Title: Mechanisms of airway protection dysfunction in Parkinson's disease

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1. Title: Mechanisms of airway protection dysfunction in Parkinson's disease

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3. Abstract:

Aspiration pneumonia (APn) occurs at a disproportionately high rate in patients with Parkinson's disease (PD) versus healthy age-matched older adults. This study aims to determine the underlying causative factors that contribute to airway protection deficits in PD in order to reduce the incidence of aspiration-related illness in these patients. Previous studies suggest that PD patients present with deficits in airway somatosensation and sensory processing and filtering. However, it remains unknown whether these changes are related to observable motor deficits of swallowing or cough in people with PD. As such, this study aims to 1) better understand the relationships between blunted airway somatosensation, reflex cough sensitivity and effectiveness, and swallowing function in people with PD, 2) determine whether measures of discriminative and affective processing of general airway sensation is/are associated with deficits in reflex cough and swallowing function, as well as 3) determine whether over-filtering of airway stimuli is associated with swallowing or cough deficits.

4. Background:

Airway somatosensation in people with PD

PD is a progressive neurologic condition that is associated with deficits in both swallowing and cough function, as well as high rates of aspiration pneumonia (APn) (1, 2). Yamanda and colleagues have shown that people with a history of APn demonstrated significantly reduced sensitivity to airway irritants, quantified as a blunted urge-to-cough (UtC) (3). The UtC is a respiratory sensation that precedes a cough bout (4, 5). The UtC is blunted in people with PD as compared with age-matched controls, and even further blunted in people with PD and concomitant dysphagia (6, 7). Our preliminary data strongly suggest that general airway somatosensation is also blunted in people with PD with and without concomitant dysphagia. We hypothesize that blunted airway somatosensation contributes to the development of swallowing and cough deficits in people with PD. The objective of this aim is to determine the relationships between blunted airway somatosensation, reflex cough sensitivity and effectiveness, and swallowing function in people with PD. Based on our preliminary studies, we predict that blunted airway somatosensation will appear earlier (years 0 – 5 post-diagnosis) in the disease process than either cough or swallowing dysfunction, and that by 6-10 years post-

diagnosis, blunted respiratory somatosensation will correlate with reduced reflexive cough sensitivity and effectiveness, and decline in swallow function and safety (Figure 1). We will determine airway somatosensitivity using resistive inspiratory loads (8). We will perform fluoroscopic studies to evaluate swallowing function, and reflex cough testing using capsaicin to determine sensitivity and effectiveness. We will perform these tests in people across a range of disease durations. The rationale is that by determining the general respiratory sensitivity, reflex cough sensitivity to an airway irritant (capsaicin), as well as swallowing and cough effectiveness, we will be able to identify whether the onset of swallowing and/or cough deficits occur after reduced respiratory somatosensation. These data will provide support to our hypothesis that reduced respiratory somatosensation underlies observable motor deficits in swallowing and cough function, as well as reduced reflex cough sensitivity. This will significantly advance our understanding of the perceptual sensory components of airway protective deficits in PD, and provide much needed information regarding why airway protection deficits develop.
Such outcomes are critically important to advancing the management of patients with PD and other neurodegenerative diseases.

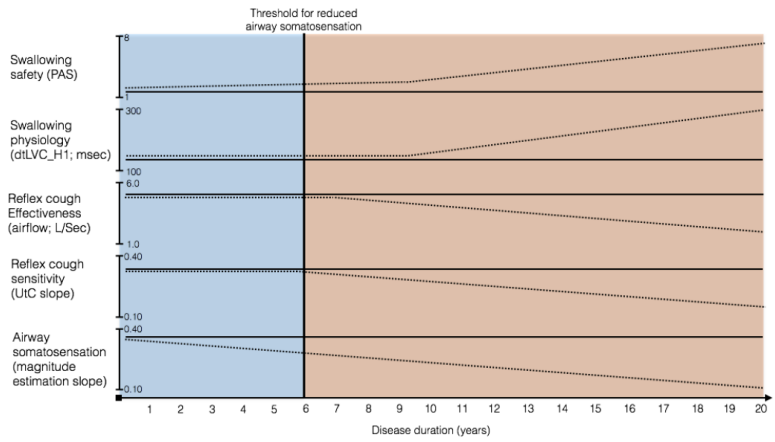

Figure 1. Hypothesized relationship between airway protective behaviors and airway somatosensation with Parkinson's disease duration. PAS is penetration-aspiration score; dtLVC_H1 is duration from initial hyoid burst (H1) to laryngeal vestibule closure (LVC), UtC is urge to cough.

