**Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice**

*Ruxandra Iancu, Paul Mohapel, Patrik Brundin and Gesine Paul\**

Neuronal Survival Unit,

 Wallenberg Neuroscience Centre, Department of Experimental Medical Science,

22184 Lund, Sweden

**\*Corresponding author:** Gesine Paul, Neuronal Survival Unit, Wallenberg

Neuroscience Centre, Department of Experimental Medical Sciences, BMC A10,

22184 Lund, Sweden

Phone: ++46-46-2220524

Fax: ++46-46-2220531

E-mail: **gesine.paul@mphy.lu.se**

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**Abstract**

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. Several toxin-induced animals models simulate the motor deficits occurring in PD. Among them, the unilateral 6-hydroxydopamine (6-OHDA) model is frequently used in rats and has the advantage of presenting side-biased motor impairments. However, the behavioral consequences of a unilateral 6-OHDA lesion have so far not been described in detail in mice. The aim of this study was to characterize mice with unilateral 6-OHDA lesions placed in the median forebrain bundle using several motor behavioral tests in order to identify the most suitable predictor of nigral cell loss. Mice underwent various drug-induced (amphetamine- and apomorphine-induced rotation) and spontaneous motor tests (cylinder, rotarod, elevated body swing, and stride length test). The amphetamine-induced rotation test, the cylinder and the rotarod test were most sensitive and reliable in detecting loss of tyrosine hydroxylase-immunoreactive cells in the substantia nigra.

This study demonstrates that substantial and stable unilateral 6-OHDA induced lesions can be established in mice, and that these lesions can be functionally assessed using several different side-bias based behavioral tests. This mouse model offers the opportunity to use transgenic mouse strains and study the interactions between genes of interest and toxins in relation to Parkinson's disease etiology in the future.

## 1. Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disorder. It is characterized by a marked loss of dopaminergic neurons of mainly the substantia nigra (SN) pars compacta leading to a reduction of dopamine (DA) in the target structure, the striatum [22]. The dopaminergic deficit results in motor disabilities such as rigidity, akinesia, tremor and postural abnormalities as well as cognitive and vegetative disturbances [16].

The most frequently used toxins in rodent models of PD are either the neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) [13]. Although MPTP is a valuable model of PD in mice and non-human primates, it is limited for several reasons: MPTP injection causes a bilateral parkinson syndrome, thereby ruling out all behavioral tests based on a side bias. Cell loss is strain, age and gender dependent in mice [47]. More importantly, a spontaneous recovery of parkinsonian symptoms has been described in both, monkeys [15,50] and mice [45,46] after MPTP administration which causes concern to use this model for an assessment of long-term therapeutic effects of a compound or neural grafts.

6-OHDA is a neurotoxin that selectively destroys catecholaminergic neurons and it is typically injected unilaterally, since bilateral injections cause high mortality. The advantage using this model is that it lends itself to more easy assessment of motor impairments by utilizing tests that examine for a side bias, e.g., drug-induced rotation tests [53] and spontaneous motor tests. Furthermore, intracerebral injection of 6-OHDA into the rat nigrostriatal pathway has been shown to permanently degenerate virtually all dopaminergic neurons in the SN pars compacta [25,26] leading to stable

motor deficits over time. The toxin can be injected intrastriatally, into the median

forebrain bundle (MFB) or directly into the SN. Only very few studies concerning

mice with 6-OHDA lesions have been published before. In these studies 6-OHDA

was injected mainly either intrastriatally [6,9,12,31] or intraventricularly,  and the

mice were subjected to relatively little behavioral assessment [3,4,7].  More recently,

new models of PD have been established that rely on genetic manipulations in mice.

A number of laboratories have now generated different mouse strains that carry

mutations in proteins or receptors that are critical for the function of the dopaminergic

system.  These include α-synuclein-overexpressing mice [32], α-synuclein knockout

mice [1], parkin knockout mice [20,24], several knockout mice for dopamine

receptors [5,33,49] and tyrosine hydroxylase (TH, the rate-limiting enzyme of

dopamine synthesis), and a mouse with a UCH-L1 gene mutation [41]. With an

increasing availability of genetically modified mice that model PD, we believe that

characterizing toxin-induced lesions in mice is highly warranted.  PD models are also

used to demonstrate therapeutic effects of neural grafts as a promising approach to

brain repair [14]. Thereby, in the future, new models could be created that combine

genetic and toxin methodologies to investigate the pathogenesis of PD or the

influence of a certain genetic background on graft properties, such as cell survival and

fiber outgrowth.


In the present study, we injected the 6-OHDA unilaterally into the MFB to

achieve retrograde dopaminergic cell loss in the SN.

We provide an extensive behavioral characterization of motor deficits by applying

multiple behavioral tests such as amphetamine- and apomorphine-induced rotation,

the rotarod test, the cylinder test, the elevated body swing test (EBST) and the stride

length test. By comparing behavioral outcome with the degree of nigral cell loss in

individual mice, we have determined which of these tests best predict SN cell loss,

and which tests should be used for selecting for adequately lesioned mice.

## 2. Material and methods

### 2.1. Animals

110 adult female CBA mice (20 g, B&K, Stockholm, Sweden) were lesioned and all animals were tested for amphetamine-induced rotation at 3 and 6 weeks post lesion. Out of these 110 mice, 53 mice were selected for the present study. This selection was based on their amphetamine rotation scores at 6 weeks after the 6-OHDA injections. We aimed to include mice with a large range of different amphetamine rotation scores for the study. The remaining mice were used in another study. Seven unlesioned adult female CBA mice (20 g, B&K, Stockholm, Sweden) were used as controls. Mice were housed 6 to a cage under a 12:12 h light-dark cycle and had free access to food and water. All experiments were performed in accordance with the Ethical Committee recommendations for animal care use of laboratory animals at Lund University, Sweden.

