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Assessing the role of dopamine in limb and cranial-ormotor control in a rat model of Parkinson's disease

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Abstract

Parkinson's disease (PD) is a neurodegenerative disorder primarily characterized by sensorimotor dysfunction. The neuropathology of PD includes a loss of dopamine (DA) neurons of the nigrostriatal pathway. Classic signs of the disease include rigidity, bradykinesia, and postural instability. However, as many as 90% of patients also experience significant deficits in speech, swallowing (including mastication), and respiratory control. Oromotor deficits such as these are underappreciated, frequently emerging during the early, often hemi-Parkinson, stage of the disease. In this paper, we review tests commonly used in our labs to model early and hemi-Parkinson deficits in rodents. We have recently expanded our tests to include sensitive models of oromotor deficits. This paper discusses the most commonly used tests in our lab to model both limb and oromotor deficits, including tests of forelimb-use asymmetry, postural instability, vibrissae-evoked forelimb placing, single limb akinesia, dry pasta handling, sunflower seed shelling, and acoustic analyses of ultrasonic vocalizations and pasta biting strength. In particular, we lay new groundwork for developing methods for measuring abnormalities in the acoustic patterns during eating that indicate decreased biting strength and irregular intervals between bites in the hemi-Parkinson rat. Similar to limb motor deficits, oromotor deficits, at least to some degree, appear to be modulated by nigrostriatal DA. Finally, we briefly review the literature on targeted motor rehabilitation effects in PD models.

Learning outcomes—Readers will: (a) understand how a unilateral lesion to the nigrostriatal pathway affects limb use, (b) understand how a unilateral lesion to the nigrostriatal pathway affects oromotor function, and (c) gain an understanding of how limb motor deficits and oromotor deficits appear to involve dopamine and are modulated by training.

Keywords

Parkinson's; 6-OHDA; Rat; Oromotor; Oral motor; Mastication; Limb use; Ultrasonic vocalizations; Exercise; Pasta

1. Introduction

Regular physical exercise is widely advocated as an important component of health and may reduce the risk of certain diseases (Nelson et al., 2007). A beneficial effect of exercise has been reported in patients with PD (Chen, Zhang, Schwarzschild, Hernan, & Ascherio, 2005). Specifically, exercise has been shown to improve motor performance (Miyai et al., 2000; Sunvisson, Lökk, Ericson, Winblad, & Ekman, 1997), increase daily activity (Miyai et al., 2000), and decrease mortality (Kuroda, Tatara, Takatorige, & Shinsho, 1992). Regular exercise may even delay the appearance of motor impairment in people diagnosed with PD (Tsai et al., 2002). Exercise is associated with a lower risk of PD, although it may be that people who exercise less already have subclinical neurodegeneration (Chen et al., 2005). Animal models may help to clarify whether some types of motor rehabilitation can protect against degeneration of DA neurons. Given the potential beneficial effects of exercise in PD, it may seem surprising that physical exercise is not always a component of therapy for PD. In fact, studies have shown that although persons with PD reduce their level of physical activity, only 12–15 percent of diagnosed individuals are referred to physical therapy for an exercise intervention (Goodwin, Richards, Taylor, Taylor, & Campbell, 2008; Thacker et al., 2008). However, optimal levels, quality, and timing of PD-specific therapies remain unclear.