Previous studies have shown that multiple airway protective behaviors are impacted in people with PD, most notably, functional decline to the mechanisms of swallowing and cough. The consequence of concurrent swallowing and cough dysfunction is uncompensated airway compromise (silent aspiration), indicative of significant sensory deficits whereby a reflexive cough response is not produced in response to aspirate material in the larynx or airway. People with PD exhibit deficits in multiple sensory modalities, including proprioception, mechanosensation and olfaction (9, 10, 11). There is a breadth of information regarding somatosensory deficits associated with decline in motor functions such as posture and gait, and much of the literature supports the hypothesis that there is a sensory origin to PD motor symptoms (12). Specific sensory deficits within the upper airway include significantly higher laryngeal somatosensory detection thresholds compared to healthy controls (13), and blunted perception of the urge-to-cough (UtC) to cough-inducing airway irritants (1, 6, 14, 15). These sensory abnormalities occur in conjunction with decreased swallowing safety and cough effectiveness (1, 6, 13). Despite this evidence that changes in upper airway sensation accompany motor deficits, the evaluation and management of airway protection in PD and other neurologic diseases continues to focus solely on observable motor deficits of swallowing.

Changes to brainstem areas involved in swallowing and cough generation are affected at the earliest points in PD (16). While severe dysphagia is typically not seen until later stages of the

disease, there are subtle changes that may occur to the pressures generated in the oropharynx during swallowing even in the early and mid-stages (Hoehn &Yahr (H&Y) 1 – 3) (17). Hammer and colleagues (2013) studied 18 patients with PD (mean disease duration: 6 years), and found a moderate correlation ($r = 0.40$, $p = 0.02$) between laryngeal somatosensory thresholds and disease severity. Interestingly, their results did not show deficits of swallowing safety in the PD group even though the PD group had significantly higher laryngeal somatosensory thresholds compared with age matched controls. These researchers suggested that "sensory abnormalities may precede penetration and aspiration deficits in the clinical progression of PD" (Hammer et al., 2013; page 4) (13), which is a hypothesis that is also supported by our preliminary studies.

Preliminary Studies:

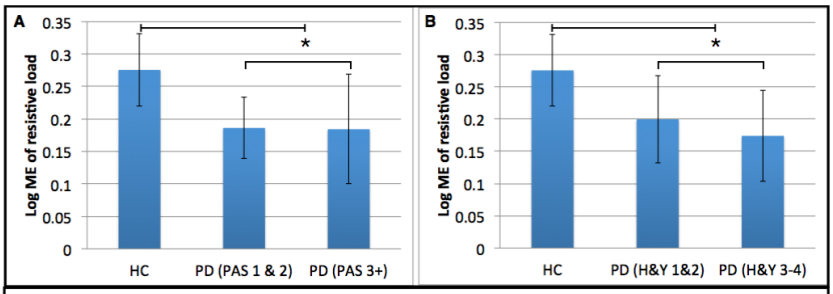

Figure 2. Significant differences (* indicates $p < .010$) between healthy controls (HC) and PD groups according to swallowing safety (PA scores; panel A) and disease severity (Hoehn & Yahr scale; panel B).

General respiratory sensation is blunted in PD, independent of disease severity or swallowing safety: In order to understand whether people with PD experience blunted respiratory somatosensation we conducted a preliminary study to examine the perception and magnitude estimated of inspiratory loads of various intensities. Our study compared 14 patients with PD to 13 age and sex matched healthy adults (HCs). The results show that people with PD exhibit blunted magnitude estimation of inspiratory resistive loads as compared with the control group regardless of whether they exhibit measureable decline in swallowing safety, or of disease severity (Figure 2 panels A and B). These data indicate general respiratory sensation is blunted even when dysphagia is not present, and in the early stages of the disease, thus lending preliminary support to the hypothesis that blunted respiratory somatosensation precedes the onset of swallowing deficits in PD.