### 2.2. Surgical procedures

To achieve unilateral lesions of the nigrostriatal system, mice received 6-OHDA injections into the right MFB. Mice were anaesthetized using hypnorm/dormicum anesthesia (Dormicum: 5mg/kg, Hypnorm 0.315 mg/kg) and placed into a stereotactic frame with nose and ear bars specially adapted for mice (David Kopf Instruments, USA). 6-OHDA (Sigma Chemical Co., St. Louis, MO) was dissolved at a concentration of 3 μg/μl saline in 0.1% ascorbic acid and injected at two different volumes resulting in final dosages 3.9 μg and 5.4 μg. The lesion was performed using a Hamilton syringe at the following coordinates: AP: -1.2 mm, ML: +/-1.1 mm, DV: -5.0 mm; TB at +/-0 mm [18]. The injection was conducted at a rate of 0.5μl/min and

the needle was left in place for another 5 min after the injection before it was slowly drawn back.


*2.3. Drug-induced behavioral tests*

All behavioral analysis was performed by an observer blinded to the group and previous performance of the mice in other behavioral tests. Tests were performed at different time points after lesion over a period of 19 weeks (Fig. 1).

*Amphetamine-induced rotation* was measured at 3, 6, 10 and 19 weeks post lesion. A minimum 3 week interval was chosen to avoid possible hypersensitisation [10]. Mice received 2.5 mg/kg metamphetamine i.p. [9] (Apoteksbolaget, Sweden), were placed in individual glass bowls with a diameter of 20 cm and attached via a specially adapted harness to an automated rotometer (Rotamex, Columbus Instruments, Columbus, OH). They were allowed to habituate to their environment for 10 min before turns contralateral and ipsilateral to the lesion were recorded over 40 min. Results were expressed as ispilateral net turns / min. Contralateral amphetamine-induced turns were separately analyzed for sessions 2 to 4 as the number of total turns over 40 min.

*Apomorphine-induced rotation* was tested at 10 and 19 weeks post-lesion with at least a 2 day period between tests.

Apomorphine was injected s.c. at a dose of 0.5 mg/kg (Apoteksbolaget) and rotation was monitored for 40 min using the same experimental set up as for amphetamine-induced rotation. Results were expressed as contralateral net turns / min.


*2.4. Spontaneous motor tests*

*2.4.1. Rotarod test*

The rotarod test was performed at 13 weeks post-lesion using a modification of a previously described procedure for rats [40] and adapted to mice. The unit consists of a rotating spindle (5 cm diameter), a power source for turning the spindle and grids beneath the rotating roller where mice can fall onto (Rotamex 4/8; Columbus Instruments, Columbus, OH). All mice were pre-trained on the rotarod apparatus in order for them to reach a stable performance. The training consisted of 3 sessions on 2 consecutive days, whereby each session included 3 separate test trials, each lasting 120 s. Mice were trained at 5, 10 and 15 revolutions per minute (rpm). On day one, mice were trained at 5 rpm.  On day 2 mice were trained once in the morning at 10 rpm and once again in the afternoon at 15 rpm. The final test (3 sessions, each lasting 180 s) was performed on the third day at 15 rpm. Between trials, mice were given at least 2 minutes of rest in order to reduce stress and fatigue.


*2.4.2. Cylinder test*

Forelimb use during explorative activity was analyzed in the cylinder test using a modified version [28,30] of a previously described test paradigm for rats [42]. The test was performed at 12 weeks post lesion during the onset of the dark cycle, when mice are more active. Mice were placed individually in a glass cylinder (11 cm diameter, 20 cm height) in darkness and were video recorded with an infrared camera (Sony Handycam, CCO-TR918E) for 3 min. No habituation was allowed before video recording. An investigator who was blinded to the group and the results of other behavioral tests analyzed all video recordings.  Only weight-bearing wall contacts made by each forelimb on the cylinder wall were scored.  Wall exploration was expressed in terms of the percentage of impaired forelimb wall contacts relative to the total number of times the mouse touched the wall with one of the forelimbs [28,30].

*2.4.3. Elevated Body Swing Test*

The Elevated Body Swing Test (EBST) was performed once a week, between 11 to 13 weeks post-lesion, at the onset of the dark cycle.  We adapted the test for unilaterally lesioned rats [8]. Each mouse was held 1 cm from the base of its tail and suspended vertically 1 cm above the ground for 60 sec. Swings were recorded whenever the mouse moved its head 30° from the vertical axis. Two examiners performed the test; one held the mouse while the other determined direction and frequency of the movement and timed the session. The number of swings to each side was expressed as percentage of the total number of swings.

*2.4.4. Stride Length*

The stride length test was performed at 15 to 17 weeks post-lesion. We adapted the stride length test described previously [17] to investigate whether we could detect unilateral motor impairments in this mouse model. In two different sessions, both the forelimbs and hindlimbs of the mouse were separately dipped in ink. The mice were placed in a passage (4.5 cm wide, 40 cm long, with walls of 12 cm height), lined with paper with a light illuminating the starting position and a dark box at the other end of the passage. Stride lengths were measured as the distance between 2 paw prints. Strides were analyzed only when mice ran with constant velocity. All strides on the first and last 7 cm of the passage were excluded due to changing velocity, as previously described [17]. The mean value of the 3 longest strides in a sequence is reported for each limb. Mice were tested 3 times over 3 weeks and the highest mean stride length out of the 3 sessions is reported.

*2.5. Tissue preparation*

Twenty weeks post lesion, mice were deeply anesthetized with sodium pentobarbital

(240 mg/kg i.p.) and perfused through the ascending aorta with 60 ml isotonic saline

followed by 80 ml ice-cold 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4).

