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Research report

Differential sensitivity of cranial and limb motor function to nigrostriatal 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.

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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 intrastriatal 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.

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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%

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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 Plexigass 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 intrastriatal 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 × 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 [(dominant forelimb touches + ½ number of both forelimbs)/(dominant forelimb touches + non-dominant forelimb touches + both) × 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 × 18 cm × 10 cm) containing a deep tray (4 cm × 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: [(# successful retrievals/the total # of reaches) × 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) × 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 sunflower 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