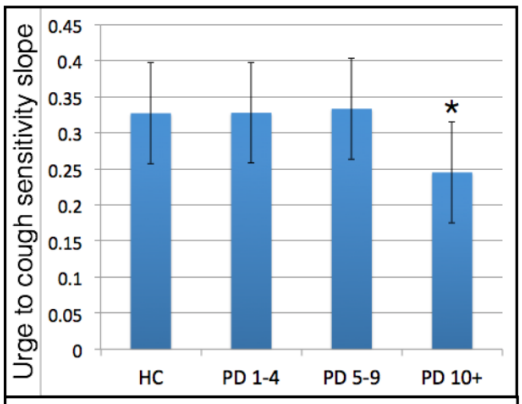

Figure 3. Urge to cough (UtC) sensitivity slope comparison between healthy controls (HC), and PD duration groups. PD1-4 is 1-4 years post PD diagnosis; PD5-9 is 5-9 years post PD diagnosis; PD 10+ is more than 10 years post PD diagnosis. * is significantly different at $p < .001$.

Reflex cough sensitivity is blunted in later PD compared to earlier PD and age-matched controls: In an ongoing study we are measuring sensitivity to the airway irritant capsaicin by examining the perceived UtC. To date, participants include 76 men and women with PD, ranging from 1 to 23 years post-diagnosis and 51 age-matched control participants. Our interim analysis show that participants within 10 years of diagnosis (Figure 3, PD 1-4 and PD 5-9 groups) do not exhibit a significant difference in the UtC sensitivity as compared to the control group, however the PD 10+ group does exhibit a significantly blunted UtC sensitivity compared to controls (Figure 3).

Airway sensory processing in people with PD

Central neural processing of sensory stimuli is critical for generating appropriate motor plans, and this sensorimotor relationship is disrupted in PD, resulting in many of the hallmark motor symptoms (9-11). Sensory processing involves both discriminative and affective processing components, and each is critical for generating an appropriate motor response. Discriminative processing refers to the ability to detect and gauge the intensity or magnitude of a stimulus, whereas affective processing refers to determination of stimulus characteristics, such as pleasantness or unpleasantness (4, 18). As well, sensory stimuli may be gated, or filtered, out from connecting to the sensory cortex, and this process is normal and expected for many somatosensory modalities to prevent an over abundance of redundant sensory stimuli (19, 20). Our preliminary data suggest that there are changes in both discriminative and affective processing and filtering of airwaysensation in people with PD as compared to an age- and sex-matched control group (21), however to date it is unknown whether these deficits are related to observable motor deficits of swallowing or cough. The objective of this aim is to determine whether measures of discriminative and affective processing of general airway sensation is/are associated with deficits in reflex cough and swallowing function. Further we aim to determine whether over-filtering of airway stimuli is associated with swallowing or cough deficits. Based on preliminary data we expect that people with PD will exhibit reduced discriminative processing components of airway stimuli. With disease progression, measures of affective processing components will decline and this will directly correlate with diminished reflex cough sensitivity and swallowing function. In order to achieve this objective, we will measure swallowing and cough function, reflex cough sensitivity, and the event-related sensory evoked potential (SEP) to an airway stimulus. We will compare the latency and amplitude of the SEP waveform component peaks to swallowing safety and physiology, and cough effectiveness and sensitivity, in people with PD across a range of disease durations. The rationale is that by determining the relationship between discriminative and affective processing of airway sensation and airway protective behaviors (swallowing and cough) we will better understand what type of sensory deficits are impacting airway protective function.

Although both swallowing and cough clearly have motor components, they are typically not considered to be classic *motor* features of PD, which traditionally includes akinesia, rigidity, and/or tremor of upper and lower extremities. While precise etiology of swallowing and cough deficits in PD remains unknown, a recent study by the Arizona Parkinson's Disease Consortium showed inclusion of alphasynuclein protein in the sensory nerve terminals of the upper airway in 10 PD patients, post-mortem, and that there was a significant association between the amount of this protein and the presence of dysphagia (22). