Published in final edited form as:

Behav Brain Res. 2018 February 26; 339: 47–56. doi:10.1016/j.bbr.2017.10.036.

Characterization of oromotor and limb motor dysfunction in the DJ1 $-/-$ model of Parkinson disease

Katie M. Yang^a, Katherine V. Blue^{b,c}, Haleigh M. Mulholland^{b,c}, Meghna P. Kurup^{b,c}, Cynthia A. Kelm-Nelson^{b,c}, and Michelle R. Ciucci^{a,b,c}

^aNeuroscience Training Program, University of Wisconsin-Madison, Madison, WI, USA, 53706

^bDepartment of Communication Sciences and Disorders, University of Wisconsin–Madison, Madison, WI, USA, 53706

^cDepartment of Surgery, Division of Otolaryngology, University of Wisconsin–Madison, Madison, WI, USA, 53706

Abstract

Parkinson disease (PD) is devastating to sensorimotor function that includes cranial/oromotor and limb motor deficits. However, the onset, progression, and neural correlates of PD-related dysfunctions are poorly understood. To address this gap, we used a genetic rat model of PD, DJ1 $-/-$, and hypothesized that motor deficits would manifest early in the disease process, be progressive in nature, and be related to pathologies in brainstem structures associated with sensorimotor function. The present study compares homozygous DJ1 $-/-$ male rats to age-matched wild type controls. Progressive cranial sensorimotor function (ultrasonic vocalizations and tongue motor performance) and limb motor function (tapered balance beam) was analyzed at 2, 4, 6, and 8 months of age. Additionally, tyrosine hydroxylase cell counts were performed in the locus coeruleus and correlated to behavioral measures. We found that compared to wild type controls, DJ1 $-/-$ show deficits in ultrasonic vocalizations as well as oromotor (tongue) deficits that were progressive. Overtime, DJ1 $-/-$ rats cross a tapered balance beam with significantly decreased speed of traversal. Additionally, in the DJ1 $-/-$, tyrosine hydroxylase positive cells in the locus coeruleus are significantly reduced and are negatively correlated to oromotor behaviors. Characterizing the DJ1 $-/-$ model of PD provides important foundational work necessary to define behavioral and early-onset biomarkers that parallels early-stage PD pathology in humans.

Keywords

Parkinson disease; DJ1; rat; vocalization; cranial-sensorimotor; ultrasonic vocalization

Corresponding Author: Michelle R. Ciucci, Ph.D., 1975 Willow Drive, Madison, WI 53706, Phone: 1-608-262-6122, Ciucci@surgery.wisc.edu.

Conflicts of Interest:

The authors have no conflicts of interest to report.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Parkinson Disease (PD), the second most common neurodegenerative disorder, affects 2% of people worldwide [1]. PD is primarily diagnosed by signs and symptoms affecting limb motor function, including resting tremor, postural instability, and bradykinesia [2]. These signs are responsive to dopamine replacement that targets loss of dopaminergic cells in the substantia nigra [3]. However, many cranial sensorimotor signs and symptoms are refractory to standard dopamine replacement treatment. These include dysarthria and dysphagia, that affect up to 90% of patients with PD [4]. Little is known about the underlying neuropathology that contribute to these common PD deficits that are devastating to health and quality of life.

There are multiple challenges to studying early-phase PD, including the inability to study onset and progression in humans. Average age of diagnosis is 60 years old [5], but a substantial preclinical phase in which deficits are subtle remains underdiagnosed [6]. Recently, genetic mutations that lead to inherited forms of PD have been identified [7–9], and these mutations have led to the development of many novel animal models of PD that can be used to study the preclinical phase [10]. Germane to this study, loss of function mutations in the PARK7 gene encoding the DJ1 protein leads to an autosomal recessive form of inherited PD [11]. The DJ1 protein has a number of functions within the cell, including self-oxidation to protect against oxidative stress [12]. In particular, DJ1 acts as an antioxidant for ROS generated by mitochondrial homeostasis as well as by damaged mitochondria. This helps to regulate fission and mitophagy, and in cases of DJ1 mutations, these processes are increased. Further, DJ1 protects against alpha synuclein aggregation, a hallmark of PD pathology, by inhibiting oligomer formation [13]. Evidence supporting the use of rodent DJ1 $-/-$ models in the study of PD is mixed. DJ1 $-/-$ mice fail to show significant loss of dopaminergic neurons or limb motor deficits, as tested using an accelerating rotarod assay, but do show progressive deficits in an adhesive removal task as well as decreases in open field locomotion [14]. These animals also show enhanced vulnerability to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposure [15]. The DJ1 $-/-$ rat shows similar patterns in gross and fine motor deficits, with no significant deficits as measured by a rotarod test but decreases in open field mobility. In contrast, the DJ1 $-/-$ rat has progressive loss of nigral dopaminergic neurons (significant at 8 (mo) months of age) but no change in tyrosine hydroxylase (TH) immunoreactivity in the striatum [10]. Other studies show a number of compensatory changes within the striatum of DJ1 $-/-$ rats, including upregulation of vesicular monoamine transporter type-2 and D2 receptor expression [16]. Together, these studies suggest that the DJ1 $-/-$ rat model may be a useful tool to study early phase pathology; however, research has yet to investigate pathology outside of traditional nigrostriatal pathway and cranial sensorimotor dysfunction.

The aim of this work was to characterize the DJ1 $-/-$ rat model's potential for studying the deficits that occur within vocal communication and orolingual function, and to examine early-onset pathology within the locus coeruleus (LC), a brainstem region affected in early PD that directly contributes to systems of sensorimotor control. Specifically, TH cell loss within the LC has been correlated to deficits in limb motor and orolingual function [17]. In this study, DJ1 $-/-$ and age-matched wild type (WT) control rats were assessed at 2, 4, 6,

and 8 mo of age for ultrasonic vocalizations, licking behavior, and limb motor function. We hypothesized that DJ1 $-/-$ rats would show progressive deficits in these three areas compared to WT animals. Additionally, the LC was examined for cell loss at 8 mo of age, and we hypothesized that DJ1 $-/-$ rats would show a loss of LC neurons that would correlate to deficits found in behavior.

2. Experimental methodology

2.1 Animals and habituation

In this study, 32 male Long-Evans rats (DJ1 $-/-$, $n=16$; WT, $n=16$) and 6 WT female stimulus rats were acquired from SAGETM Research Labs (Boyertown, PA, USA; [10]) at 6 weeks of age and same sex pair-housed in standard polycarbonate cages (290 mm $\times$ 533 mm $\times$ 210 mm) on a 12-hour (hr) reverse light cycle. Females were used as a social stimulus for ultrasonic vocalization experiments. All acclimation and testing occurred in partial red light during the animal's dark cycle except where otherwise stated. Three DJ1 $-/-$ rats did not develop appropriately prior to testing and were replaced with three new animals from SAGE labs. Rats had ad libitum access to food and water, except during tongue task acclimation and testing (described below). All animals were handled by the experimenters in the testing room and placed into testing equipment chambers each day for two weeks prior to experiments. Procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee (IACUC protocol M02505) and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH, Bethesda, MA, [18]).

2.3 Behavioral testing and analysis

Animals underwent behavioral testing at 2, 4, 6, and 8 months (mo) of age. Order of animals was randomized on each testing day.

2.3.1 Body weights—Animals were weighed (g) at each testing time point using a digital scale.

2.3.2 50-kHz ultrasonic vocalizations—Ultrasonic vocalizations were recorded using a standard paradigm in a testing room separate from colony housing [17, 19]. The ultrasonic microphone (CM16; Avisoft, Berlin, Germany) has a flat frequency response up to 150-kHz, and a working frequency response range of 10–180-kHz. The microphone tip was placed 15 cm above the animal's home cage and was used to record 50-kHz ultrasonic vocalizations at sampling rate of 250-kHz, 16-bit depth. Both rats and humans produce meaningful vocalizations through restriction of laryngeal muscles to alter egressive airflow in response to social contact[20]. Therefore, ultrasonic vocalizations provide a useful analogue to human speech and can be used as a tool to look at vocal pathology[21]. During testing, each male rat was placed alone in the home cage and a sexually receptive female stimulus rat was placed in that cage for up to five minutes (min) or 2 successful mounts. The female was removed and the subsequent male-only vocalizations of the test subject were recorded for 90 seconds (sec). Male rats typically produce affective 50 kHz vocalizations in response to positive social experiences, such as play or copulation[22]. Allowing males to interact with a receptive female prior to recording allows for the collection of vocalizations from an

individual animal alone in the home cage, ensuring that the vocalizations collected are from the test subject.

Offline acoustic analysis was performed using a customized, automated program in Avisoft SASLab Pro (CM16, Avisoft, Germany). Vocalization spectrograms were generated with the frequency resolution set to a Fast Fourier Transformation of 512 points, frame size of 100%, flat top window, and the temporal resolution set to display 75% overlap. A high-pass filter was used to eliminate noise under 25-kHz to ensure automatic detection of calls was reliable. Experienced raters, masked to genotype, analyzed the files via acoustic and visual inspection of calls in order to classify them into frequency-modulated call categories [19, 23, 24]. Within each category, the following maximum and average acoustic variables were calculated for each animal: percent complex calls, duration (ms), bandwidth (kHz), intensity (dB), and peak frequency (kHz) [19, 23, 24]. Maximum is thought to represent the animal's highest capability while average represents the animal's performance across the full 90 second testing session.

2.3.3 Tongue function—Protrusive tongue force was examined using the custom set-up, as described previously [25–27]. A specialized device acquired tongue forces that allows for modification and measurement of force during licking. As this task involves a traditional learning paradigm, rats were water-restricted with access to water for 3 hrs per day during acclimation and testing for this task. Animals were acclimated to the procedure for two weeks prior to the first test session and were subsequently re-acclimated for one week prior to testing at 4, 6, and 8 mo. During the procedure, a 1 cm × 1 cm aperture and force operandum were placed in a standard polycarbonate cage (290 mm × 533 mm × 210 mm). An operant conditioning paradigm was used: rats were trained to press the operandum with their tongue in order to receive an aliquot of water reward on a variable-ratio 5 schedule. Animals were acclimated to this task for two weeks prior to testing. On the day of testing, rats were placed individually into a polycarbonate enclosure equipped with the aperture and force operandum that delivered aliquots of water based on the individual's tongue-press behaviors. When the rat pressed with the required force at or above their threshold, an aliquot of water was delivered. The required force increased based on the rat's previous press. Testing was performed over 3 days and data from the day with the highest participation values were analyzed, consistent with previous research (see Ciucci et al., 2011).

Tongue presses were recorded at 200Hz in a custom-designed computer data-acquisition software (Matrix Product Development, Cottage Grove, WI) and analyzed with custom-designed algorithms in Matlab software (MathWorks, Natick, MA) to measure the average of force per session millinewtons (mN), the standard deviation of force (mN), and the maximal of force per session in (mN). The average force during a session is a representation of the animal's mean performance across the testing session while the maximum press is thought to represent the animal's highest capability and ability to press only as hard as necessary. Variability of tongue force is the animal's variance in performance across the testing session and is thought to be a measure of how consistently animals can regulate their tongue force.

2.3.4 Tapered balance beam—To assess overall limb motor function, gait along a 165 cm long tapered balance beam (Lafayette Instrument Company, Lafayette, IN) was assayed. Animals were acclimated to this task for two weeks prior to testing. All testing and acclimation took place in a room illuminated by natural sunlight. Animals were required to traverse the beam toward the homepage for five trials. Video recordings of each side of the beam were obtained on testing days using two cameras (HDR-CX210; Sony, New York, NY), and analysis was performed by experienced raters masked to genotype. The following variables were measured: time to traverse the whole beam (sec), time to traverse the narrowest portion of the beam (last 1/3) (sec), and the number of foot faults, defined as the animal stepping off of the upper surface of the beam [28].

2.4 Tissue processing and immunohistochemistry

Four rats from each genotype were randomly selected to be euthanized following testing at 4 mo of age (not discussed in this study); the remaining animals (n=24) were euthanized at 8 mo of age. Briefly, animals were deeply anesthetized with isoflurane and transcardially perfused with saline followed by 150 ml of 4% ice-cold paraformaldehyde. Brains were extracted, post-fixed in paraformaldehyde for 24 hr, and transferred into a 30% sucrose solution. Brains were soaked for 48 hr to equilibrate before being cut into 60 micrometer (μ m) sections using a freezing-stage microtome. Tissue slices were stored at -20°C in anti-freeze solution (30% ethylene glycol, 30% glycerol in 0.1M phosphate buffered saline (PBS)). TH immunolabeling was assessed using immunohistochemistry.

For immunohistochemical procedures, free-floating brain tissue slices were transferred into PBS overnight. Endogenous peroxidase activity was quenched with a 0.5% hydrogen peroxide solution. Sections were blocked in 20% normal goat serum in PBS-T for 1 hr to reduce non-specific antibody binding, and subsequently incubated at 4° for 24 hr in primary antibody (TH: rabbit polyclonal; Millipore, Temecula, CA (AB152) at 1:2000 diluted in 1% normal goat serum and PBS. Primary antibodies were validated using positive control tissue (TH: wild type aged 8 mo substantia nigra brain tissue). For negative controls, experimental tissue with no incubation in primary antibody or in secondary antibody yielded no visible staining.

Following PBS washes, sections were incubated in the corresponding biotinylated secondary antibody (goat anti-rabbit from Millipore, Temecula, CA (AP132B) diluted at 1:500 in 1% normal goat serum and PBS. Sections were incubated in a streptavidin-biotin-horseradish peroxidase solution (Vector, UK) and visualized with diaminobenzidine tetrahydrochloride (DAB, Sigma Aldrich, St. Louis, MO) in PBS containing 0.3% hydrogen peroxide. Sections were mounted onto gelatin-coated slides, dehydrated, and coverslipped with Cytoseal (Richard-Allen Scientific, Kalamazoo, MI) mounting medium.

2.4.1 Image acquisition and analysis—Three anatomically equivalent sections that contained the LC were selected from each animal (n=12 WT, n=12 DJ1 $-/-$) for analysis. Images were taken with a DP80 Olympus camera attached to an Olympus BX53 upright microscope (Olympus, Madison, WI) at 20x and stitched to form a composite image of the entire region of interest. Composite images were then examined by an experienced rater

masked to condition using ImageJ Cell Counter Macro (National Institutes of Health, Bethesda, Maryland) to manually count the number of TH-ir cell soma in the LC. Cell counts from each image were summed to form a total cell count for each animal.

