Published in final edited form as:

Behav Brain Res. 2011 November 20; 225(1): 97–103. doi:10.1016/j.bbr.2011.07.002.

Young and Middle-Aged Rats Exhibit Isometric Forelimb Force Control Deficits in a Model of Early-Stage Parkinson's disease

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Abstract

Deficits in manual motor control often accompany the early stages of Parkinson's disease (PD), and are often revealed through isometric force tasks. In order to determine whether similar deficits occur in a rat model of early-stage PD, young (8 months) and middle-aged (18 months) rats were trained to produce sustained press-hold-release isometric forelimb responses that allowed for analyses of force output and spectral analysis of forelimb stability and tremor. Rats then received a 6-hydroxydopamine (6-OHDA) infusion into the striatum contralateral to the trained forelimb and were tested for four weeks post-lesion. The resulting partial striatal dopamine depletions (which at $41 \pm 12\%$ and $43 \pm 6\%$ in young and middle-aged rats, respectively, did not differ between the two groups) resulted in isometric forelimb deficits. Specifically, rats exhibited significantly diminished force stability and increased high frequency (10–25 Hz) tremor, indicating potential postural disturbances and increased postural tremor respectively. Durations of press-hold-release bouts were also increased post-lesion, suggesting difficulty in task disengagement. Despite pre-lesion differences in some of the force measures, the effects of partial nigrostriatal DA depletion did not differ between the two age groups. These results support the use of the press-while-licking task in preclinical studies modeling isometric force control deficits in PD.

Keywords

Parkinson's disease; force control; isometric; forelimb; operant; behavioral; nigrostriatal; tremor; force release

1. Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disorder characterized primarily by progressive motor deficits. The cardinal symptoms, which include bradykinesia, muscle rigidity, postural disturbances and tremor, result from degeneration of

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dopamine (DA) neurons in the substantia nigra and DA depletion in the nigrostriatal pathway. The average age of onset is in the early 60s [53] and symptoms typically present with the loss of approximately 50% of DA neurons in the substantia nigra and greater than 70–80% loss of DA in the striatum [1, 12].

In addition to its cardinal symptoms, manual motor deficits are also present in PD [49]. These deficits can interfere with activities of daily living and are often experienced in early stages of disease [58]. Isometric force tasks are often used as instrumental measures of manual motor control in clinical studies [36, 37]. When executing isometric tasks, individuals with PD exhibit diminished force stability, alterations in the force frequency-power spectrum and deficits in task disengagement (i.e., force release). These deficits generally occur in the absence of frank muscle weakness. Because internally cued movements are especially affected in PD, these tasks are very sensitive indicators of parkinsonian motor decline [24, 25]. In fact, measures of isometric motor function correlate significantly with disease severity in PD [37].

Despite the use of isometric force tasks in clinical studies, to our knowledge there are no preclinical studies that have measured sustained isometric forelimb force during behavior in an animal model of PD. The goal of the current study was to determine whether partial unilateral nigrostriatal DA depletion affects isometric force control in rats in a manner similar to the effects of early-stage PD in humans. Rats were trained to produce sustained press-hold-release forelimb responses that allowed for analyses of force output and spectral analysis of stability and tremor [45, 48]. Although the effects of DA-blocking drugs have been measured in this task [13, 14, 42], the effects of nigrostriatal DA depletion on isometric force control have not been assessed in a model of PD. We also tested amphetamine-elicited rotation behavior as a more conventional assay of unilateral DA depletion [22]. Because the onset of PD typically occurs in late middle-age, we included two age groups in our study: a young group (8 months) and a middle-aged group (18 months). We hypothesized that rats with a mild unilateral DA depletion would exhibit deficits in contralateral forelimb force control and tremor, and that unlike amphetamine-elicited rotations [4] these effects would be greater in middle-aged animals.

2. Experimental procedures

Twelve male Sprague Dawley rats were obtained from Taconic (Taconic Farms, Inc., Hudson, NY). Animals were 8 (n=6) and 18 (n=6) months old and weighed 425 ± 38 g and 511 ± 49 g, respectively, at the time of the 6-OHDA lesion. Access to water was gradually restricted according to a schedule that allowed gradual weight gain. Animals were housed under a 12 hour lights on/off cycle and had access to food ad libitum. Protocols were approved by the University of Kansas Institutional Animal Care and Use Committee and procedures adhered to the Guide for the Care and Use of Laboratory Animals [33].

