ELSEVIER

Physiology & Behavior 84 (2005) 525–535

PHYSIOLOGY & BEHAVIOR

Assessment of recovery in the hemiparkinson rat: Drug-induced rotation is inadequate

Eddie Castañeda[a,*], Sheila Fleming[a,b], Melanie A. Paquette[a,b], Kim Boat[a], John Moffett[b], Ewa K. Stachowiak[b,c], David C. Bloom[d], Michal K. Stachowiak[b,c]

[a]*Molecular and Structural Neurobiology and Gene Therapy Program, Department of Psychology, Arizona State University, Tempe, Arizona 85287-1104, United States*

[b]*Molecular and Structural Neurobiology and Gene Therapy Program, Department of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013, United States*

[c]*Molecular and Structural Neurobiology and Gene Therapy Program, Department of Pathology and Anatomical Sciences, State University of New York at Buffalo, Buffalo, New York 14214, United States*

[d]*UF Powell Gene Therapy Center, Department of Molecular Genetics and Microbiology, University of Florida, College of Medicine, Gainesville, Florida 32610, United States*

Received 24 May 2004; received in revised form 3 January 2005; accepted 24 January 2005

Abstract

Recovery from apomorphine-induced rotational behavior was compared to sensorimotor and motor function in hemiparkinsonian rats receiving intrastriatal grafts of astrocytes expressing recombinant tyrosine hydroxylase (TH) or control β-galactosidase (β-gal). Rats received unilateral intranigral infusions of 6-hydroxydopamine (6-OHDA). Animals with large lesions, as determined by apomorphine-induced rotation, received grafts of astrocytes into the denervated striatum. Behavioral recovery was assessed on days 14–16 post-transplantation using apomorphine-induced rotation, somatosensory neglect, and reaching for pellets using the Montoya staircase method. Rats that received transplants of TH-transfected astrocytes showed a 34% decrease in rotational behavior, but no consistent recovery of somatosensory neglect or skilled reaching. Post-mortem histological analyses revealed survival of grafted astrocytes in host striatum and expression of TH at 17 days post-transplantation. We suggest that TH-expressing astrocytes may reverse post-synaptic dopamine (DA) receptor supersensitivity; however, sensorimotor and motor abilities are not restored due to a failure by TH-expressing astrocytes to reestablish dopaminergic circuitry. The present results demonstrate the need to utilize a variety of sensory and motor behavioral tests that cohesively provide greater interpretability than a single behavioral measure used in isolation, such as drug-induced rotational behavior, to assess the efficacy of experimental gene therapies.

Keywords: Apomorphine; Astrocytes; Gene therapy; 6-hydroxydopamine; Parkinson's disease; Recombinant tyrosine hydroxylase; Recovery of function; Sensory neglect; Skilled reaching

1. Introduction

The hemiparkinsonian rat is a model typically used to assess experimental treatments for Parkinson's disease (PD). Normalization of dopamine (DA) function is usually determined in this model by decreases in apomorphine-induced rotational behavior [1], which is often used as a sole determinant of recovery [2–19]. However, one measure of recovery may not be optimal, as several eminent researchers, including Björklund, Dunnett, Whishaw, and their colleagues, have reported a dissociation between recovery assessed by drug-induced rotation and neurologically based behaviors [20–22]. Despite this evidence, we were concerned that the use of a single behavioral

* Corresponding author. Tel.: +1 480 965 2265; fax: 1 480 965 8544.
E-mail address: eddie.c@asu.edu (E. Castañeda).

0031-9384/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2005.01.019

measure perseveres in assessment of the efficacy of potential therapies for neurological disorders, and this practice may mislead our advancement towards effective treatments. We applied a more comprehensive behavioral analysis to clarify the ameliorative effects of transgenic astrocytes in the hemiparkinsonian rat. The present investigation is significant because a large amount of research involving transgenic astrocytes limits behavioral assessment of recovery to only apomorphine-induced rotational behavior.

The use of astrocytes as vectors for delivery of potentially therapeutic genes has been a popular line of investigation. Various approaches have expressed the TH gene in vitro in rat [2,9,17] or human [4,15] astrocytes for later transplantation into the host [2,4,6,9,15,17]. There are a number of reasons to believe that astrocytes could be successful vectors for gene therapy. Astrocytes are endogenous to the striatum and thus should optimize survival when transplanted back into this environment. For this reason, astrocytes might serve as autologous transplants. In addition, transfected astrocytes show little proliferation, allowing transgenes to persist episomally. Finally, astrocytes demonstrate a propensity to accept transgenes via retroviral [2,9,17] and adenoviral vectors [6,7], as well as direct injection of a DNA-liposomal complex [13]. Unfortunately, none of the above studies utilizing TH-expressing astrocytes have incorporated a neurological approach to assess behavioral recovery; they are limited by their sole reliance on reductions in drug-induced rotational behavior as an index of recovery [2,4,6,7,13,15–17]. A comprehensive behavioral approach may better assess neurological recovery.

For the present study, we selected intrastriatal grafts of astrocytes expressing recombinant tyrosine hydroxylase (TH), the rate-limiting catecholamine biosynthetic enzyme. We were particularly interested in determining how recovery would be assessed by behavioral measures in addition to the drug-based behavioral assay. To accomplish this, we chose to compare apomorphine-induced rotation to somatosensory neglect and skilled reaching, two behavioral deficits that have been characterized in the hemiparkinsonian rat and are analogous to human PD [23–26], and thus are more likely to be predictive of recovery in human PD patients [27]. Notably, these sensorimotor and motor behaviors are dependent upon pre-synaptic events involving exocytosis, whereas apomorphine-induced rotational behavior relies solely upon activation of supersensitive post-synaptic receptors by a direct agonist. Thus, different neuronal mechanisms are involved in the expression of these behaviors. We hypothesized that TH-expressing astrocytes would promote recovery in the hemiparkinsonian rat on apomorphine-induced rotation, but not sensorimotor and motor behaviors, likely due to a failure by transgenic astrocytes to restore pre-synaptic factors of DA neurotransmission.

2. Materials and methods

2.1. Subjects

Male Fischer 344 rats weighing 250–300 g were group-housed and handled for at least two weeks prior to behavioral testing and surgery. Animals were maintained at 90% free-feeding weight during training and testing on the reaching apparatus (Montoya staircase) and were food-deprived 24 h prior to surgery. At all other times, food and water were available ad libitum. Animals were maintained on a reverse 12:12 h light–dark cycle (lights on at 1800 hours) and were tested during the dark portion. The protocol for this experiment was approved by the Arizona State University Institutional Animal Care and Use Committee in conformance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996).