2.5 Statistical analysis

All statistical analyses were conducted with SigmaPlot® 12.5 (Systat Software, Inc., San Jose, CA). Dependent variables were analyzed using a 2×4 (ultrasonic vocalization and tongue task) or 2×3 (tapered balance beam) mixed model analysis of variance (ANOVA) with genotype (DJ1 $-/-$ or WT) and age (2, 4, 6, or 8 mo; 4, 6, or 8 mo) as independent variables. Normality was determined with the Brown-Forsythe Test and equal variance with the Shapiro-Wilk Test. Main effects as well as interactions were examined, and Fisher's least significant difference was used for post-hoc testing. Effect size was calculated and reported through partial eta (η) squared values. Further, Pearson's correlations were used to examine whether a significant relationship existed between body weight and experimental variables. A mixed model ANCOVA was used in order to examine the difference between groups when body weight was a significant covariate (this was only necessary for tapered balance beam traversal). Pearson's correlations were used to test relationships between LC cell counts and behavioral variables for DJ1 $-/-$ animals. The alpha level for significance was set *a priori* to 0.05. Inter- and intra-rater reliability was tested using Intraclass Correlations (ICC) performed on randomly selected overlapping testing files (10%) of the ultrasonic vocalizations as well as the tapered balance beam behavioral assessments as well as ImageJ cell counts. The ICC was required to be 0.9 or above in order for data from that rater to be considered reliable.

3. Results

3.1 Body weight

There was a significant main effect for genotype [$F(1, 99)=48.726$, partial $\eta^2=0.3298$, $p<0.001$] (see Figure 1) and age [$F(3, 99)=250.679$, partial $\eta^2=0.8836$, $p<0.001$] (data not shown), but no significant interaction [$F(3, 99)=1.192$, partial $\eta^2=0.03485$, $p=0.317$] for bodyweight. Specifically, all animals were significantly heavier in weight at each successive timepoint (2 vs 4, 4 vs 6 mo $p<0.001$; 6 vs 8 mo $p=0.017$). Further, DJ1 $-/-$ animals were significantly heavier than age-matched WT.

3.2 Call profile

3.2.1 Percentage complex calls—There was a significant main effect for genotype (WT, DJ1 $-/-$) [$F(1, 103)=17.005$, partial $\eta^2=0.1417$, $p<0.0001$] (see Figure 2A) and age (timepoint; 2, 4, 6, 8mo) [$F(3, 103)=6.524$, partial $\eta^2=0.1597$, $p<0.001$] (see Figure 2B) but no significant interaction. [$F(3, 103)=.228$, partial $\eta^2=0.0584$, $p=0.101$]. DJ1 $-/-$ animals had a greater overall percentage of complex ultrasonic vocalizations compared to WT animals. At 6 ($p=0.003$) and 8 ($p=0.008$) mo, animals produced more complex calls than at 2 mo animals. Additionally, at 6 ($p=0.018$) and 8 ($p=0.039$) mo animals produced more complex calls than at 4 mo.

3.3 Ultrasonic vocalizations: maximum variables

3.3.1 Maximum duration—There was a significant main effect for genotype [$F(1, 103)=22.88$, partial $\eta^2 = 0.1817$, $p<0.0001$] (see Figure 3A) and of age [$F(3, 103)=3.352$, partial $\eta^2=0.0889$, $p=0.022$] (see Figure 3B) but no significant interaction [$F(3, 103)=.228$, partial $\eta^2=0.0066$, $p=0.887$]. Specifically, at 4 mo animals produced longer calls compared to 2 mo ($p=0.045$). Additionally, DJ1 $-/-$ rats had a longer maximum duration compared to WT rats.

3.3.2 Maximum bandwidth—There was a significant main effect for age [$F(3, 103)=3.298$, partial $\eta^2 = 0.0876$, $p=0.023$] (see Figure 3C), but not genotype [$F(1, 103)=0.157$, partial $\eta^2 = 0.0015$, $p=0.692$] nor a significant interaction. [$F(3, 103)=0.563$, partial $\eta^2 = 0.0161$, $p=0.640$]. Specifically, 4 mo old animals produced calls with larger maximum bandwidth than 2 mo old animals ($p=0.038$).

3.3.3 Maximum intensity—There was a significant main effect for age [$F(3, 103)=10.385$, partial $\eta^2 = 0.2322$, $p<0.001$] (see Figure 3D), but not genotype [$F(1, 103)=0.149$, partial $\eta^2 = 0.0014$, $p=0.701$] nor a significant interaction. [$F(3, 103)=.508$, partial $\eta^2 = 0.0146$, $p=0.678$]. Post hoc comparisons show significant increases in the intensity of vocalizations at 4 ($p<0.001$), 6 ($p=0.001$), and 8 ($p=0.002$) mo of age compared to 2 mo.

3.3.4 Maximum peak frequency—There was no significant main effect for age [$F(3, 103)=0.472$, partial $\eta^2 = 0.0135$, $p=0.703$], genotype [$F(1, 103)=0.144$, partial $\eta^2 = 0.0014$, $p=0.705$] or significant interaction [$F(3, 103)=0.918$, partial $\eta^2 = 0.0260$, $p=0.435$].

3.4 Ultrasonic vocalizations: average variables

3.4.1 Average duration—There was a significant main effect for genotype [$F(1, 103)=24.863$, partial $\eta^2 = 0.1950$, $p<0.001$] (see Figure 4A) but not a significant effect for age [$F(3, 103)=2.626$, partial $\eta^2=0.0711$, $p=0.054$] nor a significant interaction [$F(3, 103)=0.395$, partial $\eta^2 = 0.0114$, $p=0.757$]. DJ1 $-/-$ animals had significantly longer calls compared to WT.

3.4.2 Average bandwidth—There was a significant main effect for age [$F(3, 103)=2.988$, partial $\eta^2 = 0.0801$, $p=0.035$] (see Figure 4B), but not genotype [$F(1, 103)=0.565$, partial $\eta^2 = 0.0055$, $p=0.454$] nor significant interaction. [$F(3, 103)=0.283$, partial $\eta^2 = 0.0082$, $p=0.838$]. Specifically, at 4mo animals produced calls with larger average bandwidth than at 2 mo ($p=0.038$).

3.4.3 Average intensity—There was a significant interaction between age and genotype [$F(3, 103)=3.094$, partial $\eta^2 = 0.0827$, $p=0.030$] (see Figure 4C). Specifically, at 4 mo animals produced louder calls than at 2 mo ($p=0.018$), while at 8 mo animals produced lower average intensity calls than 4 mo ($p=0.010$). There was a significant difference between WT and DJ1 $-/-$ at 8 mo of age ($p<0.001$) in that DJ1 $-/-$ animals produced quieter calls than WT.

3.4.4 Average peak frequency—There was a significant main effect for age [$F(3, 103)=2.718$, partial $\eta^2=0.0734$, $p=0.048$] (see Figure 4D), but not for genotype [$F(1, 103)=2.704$, partial $\eta^2=0.0256$, $p=0.103$] nor significant interaction [$F(3, 103)=0.527$, partial $\eta^2=0.0151$, $p=0.665$]. In general, at 8 mo vocalizations exhibited lower average peak frequency than at 2 mo ($p=0.040$).