Brains were removed, post-fixed in the same fixative overnight and subsequently

placed in 20% sucrose/0.1M phosphate buffer. Three series of free-floating 30 μm

thick coronal sections were prepared for immunohistochemistry.

*2.6. Immunohistochemistry*

For TH-immunohistochemistry, sections were rinsed in PBS 3 times and quenched for

10 min in 3% $H_2O_2$/10% methanol in PBS, preincubated with 5% normal swine

serum/0.30% Triton X-100 in PBS and incubated overnight at room temperature (RT)

with rabbit anti-TH antibody (1:1000, Pel-Freez Biologicals, Arkansas) in 2% normal

swine serum /0.30% Triton X-100 in PBS. This was followed by incubation with

1:200 dilution of biotinylated swine anti-rabbit antibody in 0.3% Triton X-100 in PBS

for 1 hour at RT and subsequent incubation with avidin-biotin-peroxidase complex

(ABC-Elite kit, Vector Laboratories, Burlingame, CA) for 50 min at RT. The reaction

was visualized with 3,3í-diaminobenzidine tetrahydrochloride with 0.01% $H_2O_2$ in

Tris-HCl buffer, pH 7.6. Sections were mounted on gelatin-coated slides, dehydrated

in alcohol, cleared in xylene and coverslipped in DPX (BDH, England). Cresyl Violet

staining was performed to determine the site of lesion.

*2.7. Quantitative analysis*

The total number of cells in the SN and ventral tegmental area (VTA) was estimated

with unbiased stereology using an optical fractionator [54]. In mice, the entire rostro-

caudal extent of the SN is about 1200 µm. To cover this distance, we collected

sections from a point caudal to the subthalamic nucleus at -2.54 mm from the bregma

and continued caudally until the retrorubral field (A8 cell group) until -4.04 mm

(from bregma). Cells in the retrorubral nucleus were thus excluded.

To define the border between SN pars compacta (A9 cell group) and VTA (A10 cell

group), a vertical line was drawn from the medial tip of the cerebral penduncle and

counting was performed caudally until the SN pars reticulata disappeared. TH-

positive cells in SN pars compacta, pars reticulata and pars lateralis were counted as

one unit. Three series were sampled and sections cut at 30 µm. Eight sections at a

distance of approx. 100 µm from each other were counted for each brain across the

rostro-caudal extent of the SN. Sampling was performed with the Olympus CAST-

grid system (Olympus, Denmark A/S). Counting was conducted with a 40x objective

and the total number of neurons was estimated using the optical fractionator formula

[54]. The cell loss on the lesioned side is expressed as the percentage of the cell

numbers on the contralateral (unlesioned) side.


*2.8. Evaluation of behavioral tests and statistical analysis*

For each behavioral test we assessed the following: (1) the correlation with unilateral

nigral cell loss; (2) stability of behavioral performance under repeated test conditions

(if applicable); (3) the best predictor, such that which test best predicts 6-OHDA-

induced cell loss in SN.

All statistical analyses were conducted with Stat View 4.0 (Abacus Concepts,

1996). Repeated measure ANOVA was performed for the drug-induced behavioral

tests where multiple motor parameters were recorded. Statistical significance was set

at $P < 0.05$. Correlations were done using linear, polynomial and exponential

regression analysis. Here, statistical significance was set at P<0.01 to compensate for the repeated measures design. Predictive values were analyzed using a forward stepwise regression test with a single dependent variable. This stepwise model involves: (1) identifying an initial model; (2) iteratively "stepping," that is, repeatedly altering the model at the previous step by adding a predictor variable in accordance with the "stepping criteria;" and (3) terminating the search when stepping is no longer possible given the stepping criteria, or when a specified maximum number of steps has been reached. All data are expressed as means ± SEM.

3. **Results**

Fifty-three lesioned mice were included in the behavioral analysis based on their ipsilateral amphetamine-induced rotation scores ranging from –6.3 to 12.0 rotations/minute. Out of these mice, 4 were excluded due to a poor needle placement according to the analysis of the cresyl violet stains. Therefore, the statistical analysis is based on a total of 49 lesioned mice and 7 non-lesioned control mice.

*3.1. TH-cell loss*

TH-immunolabeling indicated that the unilateral MFB 6-OHDA injections reduced the number of DA neurons in both the SN and the VTA. The cell loss in the SN on the lesioned side ranged between 4 to 100% (Fig. 2) and thus gave a wide range of lesioned mice for behavioral analysis. There was no difference in TH cell numbers in the contralateral SN between lesioned mice and control mice. The 2 different doses of 6-OHDA (3.9 μg /n=12; 5.4 μg /n= 37) did not result in significantly different cell loss in the lesioned SN. The cell loss ranged between 4 - 100% in the higher dose group and 12 - 97% in the lower dose group. Cell counts of TH-positive cells in the VTA ranged between 3% - 136%. Figure 3 illustrates the morphology of the SN pars compacta in a mouse with 97% loss of TH-positive neurons (Fig. 3B) and the reduction in TH-positive fiber density in the striatum of the same mouse (Fig. 3A).

*3.2. Behavioral tests*

We performed several drug-induced and spontaneous behavioral tests to identify the most suitable predictor of nigral cell loss in the unilaterally 6-OHDA-lesioned mice.

*3.2.1. Amphetamine-induced rotation in MFB-lesioned mice*

Amphetamine-induced rotation was performed at 3, 6, 10 and 19 weeks post-lesion.

Amphetamine-induced rotational asymmetry increased linearly with greater loss of

TH-IR-cells in the SN (R= 0.57, $R^2$= 0.33, F= 23.4 (1,47); p<0.001, simple

regression, week 10, Fig. 4A). Control mice (n=7) had a mean rotation score of

0.064± 1.05. Mice with a cell loss greater than 70% demonstrated stability of the

amphetamine-induced rotational response over time such that there was no significant

change in their performance from 3 weeks onwards (repeated measures ANOVA,

p>0.05, data not shown).

In addition to analyzing the magnitude of net side bias in a direction ipsilateral

to the lesion, we also specifically examined the number of rotations performed

contralateral to the lesion (Fig. 4B) as an indicator of the lesion not being complete

and there being residual dopamine release in the striatum on the side of the 6-OHDA

lesion. The contralateral rotations correlated negatively with the cell loss (simple

regression, R= 0.54, $R^2$= 0.28, p<0.0001, 10 weeks). As expected, the contralateral

rotations correlated inversely with the net ipsilateral rotations (simple regression, R=

0.80, $R^2$= 0.65, p<0.0001, 10 weeks, Fig. 4C). A low total number of contralateral

rotations (less than 15 contralateral turns/40 min) in combination with a distinct

ipsilateral amphetamine-induced rotational response (more than 2 net ipsilateral

turns/min) selects for mice with cell loss > 80%.

*3.2.2. Apomorphine- induced rotation test*

Apomorphine-induced rotation was performed at 10 and 19 weeks post-lesion.

We detected a bimodal distribution of the response to apomorphine in mice with a

high cell loss when rotation scores were plotted against loss of TH-IR cells in the SN

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(simple regression, R=0.43, $R^2$=0.18, p=0.002, fig. 4D, week 10). Some mice showed stereotypic behavior such as sniffing and gnawing after administration of apomorphine. Control mice had a mean rotation score of 0.41± 0.34. Rotational performance was stable over time in mice with >70% cell loss (data not shown). No significant correlation between ipsilateral amphetamine-induced rotation and apomorphine-induced rotation was found (R= 0.29, $R^2$ 0.09, p=0.038, linear regression, fig. 4E, week 10).

### 3.2.3. Cylinder test

Thirteen weeks post-lesion, mice were tested for forelimb asymmetries using the cylinder test [42]. When plotting the total number of left forepaw contacts as a percentage of the total number of forepaw uses, we found a significant linear correlation with cell loss (Fig. 5A, simple regression, R= 0.52, $R^2$= 0.27, P< 0.001). Control mice (n=7) had an equal forelimb use (48.4 ± 2.6%). Six animals were excluded from the analysis because they lacked any exploratory behavior (i.e. never showed rearing behavior) and one mouse was excluded from the statistical analysis based on Dixon's *Q*-test.

### 3.2.4. The Rotarod test

The rotarod test was performed at 12 weeks post-lesion. The time spent on the rotarod at a speed of 15 rpm was used for analysis. The performance on the rotarod correlated linearly with nigral cell loss (Fig. 5B, simple regression, R= 0.54, $R^2$=0.29, p<0.0001). Control mice (n=7) stayed for a mean of 78.6 ± 20.3 sec on the rotarod.

### 3.2.5. Elevated body swing test

The EBST was performed from 11 weeks post-lesion onwards, at 3 consecutive weeks. There was no significant correlation between cell loss and performance in EBST ($p > 0.05$). Control mice had a mean side preference of $48 \pm 5.7\%$. The test was excluded from the stepwise regression test.

### 3.2.6. Stride length test

The stride length test was performed week 15 to 18 post-lesion. The overall left stride length of both forelimbs and hindlimbs was significantly shorter in all lesioned mice compared to unlesioned normal mice (data not shown). However, there was no correlation between cell loss and stride length in lesioned mice, neither for hindlimbs nor forelimbs ($p=0.53$ hindlimbs, $p=0.34$ forelimbs). The test was therefore excluded from the stepwise regression test.

### 3.3. VTA TH-cell loss

No significant correlation was observed between any of the six behavioral tests and the VTA TH-immunoreactive cell depletion (data not shown).

### 3.4. Predictive value

Based on the stepwise regression model, amphetamine-induced rotation was the best predictor of the loss of TH-immunoreactive neurons in SN, such that the rotation score uniquely predicts 34.5% of the variability in motor impairments. The unique contribution of the rotarod test was 13.8%, and the cylinder test uniquely predicted 9.1% of the variability in motor impairments. Apomorphine-induced rotation only uniquely predicts less than <1% of motor impairments.

**Discussion**

We show that it is possible to achieve a permanent loss of midbrain dopaminergic neurons after injecting 6-OHDA unilaterally into the MFB. This study identified several behavioral motor tests sensitive to detecting this unilateral nigral cell loss in mice.

As mentioned above, we selected 53 mice out of 110 mice subjected to the lesion. The selected mice showed a wide variability regarding loss of dopaminergic SN neurons probably due to the small size of the MFB that makes it difficult to target in mice. Consequently, there was no significant difference in TH-cell loss in the SN between the mice receiving the higher (5.4 µg) and the lower (3.9 µg) dose of 6-OHDA.

Behavioral tests in PD models can be used to characterize the extent of lesion and/or to detect therapeutic effects. Since toxin-lesions are often variable, these tests can also serve to detect animals with a high degree of cell loss if the relation between behavior and cell loss is established.

From all behavioral tests used, amphetamine-induced rotation showed the highest predictability of nigral TH cell loss. We therefore recommend this test as a standard for assessing TH cell loss and behavioral deficits in mice with unilateral 6-OHDA lesions. The quantification of rotational behavior after a challenge with amphetamine or a low dose of a dopamine receptor agonist such as apomorphine [53] is the most commonly used behavioral test in rats with unilateral depletion of striatal DA. Amphetamine induces release, and inhibits reuptake of dopamine in the striatum causing ipsilateral turning in animals with unilateral nigrostriatal lesions. In rats, amphetamine-induced rotation has been shown to correlate with both the extent of TH cell loss and the degree of striatal dopamine depletion [23,28,29,51,57]. However,

only a few studies on drug-induced rotational behavior in mice are published that all involve intrastriatal injections of 6-OHDA [6,9,12], which cause a loss of dopaminergic terminals in the striatum, but do not deplete the neurons in the SN as effectively; and most of these papers lack analysis of nigral cell counts.

