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Differential sensitivity of cranial and limb motor function to nigrostriatal dopamine depletion

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Abstract

The present study determined the differential effects of unilateral striatal dopamine depletion on cranial motor versus limb motor function. Forty male Long Evans rats were first trained on a comprehensive motor testing battery that dissociated cranial versus limb motor function and included: cylinder forepaw placement, single pellet reaching, vermicelli pasta handling; sunflower seed opening, pasta biting acoustics, and a licking task. Following baseline testing, animals were randomized to either a 6-hydroxydopamine (6-OHDA) ($n = 20$) or control ($n = 20$) group. Animals in the 6-OHDA group received unilateral intrastratial 6-OHDA infusions to induce striatal dopamine depletion. Six-weeks following infusion, all animals were re-tested on the same battery of motor tests. Near infrared densitometry was performed on sections taken through the striatum that were immunohistochemically stained for tyrosine hydroxylase (TH). Animals in the 6-OHDA condition showed a mean reduction in TH staining of 88.27%. Although 6-OHDA animals were significantly impaired on all motor tasks, limb motor deficits were more severe than cranial motor impairments. Further, performance on limb motor tasks was correlated with degree of TH depletion while performance on cranial motor impairments showed no significant correlation. These results suggest that limb motor function may be more sensitive to striatal dopaminergic depletion than cranial motor function and is consistent with the clinical observation that therapies targeting the nigrostriatal dopaminergic system in Parkinson's disease are more effective for limb motor symptoms than cranial motor impairments.

Keywords

6-Hydroxydopamine; Parkinson's disease; Rodent; Licking; Reaching; Cranial motor; Limb motor

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1. Introduction

Parkinson's disease (PD) is a chronic, progressive and currently non-curable neurodegenerative disease associated with substantial morbidity, increased mortality and high economic burden [37]. Approximately 1.5 million Americans are currently diagnosed with PD at a cost of \$23 billion dollars annually [37]. Although impairments in gross motor function such as gait and upper extremity movement are the most common deficits associated with PD, 90% of individuals also demonstrate cranial motor impairments in the form of a dysarthria and/or dysphagia [35] that are associated with significant reductions in patient quality of life, social interactions and mental well-being [30]. Further, aspiration pneumonia, due to swallowing impairment, is the leading cause of death in PD, resulting in a life expectancy ten years below the general population [13].

Current neurosurgical and neuropharmacological treatments are founded on the hypothesis that PD is a disease of dopaminergic depletion within the basal ganglia thalamocortical motor loop. While these interventions can be effective in alleviating general motor symptoms of PD, they have failed to provide consistent and significant benefit for cranial motor functions [15,19,20,29,31,32]. Furthermore, although some cranial motor impairments show characteristic changes with disease progression, they show no clear correlation with degradation of general motor impairment [35]. Together these results suggest that cranial motor impairments may be due to neuropathologies independent or secondary to those mediating limb motor impairments.

Animal models of PD provide the opportunity to study the relationship between specific neuropathologies associated with PD and their concomitant behavioral impairments. Virtually all animal models of PD employ genetic, pharmacological or neurotoxic manipulations directed at disrupting the midbrain dopaminergic system in rodents. The most widely used model involves injecting 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) resulting in the loss of dopaminergic neurons within the substantia nigra (SNc) and ventral tegmental area (VTA) [18]. This model has been shown to induce robust limb [4] and cranial [5,6,17,34,38] motor impairments. However, the selective effect of depleting only nigrostriatal dopamine on limb and cranial motor function is unknown. This is particularly relevant toward understanding the relationship between PD symptomology and neuropathology given that degeneration of the SNc is the most prominent pathology and the VTA shows very little degeneration [2,7,22]. The purpose of the present study was to compare the specific effects of nigrostriatal dopamine depletion on limb versus cranial motor function using a comprehensive battery of motor tests that differentially tax each motor system. Further we investigated the relationship between impairments in these two classes of motor behaviors with the level of striatal dopamine depletion. Using a well established animal model of PD, we tested the hypothesis that performance on limb and cranial motor tasks would be differentially affected by striatal dopamine depletion.