3.5 Protrusive tongue force

3.5.1 Average tongue press—There was a significant interaction between the genotype and age [$F(3, 102)=7.649$, partial $\eta^2=0.1837$, $p<0.001$] (see Figure 5A). Specifically, DJ1 $-/-$ animals had a significantly higher average press force than their WT counterparts at 2 ($p=0.013$), 4 ($p<0.001$), and 6 ($p<0.001$) mo of age. However, WT animals had a significantly higher average press force than DJ1 $-/-$ animals at 8 mo of age ($p=0.031$). There was also a significant difference between age groups with 6 ($p<0.001$, $p<0.001$) and 8 ($p<0.001$, $p<0.001$) month animals having a higher average press force than 2 and 4 month old animals.

3.5.2 Standard deviation of average tongue press—There was a significant interaction between the genotype and age [$F(3, 102)=72.854$, partial $\eta^2=0.0774$, $p=0.041$] for the standard deviation of the average tongue press (see Figure 5B). Specifically, DJ1 $-/-$ animals had a significantly increased standard deviation of average press force compared to WT at 2 ($p<0.001$), 4 ($p<0.001$), 6 ($p<0.001$), and 8 ($p=0.049$) mo of age. There was also a significant difference between age groups with 6 ($p=0.001$, $p=0.046$) and 8 ($p<0.001$, $p<0.001$) mo animals having a higher average press force than 2 and 4 mo old animals.

3.5.3 Maximum tongue press—There was a significant effect for genotype [$F(1, 102)=163.323$, partial $\eta^2=0.6156$, $p<0.001$] (see Figure 5C) and for age [$F(3, 102)=3.379$, partial $\eta^2=0.0904$, $p=0.021$] (see Figure 5D), but no significant interaction [$F(3, 102)=1.699$, partial $\eta^2=0.0476$, $p=0.172$]. Animals pressed significantly harder at 8 mo compared to 2 mo of age ($p=0.018$). Further, DJ1 $-/-$ animals had a significantly greater maximum press at all timepoints compared to WT.

3.6 Tapered balance beam

3.6.1 Average time to traverse—There was a significant main effect for genotype [$F(1, 29)=6.65$, partial $\eta^2=0.4141$, $p<0.0153$] (see Figure 6A), but not a significant main effect for age [$F(2, 40)=1.72$, partial $\eta^2=0.0210$, $p=0.1929$] nor a significant interaction between age and genotype [$F(2, 40)=1.68$, partial $\eta^2=0.0770$, $p=0.200$]. DJ1 $-/-$ animals took a significantly longer time to traverse the whole beam compared WT.

3.6.2. Average time to traverse the last 1/3—There was a significant interaction between age and genotype [$F(2, 40)=8.80$, partial $\eta^2=0.1541$, $p<0.001$] (see Figure 6B). DJ1 $-/-$ animals took significantly longer to traverse the narrowest 1/3 compared to WT (6 mo $p=0.0027$, 8 mo $p=0.0302$).

3.6.3 Foot faults on whole beam—There was a significant interaction between age and genotype [$F(2, 73)=4.634$, partial $\eta^2=0.1127$, $p=0.013$]. WT animals made significantly

more foot faults at later timepoints (4 vs 8 mo, $p=0.032$, 6 vs 8 mo, $p=0.045$). Further, they had significantly more foot faults than DJ1 $-/-$ animals at 8 mo of age ($p<0.001$) (Table 1).

3.6.4 Foot faults on last 1/3—There was a significant interaction between age and genotype [$F(2, 73)=7.136$, partial $\eta^2=0.1635$, $p=0.001$]. WT animals made significantly more foot faults than DJ1 $-/-$ animals at 8 mo of age ($p<0.001$) (Table 1).

3.7 TH cell counts

There was a significant reduction in TH-ir cell body counts in the locus coeruleus of DJ1 $-/-$ animals at 8 mo of age compared to WT ($p<0.001$) (Figure 7A). The mean TH-IR cell count of WT animals was 475 with SEM 35.54 while DJ1 $-/-$ animals had an average of 249.52 with SEM 12.52. Pearson's correlations determined that these cell counts significantly negatively correlated with standard deviation of average press ($df=12$, $r=-0.580$, $p=0.0483$) and with maximum press ($df=12$, $r=-0.692$, $p=0.0126$).

3.8 Rater reliability

The ICC Index for these data are as follows: 0.97 for duration, 0.99 for bandwidth, 0.99 for peak frequency, 0.97 for intensity, 1.0 for average time to traverse, 1.0 for average time to traverse the last third, 1.0 for average foot faults, 1.0 for foot faults for the last 1/3, and 0.99 for dopaminergic cell counts.

4. Discussion

In this study, we compared the DJ1 $-/-$ rat to age-matched WT controls at 2, 4, 6, and 8 mo of age to assess deficits in a number of motor behaviors that occur in early-stage PD. Results show that DJ1 $-/-$ rats demonstrate early and progressive motor deficits, including those in vocal, tongue, and limb motor function. Further, these deficits present themselves prior to significant loss of nigrostriatal dopaminergic neurons as reported in Dave et al 2014, which suggests contributions from other sensorimotor regions may be responsible for these observed deficits. In the present study, DJ1 $-/-$ animals show a significant reduction of TH-immunopositive neurons in the LC at 8 mo of age compared to WT. Moreover, cell counts from DJ1 $-/-$ animals significantly correlate to maximum lingual force as well as standard deviation of lingual force suggesting a significant contribution of the LC system to tongue function. Overall, these results suggest that the DJ1 $-/-$ rat shows promise in modelling the complex neuropathology of early stage orolingual and limb motor deficits seen in PD.

4.1 DJ1 $-/-$ rats show increased body weights compared to WT animals

DJ1 $-/-$ rats were significantly heavier than WT animals at each testing timepoint. However, there was not an age by genotype interaction in that, suggesting that this is a static phenotype. This finding was unexpected as previous work examining the DJ1 $-/-$ rodent models found no differences in body weight [10, 14]. Further, patients with PD have been found to report loss rather than a gain of weight following disease onset [29]. Difficulty in eating and swallowing are well-documented in PD [30] and is often associated with malnutrition [31], which contributes to weight loss in humans. However, there are also a number of neuroendocrine abnormalities found in patients with PD, specifically in hormones

related to eating such as ghrelin[32] and glucagon-like peptide 1[33]. It is possible that these hormonal abnormalities manifest within this model but in a way that increases body weight. Future work should examine energy balance and feeding pattern differences in the DJ1 $-/-$ rat model.

4.2 DJ1 $-/-$ rats show increased complexity and differences in acoustic measures of ultrasonic vocalizations

Our work found that DJ1 $-/-$ rats produced significantly more frequency-modulated ultrasonic vocalizations compared to WT counterparts. Frequency-modulated USV production positively correlates to how rewarding the eliciting incentive is [34], suggesting that DJ1 $-/-$ may have differences in incentive salience coding compared to normal animals. Likewise, because early age timepoints were evaluated, these differences may even confer some neuroprotection to degradation of vocal systems. Previous work from our laboratory shows that increased levels of socialization, via experimenter handling and female conspecific exposure, mediated the development of deficits in USVs in a Pink1 $-/-$ model of PD [35].