We found a significant correlation between amphetamine-induced rotation and nigral cell counts. Furthermore, mice with more than 70% of the nigral TH-immunopositive neurons lost showed similar rotation scores over repeated testing, indicating stability of the lesion over time. The reliability of repeated amphetamine-induced tests has been questioned due to the fact that DA agonists and DA releasing agents can have opposing effects at high dosages or with repeated testing [10]. We therefore aimed at avoiding sensitization by spreading the test sessions over a long time interval with no less than three weeks in between each test session. We also analyzed amphetamine-induced rotation in a direction contralateral to the lesion and detect a negative correlation between contralateral turns and TH-IR cell loss. We interpret contralateral turns as an indicator for a significant remaining dopaminergic innervation on the lesioned side.

In contrast, apomorphine showed a much weaker correlation with nigral cell loss than amphetamine in mice. Apomorphine is a DA receptor agonist that at low doses causes contralateral turning by stimulating both supersensitive D1 and D2 receptors preferentially on the denervated side. However, apomorphine receptor up-regulation does not occur until 90% of the DA afferents are lost [11], which could explain the poor correlation observed in our study. This test may therefore be only valid in mice with near complete dopamine lesions. Interestingly, mice with TH cell loss over 70 % showed a bimodal distribution in response to apomorphine, such that one subset rotated extensively and another did not. A similar phenomenon has been

described by Hudson and coworkers [23]. They concluded that stereotypic behavior

could be responsible for these varying responses with apomorphine testing, a

phenomenon that we also observed in some mice. This maybe due to stimulation of

the ventral striatum that initiates stereotypic behavior or may alternatively be caused

by the relatively high dose of apomorphine used in the present study (0.5 mg/kg s.c.),

which is 10x higher than the minimum dose shown to be effective in rats (for review

see [43]). Our dose was based on a previous study in intrastriatally lesioned mice,

where the authors did not report stereotypic behaviors [6]. In the present paradigm,

the inconsistency in performance of apomorphine-induced rotation leads us not to

recommend this test for selection of unilateral lesioned mice.

Among the spontaneous motor tests, the rotarod test predicted nigral cell loss best in

our study. The rotarod test is an established test used for the assessment of

neurological deficits in rodents, usually following pharmacological treatments,

genetic manipulations or brain injuries [35,36]. However, this test has also been used

as a drug-independent test for unilaterally 6-OHDA-lesioned rats to assess for akinetic

symptoms [39,55]. Our study shows that the time spent on the rotating rod correlated

inversely with the cell loss.

Similarly, we found that the cylinder test predicted nigral cell loss well. It is

used to assess forelimb asymmetries in rats [28] by assessing the innate drive to

explore a novel environment by rearing and leaning their forepaws against the wall of

the glass cylinder. Rats with unilateral 6-OHDA lesions tend to avoid the use of the

Parkinsonian forepaw.  Our results in mice correspond to those found with rats.

However, in our study we analyzed only weight-bearing forepaw use and ignored

other measurements such as use of limbs to land after rearing and simultaneous use of

both limbs for exploration or landing, as suggested by others [52]. We chose to analyze contralateral weight-bearing wall touches expressed as the total number of weight-bearing touches based on other studies [28,30] and found this test to predict cell loss well.

A test we find not sensitive enough in detecting SN cell loss in mice is the EBST, first described by Borlongan and Sanberg [8] as a measure of posture bias in unilaterally lesioned rats. The authors showed that unilaterally 6-OHDA lesioned rats would tend to swing contralateral to the side of the lesion. There are conflicting results in the literature regarding the validity of the EBST and some reports have even described an ipsilateral instead of contralateral bias [2,21,37] that would reverse after repeated testing [2].

Hypokinesia of gait with reduced stride length is characteristic for many basal ganglia related disorders. Although clinically relevant, gait performance has not widely been investigated in laboratory animals. Fernagut and his colleagues [17] described stride length performance in mice after pharmacological- and/or subacute neurotoxin-induced parkinsonism. Based on these results, we hypothesized that parkinsonian gait would express a side asymmetry after a unilateral 6-OHDA lesion. We did not detect a significant side asymmetry in lesioned mice and the small differences detected between lesioned mice did not correlate to SN cell loss. This is probably due to the fact that lesioned mice adapt their step length to the shorter step length contralateral to the lesioned side. We conclude that this test is not useful in detecting a side bias and for predicting nigral cell loss in unilaterally lesioned mice.

*Relevance of the model*

The stepwise regression model gives information on the unique prediction of each test on motor impairments based on the amount of cell loss in SN. We show that amphetamine-induced rotation has the highest predictive value (34.5%) and correlation with nigral TH cell loss. It is followed by the rotarod (13.8%) and the cylinder test (9.1%). Together, the motor impairments detected with these three tests predict almost 60% of the TH cell loss in SN.

Side-based behavioral tests can be used in this model and our study points to the most effective motor tests to be conducted with 6-OHDA lesioned mice. Behavioral deficits in MFB-lesioned mice in our study are stable once a certain degree of cell loss is achieved. In contrast, e.g. systemic injection of MPTP in mice causes bilateral parkinsonian symptoms, effects are strain-, age- and gender-dependent [47] and a spontaneous recovery of parkinsonian symptoms has been described [38,44,45,48].