2. Methods

2.1. Animals

Forty male (325–425 g) Long Evans hooded rats were used in this study (Harlan, Indianapolis, IN, USA). Animals were singularly housed in Plexiglass cages (36 cm long, 20 cm wide and 21 cm deep) with sawdust bedding and maintained on a 12:12 h light:dark cycle with controlled temperature and humidity. All behavioral testing occurred during the light period of the cycle and the experiment was conducted in compliance with the guidelines of the University of Florida Animal Care and Use Committee. Rats were given seven days to acclimatize to the housing environment upon arrival from the breeding colony

before being handled 10 min per day for five consecutive days by the experimenters prior to baseline motor testing.

2.2. Experimental timeline

Immediately following handling, all animals completed a comprehensive behavioral testing battery to assess cranial motor and limb motor function. This battery included: cylinder forepaw placement testing; single pellet reaching, vermicelli pasta handling; sunflower seed opening, and a lick-force task over approximately five-weeks. Following collection of baseline data, animals were randomized to either a 6-OHDA ($n = 20$) or control ($n = 20$) group. Animals in the 6-OHDA group immediately underwent unilateral intrastratial 6-hydroxydopamine infusions to induce striatal dopamine depletion. Following lesion surgeries, all animals remained in their home cages for six-weeks and were then re-tested on the complete behavioral testing battery to assess the impact of unilateral dopamine depletion on cranial motor vs. limb motor function. Pasta-biting acoustics was also completed at the second testing session. Completion of the behavioral testing battery was performed with animals divided into two groups (A and B) and the order of limb vs. cranial motor tests counter balanced across these groups. Animals in group A completed limb motor tests first followed by cranial motor tests while those in group B were first assessed on cranial motor tests followed by limb motor tests. Counter balancing testing order was implemented to control for any potential order effects due to timing of testing pre or post lesion. Following the completion of the experiment all animals were humanely euthanized, perfused and standard tyrosine hydroxylase (TH) immunohistochemistry performed and TH depletion determined using near infrared densitometry.

2.3. Cylinder forepaw placement test

To test voluntary forelimb use, animals were placed into a transparent cylinder (20 cm $\times$ 30 cm) for 5 min and video recorded for subsequent analysis of forelimb use during vertical exploration. The primary outcome variable for the cylinder test was the cylinder forepaw asymmetry ratio (CAR) as described by Schallert and Woodlee [33]. The CAR was calculated as an index of voluntary forepaw use $[(\text{dominant forelimb touches} + \frac{1}{2} \text{ number of both forelimbs}) / (\text{dominant forelimb touches} + \text{non-dominant forelimb touches} + \text{both}) \times 100]$. The cylinder forepaw placement test was classified as a limb motor test.

2.4. Single pellet reaching

Prior to reach training and testing, animals were placed on a restricted diet consisting of 12–18 g of standard rat chow per day (Tekland Global 2018 pellets, Harlan Laboratories). Daily monitoring of animal weights occurred throughout the entire food restriction period to ensure that animals did not fall below 90% of their original body weight prior to food restriction.”. Initially, a period of pre-training occurred in test cages (10 cm $\times$ 18 cm $\times$ 10 cm) containing a deep tray (4 cm $\times$ 5 cm) mounted to the front of the cage and filled with food pellets (45 mg; Bioserv). Rats were trained until they successfully retrieved 10 pellets in one session (approximately 1 h per day for 2–4 days). Immediately following pre-training, all animals were trained to criterion (35% accuracy over a three day period) on a single pellet reaching task (approximately two weeks). Each session was videotaped and subsequently used to assess reaching accuracy. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The primary outcome variable for this task was percentage reaching accuracy and was calculated as: $[(\# \text{ successful retrievals} / \text{the total } \# \text{ of reaches}) \times 100]$. Skilled reach testing was classified as a limb motor test.

2.5. Vermicelli handling test

Animals were presented with five 7 cm uncooked vermicelli strands (1.5 mm diameter; 0.15 g/piece; Mueller brand, distributed by American Pasta Co., Kansas City, MO) in their home cage and video recorded for subsequent analyses. To acclimate animals to pasta handling, they were given five strands of vermicelli in their home cages for several days prior to testing. The primary outcome measure for the vermicelli handling test was the vermicelli asymmetry ratio (VAR) as described by Allred et al. [1]. The VAR (%) is defined as the [(number of dominant forelimb adjustments/total number of dominant and non-dominant forelimb adjustments) $\times$ 100]. Time to eat (beginning when the pasta piece was grasped and ending when piece was released by the paws and disappeared into the mouth in seconds) was a secondary outcome measure for this task. For data analysis, the mean across the five trials was used. Vermicelli handling testing was classified as a limb motor task.