2.2. 6-OHDA lesion

Lesions were created as described previously [28,29]. The hemisphere contralateral to the forelimb dominant for skilled reaching (see Fig. 4A) was selected a priori to receive 6-OHDA [30]. Animals were anesthetized with sodium pentobarbital (Sigma, 50 mg/kg, i.p.), and supplements of chloral hydrate (Sigma, 80 mg/kg, i.p.) were administered when necessary. Atropine (Henry Schein, 5 mg/kg, s.c.) was administered prophylactically. To protect norepinephrine cells, animals were pretreated with desipramine HCl (RBI, 15 mg/kg, i.p.), a noradrenergic reuptake blocker, 30 min prior to 6-OHDA infusion. Using standard stereotaxic procedures, a 28 ga injector needle (Plastics One), connected to a 1 ml Hamilton gastight syringe by polyethylene tubing (PE 20/Clay Adams, Intramedic), was lowered to the level of the substantia nigra (SN; with bregma and lambda horizontal; AP −5.8 mm, ML +2.5 mm, DV −8.0 mm [31]. One min later, 6-OHDA HBr (RBI), dissolved in deoxygenated 0.9% saline containing 10% ascorbic acid, was infused (10 μg/2.5 μl at 0.2 μl/min over 12.5 min) via a syringe pump (Harvard Apparatus). The needle remained lowered for 1 min after the infusion to allow for diffusion from the tip. Immediately following infusion of 6-OHDA, animals were returned to their home cages for two weeks to allow a neurotoxin-induced state of chronic DA depletion to develop, in accordance with the traditional Ungerstedt paradigm [32–34].

2.3. In vitro procedures

2.3.1. Astrocyte cultures

Astrocytes were isolated from the caudate nucleus of 5–6-week-old rats. The tissue was dissociated with collagenase (0.4 mg/ml) and DNase (10 U/ml) for 5 min. Cells were then plated in astrocyte media (Clonetics) in a 75 cm^2 tissue culture flask (Corning). Astrocytes were maintained in a humidified incubator at 37 °C with 5% CO_2. Cultures were

passaged 1:2 and transfected by electroporation at passage three or four.

2.3.2. Plasmid construction

Two expression vectors based on the pBkCMV plasmids (Stratagene) were constructed. The vector pBkCMV/TH was created by inserting the gene encoding a mutated form of the human TH cDNA into the Xba I site of pBkCMV. The 2.2 kb TH cDNA (a gift from Dr. Menek Goldstein) has a substitution of Leu 40 with Ser, resulting in over a 10-fold increase in TH activity. The high activity of mutated TH is phosphorylation-independent and has been shown to contribute to the production of L-DOPA [35,36]. To construct pBkCMV/β-gal, the Lac Z gene encoding β-gal was excised from pCH110 (Pharmacia) with Bam HI and Hind III and subcloned into the Hind III/Sma I sites of pBkCMV after end-filling of the Bam HI site.

2.3.3. Transfection

The pCMV/β-gal and pCMV/TH were transfected into astrocyte cultures using electroporation as described in Stachowiak et al. [37]. Briefly, cells were trypsinized and washed in astrocyte media and 1X Hebes buffer. Cells were then placed into cuvettes (0.4 cm gap), 150 μg of pCMV/TH or pCMV/β-gal was added, and cells were electroporated at 170 mV. Electroporated cells were allowed to recover for 48 h in culture flasks before in vitro staining or transplantation.

2.3.4. Validation of transfection efficiency

Cells were plated on glass well slides (n=4) immediately after electroporation, and staining for TH or β-gal was performed 48 h later. For TH immunostaining, wells were washed with phosphate buffered saline (PBS), pH 7.4, and then fixed with PBS containing 2.5% paraformaldehyde for 15 min. Wells were washed with PBS, pH 8.4, and treated with 1% Triton X-100 for 15 min, then 3% bovine serum albumin (BSA) in PBS at 37 °C for 30 min. Cells were then incubated overnight at 4 °C with polyclonal rabbit anti-TH antibodies (Chemicon, 1:500) in 3% BSA followed by anti-rabbit IgG conjugated with alkaline phosphatase (Jackson, 1:500). The immune complexes were stained using avidin–biotin-peroxidase (Elite ABC rabbit IgG Kit, Vector) and 3,3-diaminobenzidine (DAB, Sigma), according to manufacturer's instructions. Transfection efficiency was calculated from three wells while the fourth well, lacking primary antibody, served as a control. To assess β-gal activity, the wells were washed with PBS, pH 7.4, and fixed in a formaldehyde solution [2% formaldehyde (37%), 0.2% gluteraldehyde, 0.1% NaDOC, and 0.02% NP-40 in PBS] for 15 min. Wells were washed and then incubated in the X-gal solution [2 mM $MgCl_2$, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, and 1 mg/ml of 5′-bromo-4-chloro-3-indolyl-b-D-galactoside (X-gal; Eastman Kodak Co.)] in the dark at 4 °C for up to 12 h. Transfection efficiency was calculated from three wells while the fourth well, containing non-transfected astrocytes, served as a control.

2.4. Transplantation of astrocytes

2.4.1. Cell preparation and labeling

Forty-eight hours after electroporation, when astrocytes express transgenes in vitro (see Fig. 2), astrocytes were detached by trypsinization, centrifuged at 1000 rpm for 5 min, then washed with PBS and resuspended in PBS at a density of 1×10^5 cells/μl. At this point, to further validate placement and diffusion of transgenic cells, both β-gal and TH-expressing astrocyte grafts were labeled with the carbocyanine dye diI [1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; diI-C18-(3), Molecular Probes] at a concentration of 0.1%.

2.4.2. Transplantation

Immediately after completing tests to affirm the presence of behavioral deficits (post-lesion days 14–16), on post-lesion day 17, animals received control β-gal or TH-expressing astrocytes based on pseudo-random assignment, counterbalanced for turning rate in the apomorphine-induced rotation test. Astrocytes were infused into the striatum ipsilateral to the lesion (experimental, n=8; control, n=7) using the general stereotaxic surgery procedures for the 6-OHDA lesions (see above) except that four infusions were made (3 μl/site at 0.5 μl/min to deliver 3×10^5 cells/site). Coordinates for the four sites with bregma and lambda horizontal were: 1) AP +1.8, ML +2.5, DV −4.5; 2) AP +1.2, ML +2.5, DV −6.0; 3) AP +0.7, ML +4.0, DV −6.4; 4) AP +0.2, ML +3.5, DV −7.0. Thus infusions were made in a rostral–caudal direction, with rostral infusions targeting the corpus of the striatum, while caudal infusions targeted the ventrolateral striatum, which has been shown to be important for forelimb use during food handling in rats [38]. The cannula was left in place for 5 min following each infusion. Animals were allowed two weeks post-operative recovery.

2.5. Behavioral tests

All animals (experimental, n=8; control, n=7) were tested for somatosensory neglect, skilled reaching, and apomorphine-induced rotation by an investigator blind to the experimental treatment. Somatosensory neglect and skilled reaching were assessed over three consecutive days at three time points: prior to any surgery (days 1–3 pre-lesion), two weeks after unilateral DA depletion (days 14–16 post-lesion), and two weeks after transplantation (days 31–33 post-lesion). Apomorphine-induced rotation was assessed on days 16 and 33 post-lesion, immediately after the other two tests.

2.5.1. Somatosensory neglect

Small adhesive stickers (13 mm diameter) were placed concurrently on the ulnar surface of both forelimbs of the rats, as adapted from Schallert et al. [39]. Latencies in seconds to contact and to remove the stickers were recorded separately for the left and right forelimbs [26].