Injecting 6-OHDA into the MFB has some advantages. First, one avoids injecting the toxin intrastriatally or intraventricularly [3,4,7,9,12,34] which are reported to cause inconsistent and low cell loss. Furthermore, instrastriatal lesions may cause non-specific damage at the site of injection and affect striatal output neurons that in turn may influence apomorphine-induced lesions [19,27 ]. Animal models of PD are frequently used to assess the effects of neural grafts, which are usually placed into the striatum [14]. It has been suggested that intrastriatal surgery can cause a local inflammatory response that induces several cytokines, thereby influencing the graft (for review see [56]).

Especially attractive seems the future option of combining a toxin-induced model with transgenic or knockout mice carrying genetic defects to investigate e.g. the pathogenesis of PD or the influence of a certain genetic background on graft properties, such as cell survival and fiber outgrowth.

We hope that this model will be useful in assessing the potential of new protective and restorative treatment strategies for PD, as well as for the exploration of mechanisms related to functional and structural recovery of dopaminergic neurons.

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with unilateral degeneration of the nigrostriatal dopamine system. Behav Brain Res, 1990; 39: 123-33.

**Figure legends**

Figure 1.

Time scale illustrating the experimental design of the study. Numbers along the right-hand side represent the number of weeks from the 6-OHDA lesion surgery (week 0).

Figure 2.

Distribution of TH-immunoreactive cell loss (n=49 analyzed, control mice are not included). Values are expressed as percent of lost TH-immunoreactive cells on the lesioned side compared to the non-lesioned SN.

Figure 3.

Representative microphotographs demonstrating TH-immunoreactive fibers in the striatum (A) and TH-immunoreactive cell bodies in the SN (B) of a mouse with 97% cell loss in the SN.

Figure 4 A-E.

Scatter plots representing the correlation between drug-induced rotation tests and the TH-cell loss in SN (A-C) and correlation between drug-induced behavioral tests (D, E). Control mice are not included in graphs.

A. Amphetamine-induced rotation at 10 weeks post-lesion. Amphetamine-induced rotation increased linearly with the loss of TH-IR cells in the substantia nigra (simple regression, $p<0.001$).

B. Contralateral amphetamine rotations at 10 weeks post-lesion. The number of rotations/min correlates negatively with the cell loss (simple regression, $p<0.0001$).

C. Correlation between ipsilateral amphetamine-induced rotations and contralateral (left-sided) amphetamine-induced rotations at 10 weeks post-lesion. The number of rotations correlates inversely (simple regression, p<0.0001).

D. Apomorphine-induced rotation and cell loss in the SN at ten weeks post-lesion. A bimodal distribution of mice after apomorphine administration (0.5 mg/kg s.c) is seen among the lesioned mice.

E. Amphetamine-induced rotation and apomorphine-induced rotation at 10 weeks. There was no significant correlation (p>0.01).


Figure 5 A and B.

Scatter plots representing the correlation of performance in the cylinder test and rotarod test at 11 and 13 weeks postlesion. Control mice are not included in graph. The performance is correlated with the cell loss in the lesioned SN, expressed in %.

A. The use of the left forepaw is expressed as % of the total use of both paws. Linear regression showing a positive correlation between cell loss and side bias (p< 0.001).

