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Research report

Tongue force and tongue motility are differently affected by unilateral vs bilateral nigrostriatal dopamine depletion in rats

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HIGHLIGHTS

- Unilateral and bilateral nigrostriatal dopamine depletion affect orolingual motor function in rats.
- Both unilateral and bilateral nigrostriatal dopamine depletion decrease tongue force during licking.
- Tongue motility is decreased following unilateral but not bilateral nigrostriatal dopamine depletion.
- These results reveal a dissociation between these two movement modalities in the 6-hydroxydopamine rat model of Parkinson's disease.

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ABSTRACT

In addition to its cardinal symptoms of bradykinesia, muscle rigidity, resting tremor and postural disturbances, Parkinson's disease (PD) also affects orolingual motor function. Orolingual motor deficits can contribute to dysphagia, which increases morbidity and mortality in this population. Previous preclinical studies describing orolingual motor deficits in animal models of PD have focused on unilateral nigrostriatal dopamine (DA) depletion. In this study we compared the effects of unilateral vs bilateral 6-hydroxydopamine (6-OHDA)-induced DA depletion in rats trained to lick water from an isometric force-sensing disc. Rats received either unilateral or bilateral 6-OHDA into the medial forebrain bundle and were tested for four weeks post-lesion. Dependent variables included task engagement (the number of licks per session), tongue force (mean and maximum), and tongue motility (the number of licks per second). While both lesion groups exhibited decreased tongue force output, tongue motility deficits were present in only the group that received unilateral nigrostriatal DA depletion. Task engagement was not significantly diminished by 6-OHDA. Analysis of striatal DA tissue content revealed that DA depletion was ~97% in the unilateral group and ~90% in the bilateral group. These results suggest that while nigrostriatal DA depletion affects tongue force output, deficits in tongue motility may instead result from a functional imbalance in neural pathways affecting this midline structure.

1. Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disease with characteristic symptoms that include bradykinesia, muscle rigidity, resting tremor and postural disturbances. These symptoms result primarily from degeneration of dopamine (DA) neurons in the substantia nigra, and depletion of DA in the nigrostriatal pathway [2,11]. Preclinical studies have taken advantage of this well-characterized lesion by targeting the nigrostriatal pathway with neurotoxic agents such as 6-hydroxydopamine (6-OHDA) and MPTP (reviewed in [8]). These models, which typically involve unilateral DA depletion, generally recapitulate the cardinal symptoms of PD.

In addition to its cardinal symptoms, PD can also impair orolingual and pharyngeal motor function [1,18,27,40]. These impairments can increase morbidity and mortality [25], primarily by disrupting the oral phase of swallowing [44]. In contrast to limb deficits (and with the exception of levodopa-induced oral dyskinesias [15]), preclinical attention to the primary effects of nigrostriatal DA depletion on orolingual motor function is a relatively recent

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Table 1
Baseline group means and standard errors of means for body weight and orolingual motor measures prior to lesions.

	Body weight (g)	Peak force (g)	Maximum force (g)	Frequency (licks/s)	Number of licks (licks/session)
Group					
Sham	460 ± 33	8.6 ± 2.6	21.6 ± 2.0	5.68 ± 0.54	592 ± 58
Unilateral	488 ± 9.0	8.8 ± 1.4	23.1 ± 3.4	5.69 ± 0.23	542 ± 43
Bilateral	451 ± 27	7.5 ± 0.6	20.0 ± 2.9	5.55 ± 0.29	482 ± 106

development [13,36,41,47]. These studies have reported that unilateral nigrostriatal DA depletion impairs tongue motility and protrusion force. The effects of bilateral nigrostriatal DA depletion on orolingual motor function have not been reported. This is an important consideration, not only because the tongue is a midline structure, but because onset in PD is typically unilateral, progressing bilaterally in later stages [23].

The purpose of this study was to compare the effects of bilateral and unilateral nigrostriatal DA depletion models on orolingual motor function in rats. Rats' tongue force and tongue motility were measured as they licked water from an isometric disc as described previously by our lab [43,51] and others [36,41]. Orolingual motor function was measured prior to and for four weeks after treatment with 6-OHDA. We hypothesized that rats with bilateral striatal DA depletion would exhibit greater deficits in tongue force and tongue motility than rats with unilateral lesions.

2. Materials and method

2.1. Animals

Male Sprague–Dawley rats were obtained from Harlan. Rats were 3-months-old at the time of testing, were housed individually and were maintained on a 12 h light/dark cycle. After acclimation to the facility, access to water was gradually restricted with food made available ad libitum. The water restriction schedule allowed for slow weight gain and provided rats with necessary motivation to perform the water licking task. Procedures adhered to the Guide for the Care and Use of Laboratory Animals [31], and the experiment was approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

2.2. Behavioral testing

Licking behavior was recorded using a lick force chamber as described previously [20,41,43]. Briefly, thirsty rats were placed in individual customized Gerbrands rodent operant chambers, each with a front panel containing a 6 cm^2 hole at floor level. Affixed to the square hole is a 6 cm^3 transparent enclosure that, on its lower horizontal surface, contains a 12 mm-diameter hole through which the rat can extent its tongue downward to reach the operandum. The operandum is an 18 mm-diameter aluminum disc rigidly attached to the shaft of a Model 31 load cell (0–250 g range, Sensotec, Columbus, OH). The disc is centered 2 mm below the hole in the plastic enclosure. A computer-controlled peristaltic pump (Series E at 14 rpm; Manostat Corp., New York, NY), fitted with a solid-state relay (Digikey, Thief River Falls, MN), delivers water to the center of the disc through a 0.5 mm-diameter hole. The force transducer was capable of resolving force measurements to 0.2-g equivalent weights. A PC recorded the transducer's force-time output sampled at 100 samples/s. Rats were exposed to the water-licking task during 6 min sessions until they licked reliably. The force requirement was 1 g to register a response and 12 licks were required to produce 0.05 ml of water. Each session started with a free 0.05 ml delivery of water. Rats were tested daily (5 days/week) until they achieved a stable baseline. Testing resumed within a week after surgeries and continued for four weeks post-surgery. Brains were then removed for tissue harvest.