2.6. Sunflower seed opening

To test object manipulation abilities, animals were placed into a clear plastic arena with five sunflower seeds located in the upper right hand corner. A mirror was angled at a 120° angle at the back of the enclosure to allow visualization of sunflower manipulation in the instance that an animal faced away from the experimenter and toward the back of the enclosure. To acclimate animals to testing materials, sunflower seeds were included in their diet several days leading up to testing. The primary outcome measure for sunflower seed testing was total manipulation time (TMT) and defined as the total time spent manipulating, opening and placing the seed into the mouth (starting the moment the animal touched a seed and ending the second the animal dropped the shell and released the seed into the mouth) and represented the cumulative time across all five trials. Animals were tested over two consecutive days and a two-day average TMT calculated. The sun-flower seed opening task represented a mixed task with both limb and cranial motor components.

2.7. Pasta-biting acoustics

Animals were placed in a plexiglass arena set up inside a sound-proof chamber and presented with five uncooked 5 cm vermicelli pasta strands. Audio recordings of chewing sounds were obtained using an AudioTechnica Model ATM73a unidirectional microphone digitized directly to a Marantz PMD670 recorder equipped with a built in pre-amplifier. Audio recordings were digitized and high pass filtered at 3000 kHz. Custom scripts written in MATLAB version 11 (Mathworks, Inc.) were used to calculate inter-bite duration (ms) and the mean inter-bite duration across all five pasta trials were used for data analysis purposes. These methods were adapted from Kane et al. [17]. As previously stated, this test was performed at only the post-lesion testing time point as it was an exploratory outcome measure. Pasta biting acoustics represented a cranial motor test.

2.8. Lick dynamics

A lick-force recording apparatus previously described in detail by Fowler and Wang [11] and Fowler and Mortell [10] was used for lick training and testing. Briefly, this enclosure contains a lick disk (18 mm in diameter) attached to the shaft of a force transducer with a computer controlled peristaltic pump delivering water to the center of the lick disk through a 0.5 mm diameter hole. The force transducer is capable of resolving force measurements to 0.2 g equivalent weights. Through a Labmaster interface (Scientific Solutions, Mentor, OH) a computer program recorded session force–time data at a rate of 100 samples per second allowing for force–time waveforms of each individual lick. After gradual water restriction, rats were individually introduced to the lick-force chamber for daily 2-min sessions. Biomechanically, the lapping motion of the tongue performed during this task is likely performed by contraction of the extrinsic tongue muscles (genioglossus, hyoglossus,

palatoglossus, and styloglossus) mediated by the hypoglossal and vagus nerves to protrude, depress, elevate and retract the tongue as well as the intrinsic muscles that shape and contour the tongue during these finely controlled movements. Infrahyoid musculature and muscles of mastication also contract to depress the mandible during the licking task and include the lateral pterygoid, mylohyoid, and geniohyoid mediated by the trigeminal and hypoglossal nerves. During training, the lingual force requirement for a water bolus reward was set at 2 g and continuous licking behavior reinforced by delivery of 0.06 ml of water to the lick disk surface after every 12th lick (fixed-ratio of FR12 schedule). Training occurred for fourteen consecutive days during which the animals were water restricted to ensure completion of the task. Licking outcome measures constituted the average of the final two days of training (days 13 and 14) and included: (1) Lick frequency (number of licks > 2 g during 2 min session), (2) Median peak force (g), and (3) Lick rhythm (Hz) within a 2-min testing session. Lick dynamics represented a cranial motor task.

