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Videofluorographic assessment of deglutitive behaviors in a rat model of aging and Parkinson disease

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Abstract

Dysphagia is commonly associated with aging and Parkinson disease and can have a significant impact on a person's quality of life. In some cases, dysphagia may be life threatening. Animal models may be used to study underlying mechanisms of dysphagia, but paradigms that allow adequate imaging of the swallow in combination with measurement of physiological variables have not been forthcoming. To begin development of methods that allow this, we used videofluorography to record the deglutition behaviors of 22 Fischer 344-Brown Norway rats in young adult (9 months old), old (32 months old), and parkinsonian (unilateral lesion to the medial forebrain bundle) groups. We hypothesized that the old and parkinsonian rats would manifest deficits in deglutition behaviors analogous to those found in human clinical populations. Our results supported our hypotheses in that the old group demonstrated reductions in bolus transport speeds and mastication rate, while the parkinsonian rats showed impairments in oral processing. Interpretation of these results should consider the particular animal model, lesion type and videofluorographic protocol used in this work. Future studies will link swallow imaging data of this kind with physiological and anatomical data in a manner not possible with human participants.

Keywords

deglutition; rat; aging; Parkinson disease; dysphagia

Introduction

Dysphagia can have a significant impact on a person's quality of life and is a risk factor for serious health problems including aspiration pneumonia [45, 49]. Specifically, Roy et al. reported that out of 117 seniors, 38% had a swallowing disorder at some point in their lifetime [49]. Further, 33% of elderly participants had a swallowing disorder at the time of study. Age-associated diseases, such as Parkinson disease, are also characterized by a high prevalence of dysphagia. In fact, incidence of dysphagia occurs in a range of 18.5 to 100% of patients with Parkinson disease [15]. However, the incidence of dysphagia may be underestimated because patients with Parkinson disease may not complain of dysphagia and 15% of patients with Parkinson disease may have silent aspiration [15]. Accordingly, research that contributes to an understanding of mechanisms that underlie swallowing

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deficits associated with aging and Parkinson disease is warranted and may have a significant clinical impact.

Age-related changes to swallow include increased duration of the oropharyngeal portion of the swallow [9, 14], increasing the risk of aspiration, disrupted bolus flow kinematics [44], and reduced lingual pressures [23]. Swallowing deficits in elderly people can lead to reduction in quality of life and are risk factors for serious health concerns, such as depression and aspiration pneumonia [56, 58]. Likewise, Parkinson disease, even in the early stages, can lead to significant swallow deficits that negatively impact quality of life [8, 11, 16-18, 35]. Major signs of parkinsonian dysphagia include reduced motility and increased transit times during the oropharyngeal swallow [17, 20, 35], delayed excursion of the base of the tongue and hyolaryngeal complex, and reduced airway sensory function [53], resulting in deficient laryngeal protection and contributing to an increased risk of airway compromise. The major contributor to mortality in Parkinson disease is aspiration pneumonia [25]. Despite these significant clinical problems, there is limited evidenced-based research on how to treat these conditions (for review, please see Russell et al., 2010 [65] and Baijens et al., 2009 [57]).

Historically, animal models have afforded us much of our knowledge on mechanisms underlying normal swallowing processes [1, 3, 10, 26, 31, 38, 68]. However, using animal models to study swallowing disorders and treatments is a relatively recent advance [61, 63, 69]. One of the major challenges in using animal models to study interventions for human disorders or diseases is developing behavioral assays that are relevant to the animal model and that can be easily translated to human behaviors [29]. While previous work has focused on development of behavioral assays for isolated components of the swallow in rats, such as tongue function and mastication [60, 61, 71], systematic methods for visualization and quantification of the entire swallowing behavior have not been developed. Such methods may provide information on how interventions impact functional eating.