2.3. 6-OHDA infusion

After achieving a stable baseline, rats were anesthetized with isoflurane (5% induction; 2% maintenance) and placed in a stereotaxic frame atop an isothermal heating pad. Rats in the unilateral group ($n = 8$) received 9.0 μg 6-OHDA (mixed in 0.9% NaCl with 0.02% ascorbate) into the right medial forebrain bundle (MFB; stereotaxic coordinates with respect to bregma: 6 mm posterior, 1.7 mm lateral, 7.0 mm ventral to the dural surface [34]). Rats in the bilateral lesion group ($n = 5$) received 3.0 μg 6-OHDA into the right and left MFB. The sham group ($n = 6$) received bilateral injections of saline (0.9% NaCl with 0.02% ascorbate) into the MFB. Infusion rate was 0.25 μL/min. Needles were left in place after infusions for 5 min and then slowly withdrawn. Animals were administered 0.05 mg/kg buprenorphine and 5 mg/kg ketoprofen post-surgery and were placed on a warm surface to prevent hypothermia until recovery.

2.4. Quantitative analysis

Orolingual motor function were assessed in terms of four dependent variables: (1) number of licks per session, (2) the rhythm of licking in licks/s, (3) the mean peak lick force (g), and (4) the maximum lick force (g). The number of licks was a count of the number of tongue contacts that equaled or exceeded 1 g. The lick rhythm was determined as follows: computation of the power spectra was performed by MatLab's Signal Processing Toolbox (The Math Works, Inc, Natick, MA). For this analysis, each 6-min session was divided into 35 series of 1024 samples from the lick-force transducer. With the Hanning data window selected, MatLab produced 35 corresponding power spectra. The power spectra were truncated to 25 Hz (based on prior work indicating little of behavioral interest beyond 25 Hz) and averaged together to yield a single power spectrum. A peak-find program written in Free Pascal was used to identify the peak in the averaged power spectrum, and the frequency at this peak was taken as the lick rhythm for a particular session. This method resolved lick rhythm to the nearest 0.1 Hz. The peak lick force was the mean of the peak forces exerted during a session, and the maximum lick force was the maximum force produced during a session. For statistical analyses, pre-lesion data for each variable were expressed as the mean of the values for the final three days prior to surgery. Post-lesion data were expressed as a percentage of the pre-lesion values for each week post-lesion, beginning with week 2 (due to the extent of response suppression during the first week post-lesion primarily in the bilateral group). Data for each measure were analyzed using a two-way analysis of variance (ANOVA) with group (sham vs unilateral vs bilateral) as the between-subjects variable and post-lesion time as the within-subjects repeated measure.

2.5. Analysis of dopamine content

On the 5th week post-lesion, brains were removed and placed in a chilled brain mold for sectioning as described previously [6,7,29,30]. Bilateral striatal sections were removed, weighed and frozen at −80 °C until processing. Striatal tissue was processed for analysis by high pressure liquid chromatography coupled with an electrochemical detector (HPLC-EC). Tissue samples were sonicated in 450 μL burnt, filtered citrate acetate mobile phase with 50 μL DHBA (0.1 mM) added to each sample. After sonication, tubes were placed in a cold centrifuge for 10 min at 12,000 rpm at 4 °C. Supernatant was then extracted and placed into Amicon Ultra 0.5 mL centrifugal filters and spun at 12,000 rpm for 1 h at 4 °C. HPLC-C analysis was performed on collected eluent using an isocratic HPLC coupled to a dual-channel Coulochem III electrochemical detector (ESA Inc., Chelmsford, MA, USA; Model 5011A, E_1 +0.35 mV and E_2 −0.25 mV using a 5011 dual analytical cell). The citrate acetate mobile phase was comprised octane sulfonic acid (0.07375 g/L), ethylenediaminetetraacetic acid (0.05 g/L), citric acid (14 g/L), sodium acetate trihydrate (13.8 g/L), triethylamine (0.01%), and methanol (4%). The mobile phase was made from filtered water using a Milli-Q purification system (Millipore) and was filtered through a 0.2 μm nylon membrane filter (Whatman). We used a 3 μm CAPCELL PAK reversed phase C-18 column (Shiseido). Dopamine content was expressed in ng/g tissue wet weight.

3. Results

3.1. Orolingual motor function

Pre-lesion values for body weight and the orolingual motor variables are provided in Table 1. None of the groups differed significantly with regard to body weight, tongue force, tongue motility or number of licks during these baseline measurements. Nigrostriatal DA depletion significantly attenuated tongue force in both lesion groups (Fig. 1). Although the effect for peak force did not differ significantly, maximum lick force was significantly decreased, $F = 5.236$, $p = 0.01$ (Fig. 2A and B). This effect was similar for the two 6-OHDA groups. Tongue motility was also decreased following 6-OHDA, but, unlike tongue force, only in the unilateral group, $F = 6.952$, $p < 0.01$ (Fig. 2C). The number of licks per second did