2.9. 6-OHDA infusions

Animals assigned to the 6-OHDA group received four unilateral 6-OHDA infusions into the contralateral hemisphere to their dominant limb following the protocol of Kirik et al. [18]. The dominant forelimb was determined from performance on the single pellet reaching tasks where all animals reach with a preferred paw. Rats were anaesthetized with isoflurane (4% induction, 1.5% maintenance; VIP-3000 Vaporizer, Matrix, Orchard Park, NY) and placed in a stereotaxic instrument (Kopf, Tujunga, CA) with the incisor bar set to skull flat. Infusion coordinates relative to Bregma were: (AP +1.3 mm, ML -2.6 mm; -5.0 mm DV); (AP +0.9 mm, ML -3.0 mm; -5.0 mm DV); (AP -0.4 mm, ML -4.2 mm; -5.0 mm DV); (AP -1.3 mm, ML -4.5 mm; -5.0 mm DV) in the left hemisphere. At each infusion site, a microbur (0.45 mm in diameter) was made through the skull and a pulled glass micropipette was lowered into the striatum at each coordinate. Using a computer assisted nanoinject microinfusion pump (World Precision Instruments, Sarasota, FL), 7 µg (free base weight) of 6-OHDA hydrochloride (Sigma, Oakville, ON) dissolved in 0.1% ascorbic acid was infused at a rate of 1 µl/min (total of 2 µl). The micropipette was left in place for 5 min to aid infusate diffusion before being slowly retracted from the brain. Following the four intrastriatal infusions, 4 cc of Ringers solution was given subcutaneously (s.c.), the scalp sutured and betadine applied to the suture site.

2.10. Immunohistochemistry

Animals were perfused intracardially with 200 ml of phosphate buffered saline solution followed by 400 ml of 4% paraformaldehyde and decapitated for brain extraction. Brains were post fixed in 4% paraformaldehyde for 24 h and then cut into 40-µm-thick coronal sections using a freezing microtome. Floating sections were washed with 0.01 M PBS and then treated for 15 min with 0.5% H₂O₂ + 10% methanol in 0.01 M PBS. Sections were preincubated with 3% normal horse serum (NHS) + 0.1% Triton X-100 in 0.01 M PBS, and then incubated overnight at room temperature with a 1:2000 dilution of a mouse anti-TH antibody (Chemicon, Temecula, CA). Following incubation, tissue was washed and incubated for 2 h at room temperature with a secondary antibody directed against the species in which the primary antibody was raised. The reactions were then incubated with a near infrared secondary antibody for TH densitometry, IRdye 800 (LI-COR Biosciences, Lincoln, NE). Sections were mounted on subbed slides, dehydrated in ascending alcohol concentrations, cleared in xylene, and coverslipped in permount in a dark room.

2.11. Near infrared densitometry

Serial TH-labeled sections were scanned at a resolution of 21 µm and a 5.5 sensitivity setting on a LI-COR Odyssey scanner (LI-COR Biosciences). Using the Odyssey Infrared imaging system Application Software (Version 3.0), striatal sections were outlined and the

value of the integrated intensity obtained from the right and left striatum of each section derived. TH depletion was calculated for each animal using the following formula: $[(\text{lesioned integrated intensity} / \text{intact integrated intensity}) \times 100]$ [23].

2.12. Statistical analyses

Behavioral data were analyzed using a mixed model ANOVA with time (pre-lesion vs. post-lesion) and condition (control vs. 6-OHDA) as the two factors. Post hoc analysis in the form of a Student's *T* Test was performed when significant interactions for time by condition were noted. To evaluate the differential degree of cranial motor versus limb motor dysfunction associated with unilateral dopamine depletion two subsequent analyses were performed. First, a behavioral impairment profile for the 6-OHDA animals across each task was calculated $[(\text{mean control post-lesion} - \text{mean 6-OHDA post-lesion} / \text{mean control post-lesion}) \times 100]$ and graphically depicted. Second, a series of Pearson's correlation analyses were performed between TH depletion levels (%) and the primary outcome variable for each behavioral test to specifically assess the relative degree of cranial and limb motor impairment associated with dopamine depletion. For all statistical tests, α was set at 0.05 and SPSS. Version 19.0 (Chicago, IL) was used to compute all data. All descriptive statistics are reported as means $\pm$ S.E.M. unless otherwise indicated.

3. Results

3.1. Densitometry

Three animals were excluded from the 6-OHDA group due to insufficient TH depletion (<50%). Of those animals included in the 6-OHDA group ($n = 17$), densitometry analysis revealed that Mean TH depletion was 88.27% (range = 50.6–100; SEM = 3.39). Mean (SEM) integrated intensity values were 26.35(2.49) vs. 4.36(1.51) for the intact and lesioned hemispheres respectively in 6-OHDA animals [$t(16) = -7.77$, $p = 0.000$].