DJ1 $-/-$ animals also show increases in maximum and average call duration compared to WT animals. Decreases in call duration have previously been reported to correlate with losses in striatal dopamine following 6-hydroxydopamine infusions [36]. Consistent with this report, DJ1 $-/-$ rats do not show loss of TH-immunoreactivity within the striatum through 8 mo of age, despite a significant loss of nigrostriatal dopamine neurons [10]. In contrast, DJ1 $-/-$ rats produced USV's with progressively lower average intensity compared to WT animals, though their maximum intensity was unchanged. Maximum values reflect the best performance of the animal in that particular parameter while average values reflects their overall performance over a 90-second span. Therefore, DJ1 $-/-$ rats' maximum capacity in intensity remained unchanged while average performance worsened. These deficits closely align with those experienced by patients with PD, who often report decreased loudness and difficulty being heard by family members [30, 37, 38]. However, with behavioral interventions such as LSVT-LOUD, patients with PD can be trained to increase loudness of speech, demonstrating that these deficits are responsive to exercise interventions [35, 36, 39–43].

There were a number of age effects on USV parameters that are consistent with previous work from our lab and others [17, 19, 23, 35, 36]. Specifically, 6 and 8 mo old animals produced more complex calls than 2 and 4 month old animals. Further, there were significant increases in maximum intensity from 2 mo old animals to 4, 6, and 8 month old animals. At 4 months, animals also produced calls with higher maximum intensity and higher maximum and average bandwidth compared to 2 mo old animals, which may be due to increased familiarity with the experimental environment in which USV testing occurs. Additionally, 8 mo old animals also produced calls with a lower average peak frequency than 2 month old animals. This could be due to the larger size of the animal, though body weight did not correlate with these data. Older animals experience declines in peak frequency of USVs, a parameter associated with positive affect [44].

4.3 DJ1 $-/-$ rats have increased maximum and average tongue forces as well as increased standard deviation of tongue force

DJ1 $-/-$ rats showed decreased ability to regulate tongue force in an operant conditioning lingual task. Their average press force values were significantly higher than their WT counterparts until 8 mo of age. Further, their standard deviation of average press was consistently higher than WT animals. Maximum press values were also consistently higher in DJ1 $-/-$ animals than WT animals. The protrusive tongue force deficits seen in this model are consistent with that which is seen in human patients with PD, who experience a number of orolingual tongue function deficits [30, 45–52]. Festinating tongue movement and poor bolus preparation are common signs and of dysphagia in PD [53]. These inability to properly form a bolus and transport it to the pharynx for swallowing lead to increased risk of aspiration pneumonia, the leading cause of death in PD [54]. Inabilities to grade motor control, including oromotor control, are common in PD, and so deficits that result in overshooting the target closely align with what is seen in patients [55]. Additionally, subtle changes to pressure variation from the velopharynx during swallowing in patients with early stage PD is detectable using high-resolution manometry [56]. Further, examination of changes to tongue muscle structure, including denervation and pathological changes to fiber type, as well as central neurodegeneration, may also elucidate the underlying pathology of these deficits.

Deficits in standard deviation of tongue force as well as maximum tongue force significantly negatively correlated to number of TH-immunopositive cell bodies in the LC. That is, the greater loss of neurons within the LC, the higher maximum tongue force and standard deviation of tongue force. The subcoeruleus complex synapses on protrusor motor neurons of the hypoglossal nucleus of the rat, and so loss of this modulating force may lead to deficits in tongue force regulation where animals overshoot their target [57]. Interestingly, previous work shows that nigrostriatal dopamine neuron loss in a 6-OHDA model of PD positively correlated to decreases in tongue force [17]. Differences in how these regions modulate motor behavior may account for the opposite relationships observed during neurodegeneration of these regions in PD models [58].

4.4 DJ1 $-/-$ rats take significantly longer to traverse a tapered balance beam

Consistent with other rodent models of PD, DJ1 $-/-$ rats show limb motor deficits, as measured by time to traverse a tapered balance beam. However, DJ1 $-/-$ made significantly less foot faults compared to WT animals. Due to the low number of foot faults made by animals during this task, these differences may not be biologically relevant. However, this may reflect a compensatory mechanism on the part of the DJ1 $-/-$ rats as these animals show differences in gait that could decrease the probability of an error using this task [10]. WT animals traversed the beam more quickly and thus, were more likely to make an additional foot faults due to speed. Deficits in gross motor function in PD, including bradykinesia, postural instability, and gait abnormalities, have been well-defined [2]. DJ1 $-/-$ rats show a significant decrease in TH-immunopositive cell bodies of the substantia nigra at 8 mo of age, thus, degeneration of the basal ganglia appears to contribute to the development of motor dysfunction in this model. Further, other studies have shown that DJ1

–/– rats show lower hindlimb grip strength as well as wider variability in hindlimb placement during gait analysis [10].

4.4 DJ1 –/– rats show TH-immunopositive cell loss in the LC at 8 months of age

DJ1 –/– rats showed a decrease in TH-immunopositive cell body counts in the LC at 8 mo of age compared to WT animals. According to the Braak staging model of PD, the LC shows early signs of pathology, including alpha synuclein aggregation and degeneration, that worsen as the disease progresses and ascends from the brainstem into more rostral regions [59, 60]. Though the LC has widespread projections to many regions, including the thalamus and cortical sensory regions, its contributions to PD pathophysiology are often overlooked [61, 62]. Further examination of altered neurotransmission and compensatory mechanisms of the LC would provide a broader context for understanding those contributions as we only examined loss of LC cell bodies positive for TH, not dopamine beta hydroxylase. The *Pink1* –/– rat model of PD also shows a significant loss of LC TH-immunopositive neurons, suggesting that this may be a common feature of rodent PD models [17]. Further work is needed to examine whether loss of these neurons is progressive.

4.5 Future directions and other considerations

Our work characterizing the DJ1 –/– rat model has demonstrated that it may be a useful model for studying the complex pathology underlying early stage oromotor deficits of PD. It is the first to examine this critically overlooked area of PD behavioral and neural pathology. Future studies should examine changes to central brain regions controlling oromotor behavior, including the nucleus ambiguus and hypoglossal nucleus, as well as peripheral structures such as the larynx, pharynx, and tongue. Better characterization of pathology will lead to more targeted treatments for speech and swallowing deficits in PD, and so this work provides a foundation upon which to build a better understanding of how oromotor deficits manifest in PD.

Acknowledgments

Funding:

Howard Hughes Medical Institute Gilliam Fellowship (Yang), R01 DC014358 (Ciucci), American Speech Hearing Association (Ciucci), Michael J Fox Foundation (Ciucci), Department of Surgery-Division of Otolaryngology (Ciucci), F32 DC014399 (NIDCD, National Institutes of Health) (Kelm-Nelson).

We would like to acknowledge Lauren Holt, Brooke Resch, and Angel Chavez for aiding in data collection and analysis. Further, we would like to thank John Szot for his managerial support as well as Dr. Glen Levenson and Dr. Xing Wang for their aid in statistical analysis.