2.1. Apparatus

Behavioral training and experimental sessions were conducted in modified operant chambers that were enclosed in individual sound-attenuating cubicles as previously described [14, 45]. In each chamber, a cylindrical recess provided access to the 0.5 ml cup of a solenoid-operated dipper. In the front panel of each chamber was a rectangular aperture located such that a rat could reach with its forelimb the operandum located outside the chamber. The operandum was an 18 mm diameter disc rigidly attached to a Model 31 load cell (Sensotec, Columbus, OH, USA). The disc was oriented with its flat surface parallel to the floor of the chamber. The spatial arrangement of the aperture, the operandum and the raised dipper cup was such that the trained rat could press the operandum with only its right forepaw and drink from the dipper cup at the same time (Fig. 1). A LabMaster interface

(Scientific Solutions, Solon, OH, USA) received analog signals from the load cells, converted the signals to digital form with 12 bits of resolution, and routed the signals to a computer. Computer software recorded the force output of the transducer/operandum at 100 samples/s and with a force resolution of 0.33 g equivalent weights. Force was expressed in grams because gram units were used in the daily calibrations.

2.2. Operant training

Rats were trained through successive approximations to reach through the aperture and apply downward force upon the operandum using their right forepaw. A force of at least 20 g was required to activate the dipper for water reward. When force declined below 6.7 g the dipper was returned to the reservoir to be refilled. These contingencies produced discrete press-hold-release bouts between 4–10 s in duration. After rats successfully learned the task their responses were recorded during daily 8 min sessions for 4 weeks.

2.3. Surgery

At the conclusion of the 4 week training period rats were anaesthetized with sodium pentobarbital (50 mg/kg, ip) and placed in a stereotaxic frame under aseptic conditions. Body temperature was maintained during the surgery by placing the rat on an isothermal heating pad. A midline incision was made to expose the skull and a burr hole was placed in the skull over the left striatum (AP +1 mm, ML +3 mm vs. bregma) according to coordinates by Paxinos and Watson [35]. A 26 gauge dome tipped needle connected to a microliter syringe via Teflon tubing was lowered into the left striatum (5.0 mm from the dural surface). 6 µg 6-OHDA (4.0 µl, at 1.5 µg/µl, in 0.9 % saline with 0.1% ascorbic acid) was infused at a rate of 0.25 µL/min. The needle was left in place for an additional 5 min before being slowly withdrawn. Bone wax was applied, the wound was closed with wound clips, and rats were allowed to recover in their home cages.

2.4. Behavioral testing

Behavioral testing resumed the day following lesion and continued for 4 weeks. At 2 and 4 weeks post-lesion, all rats were injected with d-amphetamine sulfate (2.5 mg/kg, s.c) and placed in a rotometer for 60 minutes. The rotometer was a 26.5 cm diameter cylinder placed atop a force-sensing actometer surface [15]. Custom software quantified the number of rotations in untethered animals. On days of rotation analysis, rotation sessions followed operant behavioral sessions.

2.5. Forelimb data analysis

Data were analyzed by computer programs that recorded data and calculated several measures of behavior from these data. Time-on-task (TOT) was a measure of task engagement defined as the amount of time the rat spent applying at least one gram of force to the operandum during the session (somewhat analogous to response rate). Raw force-time data were then parsed offline by a computer program into individual 4.36 s waveforms. The program identified a waveform as a bout initiated by at least 20 g of force output and lasting at least 4.36 s (this value was arbitrarily defined, but was selected based on sampling requirements for spectral analysis; [41]). The number and durations of individual press-hold-release bouts were calculated for each rat. Waveforms were then ensemble averaged in the time domain for the first 4.36 s of the event. Mean force output during the stationary phase of the waveform (the latter 3.36 s portion) was determined from the averaged waveforms. To quantify force-related forelimb tremor, a separate program parsed and de-trended the 3.36 s stationary portion of the waveforms (the force variation during the first second of the response precludes its use in spectral analysis). The waveforms were then subjected to fast Fourier transformation using Autosignal (Systat Software Inc., San Jose, CA). Power spectra