One of the most common methods of examining oropharyngeal swallowing in humans is videofluorography because this tool allows for visualization of anatomic and temporal parameters associated with the swallow, as well as airway protection and bolus clearance. Videofluorography has been used to describe normal swallowing in the cat [13], infant and juvenile swallowing in the pig [48], and in an ALS model in the mouse [63]. However, this technique has not been used to study age or parkinsonian-related deficits in the rat. Developing methods for visualizing the swallow in the rat would allow use of this animal model in future research. This is important because the rat has been the most frequently used species for examining the neuromuscular sequelae of aging [4, 12] and for sensorimotor deficits associated with Parkinson disease [2, 6, 22, 27, 42, 43, 46]. Because a large body of research exists in the rat, new findings on deglutitive behaviors can be placed in context with other data concerning cranial and limb sensorimotor function found abundantly within the literature [28, 30, 32, 33, 39, 47, 59, 64, 69]. [24, 27, 36].

In contrast to larger mammals, use of the rat model has many benefits, including a relatively short life span (approximately 36 months) [21] that allows physiological and morphological changes associated with aging to be realized in a relatively short period of time. Rats also respond reliably to the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) to create lesions in the nigrostriatal pathways. As noted, these particular pathways are associated with the primary pathology of PD. [46] Our work has also shown that this type of lesion causes sensorimotor deficits in vocalization and tongue function [47, 59, 64, 69]. In addition, rats are easily trained and amenable to study, which permits rigorous experimental control, measurement of multiple parameters, and examination of relationships among variables. In our laboratories, we have found that rats demonstrate physiological and morphological

changes with aging and with lesions to dopaminergic pathways that parallel human findings [47, 59, 64, 69]. Old and brain-lesioned rats can also be trained to manifest progressive increases in tongue force and other variables following behavioral interventions. However, the manner in which initial deficits in tongue function or other factors contribute to global swallowing actions, and how these global swallowing patterns may be altered with behavioral training, present substantial gaps in knowledge. Development of videofluorography procedures and systematic analyses in the rat would contribute toward this end.

The purpose of our study was to develop assays of deglutition behavior of rats using videofluorography and determine if signs of dysphagia are evident in aged and parkinsonian rats compared with healthy young adult control rats. We hypothesized that the aged and parkinsonian rats would manifest deficits analogous to those found in human clinical populations. Due to anatomical differences in laryngeal configuration and position between rats and humans, we did not predict that the aged and parkinsonian rats would demonstrate airway compromise.

Methods

Data from 22 male Fisher 344/Brown Norway are reported. Of these 22 rats, 15 were young adults (9 month old) and 7 were 32 months old. The young adult rats were further randomized into a control group (n=7) and a group that received brain lesions for creation of a Parkinson disease model (n=8). All animals were housed in pairs in standard polycarbonate cages on a 12:12 hour light-dark reversed light cycle. Rats were obtained from a National Institute on Aging animal colony 10-12 weeks prior to the start of the experiment to allow acclimation to the animal care facility reversal of light cycle, and recovery from surgery. Food and water were given ad libitum except for 18 hours prior to the videofluorographic examination. All experiments were approved by the University of Wisconsin Institutional Animal Care and Use Committee (IACUC).

PD model-surgery and behavioral and neurochemical validation

The current study employed a rodent model of PD that is well-established and used extensively to study the result of dopamine depletion in a manner consistent with the primary pathology of Parkinson disease [2, 6, 22, 27, 42, 43, 46]. This model uses the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) to create lesions in the nigrostriatal pathways. [46]. Briefly, degeneration of presynaptic dopaminergic striatal neurons was induced by unilateral infusion of 6-OHDA into the medial forebrain bundle [59]. Rats were anesthetized with 2-4% inhaled isoflurane, placed in a stereotaxic frame, and received unilateral infusions of 7 μ g 6-OHDA hydrobromide (free base weight) dissolved in 3 μ l artificial cerebrospinal fluid (composition: NaCl, KCl, CaCl₂, MgCl₂*6H₂O) containing 0.05% (w/v) ascorbic acid. Infusion coordinates were measured from bregma (-3.3 AP; $\pm$ 1.7 ML; -8.0 DV from dural surface), and infusions were delivered at a rate of . 3 μ l/min for 10 minutes. Infusions were directed into the nigrostriatal projections in the left hemisphere, creating a unilateral 6-OHDA-induced lesion to the nigrostriatal pathway.