2.5.2. Skilled reaching

The test chamber was adopted from Montoya et al. [40]. It consisted of a small Plexiglas box (285 mm long, 60 mm wide, 90 mm high) containing a platform (190 mm long, 27 mm wide). On each side were staircases (84 mm long with 6 stairs, each 14 mm wide, 6 mm high) baited with one sucrose pellet on each stair (Noyes, 94 mg). To obtain food pellets, the rats had to climb onto the platform and reach down, using the left and right forelimbs for the left and right staircases, respectively. During training, animals were acclimated to the test chamber without pellets for two days. Next, animals were food restricted to 90% of body weight and were placed in the test chamber with the staircase baited with pellets each day until they consistently reached for at least 20 pellets per day. Once this a priori criterion was achieved, testing was conducted. Daily sessions lasted 15 min, where at 5 min intervals the staircase was removed, the number of pellets collected was calculated, and the staircase was rebaited.

2.5.3. Apomorphine-induced rotation

Animals were tested for denervation supersensitivity by administration of the direct agonist apomorphine. Rats were placed in rotometers (Med Associates), which consisted of round translucent bowls with a floor diameter of 20 cm and a lip diameter of 21 cm. A 2.5 cm wide velcro harness was placed around the torso and connected to a wire rod attached to an infrared/photo diode counter 71 cm above the test floor. Rats were allowed to habituate for 15 min before receiving apomorphine HCl (RBI, 0.25 mg/kg, s.c.), and quarter turns in both directions were counted automatically for 50 min. In a pilot study, we established that DA depletions of at least 85% are predicted by two criteria collectively: 1) a dominance of at least 70% in apomorphine-evoked contraversive turning as shown in Fig. 1, and 2) at least 8 quarter turns/min throughout the 50 min test session. Therefore, only animals that displayed at least 70% dominance in contralateral turning and 8 quarter turns/min received astrocyte transplants in the current experiment.

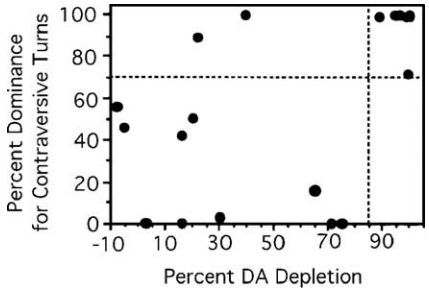

Fig. 1. In a pilot study, 21 rats received unilateral intranigral infusions of 6-OHDA. Two weeks later, the number of full (360°) turns displayed for 2 h following apomorphine (0.25 mg/kg, s.c.) was counted from video recordings. Animals with striatal DA depletions greater than 85% (n=9) relative to the intact striatum consistently displayed a minimum of 70% dominance in contraversive turning. A significant factor that distinguished rats with >85% DA depletions was their turning rate of at least 8 quarter turns/min during the 50 min test session (data not shown). DA tissue levels were determined by high pressure liquid chromatography coupled with electrochemical detection, as described previously [28].

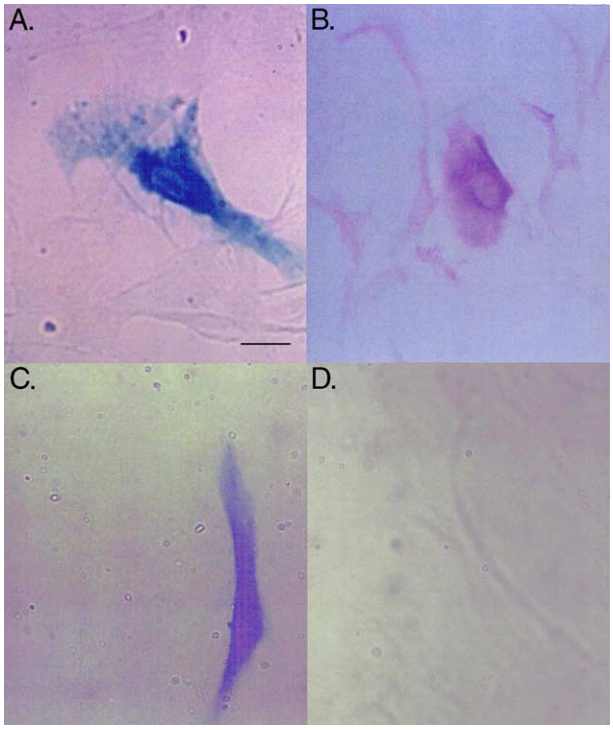

Fig. 2. Photomicrographs showing examples of in vitro staining of astrocytes. (A) Enzymatic staining for β-gal activity 48 h after transfection with pCMV/β-gal. (B) Immunostaining for TH using alkaline phosphatase 48 h after transfection with pCMV/TH. (C) Enzymatic staining for β-gal activity 14 days after transfection with pCMV/β-gal. (D) Enzymatic staining for β-gal activity in non-transfected astrocytes. Non-transfected astrocytes did not show transgene expression. Scale bar=35 μm.

2.6. Immunohistochemistry

Immediately after the last apomorphine-induced rotational test (day 33 post-lesion), animals were sacrificed under deep pentobarbital anesthesia by cardiac perfusion of ice-cold 0.9% saline in PBS followed by ice-cold 4% paraformaldehyde in PBS, pH 7.4. Brains were removed and post-fixed for 1 h in the paraformaldehyde solution, then placed in PBS with 30% sucrose. Brains were sectioned (30 μm) through the caudate nucleus using a cryostat, and each section was mounted on glass slides coated with 1% gelatin, then frozen (−20 °C) until staining.

Sections from experimental brains were immunostained for TH and those from control brains were enzymatically stained for β-gal activity. Insufficient amounts of incubating media prevented reliable visualization of TH and β-gal proteins in some of the brain sections. This procedural error in the staining process rendered 9 brains unusable. Fortunately, three brains from each group were successfully prepared after resolving this technical problem. TH sections were washed with PBS, pH 7.4, and treated with 1% Triton X-100 for 15 min, 3% hydrogen peroxide solution for 15 min, and a blocking solution of normal goat serum

(polyclonal) in PBS for 30 min at 37 °C. Sections were then incubated with polyclonal rabbit anti-TH (Chemicon, 1:500) in blocking solution overnight at 4 °C, followed by biotinylated goat anti-rabbit TH, avidin–biotin-peroxidase, and DAB. β-gal sections were washed with PBS, pH 7.4, and fixed in a formaldehyde solution [2% formaldehyde (37%), 0.2% gluteraldehyde, 0.1% NaDOC, and 0.02% NP-40 in PBS] for 1 h at 4 °C. They were then washed and treated with staining solution [0.87% NaCl, 0.1 M Na_2HPO_4, 2 mM $MgCl_2$, 0.01% NaDOC, 0.02% NP-40, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, and 1 mg/ml X-gal] in the dark at 31 °C for up to 12 h, as described in Tabbaa et al. [41]. For each brain, the section with the most staining, presumably reflecting the locus of infusion, was selected for analysis.

2.7. Statistical analyses

2.7.1. Somatosensory neglect

Latencies to contact and to remove stickers placed on the contralateral and ipsilateral forelimbs were assessed. To assess deficits following a unilateral DA depletion, an ANOVA was performed on a 2×2 within subject design, comparing limb (contralateral and ipsilateral) and time (pre-surgery and post-lesion). To determine recovery following transplantation, an ANOVA was performed on a 2×2 mixed design comparing group (TH and β-gal) and time (post-lesion and post-transplant). A priori planned comparisons were analyzed with Fisher's LSD.

2.7.2. Skilled reaching

The number of pellets collected by the contralateral and ipsilateral forelimbs was quantified. Statistical analyses were conducted as has been reported previously [21,40] and as described for somatosensory neglect.

2.7.3. Apomorphine-induced rotation

The number of contralateral quarter turns was quantified, and a square root transformation was performed on the data to achieve homogeneity of variance among groups. To determine recovery following transplantation, statistical analyses were conducted as described for somatosensory neglect.

3. Results

3.1. In vitro astrocyte transfection

Astrocyte cultures stained with antibodies to glial fibrillary acidic protein showed that approximately 95% of the cells were of astrocytic lineage (data not shown). Forty-eight hours after electroporation with pCMV/β-gal plasmids or pCMV/TH, astrocytes were stained for β-gal or TH, and typical examples of a β-gal-and TH-positive cell are shown in Figs. 2A and B, respectively. When these cells were counted and compared to the count of non-stained cells, approximately 15% of the cells transfected with pCMV/β-gal expressed active recombinant β-gal enzyme, while 20% of astrocytes transfected with pCMV/TH expressed transfected TH. Previous research using electroporation to transfect astrocytes in vitro reported a transfection efficiency of 10% [42].

In a second experiment, astrocytes were transiently transfected with pCMV/β-gal and densely plated to reduce proliferation, then β-gal activity was assessed 14 days later. Typical examples of cells that showed positive or negative staining for β-gal are shown in Figs 2C and D, respectively. Staining revealed 5% of the cells were positive for β-gal activity at two weeks, while non-transfected controls showed no staining.

3.2. Behavioral assessment

3.2.1. Somatosensory neglect

Latencies to contact the adhesive stickers placed on the forepaws were measured prior to surgery, after lesion, and after transplantation, as shown in Fig. 3. To assess somatosensory neglect following the unilateral 6-OHDA lesion, a 2×2 within subject ANOVA comparing time (pre-surgery and post-lesion) and limb (contralateral and ipsilateral) was conducted, showing significant main effects of time, $F(1,14)=15.81$, $p<0.01$, and limb, $F(1,14)=30.67$,

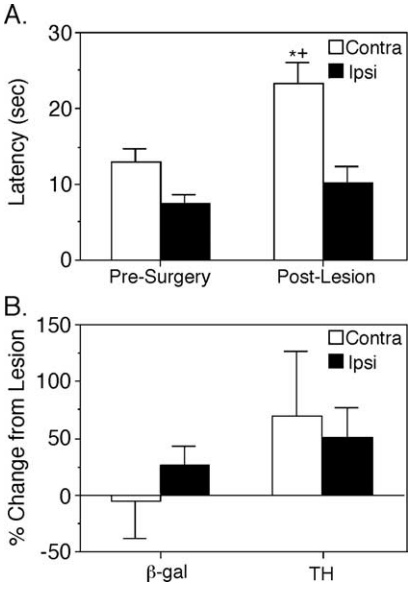

Fig. 3. (A) Mean time to make contact (s) for the contralateral (Contra, empty bars) and ipsilateral (Ipsi, solid bars) limbs before and after lesion (n=15). There were no statistically significant group differences (β-gal versus TH) during pre-surgery and post-lesion time points (t-test, $p>0.05$), so data are collapsed across groups in panel A. Post-lesion, animals showed an increased latency to use the contralateral limb. Error bars represent SEM. *, $p<0.05$ compared to post-lesion ipsilateral limb; +, $p<0.05$ compared to pre-surgery contralateral limb. (B) The change in performance from lesion to transplantation for contralateral and ipsilateral limbs. No significant differences in limb performance between β-gal (n=7) and TH (n=8) groups were found.

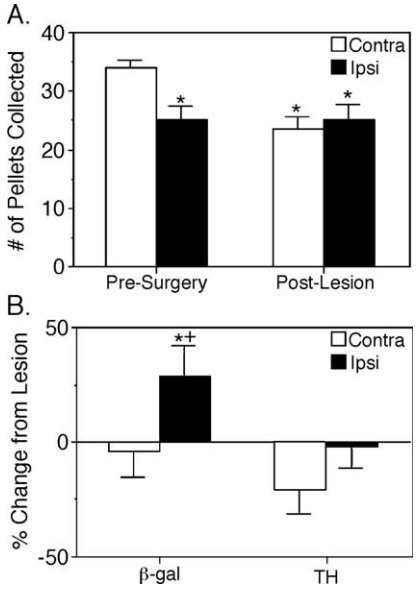

Fig. 4. (A) The mean number of pellets collected for the contralateral (Contra, empty bars) and ipsilateral (Ipsi, solid bars) limbs before and after a unilateral 6-OHDA-induced lesion (n=15). There were no statistically significant group differences (β-gal versus TH) during pre-surgery and post-lesion time points (t-test, p>0.05), so data are collapsed across groups in panel A. To be conservative, the hemisphere contralateral to the dominant limb was selected for 6-OHDA infusion. Post-lesion, animals showed a significant decrease in contralateral limb use. Error bars represent SEM. *, p<0.05 compared to pre-surgery contralateral limb. (B) The percent change in performance from lesion to transplantation for the contralateral and ipsilateral limbs. Animals with control β-gal grafts (n=7) increased the use of the ipsilateral limb (*, p<0.05), while animals with TH grafts (n=8) showed no significant change. Error bars represent SEM. +, p<0.05 compared to the contralateral limb of the β-gal group and both the contralateral and ipsilateral limbs of the TH group.

p<0.01, in latency to make contact. Planned comparisons revealed an increase in the time to contact the contralateral paw following the 6-OHDA lesion relative to baseline for all animals (p<0.01) and a significant difference between the ipsilateral and contralateral limbs following a 6-OHDA lesion (p<0.01), as shown in Fig. 3A.

To assess recovery in somatosensory neglect after transplantation, a 2×2 mixed ANOVA comparing limb (contralateral and ipsilateral) and group (TH and β-gal) was conducted. There were no changes in the latency to make contact in either the TH or β-gal groups, as shown in Fig. 3B. The measure of latency to remove the sticker is not reported because rats quickly habituated and subsequently ignored the stimulus, in part because they may have partially removed the sticker at first contact thus producing a confound.

3.2.2. Skilled reaching

To assess deficits in collecting pellets following the unilateral 6-OHDA lesion, a 2×2 within subject ANOVA comparing time (pre-surgery and post-lesion) and limb (contralateral and ipsilateral) was conducted, revealing a significant main effect of time, F(1,14)=14.11, p<0.01, and a significant interaction of time and limb, F(1,14)=14.62, p<0.01. Planned comparisons revealed a significant decrease in the number of pellets collected with the contralateral limb following the 6-OHDA-induced lesion compared to pre-surgery scores (p<0.01), as shown in Fig. 4A. There was no change in the number of pellets collected with the ipsilateral limb after the lesion.