were ensemble averaged in the frequency domain for each rat. Power was then integrated by calculating the area under the curve for the power spectrum for two frequency bands: 0–5 Hz and 10–25 Hz. This frequency band lies below the range of the natural frequency of the apparatus under unloaded (160 Hz) and loaded (>30 Hz) conditions as described previously [13]. The portion of the power spectrum below 5 Hz likely reflects slow postural and/or paw adjustments during the hold portion of the response. The 10–25 Hz frequency band reflects physiological tremor associated with isometric force [48]. Because force output influences power in this frequency band, isometric force-related tremor was expressed as the ratio of power in the 10–25 Hz band to mean force in order to account for elevated force in the older animals. Frequencies between 5 and 10 Hz are attributable to licking behavior [13], so this frequency band was excluded from the above analyses. We did, however, determine the dominant frequency in the 5–10 Hz band in order to determine whether licking rhythm (licks/second) was affected by age or by 6-OHDA.

2.6. Whole tissue levels of DA

At the end of the study, brains were removed and placed into a chilled brain mold. One-mm sections were made and bilateral striata were dissected freehand. Tissue from the left (lesioned) and right (unlesioned) side of the striatal section approximately 0–1 mm anterior to bregma were processed and analyzed for DA content as described previously [4, 11]. Briefly, DA levels were quantified using an isocratic high pressure liquid chromatography system coupled to a dual-channel electrochemical array detector (ESA Inc., Chelmsford, MA; Model 5100A, $E_1 + 0.35$ mV and $E_2 - 0.25$ mV using a 5011 dual analytical cell). The unlesioned striatum served as the control for calculating DA depletion.

2.7. Statistical analysis

For pre-lesion age effects, data for each behavioral measure were analyzed using independent samples t-tests. For post-lesion effects, data were analyzed using repeated-measures 2-way analyses of variance with age group (8 vs 18 months) as the between-subjects variable and time (pre-lesion, two weeks post-lesion and four weeks post-lesion) as the within-subjects repeating variable. DA depletion was calculated by dividing DA content in the lesioned striatum by DA content in the unlesioned striatum. Neurochemical data were analyzed using a one-way ANOVA with age as the between-subjects variable. In addition, Pearson's correlation coefficients were calculated for relationships between striatal DA depletion and each of the behavioral variables. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Operant task engagement and amphetamine-elicited rotation behavior

Prior to lesion, middle-aged animals exhibited decreased task engagement compared to young rats, $t(1,10)=7.33$, $p<0.05$ (Fig. 2a). Despite a ~50% post-lesion reduction in TOT in the young group, rats generally continued to engage in the task. The significant post-lesion decrease in TOT in the young, but not middle-aged, animals led to a significant age by time point interaction, $F(2,18)=6.45$, $p<0.01$. The number of amphetamine-elicited rotations did not differ between the two age groups at either 2 or 4 weeks post-lesion (Fig. 2b).

3.2. Force control

Representative raw force-time waveforms for a middle-aged rat prior to 6-OHDA and at two and four weeks post-6-OHDA are presented in Figure 3. Averaged force-time waveforms and power spectra for this rat are presented in Figure 4. Forelimb force output was greater for middle-aged rats than for young rats pre-lesion, $t(1,10)=8.80$, $p=0.01$ (Fig. 5a), likely due to greater body weight in the older animals. Forelimb force was significantly diminished