To confirm the degree of lesion in vivo, two behavioral tests were performed, forelimb-use asymmetry and apomorphine rotation. Forelimb-use asymmetry is the association between forelimb use and striatal dopamine depletion [24]. Briefly, rats were placed in an upright acrylic cylinder (20 cm diameter) to encourage rearing and exploration with the forepaws. The number of contacts made by either forelimb or by both forelimbs simultaneously was recorded. The ratio of contacts made by the nonimpaired forelimb relative to the total number of contacts was calculated using the formula: ipsilateral limb contacts minus both [simultaneous or rapidly alternating limb contacts] divided by the total number of contacts.

Scores above 50% indicate a greater reliance on the ipsilateral limb for voluntary movement. This test was performed at baseline and 8 weeks after lesion. During apomorphine rotation (8 weeks after lesion), the rats were given subcutaneous injections of 0.5 mg/kg apomorphine hydrochloride, and the net number of contraversive quarter turns, during a 2-min trial was recorded 25-min post-injection [6].

To examine the brains of the parkinsonian animals and document the extent of lesions, the following procedures were used. After completion of the study, all parkinsonian rats were deeply anesthetized with 2.5–4.0% isoflurane and intra-aortically perfused with 250 mL physiological saline 1 minute after an intracardial injection of 100 units of heparin and 500 mL of ice cold 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) was perfused to fix brain tissue. Whole brains were removed and postfixed in ice cold fixative for 1–4 hours. Brains were then cryoprotected for 48–96 hours in a 20% sucrose/5% glycerol solution in 0.1M PBS at 4°C. Brains were mounted on a freezing microtome and one of every five 60 µm coronal slices throughout the basal ganglia were harvested and stored in PBS with 0.02% Na₃N at 4°C. Free-floating slices were probed for tyrosine hydroxylase using a rabbit anti-tyrosine hydroxylase primary antibody (1:2000 dilution, Millipore, Billerica, MA, USA) and a biotinylated goat anti-rabbit secondary antibody (1:500 dilution, Millipore, Billerica, MA, USA). The signal was amplified using the VECTASTAIN Elite ABC avidin-biotin system (Vector Laboratories, Burlingame, CA, USA). Slices were incubated in primary antibody for 16 hours, in secondary for 3 hours, and avidin-biotin solution for 1 hour at room temperature. Labeling was visualized with 3,3'-diaminobenzidine (DAB) chromogen developed with a peroxidase reaction for 90 seconds. Slices were quenched, counterstained with hematoxylin, and mounted on gelatin-coated slides. Brain slices were imaged on an Epson Perfection V500 Photo Scanner and uploaded to a computer (Dell Optiplex 960) and analyzed using ImageJ software (National Institutes of Health, Bethesda, MD). A custom-designed software program was used in Image J to detect the optical density of thresholded values for neurons that were positive for tyrosine hydroxylase immunoreactivity. The region of interest (striatum in each hemisphere) was identified manually, run through the analysis script, and values were expressed as a percent of thresholded values compared with the non-injured hemisphere. The injured hemisphere severity level is expressed as percent loss of dopaminergic neurons in the striatum.

Videofluorographic studies

Videofluorographic studies for the parkinsonian rats occurred 8 weeks after neurotoxin infusion. Thus, rats were 9 months old (Young), 9 months old (Parkinsonian), and 32 months old (Old) at the time of testing. A custom designed L-shaped apparatus was affixed to the interior of the animal's plastic home cage at the level of the mouth (Figure 1). The rats were moved freely within the home cage and were recorded individually while ingesting a mixture of 5 mL peanut butter mixed with 5 mL barium (EZ-M Varibar Nectar) *ad libitum* for approximately 5 minutes to obtain 3 swallows with sufficient radiographic quality for analysis. The peanut butter - barium mixture was a pudding consistency.

Images were obtained onto S-VHS tape using a C-ARM fluoroscope model OEC 9800 (GE Medical Systems-OEC, Salt Lake City, UT) at a rate of 30 frames per second. Video images were later digitized for frame-by-frame analysis and measured using HCImage (Compix, Sewickley, PA). A 1.8 cm disk was placed into the field of view for calibration purposes.