Animal models have provided a means for investigating the potentially positive effects of exercise on the brain, including neurogenesis, synaptogenesis, angiogenesis, increased neurotrophic factors, and increased levels of DA (Faherty, Shepherd, Herasimtschuk, & Smeyne, 2005; Jones & Schallert, 1994; Poulton & Muir, 2005; Schallert & Jones, 1993; Swain et al., 2003; van Praag, Shubert, Zhao, & Gage, 2005). Animal models of stroke have been instrumental in helping to guide the experimental use of exercise therapy by suggesting that forced use of the upper limb improves motor recovery and neural plasticity (Jones, Kleim, & Greenough, 1996; Jones & Schallert, 1994; Kleim, Jones, & Schallert, 2003; Nudo, Miliken, Jenkins, & Merzenich, 1996). However, very intense or prolonged levels of motor training very early after injury may be toxic (Kozlowski, James, & Schallert, 1996; Schallert, Fleming, & Woodlee, 2003). Animal models of PD afford us the opportunity to examine experience-dependent plasticity in the nervous system as a result of sensorimotor training. Our labs focus on a rat model of PD that is created by unilateral infusion of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. During a short window, this procedure leads to death of DA neurons that have their cell bodies in the substantia nigra and terminate in the striatum, which models primary disease pathology of PD (Fulceri et al., 2006; Marshall, 1979; Tillerson et al., 2001; Ungerstedt & Arbuthnott, 1970). Several studies suggest that intensive training that includes behaviors that are highly vulnerable to DA neurotoxins may reverse or slow disease progression (Anstrom, Schallert, Woodlee, Shattuk, & Roberts, 2007; Cohen, Tillerson, Smith, Schallert, & Zigmond, 2003; Smith & Zigmond, 2003; Tillerson et al., 2001, 2002). For example, in unilateral 6-OHDA models, rats show deficits in forelimb use (Allred et al., 2008; Calne & Zigmond, 1991; Schallert, Fleming, Leasure, Tillerson, & Bland, 2000), but forced use of an impaired forelimb yields behavioral sparing in that limb and may prevent the degeneration of dopaminergic neurons when training is initiated before or early enough after introduction of the neurotoxin (Anstrom et al., 2007; Cohen et al., 2003; Tillerson et al., 2001, 2002).

However, if initiation of intervention is delayed by 7 days, then the effect of behavioral sparing is not apparent or as robust (Tillerson et al., 2001, 2002).

Until recently, the body of animal model rehabilitation literature has focused on the forelimb. However, even in the early stages of PD, significant voice and swallowing deficits emerge that negatively impact quality of life, often leading to loss of employment and social isolation (Athlin et al., 1989; Bird et al., 1994; Fuh, Lee, & Wang, 1997; Miller et al., 2006). Up to 90% of people with PD experience a voice or swallowing problem and as the disease progresses, these deficits can cause debilitating health complications, including aspiration pneumonia, which is the leading cause of death in PD (Beyer, Herlofson, Arslan, & Larsen, 2001; D'Amelio et al., 2006; Fox, Morrison, Ramig, & Sapir, 2002; Ho, Iansek, Marigliani, Bradshaw, & Gates, 1998; Plowmann-Prine et al., 2009). Despite these significant clinical issues, there is not a clear understanding of the underlying neurobiological mechanisms of voice and swallowing disorders in regard to PD. Thus, a goal of this paper is to review tests commonly used in our labs to model PD deficits, focusing on those that involve DA, and tests of oromotor functions including voice, mastication and swallowing. New preliminary data on the control of biting will be presented in some detail.

2. Methods, preliminary results and discussion

2.1. Parkinson's disease model

Moderate to severe degeneration of presynaptic dopaminergic striatal neurons is induced by unilateral infusion of 6-OHDA (a catecholamine neurotoxin; Sigma) into the medial forebrain bundle (Fulceri et al., 2006; Marshall, 1979; Tillerson et al., 2001; Ungerstedt & Arbuthnott, 1970). Rats are anesthetized with a combination of ketamine (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and placed in a stereotaxic frame. Pre-operative analgesia [5 mg/kg 0.25% (w/v) Marcaine] is injected underneath the scalp prior to making the initial incision. Animals receive unilateral infusions of 7 µg (free base weight) 6-OHDA hydrobromide dissolved in 3 µl artificial cerebrospinal fluid (composition: NaCl, KCl, CaCl₂, MgCl₂·6H₂O) containing 0.05% (w/v) ascorbic acid. Injection coordinates are measured from bregma (−4.3 AP; ±1.5 ML; −8.0 DV from dural surface), and the solution is infused at a rate of 0.5 µl/min for a total of 6 min. At the end of the 6-min infusion, the needle is left in place for an additional 2 min before being slowly retracted. Infusions are directed into the nigrostriatal projections in the hemisphere opposite to the preferred forelimb as determined from baseline scores on the limb-use asymmetry test (see below). Post-operative analgesia (0.05 mg/kg buprenorphine, s.c.) is administered after suturing. Following surgery, animals are allowed to recover in a humidified incubator and, upon waking, are returned to their home cages.