3.2. Cylinder forepaw asymmetry ratio

A mixed model ANOVA revealed a significant time by condition interaction $F(1) = 92.89$, $p < 0.0001$. Subsequent post hoc analysis (*T*-test; $p < 0.05$) revealed that post lesion, 6-OHDA animals demonstrated a significant reduction in CAR with respect to both pre-lesion levels [$t(16) = 10.04$, $p < 0.0001$] and in comparison to controls at the post-lesion test [$t(36) = -10.20$, $p < 0.0001$]. Fig. 1 A plots mean CAR pre and post-lesion for control and 6-OHDA groups.

3.3. Single pellet reaching

A significant time by condition interaction was revealed for skilled reaching accuracy, $F(1) = 20.53$, $p < 0.0001$. Subsequent post hoc analysis showed the 6-OHDA animals had significantly lower reaching accuracies post-lesion in comparison to both pre-lesion reaching [$t(16) = 5.03$, $p < 0.0001$] and control animals post-lesion [$t(36) = -5.53$, $p < 0.0001$]. Fig. 1B plots mean reaching accuracy for control and 6-OHDA animals across testing points.

3.4. Vermicelli handling test

3.4.1. Vermicelli asymmetry ratio—A significant time by condition interaction was also found for the VAR [$F(1) = 12.52$, $p = 0.001$]. Subsequent post hoc testing revealed a significant post-lesion reduction in VAR for 6-OHDA animals in comparison to both their pre-lesion levels [$t(16) = 4.91$, $p < 0.0001$], and control animal post-lesion VAR scores [$t(36) = -4.56$, $p < 0.0001$]. Mean VAR for control and 6-OHDA animals is plotted in Fig. 1C.

3.4.2. Time to eat—A significant time by condition interaction was observed for time to eat, [$F(1) = 12.52, p < 0.01$]. Subsequent post hoc testing revealed that the 6-OHDA animals took a significantly longer time to eat the pasta compared to the control animals at the post-lesion pasta testing, [$t(36) = 1.93, p < 0.05$], with no significant differences compared to 6-OHDA pre-lesion pasta eating times. Fig. 1D plots mean pasta consumption times across time points for control and 6-OHDA animals.

3.5. Sunflower seed opening

A significant time by condition interaction was observed for the total manipulation time for sunflower seed opening, [$F(1) = 5.19, p < 0.03$]. Subsequent post hoc testing revealed a significant post-lesion increase in sunflower seed total manipulation time in the 6-OHDA animals in comparison to both pre-lesion levels [$t(16) = -2.27, p < 0.04$], and control animals post-lesion manipulation times [$t(36) = 2.19, p < 0.04$]. Mean sunflower seed opening times for control vs. 6-OHDA animals are displayed in Fig. 1E.

3.6. Pasta biting acoustics

Acoustic analysis of pasta biting at the single time point (post-lesion) revealed a significantly longer inter-bite duration for the 6-OHDA animals (m : 21 ms, SEM: 1.50) compared to control animals (m : 17 ms, SEM: 0.80), $t(38) = 2.39, p < 0.03$.

3.7. Licking task

3.7.1. Lick force—A significant time by condition interaction was revealed for lick force [$F(1) = 4.36, p = 0.04$]. Subsequent post hoc analysis revealed a significant post-lesion reduction in lick force for the 6-OHDA animals compared to their pre-lesion licking performance, [$t(16) = 4.04, p = 0.001$].

3.7.2. Lick frequency—A significant interaction for time by condition was revealed for lick frequency [$F(1) = 47.86, p < 0.001$]. Subsequent post hoc testing revealed a significant post-lesion reduction in lick frequency for 6-OHDA animals in comparison to both their pre-lesion lick frequency [$t(16) = 9.31, p < 0.001$], and control animals post-lesion licking frequency [$t(36) = -4.63, p < 0.001$].