Preliminary Data Analysis

To develop measurements appropriate for use in the rat model, three masked viewers (JR, MC, and MH) conducted a preliminary viewing of all videos. For rodent deglutition, different terminology was necessary than that used for humans. We observed that there were

two phases of bolus management by the rats that consisted of transport and processing that has been described previously in rodents [63]. In general, we observed that the oral transport phase was characterized by variable, small, irregular jaw movements, procurement of the bolus was initiated by the incisors, and oral processing was slow with large and rhythmic jaw movements used to form discrete boluses. The preliminary viewing yielded an assessment of the oropharyngeal swallows that included qualitative, quantitative temporal, and quantitative spatial measures (Table 1). All quantitative measures were conducted during the processing phase of bolus management.

Within this context, we piloted several measures that captured bolus processing, and oropharyngeal transit. We also noted any behaviors that appeared inconsistent among rats. These behaviors included any approach or postural abnormalities, coughing or gagging, extraneous head movement, or use of forelimbs instead of tongue to procure the bolus. Timing and excursion variables were derived for events that were the most salient according to our observations and have been reported in the literature [63]. From our larger set of observations, we reduced the data set into the 4 qualitative and 4 quantitative measures described below. This was done to limit the number of dependent measures. Variables were chosen based on their functional significance and what we predicted would likely manifest as a deficit in the age and parkinsonian rats based on findings from previous studies [55, 62, 69, 71].

Data Analysis

There were four qualitative measures in this study and their presence/absence was noted if they occurred at any time during the study: 1) Aberrant bolus movement was defined as festinated tongue movement without successful bolus transport, and was used to indicate any problem in the oral processing phase of the rat; 2) Dorsal head compensation during bolus transport was defined as head movement that was judged to aid in the swallowing process; 3) Abnormal posture was defined as the rats inability to properly position the body and therefore limited the rat from procuring the bolus, and 4) Cough/gag during or immediately after the pharyngeal swallow. Cough and gag were placed into the same category because they were not radiologically distinct. To ensure reliability of judgments, qualitative measures were made jointly with 100% agreement by three investigators (JR, MC, MH) on 50% of the trials. One investigator (JR) made the remaining judgments.

Quantitative temporal measures were averaged for 3 swallows when the rat was in the lateral view and the entire deglutitive process could be observed. The two quantitative temporal measures conducted were: 1) Bolus Speed (mm/s) - the speed at which the head of the bolus traveled from the initiation point to the fourth cervical vertebra (C4); and, 2) Mastication rate (cycles/sec) - the number of complete jaw open and close cycles over a period of at least 5 seconds during mastication of the bolus. Quantitative spatial measures were also made on 3 distinct swallows and averaged. The two spatial measures were: 1) Jaw excursion (mm) - maximum jaw opening during chewing; 2) Bolus area (mm²) - bolus size measured after swallow initiation and before the head of the bolus reached C4.

Statistical Analysis

All statistical analyses were performed with SAS (SAS Institute, Inc, Cary, NC). A one-way analysis of variance (ANOVA) was used for all quantitative measures to test for differences across the three groups. Pair-wise comparisons were made between groups using Fisher's protected least significant difference tests (LSD). To analyze the frequency of occurrence in the qualitative data, a Fisher's Exact Test was used to test for differences across groups. The alpha level was set at .05 for both the quantitative and qualitative analyses. Paired t-tests were used to compare forelimb use asymmetry at baseline and after lesion. Experiment-wise

error was not controlled with an alpha correction due to the exploratory nature of this investigation.

Results

Validation of Lesion in parkinsonian rats

The percent loss of dopamine neurons in the injured striatum of the parkinsonian rats was $97 \pm 3\%$, indicating severe hemi-parkinson lesions (Figure 2). The mean proportion of contralateral forelimb use (unimpaired limb) was 93 post-lesion, indicating a severe unilateral parkinsonian deficit [41]. There was a statistically significant difference in forelimb use asymmetry ratio after 6-OHDA-induced lesion as compared to baseline measures ($t = -15.08$, $df = 14$, $p < .001$). The average number of revolutions per minute after 6-OHDA-induced lesion was 11, which is consistent with the number of rotations found in other studies with severe striatal dopamine loss. Taken together, these scores confirm that unilateral striatal dopamine depletion was severe [6].

