine administration in control and 6-OHDA lesioned animals. There was a clear group difference, but MT levels of both groups did not change following increasing doses of *d*-amphetamine. This was surprising since MT as well as RT were increased by the DAergic lesion. The information processing of motor initiation (i.e., RT) was improved by administering *d*-amphetamine, but motor performance (i.e., MT) did not benefit from *d*-amphetamine. Thus, again these data support the existence of separate pathways within the basal ganglia, which are responsible for both functions. It has to be noted that *d*-amphetamine was injected peripherally and hence the drug may influence other brain sites than the CPu alone. Consequently, DA availability was most probably elevated in all four major DAergic pathways rather than in the nigrostriatal pathway only. Furthermore, *d*-amphetamine does not only act on DA but also on á1-adrenergic receptors (Darracq et al., 1998). Besides the basal ganglia, motor executive functions are also mediated by cortical pathways. An increase of DA availability in the rat CPu could be beneficial for MT performance in the rats. An additional increase of DA availability in the cortex (via the mesocortical DAergic pathway) might have a negative effect on MT performance. Thus, the overall effect of *d*-amphetamine might be unaltered MT in the rats.

PR were similarly increased in both groups at increasing doses of *d*-amphetamine, as was found in a previous studies (Blokland, 1998; Blokland et al., 2005). Interestingly, restoration of the DA availability in the rat CPu, by means of *d*-amphetamine injection, did not decrease the number of PR. Instead, the number increased further. This might be explained by the fact that high doses of *d*-amphetamine may cause an over excitation of frontal circuits important for inhibition of inadequate responses.

Increasing doses of DOI improved the RT performance of 6-OHDA lesioned rats, whereas control rats maintained the same performance, most likely due to a floor-effect. After injection of the highest dose, 6-OHDA lesioned animals performed at the same levels as controls. Thus, administration of the 5-$HT_{2a/2c}$ receptor agonist DOI resulted in a similar outcome on this behavioral parameter as administration of *d*-amphetamine, suggesting that 5-HT and DA are agonistically coupled in the CPu. DOI increased the mean MT in a dose-dependent manner. This effect was observed in both 6-OHDA lesioned animals and control rats. This effect is not likely to be related to sedation or a lack of motivation since the mean RT became faster and the proportion of PR increased after DOI administration. The number of PR increased with increasing doses of DOI in both 6-OHDA lesioned and control rats and has been described previously in a similar reaction time task (Blokland et al., 2005; Koskinen and Sirvio, 2001). This effect of DOI equals again the effect of *d*-amphetamine administration. This is also in agreement with an agonistic interaction between the 5-HT and DA neurotransmitter systems in the rat brain.

The 5-$HT_{2a/2c}$ receptor antagonist ketanserin was tested in the post-lesion weeks 11 and 12. At that moment, a clear difference in RT performance between the lesion and control group was not present anymore, most likely due to compensatory and/or recovery mechanisms. However, a significant baseline difference between both groups was still present for MT and PR at this time point. Increasing doses of ketanserin worsened RT performance in both groups and all animals had slower RT at increasing doses of ketanserin. This finding is opposite to the effect of the antagonist DOI. MT also increased at rising doses of the drug, affecting both the control and the lesioned group in the same way. There was a clear difference between both groups, which remained the same regardless the dose of ketanserin. The increase in MT was not caused by a sedative effect of the drug since there was no impairment in the total number of trials performed in one session of 30 min.

At increasing doses of ketanserin, PR remained largely unaffected. This is surprising since the 5-HT_{2a} receptor agonist DOI increased the number of PR in both groups and an opposite response would be expected for ketanserin. This indicates that the relation between cerebral 5-HT and aspects of behavior like motivation and impulsivity are rather complex.

4.3. Anatomy

Injection of 6-OHDA into the CPu of the rats introduced a significant reduction of TH immunopositive fibers. However, this lesion was not permanent. Over time, a clear recovery of the lesioned CPu was observed, indicated by an increase in TH-immunopositive fibers (as can be seen in Fig. 6), which coincided with the behavioral recovery of RT, but not of MT. Thus, the recovery of the lesioned CPu, possibly due to collateral sprouting, might be an explanation for the recovery of the behavioral parameters. However, since MT did not show signs of recovery in the present experiment, it can be stated that not all aspects of motor behavior are dependent on the circuits and structures, which were lesioned by the 6-OHDA.