post-lesion in middle-aged (e.g., Fig. 4a), but not in young animals. This led to a significant age by time point interaction for this measure, $F(2,18) = 3.86$, $p < 0.05$ (Figure 5a). The disruption in isometric force steadiness following 6-OHDA is apparent in the force-time waveforms and power spectra. This instability, which increased power in the < 5 Hz region of the power spectrum (Fig. 4b), was exhibited by both age groups; $F(2,18) = 24.31$, $p < 0.0001$ (Fig. 5b). There were no pre- or post-lesion differences in this measure between the two age groups. Middle-aged rats exhibited decreased force-related tremor (power in the 10–25 Hz band normalized for mean force) compared to young rats pre-lesion, $t(1,10) = 14.28$, $p < 0.005$ (Fig. 6a), but this age effect was no longer significant following DA depletion. Force-related tremor increased in both groups post-lesion; $F(2,28) = 15.97$, $p < 0.0001$ (Fig. 6a). In addition to forelimb unsteadiness and tremor, increased durations of press-hold-release bouts are also apparent in Figure 3. Bout durations, which were significantly shorter in middle-aged rats pre-lesion, $t(1,10) = 5.57$, $p < 0.05$ (Fig. 6b), were increased in both groups following DA depletion; $F(2,12) = 11.11$, $p < 0.01$. The dominant (licking) frequency between 5–10 Hz did not differ between the two age groups nor was it affected by 6-OHDA (data not shown).

3.3. Dopamine depletion

DA depletion was $41.2 \pm 12.1\%$ for the young rats and $42.6 \pm 5.6\%$ for the middle-aged rats. These values did not differ significantly.

3.4. Relationships between behavioral variables and striatal DA depletion

Pearson's correlation coefficients calculated for relationships between striatal DA depletion and behavioral variables at the 4-week time point are provided in Table 1. The only variable that correlated significantly with DA depletion 4-weeks post-lesion was low frequency (< 5 Hz) power ($R^2 = 0.35$, $p < 0.05$; Fig. 7).

4. Discussion

We report here for the first time that, like individuals with PD, rats with partial nigrostriatal DA depletion exhibit deficits in isometric force control and increased forelimb tremor. Specifically, both young and middle-aged rats exhibited increases in the low (< 5 Hz) and high (10–25 Hz) frequency bands of the force-power spectrum, and increased durations of press-hold-release bouts. Force output was decreased post-lesion in middle-aged, but not in young animals, while task engagement was decreased in young, but not in middle-aged rats following 6-OHDA. There were no age-related differences in the number of amphetamine-elicited rotations or in the degree of striatal DA depletion following intrastratial 6-OHDA. Correlation analysis revealed that low frequency power exhibited the strongest relationship with striatal DA depletion at the 4-week time point.

Despite numerous reports of impaired forelimb function in animals following partial striatal DA depletion [2, 9, 28, 29, 54, 56], isometric force control has not been measured in a preclinical model of PD. Clinical studies report that individuals with PD experience early deficits in isometric force control [24, 25] and that these deficits correlate significantly with severity of disease [37]. In our study, rats with striatal DA depletion exhibited force control deficits that are analogous to those that have been reported in the clinic. It is important to note that due to the spatial arrangement of the apparatus, rats were only able to use their right (affected) forelimb to perform the task. The decreased steadiness, which increased power in the < 5 Hz region of the power spectrum, likely resulted from postural instability and increased paw adjustments during the execution of the learned forelimb response. Previous studies report abnormalities in postural reactions following unilateral 6-OHDA under diverse testing conditions, including spontaneous home cage behaviors [23],

investigator-mediated forelimb adjustment assays [40], and skilled forelimb reaching movements [32]. The elevated power in the low frequency band of the power spectrum, and the fact that this variable exhibited the strongest relationship with striatal DA depletion at the 4-week time point, is consistent with such phenomena.

The prolonged durations of the press-hold-release bouts parallel the difficulties in force release and task disengagement that have been reported in PD patients [25, 27]. It is unclear whether this deficit is related to prolonged agonist muscle activity during force production [55], disruption of agonist/antagonist muscle EMG relationships between the hold and release phases of the task [25], or to more central processes involved in sensorimotor processing and switching between individual components of learned motor sequences [3]. We can exclude as a possibility that it took longer for rats to lick the water from the dipper cup post-lesion, because there were no significant between-groups or post-lesion changes in the dominant forelimb frequency that is attributable to licking behavior. Furthermore, increased task engagement did not accompany increased response durations post-lesion. Unlike analogous clinical tasks that use external cues to indicate when force is to be released, force release in the press-while-licking task is under voluntary control. Because internally-cued movements are more vulnerable to nigrostriatal DA depletion, we believe that the increased durations of press-hold-release bouts reflect an impairment of sensorimotor processing by the basal ganglia [39]. Under these conditions, an internal cue indicating the need to release force to refill the dipper when the water had been consumed was likely disrupted. The fact that similar effects occur in this task when rats are administered DA receptor-blocking antipsychotic drugs [42, 43, 44] is consistent with a mechanism that involves disruption of nigrostriatal DA neurotransmission.