To assess recovery in collecting pellets after transplantation, a 2×2 mixed ANOVA comparing limb (contralateral and ipsilateral) and group (TH and β-gal) was conducted, revealing a significant main effect of limb, F(1,14)=16.47, p<0.01. Planned comparisons revealed a significant increase in the number of pellets collected by the ipsilateral limb in the β-gal group compared to their post-lesion scores (p<0.01) as well as compared to the ipsilateral and contralateral limbs of the TH group (p's<0.01), as shown in Fig. 4B. No change in the number of pellets collected was observed with the contralateral limb of control animals, and no significant changes were seen in the TH group.

3.2.3. Apomorphine-induced rotation

Animals were included in the study if they met the two a priori criteria for lesion size: 1) a minimum of 70% dominance in contraversive turning, and 2) at least 8 quarter turns/min during the 50 min test. Animals were placed in groups counterbalanced for turning rate and received transplants of TH or β-gal-transfected astrocytic cultures. On day 16 post-transplant, apomorphine-induced rotation was re-assessed. A 2×2 mixed ANOVA comparing direction (contraversive and ipsiversive) and group (TH and β-gal) was conducted, revealing a significant main effect of time, F(1,14)=6.49, p<0.05, and a significant interaction between group (TH or β-gal astrocytes) and time, F(1,14)=5.61, p<0.05. Planned comparisons revealed that turning in TH-treated animals was 34% lower than their pre-graft values

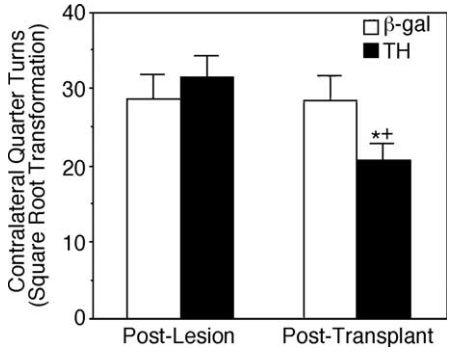

Fig. 5. The mean number of contralateral turns executed in the 50 min following apomorphine administration (0.25 mg/kg, s.c.) 16 days after a unilateral 6-OHDA-induced lesion (Post-Lesion) and 16 days after transplantation (Post-Transplant). β-gal controls (empty bars, n=7) and TH animals (solid bars, n=8) demonstrated similar apomorphine-induced rotation 16 days post-lesion. However, 16 days post-transplant, TH animals displayed significantly reduced apomorphine-induced rotation, while β-gal controls showed no change from post-lesion rotation. Error bars are SEM. *, p<0.01 compared to post-lesion and post-transplantation β-gal and +, p<0.05 compared to post-lesion TH.

(p<0.01) and also significantly reduced compared to β-gal control performance measured at post-lesion and post-transplantation time points (p's<0.05), as shown in Fig. 5.

3.3. In vivo transplant evaluation

In vivo staining results are shown in Fig. 6. Visualization of diI-labeled transplants by fluorescence microscopy revealed that injection coordinates targeted the striatum, as shown in Fig. 6C. Our TH immunostaining procedure was validated by immunostaining of an intact SN, shown in Fig. 6D. Due to technical difficulties, only three brains from each group were successfully processed. For the three brains from animals that received transplants of astrocytes expressing TH, immunohistochemical staining for TH in striata revealed positive staining 14 days post-transplantation, and one representative brain is shown in Fig. 6E. For the three brains from animals that received β-gal-transfected astrocytes, no positive TH staining was observed, and one representative brain is shown in Fig. 6F.

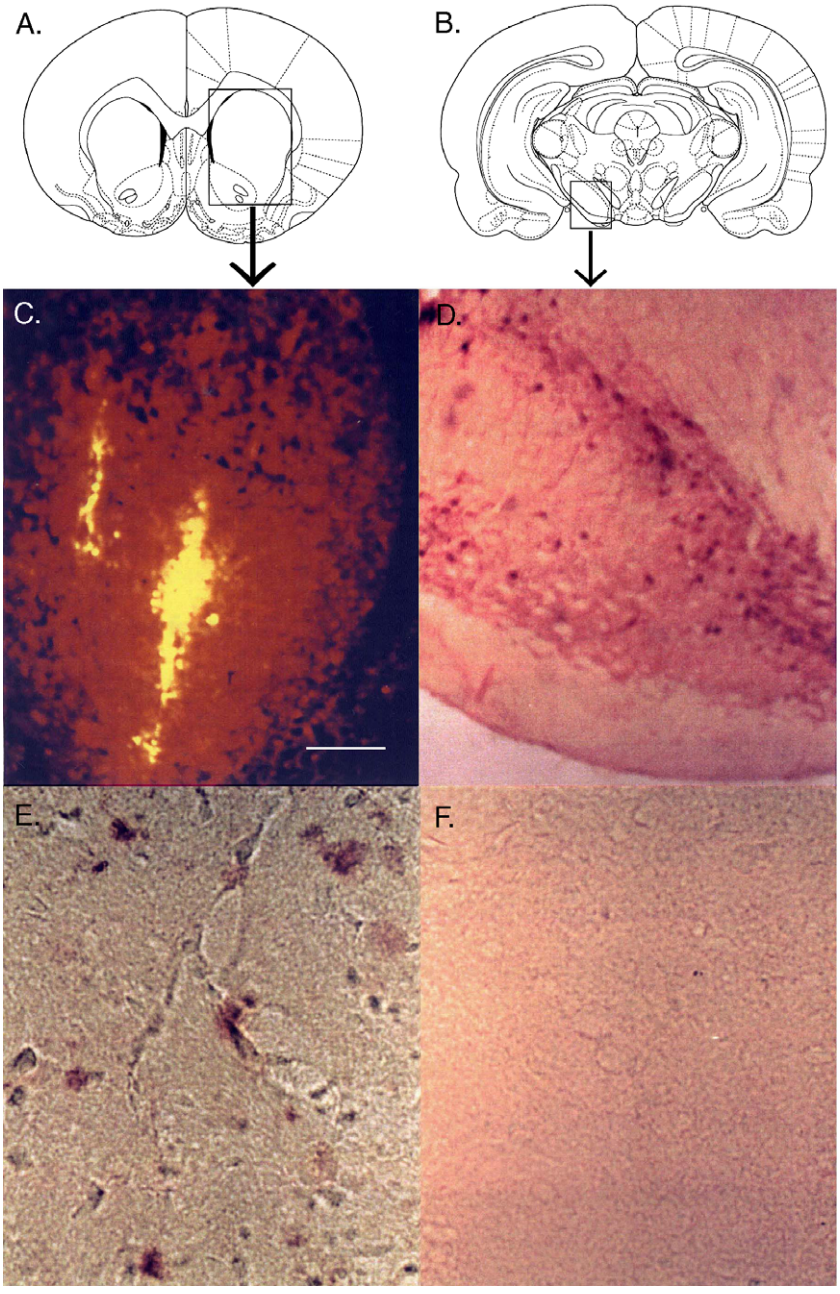

Fig. 6. Photomicrographs of in vivo staining. (A) Coronal section at 1.6 mm Bregma, with box illustrating the area of the striatum magnified in Panel C. (B) Coronal section at −5.3 mm Bregma, with box illustrating the area of the SN magnified in Panel D. (C) Visualization of DiI in the striatum 17 days after transplantation of DiI-labeled astrocytes. (D) Immunostaining for TH in the SN of an intact animal. (E) Immunostaining for TH in the denervated striatum 17 days after transplantation of TH-transfected astrocytes. (F) Immunostaining for TH in the denervated striatum 17 days after transplantation of β-gal-transfected astrocytes. No TH positive cells were detected in animals with β-gal grafts. Scale bars=1 mm (C and D) or 250 μm (E and F).