3.7.3. Lick rhythm—Although a time by condition interaction approached significance ($p = 0.06$), no significant differences were revealed for licking rhythm. Mean (SEM) lick rhythm for 6-OHDA animals pre and post-lesion were 6.27 Hz (0.07) and 5.91 Hz (0.16) respectively. Mean lick rhythm for control animals were 6.22 Hz (0.06) and 6.23 Hz (0.14) for pre and post-lesion testing. Fig. 1 illustrates mean and SEM data for each of the behavioral tasks across pre and post-lesion testing points. Mean data for control and 6-OHDA groups across testing time points are displayed in Fig. 1F–H.

3.8. Limb motor vs. cranial motor impairment

Fig. 2 A summarizes the observed motor impairment profile for 6-OHDA animals in comparison to controls. Although significant motor impairments were noted across all behavioral assessments, deficits were greatest in those tasks relying more heavily on the limb motor system than cranial motor tasks.

3.9. Relationship between TH depletion and motor impairment

Significant negative correlations ($p < 0.05$) were revealed between TH depletion and forelimb performance on the cylinder ($r = -0.77, p = 0.000$), reaching ($r = -0.69, p = 0.001$), and pasta handling ($r = -0.58, p = 0.006$) tasks. No other correlations between TH depletion levels and behavioral performance were observed ($p > 0.05$). Fig. 2B plots the Pearson's R

value for each of the behavioral tasks and illustrates the finding that tasks relying more heavily on limb motor function were significantly related to degree of TH depletion while those relying on the cranial motor system were not correlated to levels of TH depletion. Scatter plots for individual behavioral tasks and TH depletion are provided in Fig. 3.

4. Discussion

The present study is the first to directly compare the specific effects of nigrostriatal dopamine depletion on limb versus cranial motor function in an animal model of Parkinson's disease. The effect of dopamine depletion on motor performance was assessed by comparing the mean performance of animals in the 6-OHDA condition to both their pre lesion levels and to that of control animals. In addition, the correlation between post lesion performance on each task and level of TH staining in the striatum was also examined. The results demonstrate that tasks relying heavily on limb use were more severely affected by nigrostriatal dopamine depletion than those relying on function of the tongue and cranial motor circuits. Further, impairment on limb motor tasks was highly correlated with the level of dopamine depletion in the striatum. Conversely, performance on cranial motor tasks was only mildly impaired and not correlated with the level of striatal dopamine depletion. Our results demonstrate a clear role for the nigrostriatal dopaminergic system in limb motor control but suggest a secondary or tertiary role for this system in cranial motor control. These data provide some insight into the apparent resistance of cranial motor impairments and dopaminergic therapy observed in individuals with PD. They also have implications for the role of the midbrain dopaminergic systems in motor control and for animal studies attempting to model the complex array of motor impairments associated with PD.

4.1. Dissociating limb and cranial motor impairments

The behavioral tests used in this study were chosen because of the differential behavioral demands they placed on cranial motor versus limb motor function. They ranged from those explicitly testing forelimb function (cylinder task) to those explicitly testing tongue function (licking). The single pellet reaching, sunflower seed opening and vermicelli handling and biting tests were also included because they involved varying degrees of both forelimb and tongue/jaw function depending on the measure of performance assessed. In single pellet reaching the animals are required to target the food pellet with the forelimb, grasp the pellet with the paw and bring it back to the mouth for consumption. Thus, while impairments in jaw and tongue movements could cause an increase in errors as the animals consume the pellet, the task is clearly more sensitive to forelimb impairments. The sunflower seed opening task more heavily involves the use of the tongue and jaw than the single pellet reaching task as the animals work to open the seeds. The task also relies on the forelimbs to adjust the position of the seed so they can bite off pieces of shell thus it represents a mixed task. The vermicelli handling test can be used to test both limb and cranial motor function depending on the specific measure examined. The vermicelli forelimb asymmetry measure is more sensitive to forelimb impairments while the use of acoustic analysis of pasta biting is more sensitive to jaw and tongue (i.e. cranial motor) impairments. This comprehensive battery of tests and measures allowed for a direct examination of how striatal dopamine depletion affected cranial versus limb motor function.