Our finding regarding elevated power in the 10–25 Hz band of the force-power spectrum following 6-OHDA is at odds with some previous studies in humans [21, 37, 52]. However, task- and species-related differences may account for this difference. For example, while continuous forelimb force was required as rats actively consumed water in our task, the human studies involved achieving and/or maintaining a target isometric force in isolation from other behavior. Because of this, it is possible that the demands of the press-while-licking task activate more postural mechanisms in quadrupedal rats than analogous hand and forearm-based tasks in humans. In this context, the increased high frequency power is consistent with increased high frequency, low amplitude postural or physiological tremor that is believed to reflect increased corticomuscular coherence in PD [7, 10]. Interestingly, the middle-aged rats exhibited lower force-related tremor pre-lesion than the young rats. This is consistent with human studies reporting decreases in force-related electro- and mechanomyography with age [51, 57]. Factors that may contribute to this effect include age-related decreases in type II muscle fibers, altered motor unit activity, changes in subcutaneous tissue composition, or increased intramuscular fat [26].

We did not challenge rats with increased force requirements as we have in earlier studies [48], so we cannot attribute the post-lesion decline in force exhibited by the middle-aged group to weakness. Many PD patients also show a decrease in force output [16, 34]. It is possible that striatal DA depletion and age-related changes in nigrostriatal and mesolimbic DA function [46] combined to affect effort-related processes [38] in the middle-aged group. The decrease in task engagement (TOT) in the young group may have resulted from similar processes manifested differently, since striatal DA depletion shifts the effort/reward-response curve to the left. It may have been that the respective age-related differences in task engagement and force output in the young and middle-aged groups prior to 6-OHDA were manifestations of excessive effort that were decreased following striatal DA depletion. Alternatively, striatal DA depletion may have affected postural processes or response

topography differently in the two age groups, leading to different effects on these measures. Future studies are necessary to test these hypotheses.

Our findings that partial striatal DA depletion disrupted forelimb force control similarly in young and middle-aged rats were consistent with previous studies comparing motor deficits in young and older 6-OHDA-lesioned rats [30, 50]. Despite age-related changes in nigrostriatal function [46], the ability of residual nigrostriatal terminals to increase activity following partial injury remains intact with increased age [31]. The absence of age-related differences in the total number of amphetamine-elicited rotations and striatal DA depletion following intrastratial 6-OHDA was likewise consistent with our recent work [4]. It could be argued that by analyzing an entire 1 mm section of striatum for DA content, we underestimated the amount of depletion at the injection site. However, we attempted to target the injection site for tissue analysis and our results were consistent with previous studies using intrastratial 6-OHDA in different age groups. Despite studies reporting enhanced DA depletion in older rats following intracerebroventricular or midbrain delivery of 6-OHDA [6, 31], intrastratial delivery produces a different result, at least at this time point [4, 30]. It is possible that the greater toxicity of 6-OHDA in the older brain was counteracted by age-related decreases in both the number and function of DA transporters [19, 20], resulting in diminished uptake of 6-OHDA into striatal DA terminals. These previous studies report significant decreases in striatal DA transporter binding sites and uptake capacity between 12–18 months of age in F344 rats. We did not measure DA transporters in this study, so testing this hypothesis awaits future studies.

Several weaknesses of our study should be mentioned. One potential weakness is that we did not sample discrete striatal subregions for tissue DA analysis [8]. Due to functional striatal heterogeneity, such analyses may have revealed more complex relationships between motor variables and DA depletion. Another weakness is that we did not test a therapeutic intervention in an attempt to ameliorate the motor effects of 6-OHDA. Atropine and L-dopa normalize postural deficits in rats following 6-OHDA [40], and anticholinergics reversed elevated 10–25 Hz force-related tremor in haloperidol-treated rats in the press-while-licking task used here [14]. Based on these findings, antiparkinson drugs should normalize power in the low and high frequency bands of the force-power spectrum. However, effects on prolonged response durations are hard to predict, since latency to release force in an isometric task has been reported to be resistant to treatment in PD patients [25]. Future studies testing antiparkinson drugs or neurotrophic factors such as GDNF [17] are warranted to further support the model.