2.2. Limb behavioral tests

2.2.1. Limb-use asymmetry (cylinder test)—To estimate the degree of 6-OHDA induced dopaminergic degeneration and the severity of parkinsonism, a forelimb-use asymmetry test is performed by placing the rat in an upright acrylic cylinder (diameter 20 cm, height 30 cm) to encourage rearing and exploratory movements with the forepaws (Schallert & Tillerson, 2000; Schallert & Woodlee, 2005; Schallert et al., 2000). The numbers of wall contacts made by either forelimb or by both forelimbs simultaneously or in alternation are recorded. The ratio of contacts made by the ipsilateral (non-impaired) forelimb relative to the total number of contacts is calculated using the formula: [(ipsilateral limb contacts + 0.5 both limb contacts)/total number of contacts including contralateral limb]. Scores above 0.5 indicate a greater reliance on the ipsilateral (non-impaired) limb. Limb use asymmetry can be detected in rats with as low as 50% DA depletion and is highly

correlated with the degree of nigrostriatal DA depletion over a wide range (Ariano et al., 2005; Schallert et al., 2000).

2.2.2. Postural instability test (PIT)—Rats are held almost vertically (in a “wheelbarrow”-like position) over a sandpaper-covered surface alongside a ruler (Woodlee, Kane, Chang, Cormack, & Schallert, 2008). This rough surface material induces stepping (Woodlee et al., 2008) rather than dragging or bracing (Schallert, De Ryck, Whishaw, Ramirez, & Teitelbaum, 1979; Schallert et al., 1982), in response to imposed weight shifts (Schallert et al., 1979, 1982). Viewed from above, the tip of the rat’s nose is aligned with the zero line of the ruler, and one forelimb is gently restrained against the animal’s torso by the experimenter while the animal is moved forward over the single planted forelimb until making a “catch-up” step to regain its center of gravity. The new position of the nose tip indicates the displacement of the body (in cm) needed to trigger a catch-up step in the unrestrained supporting forelimb. We examine each forelimb independently while slowly shifting the center of gravity during weight support and quantify the size of the adjusting response used to regain center of gravity. We perform three trials on each forelimb on a given day of testing (Woodlee et al., 2008). Longer steps (i.e., larger displacement values) are associated with higher degrees of DA degeneration (Woodlee et al., 2008). The test may model the push-pull test used to assess postural stability in PD patients. Chronic deficits can be found with about 80% or greater depletion.

2.2.3. Vibrissae-evoked placing test—As described in more detail elsewhere (Woodlee et al., 2005), rats are held aloft and the vibrissae on one side are brushed against the edge of a tabletop to trigger a forelimb placing response. The ipsilateral forelimb places in response to stimulation of either the ipsilateral or contralateral vibrissae. In contrast, if the level of DA depletion is sufficiently severe (80% or more depletion of striatal DA) the contralateral forelimb fails to place regardless of whether the ipsilateral or contralateral vibrissae are stimulated. These data indicate that the deficit is primarily motor rather than sensory (Woodlee et al., 2005). We recently developed a graded rating scale for limb placing reactions that is highly useful for assessing varying levels of sensorimotor dysfunction with about 70% or more DA depletion (Anstrom et al., 2007; Ciucci, Ma, Kane, Ahrens, & Schallert, 2008).

2.2.4. Single-limb akinesia test—The hindquarters of the animal are suspended while the animal supports its weight on only one forelimb (Schallert, Norton, & Jones, 1992; Tillerson et al., 2001). The animal is allowed to self-initiate stepping movements in a 10-s period for one forelimb and then the other forelimb in a balanced order. Stepping measurements for both limbs are recorded and an ipsilateral asymmetry score is derived [(ipsilateral steps/ipsilateral plus contralateral steps) – (contralateral steps/ipsilateral plus contralateral steps)]. Chronic maximum deficits in this test are found in rats with about 85% striatal DA depletion.

2.2.5. Pasta handling test—Rats are given 7-cm long pieces of uncooked vermicelli (1.5 mm diameter; Creamette brand, distributed by New World Pasta Co., Harrisburg, PA). This length was determined based on previous studies (Allred et al., 2008) as pieces longer than 7 cm led to the strategy of breaking the pasta into smaller pieces prior to eating, which confounded the test. All rats are habituated and tested in the home cage (isolated from cagemate) to familiarize the animals to pasta handling prior to testing and to reduce neophobic responses to a new food source. Rats are given five trials per testing session with pasta pieces given one at a time. Trials are videotaped for further analysis. Data are recorded such that the digits and joints of the animals’ metacarpals and phalanges (knuckles) of both forepaws are clearly visible to the experimenter. The main quantitative variable is the

number of adjustments made with each forepaw (only after eating begins) per 7-cm piece of pasta. Time of consumption is measured from the initiation of pasta grasping and placement into the mouth until the pasta is released by the forepaws and disappears into the mouth. Animals that have partial DA depletions (approximately 40% dopamine remaining in the lesioned hemisphere) as well as those with severe depletions (less than 10% DA remaining in the lesioned hemisphere) reduce adjustments with the contralateral (impaired) forepaw compared to controls (Allred et al., 2008). With severe depletions, in replication of Allred et al. (2008) there is also a significant increase in the number of adjustments made with the ipsilateral (non-impaired) forepaw and time to eat is increased (Fig. 3).