Qualitative Measures

Results from the qualitative measures are summarized in Table 2. Five of the 8 rats in the parkinsonian group and 1 of the old rats were identified as having aberrant bolus movement, with the parkinsonian group having significantly more aberrant movement than both the young adult and old groups ($p = .012$ on 2-tailed Fisher's Exact Test). There were no significant differences among groups for in the compensatory head movement, although we observed this in four of the young adult rats, two of the old rats, and three of the parkinsonian rats. One old rat and one parkinsonian rat exhibited a cough or gag, but there was no significant difference across groups.

Quantitative Temporal Measurements

Results for bolus speed and mastication rate measures are summarized in Table 3. There was a significant difference in mastication rate, $F(2,20) = 3.99$, $p = .03$, $LSD\ p = .01$, with the old group having a significantly slower mastication rate than the young adult group (Figure 2). One animal repeatedly swallowed outside of the videofluorographic view and thus bolus speed could not be measured. There was a significant difference in bolus speed among groups, $F(2,19) = 8.39$, $p = .003$ (Figure 3). The old group had the slowest bolus speed and was significantly slower than both the young adult and parkinsonian groups, $LSD\ p < .001$ and $p = .019$ respectively.

Quantitative Spatial Measurements

The quantitative spatial findings are summarized in Table 3. There were no significant differences in the degree of mouth opening during mastication among groups, $F(2,20) = .71$, $p = .51$. Two rats positioned themselves outside of the videofluorographic view during the swallow and thus there were two missing data points for bolus area. There was a significant difference in the size (area) of the bolus created and swallowed across all groups $F(2,18) = 58.82$, $p < .001$ (Figure 4), with the parkinsonian group having significantly smaller bolus areas compared with both the young adult and old groups, $LSD\ p < .001$ and $p = .042$, respectively. In addition, the old group had a significantly smaller bolus area than the young adult group $LSD\ p < .001$.

Discussion

The purpose of this study was to develop assays of deglutition behavior in rats using videofluorography and to determine if signs of dysphagia would be evident by videofluorography in aged and parkinsonian rats. Parkinsonian rats showed impairment in

the oral processing stage of deglutition, evident by the increase in aberrant tongue movement without successful bolus transit to the pharynx. The old rats manifested significantly slower mastication rates and reductions in bolus speed. However, regardless of the deficiency, both the old and parkinsonian rats self-selected smaller bolus sizes compared with the young adult rats. Thus, using measures that reflect parameters that are typical of rat bolus procurement and swallowing, we discovered that both aged and parkinsonian rats display oral and pharyngeal deficits and thus present a good model for use in studies aimed at uncovering biological correlates of dysphagia in these disease/disorder groups.

Parkinson disease has negative effects on several of the of oral stage components of swallowing [40], although measurement of swallowing deficits with videofluorography in patients with Parkinson disease can be variable [66]. Common measures in videofluorography of humans to capture these deficits include number of swallows per bolus, festinated tongue movement, range of tongue movement, oral residue, and premature loss of bolus to pharynx, and disordered jaw movements for mastication and bolus transport [50] [11, 16-18, 35]. In the rat model, we have also shown biting and chewing deficits in a 6-OHDA model in rats while eating pasta [71]. In the current study, the parkinsonian rats also demonstrated aberrant tongue movements to process the bolus significantly more than both the young adult and old groups. This finding suggests that the current 6-OHDA model of Parkinson disease does indeed manifest oral processing deficits in rats. Contrary to reduced pharyngeal transit time observed in humans with Parkinson disease, the parkinsonian rats did not demonstrate slower bolus speeds [35, 37]. In fact, in other studies of cranial sensorimotor deficits in this model, not all aspects of a behavior are equally compromised [60, 69]. For example, during a complex tongue protrusion test, average, but not maximal force generation was affected by 6-OHDA lesions [69]. In the current study, the parkinsonian rats self-selected smaller bolus volumes, perhaps as a compensatory mechanism to the oromotor deficits associated with the nigrostriatal dopamine depletion, such as reduced tongue force [69]. To our knowledge, most studies examining pharyngeal transit in aged humans used discrete, rather than self-selected, bolus sizes [9, 44]. Perhaps bolus speeds would have been altered in a similar pattern to humans if the rats were presented with controlled bolus sizes of larger volumes.

In the current study, both the mastication rate and bolus speed were significantly slower for the old rats. Aging is associated with many muscle changes that include reductions in muscle mass and a transformation of muscle fiber types [5, 34]. The speed of a muscle fiber contraction is closely related to myosin heavy chain (MHC) composition within a muscle [7]. Recent studies have demonstrated a shift from a fast (type IIb) to slow (type I) fiber type in the aging tongue [73], as well as slower contraction times for muscles of the tongue in old rats [51]. Perhaps the physiological changes observed in the cranial muscles of old rats contribute to kinematic changes of reduced jaw and tongue movement speed, thereby reducing the speed and force of bolus propulsion.

There are some limitations to consider when translating the current videofluorographic findings in the rat to standard videofluorography in humans. There are a number of anatomic and behavioral differences that exist between the rat and human. Due to these differences, alternative measures of deglutition are necessary for defining swallowing parameters in this animal model. It is outside of the scope of this paper to present the many different measures and measurement methods proposed for quantifying videofluorography in humans [54]. However, the necessity of different measures from those used for human swallowing does not negate the validity of this animal model. Albeit imperfect, an appropriate animal model can provide critical insights into putative mechanisms underlying health and disease in humans, and is in fact, one of the only ways to do so. Invasive procedures are often required to investigate mechanism (for example changes in neurochemistry or muscle proteins) and

cannot be performed with humans. Thus, the use of an animal models is required to answer many specific experimental questions. Any model, in the best of circumstances, can only serve as an approximation to the human clinical situation it is designed to reflect.

Anatomical differences relevant to deglutition between the rat and human are found in the oral cavity, pharynx and larynx. The oral cavity geometry of the rat is less curvilinear and longer than that of the human. In addition, the rat has a shorter pharynx, placing the laryngeal complex in a more rostral position. The ratio of cartilaginous to membranous vocal fold in the rat is 2:1 (whereas this ratio in human is 1:2). These differences in the position and configuration of the larynx may reduce the probability of aspiration in rats [19, 52, 72]. With regard to behavioral differences in deglutition between rats and humans, rats typically procure a bolus using a licking and a biting motion while humans commonly use their hands or a utensil. Rats also typically perform successive swallows with a significant part of the bolus remaining in the oral cavity, rather than taking discrete bites and performing single swallows. As a result, part of the bolus remains in the oral cavity after each swallow. This pattern of feeding and swallowing behaviors is distinct from human swallowing in traditional videofluorographic studies, where the bolus is presented for a single swallow and oral residue is considered abnormal, with the exception of continuous eating. Humans have longer pharyngeal ‘dwell’ times before triggering of a pharyngeal swallow and oral residue during larger bolus presentations or continuous eating and drinking [67, 70]. However, certain types of deglutitive deficits found in the aging and parkinsonian rats may parallel deficits found in the human. Thus, our measures in the rat model provide important insights that may further our understanding of Parkinson disease, the process of aging, and the influence of these on the intricate act of deglutition.

In conclusion, videofluorography can be a useful tool for assessment of rat deglutition for research purposes. Instead of applying commonly used measures of the human swallow, which don’t directly translate to the rat, we focused on more specific measures of rat deglutition. Both the aging and 6-OHDA parkinsonian rat models manifested changes to the oropharyngeal swallow and these changes were identified by videofluorography. Future research could examine the effects of antiparkinsonian medications using this model, or the effect of aging combined with parkinsonian models. In addition, we will use videofluorography to test hypotheses regarding deglutition behaviors by providing treatments and examining how these treatments relate to function with the ultimate goal of translating these to human trials.

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Figure 1.
Representative still frame from a rat undergoing videofluorography. The bolus area is outlined in white. C4 is noted by the arrow.

Figure 2.