4.4. The partial bilateral striatal 6-OHDA model

PD is slowly progressive in humans, and neurodegeneration of the DAergic and other systems leads to worsening of PD symptoms and increasing loss of DA levels in the dorsal part of the striatum. With regard to the time course, the rat model used in the present study is in contrast with the human condition. When inducing a DAergic lesion in the rat, the rat will go from a state of having an intact nigrostriatal pathway (before the lesion) to a state of having severe neurodegeneration (after the lesion) within a short time period. The rat brain activates compensating mechanisms in response to this neurodegeneration to antagonize neurobiological deficits. It was observed that, over time, the behavioral and neurochemical deficits were, to some extent, alleviated in the rat model. This recovery can probably be overcome by injecting 6-OHDA at more locations throughout the CPu, or increasing the dose of 6-OHDA (Roedter et al., 2001; Kirik et al., 1998). Besides increasing the dose and striatal locations of 6-OHDA injection, using parallel groups regarding the pharmacological testing could possibly diminish the potential confounding effects of serial administration of the tested ligands.

PD is a progressive disease with subsequent stages that are characterized by more extensive loss of DAergic neurons and more severe PD symptoms. Since PD is progressive, modeling this disease in the rat implies that the induced syndrome will resemble the human situation at a certain phase of the disease. On the other hand, the activated compensation mechanisms in the rat brain will decrease the severity of the syndrome in the rat, so that, over time, the rat model will resemble earlier PD phases (Deumens et al., 2002). Although RT showed a recovery over time, this was not observed for parameters such as MT and PR. This has also been found in other studies where 6-OHDA lesion models were used (Smith et al., 2002; Barneoud et al., 1999). The recovery of function is possibly due to compensatory mechanisms, like sprouting of remaining DAergic fibers (Blanchard et al., 1996). It could also be possible that other neurotransmitter systems (for instance ACh) play a significant role in compensation (an overview of the different systems influencing the CPu has been provided in Hauber, 1998). Also, it should be noted that next to sprouting other compensatory mechanisms (e.g., elevated DA biosynthesis, metabolism, changed receptor levels and release by the remaining DAergic neurons) could contribute to the recovery of function.

5. Conclusions

RT (motor initiation) and MT (motor performance) were impaired in our partial bilateral striatal 6-OHDA lesioned animal model of PD. However, RT was subject to recovery over time, which was paralleled by a recovery in TH staining. Contrary to RT, MT did not recover. Thus, when using this 6-OHDA model, experiments should only be performed within a specific timeframe of the first 8 weeks after lesioning.

With regard to the role of 5-HT in the 6-OHDA lesioned rat model, 5-HT_{2a} and DA are agonistically linked to each other as based on the finding that the behavioral results of the 5-HT_{2a} receptor agonist DOI and the partial DA agonist *d*-amphetamine were similar. The behavioral results of the 5-HT_{2a} receptor antagonist ketanserin showed opposing results, which argues further towards an agonistic relation between 5-HT and DA. There was no evidence for a differential effect of the drugs in the two groups of animals. Thus, an agonistic relationship between the 5-HT and DA neurotransmitter systems could be observed in both 6-OHDA lesioned rats and controls. The 5-HT_{2a} receptor may play a role in this relation.

The observed agonistic relation between 5-HT and DA was not expected, since in human research an antagonistic relation is generally considered. The present experiments started out from an antagonistic relation (Ng et al., 1999; Sershen et al., 2000). DOI and ketanserin also partially, but less strongly, influence the 5-HT_{2c} receptor subtype. Recently, it has been suggested that 5-HT_{2a} might have no effect on basal DA release in the rat CPu, while the remaining antagonistic relation of 5-HT_{2c} with basal DA release in the CPu should actually be an agonistic one (Lucas and Spampinato, 2000). It should also be noted that the

injections were given peripherally; thus, other areas besides the CPu might be influenced.

Contrary to the general consideration in human research, 5-HT and DA appear to be agonistically linked in the investigated animal model of PD. However, the exact role of 5-HT in PD remains unclear, and needs to be examined in further studies.

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