References

1. de Lau LM, Giesbergen PC, de Rijk MC, Hofman A, Koudstaal PJ, Breteler MM. Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. *Neurology*. 2004; 63(7):1240–4. [PubMed: 15477545]
2. Lees AJ, Hardy J, Revesz T. Parkinson's disease. *Lancet*. 2009; 373(9680):2055–66. [PubMed: 19524782]
3. Marsot A, Guilhaumou R, Azulay JP, Blin O. Levodopa in Parkinson's Disease: A Review of Population Pharmacokinetics/Pharmacodynamics Analysis. *J Pharm Pharm Sci*. 2017; 20(0):226–238. [PubMed: 28719359]

4. Baijens LW, Speyer R. Effects of therapy for dysphagia in Parkinson's disease: systematic review. *Dysphagia*. 2009; 24(1):91–102. [PubMed: 18931877]
5. Pagano G, Ferrara N, Brooks DJ, Pavese N. Age at onset and Parkinson disease phenotype. *Neurology*. 2016; 86(15):1400–7. [PubMed: 26865518]
6. Noyce AJ, Lees AJ, Schrag AE. The prediagnostic phase of Parkinson's disease. *Journal of neurology, neurosurgery and psychiatry*. 2016; 87(8):871–8.
7. Bonifati V. Autosomal recessive parkinsonism. *Parkinsonism & related disorders*. 2012; 18(Suppl 1):S4–6. [PubMed: 22166450]
8. Bonifati V, Dekker MC, Vanacore N, Fabbri G, Squitieri F, Marconi R, Antonini A, Brustenghi P, Dalla Libera A, De Mari M, Stocchi F, Montagna P, Gallai V, Rizzu P, van Swieten JC, Oostra B, van Duijn CM, Meco G, Heutink PN. Italian Parkinson Genetics, Autosomal recessive early onset parkinsonism is linked to three loci: PARK2, PARK6, and PARK7. *Neurol Sci*. 2002; 23(Suppl 2):S59–60. [PubMed: 12548343]
9. Guo JF, Wang L, He D, Yang QH, Duan ZX, Zhang XW, Nie LL, Yan XX, Tang BS. Clinical features and [11C]-CFT PET analysis of PARK2, PARK6, PARK7-linked autosomal recessive early onset Parkinsonism. *Neurol Sci*. 2011; 32(1):35–40. [PubMed: 20607337]
10. Dave KD, De Silva S, Sheth NP, Ramboz S, Beck MJ, Quang C, Switzer RC Iii, Ahmad SO, Sunkin SM, Walker D, Cui X, Fisher DA, McCoy AM, Gamber K, Ding X, Goldberg MS, Benkovic SA, Haupt M, Baptista MAS, Fiske BK, Sherer TB, Frasier MA. Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. *Neurobiology of Disease*. 2014; 70(0):190–203. [PubMed: 24969022]
11. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science*. 2003; 299(5604):256–9. [PubMed: 12446870]
12. Jiang T, Sun Q, Chen S. Oxidative stress: A major pathogenesis and potential therapeutic target of antioxidative agents in Parkinson's disease and Alzheimer's disease. *Prog Neurobiol*. 2016; 147:1–19. [PubMed: 27769868]
13. Ryan BJ, Hoek S, Fon EA, Wade-Martins R. Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. *Trends Biochem Sci*. 2015; 40(4):200–10. [PubMed: 25757399]
14. Chen L, Cagniard B, Mathews T, Jones S, Koh HC, Ding Y, Carvey PM, Ling Z, Kang UJ, Zhuang X. Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice. *J Biol Chem*. 2005; 280(22):21418–26. [PubMed: 15799973]
15. Kim RH, Smith PD, Aleyasin H, Hayley S, Mount MP, Pownall S, Wakeham A, You-Ten AJ, Kalia SK, Horne P, Westaway D, Lozano AM, Anisman H, Park DS, Mak TW. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and oxidative stress. *Proc Natl Acad Sci U S A*. 2005; 102(14):5215–20. [PubMed: 15784737]
16. Sun J, Kouranova E, Cui X, Mach RH, Xu J. Regulation of dopamine presynaptic markers and receptors in the striatum of DJ-1 and Pink1 knockout rats. *Neurosci Lett*. 2013; 557(Pt B):123–8. [PubMed: 24157858]
17. Grant LM, Kelm-Nelson CK, Hilby BL, Blue KV, Rajamanickam ESP, Pultorak J, Fleming SM, Ciucci MR. Evidence for early and progressive ultrasonic vocalization and oromotor deficits in a PINK1 knockout rat model of Parkinson disease. *J Neurosci Res*. 2015; 93(11):1713–27. [PubMed: 26234713]
18. N.R. Council. Guide for the care and use of laboratory animals. National Academy Press; Washington D.C: 1996.
19. Ciucci MR, Ma ST, Fox C, Kane JR, Ramig LO, Schallert T. Qualitative changes in ultrasonic vocalization in rats after unilateral dopamine depletion or haloperidol: A preliminary study. *Behavioural Brain Research*. 2007; 182(2):284–289. [PubMed: 17397940]
20. Riede T. Subglottal pressure, tracheal airflow, and intrinsic laryngeal muscle activity during rat ultrasound vocalization. *J Neurophysiol*. 2011; 106(5):2580–92. [PubMed: 21832032]