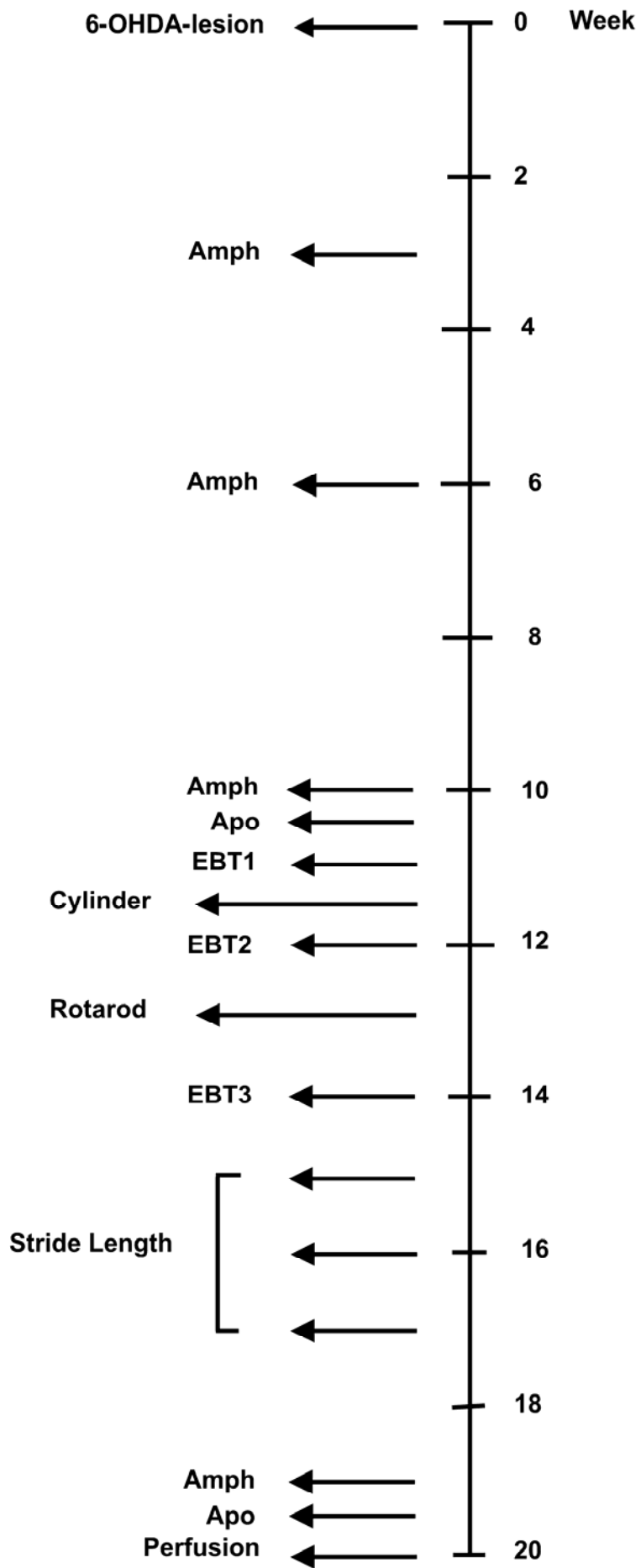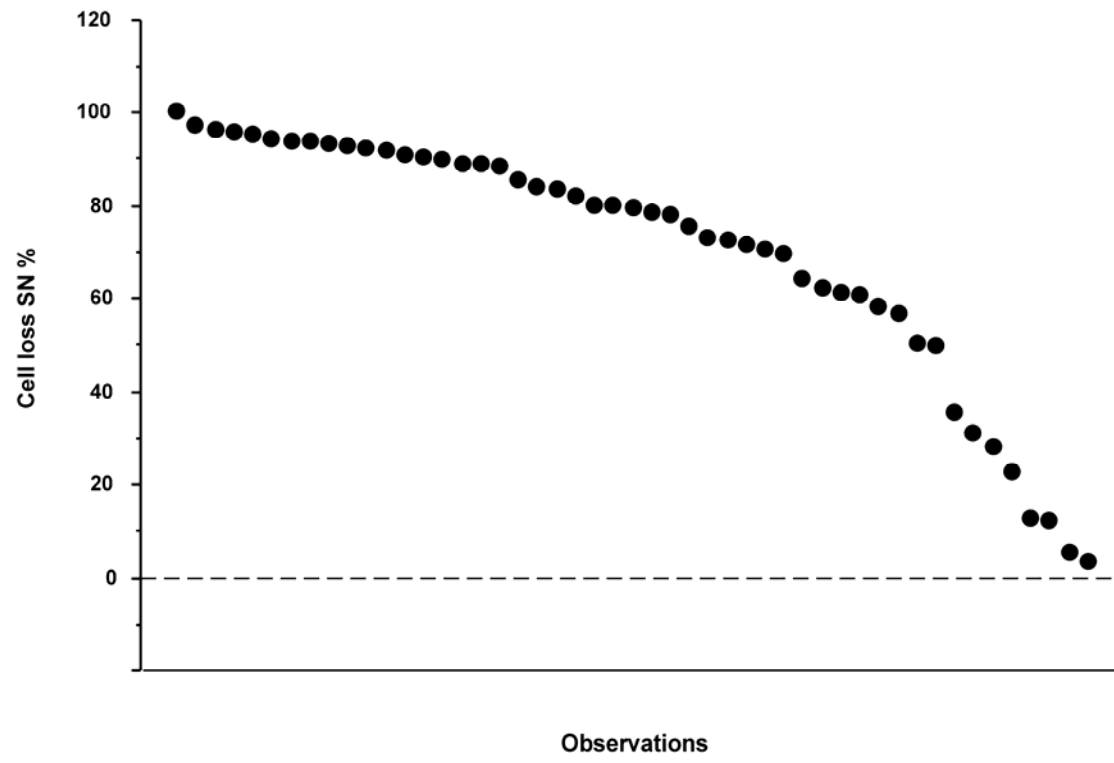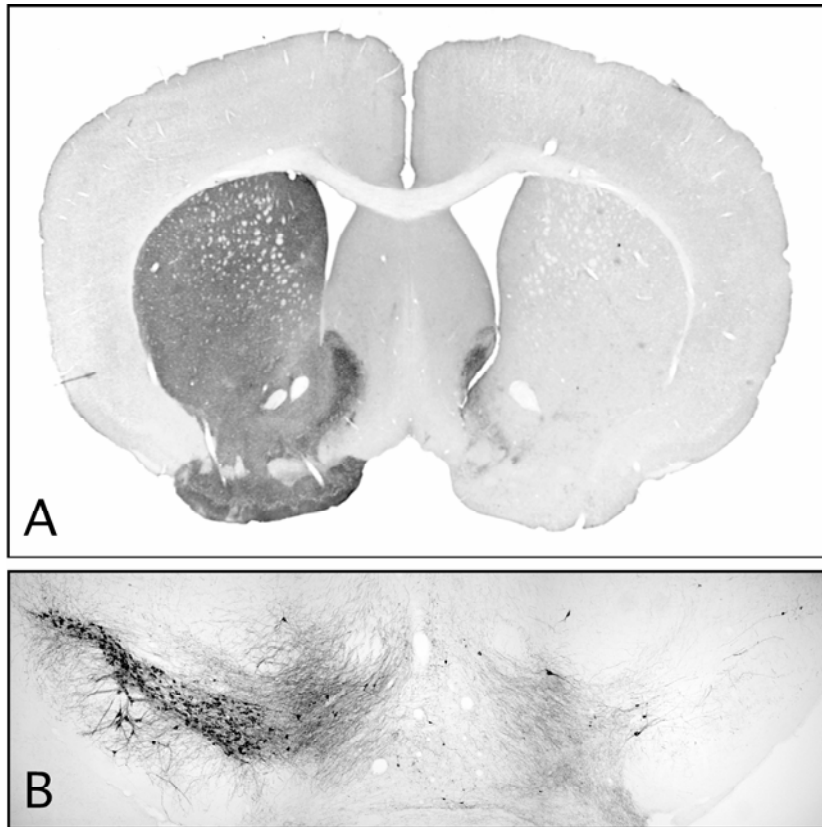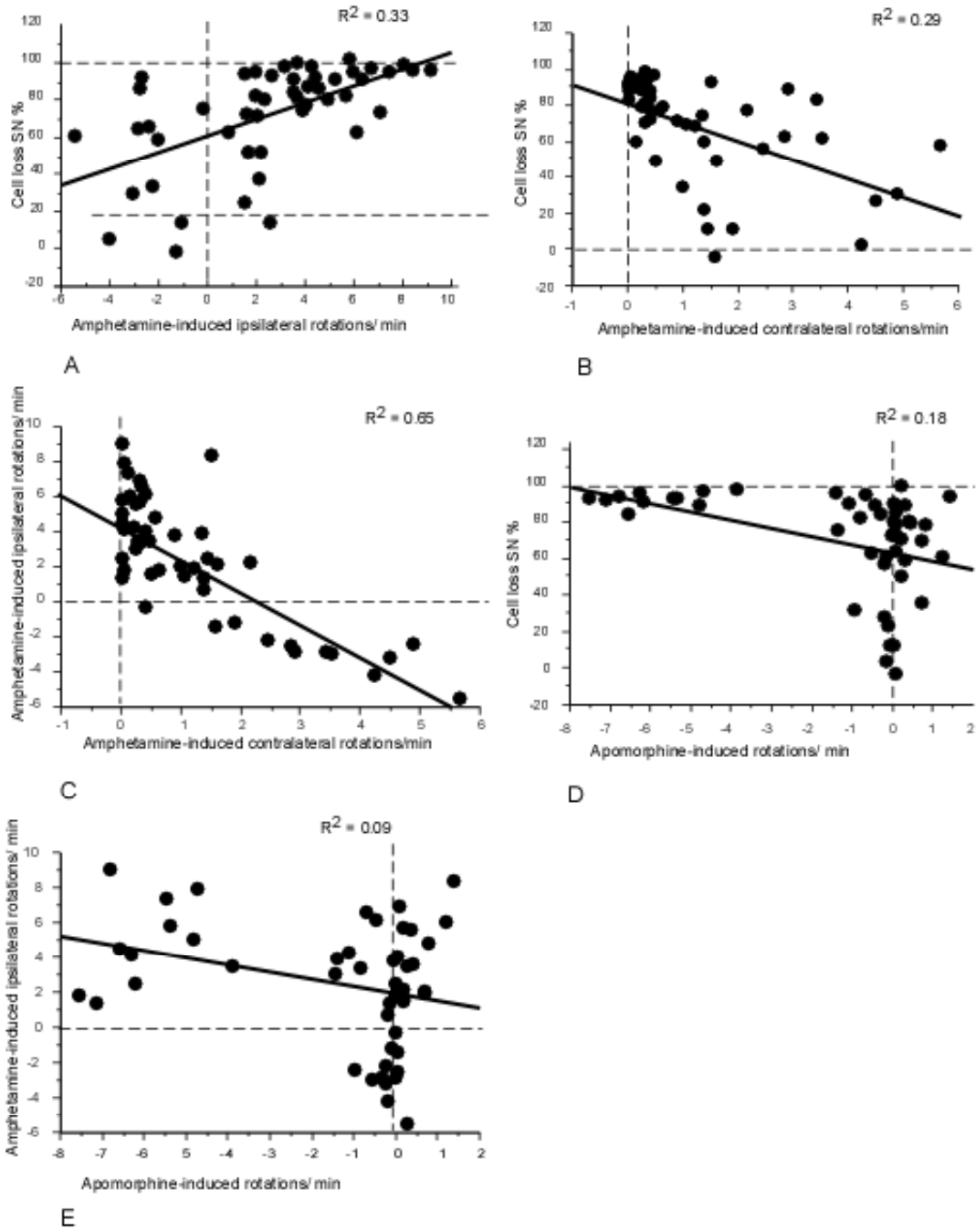B. Linear regression