The results demonstrated that although both classes of motor behaviors were significantly impaired in 6-OHDA animals, there were important differences with respect to the severity of impairment and relationship to the level of striatal dopamine depletion. Forelimb use during vertical exploration, single pellet reaching accuracy, vermicelli pasta handling and sunflower seed manipulation abilities, were all seen to be significantly impaired in the 6-OHDA animals. These animals exhibited a reduction in the amount of forepaw use of the affected limb in both the cylinder and vermicelli handling tasks, as well as lower skilled

reaching accuracies and a greater amount of time required to manipulate and open sunflower seeds. Further, the degree of dopamine depletion within the striatum significantly correlated with the level of impairment in skilled reaching accuracy and forelimb asymmetry in both the cylinder and vermicelli handling tasks. These findings are consistent with several other reports using this same model of striatal dopamine depletion [18,39,40] and a recent report correlating such impairments with a loss of forelimb movement representations in the motor cortex [28]. It is also consistent with the basal ganglia thalamocortical model of dysfunction in PD patients whereby a loss of nigrostriatal dopamine results in hyperactivity of inhibitory basal ganglia output to thalamic nuclei, inhibition of thalamocortical projections and reduced cortical excitability resulting in limb hypokinesia [8].

Mean lingual strength was reduced in 6-OHDA animals post injection and lick frequency was reduced across testing sessions and compared to control animals at the post-lesion time point. These results are consistent with prior studies that have examined cranial motor function after medial forebrain bundle (MFB) 6-OHDA infusions. Skitek et al. [34] and Ciucci et al. [6] reported similar deficits in both temporal and force lingual function following unilateral infusions of 6-OHDA into the MFB. However, the average mean impairment level in the present study (88%) was less than what has previously been reported in these earlier studies (91–92%). Further, despite an overall difference in means between conditions on cranial motor tasks in the current study, there was no correlation between level of dopamine depletion and performance on any of the cranial motor tasks. These findings likely reflect the fact that intrastriatal infusions of 6-OHDA produce more variable levels of striatal dopamine depletion than infusions of 6-OHDA into the MFB. MFB lesions typically result in either severe depletion (over 90%) or very mild depletion (under 20%) and animals with mild dopamine depletion are typically removed from these studies. This is consistent with Skitek et al.'s [34] finding that following MFB lesions, only animals with 75% or greater striatal dopamine depletion demonstrated licking impairments. The current data suggest that only severe levels of dopamine depletion affect cranial motor function while mild to moderate levels of dopamine depletion can affect limb motor function.

4.2. The role of midbrain dopaminergic systems in motor control

All prior studies examining cranial motor impairments in rodent models of PD have utilized the MFB model of nigrostriatal dopamine depletion. This model destroys dopaminergic neurons in the substantia nigra, striatum and ventral tegmental area (VTA) and has been documented to produce robust impairments in licking performance [6,34]. By contrast, the intrastriatal 6-OHDA infusion model of PD used in the present study spares the VTA dopaminergic system and did not induce severe licking impairments. Anatomically, the substantia nigra pars compacta (SNc) projects to the caudate, putamen and globus pallidus, while the VTA innervates the frontal cortex, accumbens and hippocampal formation [9,12,21]. Thus these two systems may have differential roles in modulating motor control both within and across motor domains. For example, Hosp et al. [14] demonstrated that the dopaminergic projections from the VTA to the frontal cortex are necessary for the acquisition but not the performance of skilled reaching. The present experiment shows that the nigrostriatal system is critical for reaching performance given that the animals were trained to criterion prior to 6-OHDA injections (i.e. had already acquired the skill). The nigrostriatal system likely influences forelimb behavior by modulating activity of neurons within the dorsolateral striatum that are critical for performing limb movement [27]. Pioli et al. [26] have suggested that SNc lesions caused impairments in fine motor control while VTA lesions spared motor performance but increased perseverative behavior related to a dysfunction in motivation.

The neural circuitry controlling licking behavior in the rat involves a complex network of highly interconnected areas of the brainstem and medulla [36]. This system may be

modulated by both the VTA and SNc as infusions of 6-OHDA into the globus pallidus, [3] and dopamine antagonists into the accumbens [16] were both seen to cause reductions in tongue extension and lap volume during a licking task. Thus it is plausible to suggest that intrastriatal infusions of 6-OHDA, destroying dopaminergic neurons in only the SNc, might result in less severe licking impairments than MFB infusions that destroy neurons in both the SNc and VTA.