2.3. Oromotor function

2.3.1. Ultrasonic vocalizations—Until recently, the manner and degree that the nigrostriatal dopaminergic pathways contribute to voice and swallowing function has been unclear, especially because medical and surgical interventions that target these pathways have been unsuccessful with voice and swallowing disorders (Ciucci, Barkmeier-Kraemer, & Sherman, 2008; Fuh et al., 1997; Hunter, Cramer, Austin, Woodward, & Hughes, 1997; Narayana et al., 2009; Potulska, Friedman, Krolicki, & Spychala, 2003). However, a severe unilateral lesion to the medial forebrain bundle (nigrostriatal pathway) leads to decreased complexity, bandwidth, intensity, and duration of 50 kHz ultrasonic vocalizations (USVs) in male rats reacting to the odor cues of receptive female rats. These deficits do not affect latency to mount once the rats have access to the females, so motivational effects cannot account for the impairments (Ciucci et al., 2007, 2009). Thus, in the unilateral PD animal, these vocal sensorimotor deficits appear to be at least partly modulated by a compromised nigrostriatal dopaminergic system. The threshold level of DA loss for producing vocalization deficits has not yet been established. Moreover, it remains to be determined whether the DA deficiency component of PD disease pathology can influence speech quality in humans (Ciucci et al., 2009).

The link between 50-kHz USVs and mesolimbic dopamine is further supported by the ability of psychostimulants, such as amphetamine and cocaine, to increase USV production (Ahrens, Ma, Maier, Duvauchelle, & Schallert, 2009; Mu et al., 2009). These drugs increase synaptic DA levels in the nucleus accumbens and dorsal striatum, and produce a dose-dependent increase in 50-kHz USVs that can be blocked by DA receptor antagonists (Barker et al., 2010; Thompson, Leonard, & Brudzynski, 2006; Williams & Undieh, 2010; Wright, Gourdon, & Clarke, 2010). Repeated exposure to amphetamine is known to permanently sensitize DA circuitry, so that subsequent administration of the same dose of amphetamine causes greater behavioral effects compared to the initial exposure. This process of sensitization is thought to underlie some of the reinforcing properties of addictive drugs and contributes to relapse after periods of drug abstinence (Robinson & Berridge, 1993). Our lab has found that repeated intermittent exposure to intravenous amphetamine causes a sensitized increase in the production of 50-kHz USVs, with later same-dose drug trials eliciting more 50-kHz calls than the first trial, even after a 2-week drug-free period. The majority of amphetamine-induced 50-kHz calls were of the frequency-modulated trill subtype, and only frequency-modulated calls (but not flat 50-kHz calls) showed sensitization with repeated treatment. This suggests that frequency-modulated 50-kHz calls are more sensitive to the heightened DA activity produced by amphetamine [(Fig. 1); Ahrens et al., 2009].

2.3.2. The sunflower seed test—Because eating involves complex interactions among sensorimotor actions for food handling, procurement of food, and preparation of the bolus, we choose measures that represent feeding in the context of both limb and oropharyngeal function. One such technique is the Sunflower Seed Test (Gonzalez & Kolb, 2003; Nemati

& Kolb, 2010; Whishaw & Coles, 1996; Whishaw, Sarna, & Pellis, 1998). Rats typically manipulate a sunflower seed with their forelimbs, bite a corner of the seed, split it longitudinally (into two pieces), and then eat the seed (Whishaw et al., 1998). An intact rat can typically shell and eat five seeds in approximately 30–35 s with an average of 11 leftover shell pieces (Gonzalez & Kolb, 2003). However, limb and oromotor deficits can interfere with this process, leading to increased shell pieces and increased time to complete the task (Gonzalez & Kolb, 2003). In this test, animals are placed in a clear Plexiglas box and are filmed from below to allow the experimenter to view the animal's behavior. Five sunflower seeds are placed in one of the corners of the box. The time the rat spends handling, opening, and consuming all five seeds is assessed and recorded as well as the number of shells the rat has to break in order to retrieve the seed. The experimenter reviewing the videotape starts the timer the instant the animal contacts the first seed and stops it each time the animal diverts his attention away from the seed. If an animal spends more than 5 min retrieving the seeds, a maximum of 300 s is recorded and the trial is stopped. Additionally, if the animal tears apart the shell into pieces that are too small to count, a maximum of 30 shell pieces is recorded.