between cell count in the SN and time spent on the rotarod at 15 rpm (P<0.001).
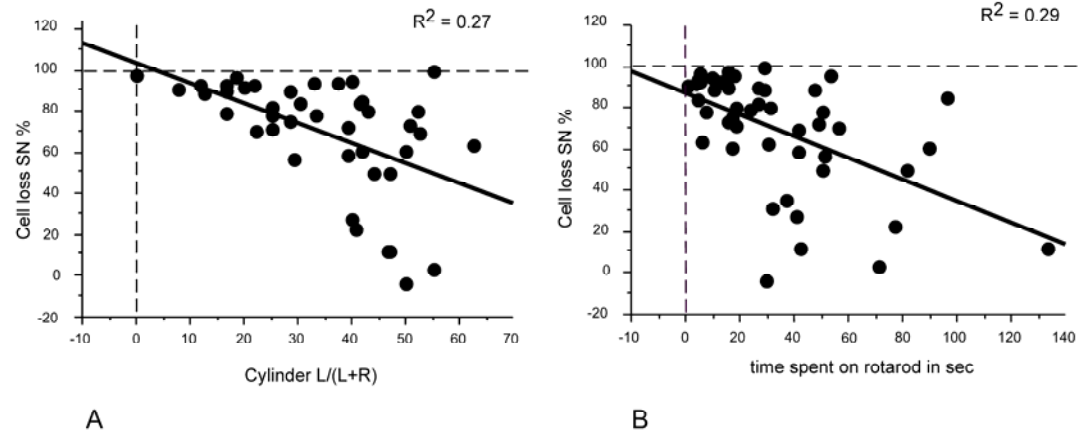
Figure 1.

Figure 2.

Figure 3.

Figure 4 A-E.

Figure 5 A and B