5. Conclusions

Overall, these findings support the press-while-licking task as a translatable preclinical model of isometric force deficits in PD. As in PD, rats with partial striatal DA depletion exhibited forelimb force instability, deficits in force release and increased force-related tremor. Because these deficits were similar for middle-aged as for young rats, our findings suggest that early-stage isometric force deficits in PD can be sufficiently modeled in young adult animals.

Acknowledgments

This study was supported by NIH grants AG023549, AG026491, a Lied Endowed Basic Science Research Grant, and the Kansas Intellectual and Developmental Disabilities Research Center (HD02528). The authors would like to thank Cassi Johnson for editorial assistance with the manuscript.

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Figure 1.

(A) Time on task (TOT) and (B) amphetamine-elicited rotation behavior as a function of age and lesion. Prior to lesion, task engagement was significantly greater in young rats than in middle-aged rats ($*p<0.05$). Partial striatal DA depletion diminished TOT in young, but not middle-aged rats, leading to a significant age X lesion interaction ($**p<0.01$). The mean number of d-amphetamine-elicited rotations at post-lesion days 15 and 30 did not differ between the two age groups. Error bars = SEM.

Figure 2.

Raw force-time waveforms produced by a representative rat in the middle-aged group. Each row is a 14-second sample (time in seconds is on the abscissa) from sessions (A) prior to lesion and at (B) two weeks and (C) four weeks post-lesion. Aside from the regular ~7 Hz oscillations (produced by the rat's licking movements as they were transmitted to his forelimb muscles), the waveforms produced during the pre-lesion session were relatively flat and were ~7 seconds in duration. Notice how the waveforms became more irregular and their durations lengthened during the 2- and 4-week post-lesion sessions.

Figure 3. Averaged (A) force-time waveforms and (B) resulting power spectra from testing sessions sampled and depicted in Figure 2. These waveforms show the decrease in forelimb force by this representative middle-aged rat. They also show the post-lesion increased power in the low (<5 Hz) and higher (10–25 Hz) regions of the frequency band.

Figure 4.

Mean (A) hold force and (B) low frequency power (expressed in arbitrary units) as a function of age and lesion. Prior to lesion, forelimb force output was significantly greater in middle-aged rats (** $p < 0.01$). Partial striatal DA depletion diminished force in middle-aged but not in young rats, leading to a significant age X lesion interaction (* $p < 0.05$). Rats in both age groups exhibited increased power in the < 5 Hz region of the power spectrum post-lesion (** $p < 0.0001$). Error bars = SEM.

A **B** **Figure 5.**

Mean (A) force-related tremor (expressed as the ratio of integrated power in the 10–25 Hz band to mean force for each press-hold-release bout) and (B) duration of press-hold-release bouts as a function of age and lesion. Middle-aged rats exhibited decreased force-related tremor pre-lesion (** $p < 0.01$). Both age groups exhibited increased force-related tremor post-lesion (** $p < 0.0001$). Bout durations were significantly shorter in middle-aged rats pre-lesion (* $p < 0.05$), but were increased in both age groups post-lesion (** $p < 0.01$). Error bars = SEM.

Figure 6. Comparing relationships between striatal dopamine depletion and (A) rotation behavior, and (B) low frequency power at the 4-week time point. Unlike amphetamine-elicited rotation behavior, power in the < 5Hz region of the power spectrum correlated significantly with striatal DA depletion ($p < 0.05$).

Fig. 7. Comparing relationships between striatal dopamine depletion and (A) rotation behavior, and (B) low frequency power at the 4-week time point. Unlike amphetamine-elicited rotation behavior, power in the <5 Hz region of the power spectrum correlated significantly with striatal DA depletion ($p < 0.05$).

Table 1

Correlation coefficients (r) between striatal DA depletion and selected behavioral variables at the 4-week time point.

	Rotations	TOT	Force	Force-related tremor	Durations	Power < 5Hz
% DA depletion	-0.01	-0.12	0.17	0.07	-0.06	0.59 *

* p<0.05