2.3.3. Pasta biting—As noted above, rats in a unilateral 6-OHDA model exhibit forelimb deficits while eating pasta (Allred et al., 2008). An exciting finding from this pasta-handling paradigm was that the intensity and regularity of chewing appeared to be impaired when listening to the audio signal. Thus, we explored biting deficits associated with the 6-OHDA model. The recording microphone [Audio-Technica AT2050 condenser microphone (cardioid setting)] was suspended 1 cm above the home cage, which yielded a distance from the rat's mouth to the microphone equal to 8–14 cm from any given spot within the testing area, although the rat tends to remain in one location while eating. For each recording session, the animal received three habituation pieces (warm-up trials) prior to testing. Rats were then given five trials of 7 cm length pieces of pasta given one at a time. Pasta biting acoustic signals were recorded using an MBox 2 Mini (Digidesign; Burlington, MA) and ProTools LE software (Digidesign: Version 8.0.3) with a sampling rate of 44.1 kHz and 16-bit depth. Files were noise-reduced with WAVES "X-Noise" Noise Reduction plug-in (AudioSuite-Burlington, MA) exported in .wav format, and analyzed with a custom code written for the software program Matlab.

The top and bottom panels of Fig. 2 show a full trial for the parkinsonian and control animals, respectively. A few features of the data are evident by comparing these plots. First, the overall intensity of the bites is much higher in the control animal (note that the y-axes are scaled identically). Based on a bi-modality that was evident in bite intensities from the control animal data, we have colored each bite (peak in intensity) either green or red depending on whether it was above or below the break between the modes (what was at about 0.15). Clearly many of the parkinsonian bites are below this threshold (red points), and even the stronger bites (green points) do not seem to form a separate band like they do in the lower (control) plot. It also appears that the parkinsonian animal bites intermittently (bursts and gaps), whereas the control animal bites in a more sustained manner throughout the trial. An additional feature of the data becomes obvious from looking at the x-axis; the parkinsonian animal took over twice the amount of time to finish the pasta than the control animal did.

Because of the dramatic difference in completion times, it is difficult to make an intuitive visual comparison of the data in the top and bottom panels. The middle panel thus shows the first 20 s of the parkinsonian trial, so both y (intensity) and x (time) axes are scaled identically. In this comparison, the contrast between the behaviors is stark, qualitative, and easy to appreciate.

Fig. 3 shows the mean time (s) it took the control (left) and parkinsonian (right) animals to consume a single piece of pasta across all trials. The error bars show 95% confidence intervals around the means. As was evident in the example data shown in Fig. 2, the parkinsonian animals took about twice as long to finish their pasta as the control animals did. Even more striking, perhaps, is the dramatically increased variability in the completion times for the parkinsonian group (this was true both within and across the parkinsonian animals). The variability of behavior can be as indicative as a change in that behavior.

Fig. 4 shows the mean bite strength as indicated by the relative intensity of the acoustic signal measured at the microphone for the controls and parkinsonian animals. As in Fig. 3, the error bars show the 95% confidence intervals around the mean. The overall intensity in the control animals is almost double that of parkinsonian animals, and the difference is remarkably large relative to the variability in the data (i.e., effect size; see figure legends for statistics). As mentioned above, there was a seeming bimodality in the histogram of bite strengths for the control animals (data not shown), and the parkinsonian bites fit nicely into the weaker mode of the control data (see also Fig. 2). Whether this is of fundamental import or merely coincidence we cannot yet say. Regardless, it is clear that – and this is the main point we wish to make here – by almost any measure we care to extract from the pasta biting test, the parkinsonian animals are easily and readily distinguished from the controls.