21. Johnson AM, Grant LM, Schallert T, Ciucci MR. Changes in Rat 50-kHz Ultrasonic Vocalizations During Dopamine Denervation and Aging: Relevance to Neurodegeneration. *Curr Neuropharmacol*. 2015; 13(2):211–9. [PubMed: 26411763]
22. Wohr M, Schwarting RK. Ultrasonic communication in rats: can playback of 50-kHz calls induce approach behavior? *PLoS One*. 2007; 2(12):e1365. [PubMed: 18159248]
23. Johnson AM, Doll EJ, Grant LM, Ringel L, Shier JN, Ciucci MR. Targeted Training of Ultrasonic Vocalizations in Aged and Parkinsonian Rats. *Journal of Visualized Experiments*. 2011; (54):e2835.
24. Ciucci MR, Ahrens AM, Ma ST, Kane JR, Windham EB, Woodlee MT, Schallert T. Reduction of dopamine synaptic activity: Degradation of 50-kHz ultrasonic vocalization in rats. *Behav Neurosci*. 2009; 123(2):328–336. [PubMed: 19331456]
25. Ciucci MR, Connor NP. Dopaminergic influence on rat tongue function and limb movement initiation. *Exp Brain Res*. 2009; 194(4):587. [PubMed: 19247644]
26. Connor NP, Russell JA, Wang H, Jackson MA, Mann L, Kluender K. Effect of tongue exercise on protrusive force and muscle fiber area in aging rats. *J Speech Lang Hear Res*. 2009; 52(3):732. [PubMed: 18723593]
27. Ciucci MR, Russell JA, Schaser AJ, Doll EJ, Vinney LM, Connor NP. Tongue force and timing deficits in a rat model of Parkinson disease. *Behavioural brain research*. 2011; 222(2):315–320. [PubMed: 21459116]
28. Fleming SM, Ekhatior OR, Ghisays V. Assessment of Sensorimotor Function in Mouse Models of Parkinson's Disease. *Journal of Visualized Experiments*. 2013; (76):e50303.
29. Beyer PL, Palarino MY, Michalek D, Busenbark K, Koller WC. Weight change and body composition in patients with Parkinson's disease. *J Am Diet Assoc*. 1995; 95(9):979–83. [PubMed: 7657912]
30. Ciucci MR, Grant LM, Rajamanickam ES, Hilby BL, Blue KV, Jones CA, Kelm-Nelson CA. Early identification and treatment of communication and swallowing deficits in Parkinson disease. *Seminars in speech and language*. 2013; 34(3):185–202. [PubMed: 24166192]
31. Sheard JM, Ash S, Silburn PA, Kerr GK. Prevalence of malnutrition in Parkinson's disease: a systematic review. *Nutr Rev*. 2011; 69(9):520–32. [PubMed: 21884132]
32. Song N, Wang W, Jia F, Du X, Xie A, He Q, Shen X, Zhang J, Rogers JT, Xie J, Jiang H. Assessments of plasma ghrelin levels in the early stages of parkinson's disease. *Movement disorders: official journal of the Movement Disorder Society*. 2017
33. Li Y, Perry T, Kindy MS, Harvey BK, Tweedie D, Holloway HW, Powers K, Shen H, Egan JM, Sambamurti K, Brossi A, Lahiri DK, Mattson MP, Hoffer BJ, Wang Y, Greig NH. GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. *Proc Natl Acad Sci U S A*. 2009; 106(4):1285–90. [PubMed: 19164583]
34. Willuhn I, Tose A, Wanat MJ, Hart AS, Hollon NG, Phillips PE, Schwarting RK, Wohr M. Phasic dopamine release in the nucleus accumbens in response to pro-social 50 kHz ultrasonic vocalizations in rats. *J Neurosci*. 2014; 34(32):10616–23. [PubMed: 25100595]
35. Kelm-Nelson CA, Yang KM, Ciucci MR. Exercise Effects on Early Vocal Ultrasonic Communication Dysfunction in a PINK1 Knockout Model of Parkinson's Disease. *J Parkinsons Dis*. 2015; 5(4):749–63. [PubMed: 26577653]
36. Kelm-Nelson CA, Brauer AF, Ciucci MR. Vocal training, levodopa, and environment effects on ultrasonic vocalizations in a rat neurotoxin model of Parkinson disease. *Behav Brain Res*. 2016; 307:54–64. [PubMed: 27025445]
37. Rusz J, Cmejla R, Ruzickova H, Ruzicka E. Quantitative acoustic measurements for characterization of speech and voice disorders in early untreated Parkinson's disease. *J Acoust Soc Am*. 2011; 129(1):350–67. [PubMed: 21303016]
38. Stewart C, Winfield L, Hunt A, Bressman SB, Fahn S, Blitzer A, Brin MF. Speech dysfunction in early Parkinson's disease. *Movement disorders: official journal of the Movement Disorder Society*. 1995; 10(5):562–5. [PubMed: 8552106]
39. Atkinson-Clement C, Sadat J, Pinto S. Behavioral treatments for speech in Parkinson's disease: meta-analyses and review of the literature. *Neurodegener Dis Manag*. 2015; 5(3):233–48. [PubMed: 26107322]

40. Fox CM, Ramig LO, Ciucci MR, Sapir S, McFarland DH, Farley BG. The science and practice of LSVT/LOUD: neural plasticity-principled approach to treating individuals with Parkinson disease and other neurological disorders. *Seminars in speech and language*. 2006; 27(4):283–99. [PubMed: 17117354]
41. Ramig LO, Sapir S, Countryman S, Pawlas AA, O'Brien C, Hoehn M, Thompson LL. Intensive voice treatment (LSVT) for patients with Parkinson's disease: a 2 year follow up. *Journal of neurology, neurosurgery and psychiatry*. 2001; 71(4):493–8.
42. Russell JA, Ciucci MR, Connor NP, Schallert T. Targeted exercise therapy for voice and swallow in persons with Parkinson's disease. *Brain Res*. 2010; 1341:3–11. [PubMed: 20233583]
43. Sapir S, Spielman JL, Ramig LO, Story BH, Fox C. Effects of intensive voice treatment (the Lee Silverman Voice Treatment [LSVT]) on vowel articulation in dysarthric individuals with idiopathic Parkinson disease: acoustic and perceptual findings. *J Speech Lang Hear Res*. 2007; 50(4):899–912. [PubMed: 17675595]
44. Basken JN, Connor NP, Ciucci MR. Effect of aging on ultrasonic vocalizations and laryngeal sensorimotor neurons in rats. *Exp Brain Res*. 2012; 219(3):351–61. [PubMed: 22562586]
45. Fuh JL, Lee RC, Wang SJ, Lin CH, Wang PN, Chiang JH, Liu HC. Swallowing difficulty in Parkinson's disease. *Clin Neurol Neurosurg*. 1997; 99(2):106–12. [PubMed: 9213054]
46. Kalf JG, de Swart BJ, Bloem BR, Munneke M. Prevalence of oropharyngeal dysphagia in Parkinson's disease: a meta-analysis. *Parkinsonism & related disorders*. 2012; 18(4):311–5. [PubMed: 22137459]
47. Leopold NA, Kagel MC. Laryngeal deglutition movement in Parkinson's disease. *Neurology*. 1997; 48(2):373–6. [PubMed: 9040724]
48. Leopold NA, Kagel MC. Pharyngo-esophageal dysphagia in Parkinson's disease. *Dysphagia*. 1997; 12(1):11–8. discussion 19–20. [PubMed: 8997827]
49. Nagaya M, Kachi T, Yamada T, Igata A. Videofluorographic study of swallowing in Parkinson's disease. *Dysphagia*. 1998; 13(2):95–100. [PubMed: 9513304]
50. Robbins JA, Logemann JA, Kirshner HS. Swallowing and speech production in Parkinson's disease. *Ann Neurol*. 1986; 19(3):283–7. [PubMed: 3963773]
51. Troche MS, Sapienza CM, Rosenbek JC. Effects of bolus consistency on timing and safety of swallow in patients with Parkinson's disease. *Dysphagia*. 2008; 23(1):26–32. [PubMed: 17551793]
52. Wintzen AR, Badrising UA, Roos RA, Vielvoye J, Liauw L. Influence of bolus volume on hyoid movements in normal individuals and patients with Parkinson's disease. *Can J Neurol Sci*. 1994; 21(1):57–9. [PubMed: 8180907]
53. Ertekin C, Tarlaci S, Aydogdu I, Kiylioglu N, Yuceyar N, Turman AB, Secil Y, Esmeli F. Electrophysiological evaluation of pharyngeal phase of swallowing in patients with Parkinson's disease. *Movement disorders: official journal of the Movement Disorder Society*. 2002; 17(5):942–9. [PubMed: 12360543]
54. Beyer MK, Herlofson K, Arslan D, Larsen JP. Causes of death in a community-based study of Parkinson's disease. *Acta Neurol Scand*. 2001; 103(1):7–11. [PubMed: 11153892]
55. Rosen KM, Kent RD, Duffy JR. Task-based profile of vocal intensity decline in Parkinson's disease. *Folia Phoniatr Logop*. 2005; 57(1):28–37. [PubMed: 15655339]
56. Jones CA, Ciucci MR. Multimodal Swallowing Evaluation with High-Resolution Manometry Reveals Subtle Swallowing Changes in Early and Mid-Stage Parkinson Disease. *J Parkinsons Dis*. 2016; 6(1):197–208. [PubMed: 26891176]
57. Aldes LD. Topographically organized projections from the nucleus subceruleus to the hypoglossal nucleus in the rat: a light and electron microscopic study with complementary axonal transport techniques. *J Comp Neurol*. 1990; 302(3):643–56. [PubMed: 1702122]
58. Kane JR, Ciucci MR, Jacobs AN, Tews N, Russell JA, Ahrens AM, Ma ST, Britt JM, Cormack LK, Schallert T. Assessing the role of dopamine in limb and cranial-ormotor control in a rat model of Parkinson's disease. *J Commun Disord*. 2011; 44(5):529–37. [PubMed: 21820129]
59. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of aging*. 2003; 24(2):197–211. [PubMed: 12498954]

60. Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. *Cell and tissue research*. 2004; 318(1):121–34. [PubMed: 15338272]
61. Del Tredici K, Braak H. Dysfunction of the locus coeruleus-norepinephrine system and related circuitry in Parkinson's disease-related dementia. *Journal of neurology, neurosurgery and psychiatry*. 2013; 84(7):774–83.
62. Del Tredici K, Braak H. Lewy pathology and neurodegeneration in premotor Parkinson's disease. *Movement disorders: official journal of the Movement Disorder Society*. 2012; 27(5):597–607. [PubMed: 22508278]

Highlights

- DJ1 $-/-$ rats develop early and progressive vocalization and oromotor deficits
- DJ1 $-/-$ rats are slow to traverse a tapered beam
- At 8 months of age DJ1 $-/-$ rats have reductions in tyrosine hydroxylase cells in the locus coeruleus

Figure 1. Body weight is increased in DJ1

-/- rats. DJ1 -/- animals (dark gray bar) were significantly heavier in weight (grams) compared to wild type controls (light gray bar). For box plots: the 25th percentile (line closest to 0), the median (line within the box), the 75th percentile (line furthest from 0), 10th and 90th percentiles (whiskers above and below the box), and outlying data points (open circles below and above the whiskers). Asterisks represents statistical significance (***) ($p < 0.001$).

Figure 2. Percentage of complex ultrasonic calls is increased in DJ $-/-$ rats

(A) Percentage of complex frequency-modulated calls by age (white bars, data collapsed at each testing timepoint). (B) Percentage complex frequency-modulated calls (bars collapsed by genotype). Wild type is gray bars, DJ1 $-/-$ is dark gray. For box plots: the 25th percentile (line closest to 0), the median (line within the box), the 75th percentile (line furthest from 0), 10th and 90th percentiles (whiskers above and below the box), and outlying data points (open circles below and above the whiskers). Lines represent statistical significance between two testing timepoints. Asterisks represents statistical significance (*** $p < 0.001$, * $p < 0.05$).

Figure 3. Maximum acoustic USV metrics are dependent on age and genotype

(A) Maximum USV duration by age (white bars, data collapsed at each testing timepoint). (B) Maximum USV duration collapsed by genotype. Wild type is gray bars, DJ1 $-/-$ is dark gray. (C) Maximum bandwidth by age. (D) Maximum intensity by age. For box plots: the 25th percentile (line closest to 0), the median (line within the box), the 75th percentile (line furthest from 0), 10th and 90th percentiles (whiskers above and below the box), and outlying data points (open circles below and above the whiskers). Lines represent statistical significance between two testing timepoints. Asterisks represents statistical significance (***) $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 4. Average acoustic USV metrics are dependent on age and genotype

(A) Average duration by genotype. Wild type is gray bars, DJ1^{-/-} is dark gray. (B) Average bandwidth by age (white bars, data collapsed at each testing timepoint). (C) Average intensity by genotype and age; there was a significant interaction between age and genotype. Solid line is wild type, dashed line is DJ1^{-/-}. Bars represent standard error of the mean (SEM) at each testing timepoint. (D) Average peak frequency by age. For box plots: the 25th percentile (line closest to 0), the median (line within the box), the 75th percentile (line furthest from 0), 10th and 90th percentiles (whiskers above and below the box), and outlying data points (open circles below and above the whiskers). Lines represent statistical significance between two testing timepoints. Asterisks represents statistical significance (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 5. DJ1^{-/-} animals show deficits in licking force and timing

(A) Average tongue force by genotype and age. (B) Standard deviation of average tongue force by genotype and age. Solid line is wild type, dashed line is DJ1^{-/-}. Bars represent standard error of the mean (SEM) at each testing timepoint. (C) Maximum tongue force by genotype. Wild type is gray bars, DJ1^{-/-} is dark gray. (D) Maximum tongue force by age (white bars, data collapsed at each testing timepoint. For box plots: the 25th percentile (line closest to 0), the median (line within the box), the 75th percentile (line furthest from 0), 10th and 90th percentiles (whiskers above and below the box), and outlying data points (open circles below and above the whiskers). Lines represent statistical significance between two testing timepoints. Asterisks represents statistical significance (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 6. DJ1^{-/-} animals are slow to traverse the tapered balance beam
 (A) Time to traverse the whole beam (sec). Wild type is gray bars, DJ1^{-/-} is dark gray. (B) Average time to traverse the narrowest third of the beam (sec) over testing timepoints $\pm$ standard error of the mean (SEM). Solid line is wild type, dashed line is DJ1^{-/-}. For box plots: the 25th percentile (line closest to 0), the median (line within the box), the 75th percentile (line furthest from 0), 10th and 90th percentiles (whiskers above and below the box), and outlying data points (open circles below and above the whiskers). Asterisks represents statistical significance (*** p < 0.001, ** p < 0.01, * p < 0.05).

Figure 7. Reductions in locus coeruleus tyrosine hydroxylase-labeled cells are correlated to lingual function in DJ1 $-/-$ rats

At 8 months of age, (A) Tyrosine hydroxylase (TH) immunoreactive cell counts in the locus coeruleus of wild type (light gray bar) and DJ1 $-/-$ (dark gray bar) rats. (B) Representative photomicrographs taken at 20X magnification in a wild type and DJ1 $-/-$ rat. Scale bar in DJ1 $-/-$ photo is 50 μ m. Abbreviation: 4V: fourth ventricle. (C) Average standard deviation of the average press and locus coeruleus cell counts. (D) Average maximum press and locus coeruleus cell counts. Red line indicates significant correlation. Correlation r -values and p values are described in the results section. Each dark circle represents one individual DJ1 $-/-$ rat. For box plots: the 25th percentile (line closest to 0), the median (line within the box), the 75th percentile (line furthest from 0), 10th and 90th percentiles (whiskers above and below the box), and outlying data points (open circles below and above the whiskers). Asterisks represents statistical significance (***) $p < 0.001$.

Table 1

Tapered balance beam foot fault data. Means (SEM) at each testing timepoint.

	Genotype	4 months	6 months	8 months
Whole Beam Foot Faults	WT	0.867 (0.20)	0.817 (0.136)	1.8 (0.338)
	DJ1 $-/-$	0.7875 (0.143)	0.467 (0.086)	0.45 (0.096)
Last Third Foot Faults	WT	0.507 (0.108)	0.65 (0.128)	1.25 (0.224)
	DJ1 $-/-$	0.65 (0.15)	0.417 (0.076)	0.23 (0.048)