Table 1
TH immunostaining and behavioral recovery following transplantation

Subject	TH Staining	Turning	SS Neglect	Reaching
TH1	***	+++	0	0
TH2	*	++	0	0
TH3	**	++	++	+
β-gal1	0	0	0	0
β-gal2	0	0	0	0
β-gal3	0	0	0	0

Animals that showed positive TH immunostaining and reduction in apomorphine-induced rotation did not consistently show improvement from contralateral SS neglect or contralateral reaching deficits. * Represents positive staining for TH, with the number of asterisks indicating staining intensity where * is least and *** is most intense. + represents the extent of improvement following transplantation, where + is mild, ++ is moderate, and +++ is greatest improvement. 0 indicates no behavioral improvement. SS, somatosensory.

Table 1 summarizes the behavioral data from these six animals for which in vivo staining was successful. Rats receiving TH-expressing astrocytes showed reduced apomorphine-induced turning, but displayed no consistent recovery in the measures of somatosensory neglect or skilled reaching. Control rats grafted with β-gal-expressing astrocytes showed no TH-expressing cells in striatum nor improvement in any of the tasks.

4. Discussion

The hemiparkinsonian rat model has been a historically valuable tool for reaping extensive insights about the underlying neurophysiology of Parkinson's disease. Furthermore, the long-standing acceptance of drug-induced rotation as a behavioral assay makes this easy-to-use, automated procedure an attractive choice to predict recovery of behavioral function following experimental treatments. However, with transgenic astrocytes as an experimental intervention, we report a dissociation between recovery assessed by apomorphine-induced rotational behavior and neurologically-relevant, integrative behaviors. In the present study, rats that received transplants of TH-transfected astrocytes showed a 34% decrease in rotational behavior, but no consistent recovery of somatosensory neglect or skilled reaching at a time when the grafted astrocytes were expressing TH. The current findings have ubiquitous application for Parkinson's disease research, emphasizing the importance of a thorough behavioral assessment in these types of multidisciplinary projects, regardless of the experimental treatment being evaluated.

The advantages of using transgenic astrocytes include enhanced viability in the striatum due to their endogenous nature, decreased proliferation that may optimize transgene expression, and a propensity to accept transgenes. However, some issues that may have precluded better recovery in the present study include whether an optimal density of cells was infused, the physical damage potentially created by the infusion of the grafts [43], whether other subregions of striatum should have received grafts based on its somatotopic organization [38,44–46], the extent of graft diffusion, co-factor availability, a more clearly defined time-course and extent of TH expression, and whether DA itself was actually replenished.

While additional research is required to resolve these questions and to further clarify the potential of astrocytes as vectors to deliver therapeutic genes, the present behavioral findings remain critically important. We report a dissociation between behavioral recovery assessed by apomorphine-induced rotation and by sensorimotor and motor behaviors. These results are consistent with other studies that did not find an improvement in integrative behaviors, like reaching, following transplantation of fetal tissue into the hemiparkinson rat [20–22,47]. This dissociation may be explained by different underlying substrates. Specifically, apomorphine-induced rotational behavior reflects asymmetric post-synaptic receptor supersensitivity. Receptor supersensitivity in the denervated hemisphere is produced by low tonic extracellular DA levels [48,49]. The TH-expressing astrocytes likely alleviated this asymmetry by: 1) elevating tonic extracellular DA levels, and 2) in the absence of an efficient reuptake system, allowing astrocyte-derived DA to diffuse over larger distances on a cellular scale (i.e., volume transmission). In contrast, sensorimotor integration requires pre-synaptic mechanisms that produce phasic surges of DA release [50–52]. Therefore, an intact circuitry is necessary for expression of these latter behaviors. In this light, there continues to be a prevalence of studies that use drug-induced rotation only [2–19], a behavioral test that literally assays only post-synaptic receptor function. Our present data renew the argument [20–22] for a comprehensive analysis to assess recovery of function based upon behaviors that are dependent on pre-synaptic mechanisms of DA neurotransmission (e.g., Refs. [53–55]). We suggest that astrocytes were unable to integrate into the basal ganglia circuitry sufficiently to restore phasic DA release. The unmistakable inference is that restoration of pre-synaptic mechanisms that mediate the phasic, or pulsatile, nature of neurotransmission may be most fruitful for producing significant recovery [56–61].

Other questions do remain in the present study. Rats experienced extensive training and testing in the behavioral protocols that could potentially confound the present results. Exercise is known to affect circuit function [62,63], and this could render the host unable to benefit from the transplants due to a masking effect from practice. Past research has shown that pre- and post-lesion exercise mitigates both 6-OHDA-induced decreases in neurotransmitter levels and functional impairment [64,65]. As a result, a dissociation in recovery between sensorimotor and drug-induced behaviors might ensue. Nonetheless, this remains to be more fully explored by minimizing training in future experiments.

Another question concerns the skilled reaching task in which control animals successfully retrieved a greater number of pellets with their ipsilateral limbs post-transplant.

Past research has shown such behavioral compensation after unilateral 6-OHDA, namely that rats show a preference to use the ipsilateral limb [24,65,66]. In contrast, the group receiving TH-expressing astrocytes did not develop this pattern of reaching. One might speculate that the TH-expressing astrocytes produced recovery in the contralateral limb, abolishing the need for behavioral compensation by the ipsilateral limb. It is too early to be confident about this interpretation. It is just as likely that the control group was reaching more but with less success, possibly due to the additional damage produced by the transplant procedure. An index of efficiency to retrieve pellets (number of pellets collected as a function of the number of attempts) could have resolved this issue.

Finally, another question is posed by the possibility for additional damage produced by the transplant procedure. Damage from the infusion volume that destroys post-synaptic receptors on neurons intrinsic to the striatum would decrease apomorphine-induced rotation, as well as prevent recovery in motor and sensorimotor abilities. If this is true, then there is no dissociation, and our results reflect post-synaptic damage. Given the limited number of infusions (4 injections, 3 μl/site) into the relatively large striatum, we maintain that the explanation of dissociation is more viable. Indeed, the possibility for volume transmission produced by the transplants means that a healthy area beyond the locus of damage could be affected. However, only further investigation will resolve this issue, for example by minimizing infusion volumes using optimized cell densities.

In summary, transplants of astrocytes expressing TH were demonstrated to reduce apomorphine-induced rotation but did not alleviate deficits in somatosensory neglect or skilled reaching despite survival of grafts and expression of TH. We surmise that transfected astrocytes functioned as tonic pumps, with no impact upon pre-synaptic mechanisms. The overarching significance of these results is in the message that changes in drug-induced rotation alone are not sufficient to demonstrate recovery of function by any putatively therapeutic approach. A thorough assessment across a wide profile of abilities such as sensory, motor, self-regulatory, and motivated behaviors would provide stronger evidence for the potential of experimental treatments to alleviate PD and other neurodegenerative disorders [66]. Moreover, such a "neuropsychological" approach in tandem with apomorphine-induced rotation is a powerful strategy for dissecting molecular changes, such as pre-synaptic versus post-synaptic function.