3. Conclusions

PD is a debilitating condition that affects virtually all aspects of movement, including cranial-omomotor function such as speech and swallowing. Excitingly, more attention is being given to the oromotor system in recent scientific investigations. Exploring basic mechanisms underlying the disease process and recovery from deficits, including the effects of deficit targeted training, can be facilitated through animal models. Accordingly, we have developed several methods to explore these deficits in a PD model that involves unilateral lesions to the nigrostriatal DA pathway. This paper reviews our established tests of limb use, and introduces the reader to our more recent methods examining vocalization, biting, and eating behaviors. Forelimb, vocalization and biting deficits appear to be modulated, at least in part, by forebrain DA, and are vulnerable to the primary pathology in PD.

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Appendix A. Continuing education

1. A lesion to the left striatum would cause impairments:
 - a. On the right side of the body (contralaterally).
 - b. On the left side of the body (ipsilaterally).
 - c. On both sides of the body (bilaterally).
 - d. None of the above.
2. The leading cause of death for patients with Parkinson's disease is:
 - a. Falls.
 - b. Aspiration pneumonia.
 - c. Stroke.
 - d. None of the above.
3. Parkinson's disease affects:
 - a. Limb use.
 - b. Posture.
 - c. Swallowing.
 - d. Voice.
 - e. All of the above.
4. Although PD affects many areas of the brain, the primary disease pathology is degeneration of:
 - a. Dopamine neurons in the cortex.
 - b. Dopamine neurons in the substantia nigra.
 - c. Dopamine neurons in the vagus nerve.
 - d. Dopamine neurons in the hypothalamus.
5. Rats that have undergone infusion of 6-OHDA to the medial forebrain bundle show deficits in:
 - a. Vocalization.
 - b. Posture.
 - c. Biting.
 - d. Skilled paw movements.
 - e. All of the above.

Fig. 1.

Frequency-modulated 50-kHz USVs are increased by i.v. amphetamine and are sensitized by repeated amphetamine exposure. Results are mean $\pm$ S.E.M. of the number of 50-kHz USVs categorized as frequency-modulated (trill type calls) recorded in 5 min after i.v. infusions of saline or amphetamine. In Trial 1, amphetamine elicited more frequency-modulated calls than saline ($\dagger p < .05$). Re-exposure to the same dose of amphetamine in Trials 2 and 3 elicited a greater increase in frequency-modulated 50-kHz USVs than Trial 1 ($*p < .05$, $**p < .01$). Two weeks after Trial 3, a challenge dose of amphetamine elicited a greater increase in frequency-modulated calls than the initial exposure ($\#p < .05$).

Fig. 2.

Example pasta eating trials for one hemi-parkinsonian and one control animal. All graphs show the relative instantaneous sound intensity (blue traces) in arbitrary units as a function of time (s). The green and red circles show local intensity maxima corresponding to teeth striking pasta. Histograms (not shown) of the control data showed bimodality in intensity that corresponds to “bites” – hard bites that crack the pasta – and “taps” – softer bites that do not crack the pasta. The split between the modes was generally around 0.175, and the green circles show the “bites” that exceeded this intensity, while the red circles show the “taps” that did not. The upper and lower panels show entire trials (i.e., the time it took the animal to finish one 7 cm length of pasta). The control animal (bottom) finished in less than 20 s while the parkinsonian animal (top) took over twice that. The middle panel shows the first 20 s of the parkinsonian animal’s trial and is thus scaled identically to the control data beneath it. Clearly, the parkinsonian bites were much weaker over all, the bites less frequent, and there were longer pauses taken between bursts of biting.

Fig. 3.

Mean time taken to consume one 7 cm length of pasta. The error bars show $\pm 95\%$ confidence intervals across animals ($t(28) = 4.5$, $p = 1.3 \times 10^{-4}$). The PD animals take almost exactly twice as long, on average, to finish the pasta (effect size = 1.45). Perhaps even more interestingly, the normal animals finish within a very small interval – their time to completion is virtually a constant – whereas the PD animals are quite variable (for the ratio of the PD to Control variance, $F(15,14) = 48.2$, $p = 2.0 \times 10^{-9}$).

Fig. 4. Mean bite strength for the control and parkinsonian (PD) animals. The error bars show $\pm 95\%$ confidence intervals computed across animals ($t(28) = 6.1, p = 2.01 \times 10^{-6}$). The PD animals have markedly lower bite strength, as measured by the sound intensity (see also Fig. 2). While we do not know the exact relationship between bite force and sound intensity, the effect size is 1.6, which is considered extremely large.