A representative coronal slice of rat brain showing immunoreactivity to tyrosine hydroxylase in the striatum following 6-OHDA injection into the median forebrain bundle to create a model of Parkinson disease. Note the absence of staining in the left hemisphere (shown on the right side of the slice), indicating severe striatal dopamine depletion.

Figure 3.

Box-and-whisker plot depicting mastication rate during the processing phase. The dark line represents the median. The box contains the interquartile range (IQR) of the data, and the whiskers extend to the last observation within 1.5x the IQR. The open circles are observations beyond 1.5 x the IQR. The mastication rate was significantly faster for the young adult group compared with old and parkinsonian groups ($p < .05$).

Figure 4. Box-and-whisker plot of bolus speed during swallowing. The old rats had significantly slower bolus speeds compared to the young adults and parkinsonian rats ($p < .05$).

Figure 5.

Box-and-whisker plot of bolus area for the different rat groups. The parkinsonian rats swallowed significantly smaller boluses than both the young adult and old rats. In addition, the old rats swallowed smaller boluses than the young adult rats ($p < .05$).

Table 1

Videofluorographic measures considered during preliminary viewing of all videos. PB = Peanut butter/barium mixture.

Measures	Description
*Bolus Speed	Speed of bolus (mm/s) starting from swallow onset to head of bolus reaching C4. Measured 3 different swallows for each rat and averaged.
*Bolus Area	Size of the bolus (mm ²)
Swallow Rate	Following PB engagement, the number of swallows over 15 sec. <i>This measure was not included among our final variables because rats would often move off screen, making assessment difficult.</i>
*Jaw Rate	Following PB engagement, the number of jaw open/close cycles over 5 sec period
*Jaw Excursion	Following PB engagement, extent of jaw opening (mm) during chewing measured from the edge of the incisors. Measured 3 times for each rat and averaged.
*Aberrant Bolus movement	Movement of the bolus back and forth within the oral cavity during ingestion (Yes or No)
*Head Compensation	Additional head movements used to aid in swallowing (Yes or No)
*Cough/Gag	Cough or gag observed (Yes or No)
Limb Stabilization	Use of limbs for postural stabilization while attempting to obtain the PB. (Yes or No) <i>This measure was not included among our final variables because stabilization was used in all groups and appeared to be variable by rat.</i>
*Abnormal Posture	Abnormal posture noted (Yes or No)
Head distance	Distance (cm) of rat head from the bottom of the cage during the swallowing process. <i>This measure was not included among our final variables because it was highly variable within and between rats. Instead, a qualitative assessment was used.</i>
First Swallow	The time it takes from first engagement of the PB until first swallow. <i>This measure was not included among our final variables because rats would often move out of view limiting our ability to make this measure.</i>

* measure used in final analysis.

Table 2

Qualitative results for aberrant bolus movement, head compensation, cough/gag, and abnormal posture. Values are reported as the number of animals in each group that elicited the condition. The parkinsonian rats had significantly more aberrant bolus movement than both the young adult and old rats.

Measures	Young	Old	Parkinsonian	P-Value
Aberrant Bolus Movement	0	1	5	<.05
Head Compensation	4	2	3	NS
Cough/Gag	0	1	1	NS
Abnormal Posture	0	0	3	NS

Quantitative results for jaw excursion, mastication rate, bolus speed, and bolus area

Table 3

Values are reported as means and standard deviations (SD). There was no significant difference in jaw excursion among groups. Young adult rats had significantly faster mastication rates ($p<.05$). Old rats elicited slower bolus speeds than both the young and parkinsonian rats ($p<.05$). Parkinsonian rats swallowed the smallest bolus size ($p<.001$).

Measures	Young		Old		Parkinsonian		P-value
	Mean	SD	Mean	SD	Mean	SD	
Jaw Excursion (mm)	14.0	1.5	14.8	1.3	14.4	1.3	NS
Mastication Rate (times/sec)	4.7	0.4	4.1	0.4	4.4	0.4	<.05
Bolus Speed (mm/sec)	59.9	9.6	45.0	3.1	54.1	6.2	<.05
Bolus Area (mm ²)	36.1	3.7	23.3	3.1	19.8	1.7	<.001