Acknowledgements

This research was supported by NINDS/NIH to MKS, Arizona Disease Control Research Commission Contract #9834, the Office of the Vice Provost for Research at ASU, and the ASU Hispanic Research Center. We thank Victor Luevano for technical assistance and Dr. Cheryl Conrad as well as the anonymous reviewers for constructive feedback that significantly improved the quality of this manuscript.

References

[1] Schwarting RK, Huston JP. The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. Prog Neurobiol 1996;50:275–331.
[2] Cortez N, Trejo F, Vergara P, Segovia J. Primary astrocytes retrovirally transduced with a tyrosine hydroxylase transgene driven by a glial-specific promoter elicit behavioral recovery in experimental parkinsonism. J Neurosci Res 2000;59:39–46.
[3] Fisher LJ, Jinnah HA, Kale LC, Higgins GA, Gage FH. Survival and function of intrastriatally grafted primary fibroblasts genetically modified to produce L-dopa. Neuron 1991;6:371–80.
[4] Fitoussi N, Sotnik-Barkai I, Tornatore C, Herzberg U, Yadid G. Dopamine turnover and metabolism in the striatum of parkinsonian rats grafted with genetically-modified human astrocytes. Neuroscience 1998;85:405–13.
[5] Harvey BK, Mark A, Chou J, Chen GJ, Hoffer BJ, Wang Y. Neurotrophic effects of bone morphogenetic protein-7 in a rat model of Parkinson's disease. Brain Res 2004;1022:88–95.
[6] Hida H, Hashimoto M, Fujimoto I, Nakajima K, Shimano Y, Nagatsu T, et al. Dopa-producing astrocytes generated by adenoviral transduction of human tyrosine hydroxylase gene: in vitro study and transplantation to hemiparkinsonian model rats. Neurosci Res 1999;35:101–12.
[7] Horellou P, Vigne E, Castel MN, Barneoud P, Colin P, Perricaudet M, et al. Direct intracerebral gene transfer of an adenoviral vector expressing tyrosine hydroxylase in a rat model of Parkinson's disease. Neuroreport 1994;6:49–53.
[8] Kemmerer ES, Desmond TJ, Albin RL, Kilbourn MR, Frey KA. Treatment effects on nigrostriatal projection integrity in partial 6-OHDA lesions: comparison of L-DOPA and pramipexole. Exp Neurol 2003;183:81–6.
[9] Lundberg C, Horellou P, Mallet J, Björklund A. Generation of DOPA-producing astrocytes by retroviral transduction of the human tyrosine hydroxylase gene: in vitro characterization and in vivo effects in the rat Parkinson model. Exp Neurol 1996;139:39–53.
[10] Maesawa S, Kaneoke Y, Kajita Y, Usui N, Misawa N, Nakayama A, et al. Long-term stimulation of the subthalamic nucleus in hemiparkinsonian rats: neuroprotection of dopaminergic neurons. J Neurosurg 2004;100:679–87.
[11] Olds ME, Jacques DB, Kopyov O. Behavioral and subthalamic effects of combining a fetal ventral mesencephalic transplant in striatum with an electrolytic lesion of the entopeduncular nucleus in the rat with a unilateral 6-OHDA lesion of substantia nigra. Synapse 2003;48:90–9.
[12] Olds ME, Jacques DB, Kopyov O. Behavioral/neurophysiological investigation of effects of combining a quinolinic acid entopeduncular lesion with a fetal mesencephalic tissue transplant in striatum of the 6-OHDA hemilesioned rat. Synapse 2003;49:1–11.
[13] Segovia J, Verara P, Brenner M. Astrocyte-specific expression of tyrosine hydroxylase after intracerebral gene transfer induces behavioral recovery in experimental parkinsonism. Gene Ther 1998; 5:1650–5.
[14] Shen Y, Muramatsu SI, Ikeguchi K, Fujimoto KI, Fan DS, Ogawa M, et al. Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease. Hum Gene Ther 2000;11:1509–19.
[15] Tornatore C, Baker-Cairns B, Yadid G, Hamilton R, Meyers K, Atwood W, et al. Expression of tyrosine hydroxylase in an immortalized human fetal astrocyte cell line: in vitro characterization