A unilateral model of dopamine depletion was utilized in this study to serve as a surrogate for mild to moderate PD. While it is possible to administer bilateral lesions of 6-OHDA, these are typically associated with profound morbidity and a high degree of rodent mortality. One important consideration when interpreting our results is the fact that dopamine depletion was unilateral and the cranial motor system and corticobulbar tracts are less lateralized than the limb motor system and corresponding corticospinal projections. Therefore, it is possible that the cranial motor system displayed less severe deficits than the limb motor system due to the intact functioning of the non-lesioned hemisphere that may have provided an inherent 'reserve' or compensation for maintenance of cranial motor function. Recent findings by Nuckolls et al. [24], however, would suggest that this is not a likely variable. These authors studied the effects of unilateral vs. bilateral dopamine depletion on tongue force and tongue motility during a licking task in rats and reported that while both lesion groups demonstrated significant and similar reductions in lick force, only the unilateral group demonstrated tongue motility deficits. Nuckolls et al. [24] suggested that the etiology of this later finding resulted from the presence of a functional imbalance in neural pathways affecting motility of the midline structure. Given these results, the mild impairments in cranial motor function observed in the present experiment are not likely due to the increased potential for functional compensation by contralesional cranial motor circuits.

4.3. Clinical implications

Although PD has historically been thought of as a disease of the basal ganglia resulting from the loss of dopaminergic neurons within the substantia nigra, there is good reason to suggest that not all manifestations of PD result solely from dopaminergic loss within the nigrostriatal pathway. Indeed, PD is increasingly being recognized as a multi-system neurodegenerative disease [2] that includes the widespread loss of cholinergic, serotonergic, and noradrenergic neurons within the brainstem, spinal cord and peripheral nervous system [25]. The current finding of differential sensitivities to dopamine depletion across classes of behavior suggests that cranial motor function is less dependent on striatal dopamine transmission than the limb motor system in a rodent model. The present findings are consistent with the clinical observation that therapies targeting the nigrostriatal dopaminergic system are more effective for limb motor symptoms than cranial motor impairment in PD. It also supports the notion that cranial motor function may be more dependent on other neurotransmitter systems and brain structures. Future work needs to evaluate the differing effects of therapies on limb vs. cranial motor function in PD and the relationship between cranial motor function and other neurotransmitter systems in PD.

Our work demonstrates that (1) unilateral striatal dopamine depletion significantly impairs both limb motor and cranial motor function; (2) limb motor impairments are more severe than cranial motor impairments; and (3) only limb motor function was correlated to degree of dopamine depletion. These findings suggest differential sensitivities to dopamine depletion in a rodent model of PD. Limb motor function appears more sensitive to dopamine transmission and cranial motor function may be due to dysfunction within other neurotransmitter systems and structures in PD. Future work is required to explore this possibility and evaluate differences in a bilateral model of dopamine depletion.

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HIGHLIGHTS

- Unilateral dopamine depletion impairs limb and cranial motor function.
- Limb motor impairments are more severe than cranial motor impairments.
- Only limb motor function was correlated to degree of dopamine depletion.
- Suggesting differential sensitivities to dopamine depletion in a rodent model of PD.

Fig. 1.

Mean (SEM) behavioral performance for control (open circle) and unilateral 6-OHDA (closed square) groups across pre-lesion and post-lesion testing points. (A) cylinder asymmetry ratio; (B) single pellet reaching accuracy; (C) vermicelli pasta handling asymmetry ratio; (D) vermicelli pasta handling time; (E) sunflower seed manipulation time; (F) lick force; (G) lick frequency; (H) lick rhythm.

Fig. 2.

(A) Summary motor impairment profile demonstrates that impairment on limb motor tasks was more severe than cranial motor impairment in unilateral 6-OHDA animals; (B) summary of Pearson's R values across all behavioral tasks with TH depletion demonstrates the finding that TH depletion level is correlated with limb but not cranial motor function.

Fig. 3.

Scatterplots for individual behavioral tasks and TH depletion levels in the 6-OHDA animals ($n = 20$). (A) cylinder asymmetry ratio; (B) single pellet reaching accuracy; (C) vermicelli pasta handling asymmetry ratio; (D) sunflower seed manipulation time; (E) pasta biting acoustics; (F) lick force; (G) lick frequency; (H): lick rhythm. Note that for these scatter plots and correlation analyses all animals infused with 6-OHDA were used, including the three excluded animals with TH depletion less than 50%.