and engraftment into the rodent striatum. Cell Transplant 1996; 5:145–63.
[16] Wang Y, Chang CF, Morales M, Chiang YH, Harvey BK, Su TP, et al. Diadenosine tetraphosphate protects against injuries induced by ischemia and 6-hydroxydopamine in rat brain. J Neurosci 2003;23: 7958–65.
[17] Wang ZH, Ji Y, Shan W, Zeng B, Raksadawan N, Pastores GM, et al. Therapeutic effects of astrocytes expressing both tyrosine hydroxylase and brain-derived neurotrophic factor on a rat model of Parkinson's disease. Neuroscience 2002;113:629–40.
[18] Wolff JA, Fisher LJ, Xu L, Jinnah HA, Langlais PJ, Iuvone PM, et al. Grafting fibroblasts genetically modified to produce L-dopa in a rat model of Parkinson's disease. Proc Natl Acad Sci 1989;86:9011–4.
[19] Zhou M, Xu DH, Cao L, Xu LF, Yu FR, Zheng ZC, et al. Long term gene therapy of Parkinson's disease using immortalized rat glial cell line with tyrosine hydroxylase gene. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao 2003;35:1066–71.
[20] Abrous DN, Shaltot AR, Torres EM, Dunnett SB. Dopamine-rich grafts in the neostriatum and/or nucleus accumbens: effects on drug-induced behaviours and skilled paw-reaching. Neuroscience 1993;53: 187–97.
[21] Dunnett SB, Whishaw IQ, Rogers DC, Jones GH. Dopamine-rich grafts ameliorate whole body motor asymmetry and sensory neglect but not independent limb use in rats with 6-hydroxydopamine lesions. Brain Res 1987;415:63–78.
[22] Olsson M, Nikkhah G, Bentlage C, Björklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J Neurosci 1995;15:3863–75.
[23] Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. J Neurol Sci 1973;20:415–55.
[24] Miklyaeva EI, Castañeda E, Whishaw IQ. Skilled reaching deficits in unilateral dopamine-depleted rats: impairments in movement and posture and compensatory adjustments. J Neurosci 1994;14:7148–58.
[25] Miklyaeva EI, Martens DJ, Whishaw IQ. Impairments and compensatory adjustments in spontaneous movement after unilateral dopamine depletion in rats. Brain Res 1995;681:23–40.
[26] Schallert T, Upchurch M, Lobaugh N, Farrar R, Spirduso W, Gilliam P, et al. Tactile extinction: distinguishing between sensorimotor and motor asymmetries in rats with unilateral nigrostriatal damage. Pharmacol Biochem Behav 1982;16:455–62.
[27] Metz GA, Whishaw IQ. Drug-induced rotation intensity in unilateral dopamine-depleted rats is not correlated with end point or qualitative measure of forelimb or hindlimb motor performance. Neuroscience 2002;111:325–36.
[28] Castañeda E, Whishaw IQ, Robinson TE. Changes in striatal dopamine neurotransmission assessed with microdialysis following recovery from a bilateral 6-OHDA lesion: variation as a function of lesion size. J Neurosci 1990;10:1847–54.
[29] Zhang WQ, Tilson HA, Nanry KP, Hudson PM, Hong JS, Stachowiak MK. Increased dopamine release from striata of rats after unilateral nigrostriatal bundle damage. Brain Res 1988;461:335–42.
[30] Metz GA, Gonzalez CL, Piecharka DM, Whishaw IQ. Acute alcohol administration improves skilled reaching success in intact but not 6-OHDA dopamine depleted rats: a subsystems analysis of the motoric and anxiolytic effects of alcohol. Behav Brain Res 2003;142(1-2): 167–74.
[31] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. San Diego: Academic Press, Inc.; 1986.
[32] Hokfelt T, Ungerstedt U. Specificity of 6-hydroxydopamine induced degeneration of central monoamine neurones: an electron and fluorescence microscopic study with special reference to intracerebral injection on the nigro-striatal dopamine system. Brain Res 1973;60: 269–97.
[33] Ungerstedt U. 6-hydroxydopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 1968;5:107–10.
[34] Ungerstedt U, Ljungberg T, Steg G. Behavioral, physiological, and neurochemical changes after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine neurons. Adv Neurol 1974;5: 421–6.
[35] Harada K, Wu J, Haycock JW, Goldstein M. Regulation of L-DOPA biosynthesis by site-specific phosphorylation of tyrosine hydroxylase in AtT-20 cells expressing wild-type and serine 40-substituted enzyme. J Neurochem 1996;67:629–35.
[36] Wu J, Filer D, Friedhoff AJ, Goldstein M. Site-directed mutagenesis of tyrosine hydroxylase. J Biol Chem 1992;267:25754–8.
[37] Stachowiak MK, Goc A, Hong JS, Poisner A, Jiang HK, Stachowiak EK. Regulation of tyrosine hydroxylase gene expression in depolarized non-transformed bovine adrenal medullary cells: second messenger systems and promoter mechanisms. Mol Brain Res 1994;22: 309–19.
[38] Salamone JD, Mahan K, Rogers S. Ventrolateral striatal dopamine depletions impair feeding and food handling in rats. Pharmacol Biochem Behav 1993;44:605–10.
[39] Schallert T, Upchurch M, Wilcox RE, Vaughn DM. Posture independent sensorimotor analysis of inter-hemispheric receptor asymmetries in the neostriatum. Pharm Biochem Behav 1983; 18:753–9.
[40] Montoya CP, Campbell-Hope LJ, Pemberton KD, Dunnett SB. The "stair-case test": a measure of independent forelimb reaching and grasping abilities in rats. J Neurosci Methods 1991;36:219–28.
[41] Tabbaa S, Goulah C, Tran RK, Lis A, Korody R, Stachowski B, et al. Gene transfer into the central nervous system using Herpes Simplex Virus-1 vectors. Folia Morphol 2000;59:221–32.
[42] Mertz KD, Weisheit G, Schilling K, Luers GH. Electroporation of primary neural cultures: a simple method for directed gene transfer in vitro. Histochem Cell Biol 2002;118(6):501–6.
[43] Martin-Iverson MT, Altar CA. Spontaneous behaviours of rats are differentially affected by substantia nigra infusions of brain-derived neurotrophic factor and neurotrophin-3. Eur J Neurosci 1996;8:1696–706.
[44] Alexander GE, Crutcher MD, DeLong MR. Basal ganglia–thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 1990;85:119–46.
[45] Pisa M. Motor functions of the striatum in the rat: critical role of the lateral region in tongue and forelimb reaching. Neuroscience 1988;24:453–63.
[46] Pisa M, Schranz JA. Dissociable motor roles of the rat's striatum conform to a somatotopic model. Behav Neurosci 1988;102:429–40.
[47] Montoya CP, Astell S, Dunnett SB. Effects of nigral and striatal grafts on skilled forelimb use in the rat. Prog Brain Res 1990;82:459–66.
[48] Bunney BS, Walters JR, Roth RH, Aghajanian GK. Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. J Pharmacol Exp Ther 1973;185:560–71.
[49] Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 1984;4:2866–76.
[50] Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 1991;41:1–24.
[51] Grace AA. The tonic/phasic model of dopamine system regulation: its relevance for understanding how stimulant abuse can alter basal ganglia function. Drug Alcohol Depend 1995;37:111–29.
[52] Grace AA. The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. Addiction 2000;95:S119–28.
[53] Kirik D, Georgievska B, Rosenblad C, Björklund A. Delayed infusion of GDNF promotes recovery of motor function in the partial lesion model of Parkinson's disease. Eur J Neurosci 2001;13: 1589–99.
[54] Kirik D, Rosenblad C, Björklund A. Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. Exp Neurol 1998;152:259–77.

[55] Kirik D, Winkler C, Björklund A. Growth and functional efficacy of intrastriatal nigral transplants depend on the extent of nigrostriatal degeneration. J Neurosci 2001;21:2889–96.
[56] Chiodo LA, Caggiula AR, Antelman SM, Lineberry CG. Sensory stimuli alter the discharge rate of dopamine (DA) neurons: evidence for two functional types of DA cells in the substantia nigra. Brain Res 1980;189:544–9.
[57] Keller RW, Stricker EM, Zigmond MJ. Environmental stimuli but not homeostatic challenges produce apparent increases in dopaminergic activity in the striatum: an analysis by in vivo voltammetry. Brain Res 1983;279:159–70.
[58] Mirenowicz J, Schultz W. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 1996;379:449–51.
[59] Salamone JD, Keller RW, Zigmond MJ, Stricker EM. Behavioral activation in rats increases striatal dopamine metabolism measured by dialysis perfusion. Brain Res 1989;487:215–24.
[60] Schultz W. Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. J Neurophysiol 1986;56:1439–61.
[61] Schultz W, Romo R. Dopamine neurons of the monkey midbrain: contingencies of responses to stimuli eliciting immediate behavioral reactions. J Neurophysiol 1990;63:607–24.
[62] Kolb B, Brown R, Witt-Lajeunesse A, Gibb R. Neural compensations after lesion of the cerebral cortex. Neural Plast 2001;8:1–16.
[63] Schallert T, Leasure JL, Kolb B. Experience-associated structural events, subependymal cellular proliferative activity, and functional recovery after injury to the central nervous system. J Cereb Blood Flow Metab 2000;20:1513–28.
[64] Cohen AD, Tillerson JL, Smith AD, Schallert T, Zigmond MJ. Neuroprotective effects of prior limb use in 6-hydroxydopamine-treated rats: possible role of GDNF. J Neurochem 2003;85:299–305.
[65] Tillerson JL, Cohen AD, Philhower J, Miller GW, Zigmond MJ, Schallert T. Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine. J Neurosci 2001;21:4427–35.
[66] Whishaw IQ, Haun F, Kolb B. Analysis of behavior in laboratory rodents. In: Windhorst U, Johansson H, editors. Modern Techniques in Neuroscience. Berlin: Springer-Verlag; 1999. p. 1243–75.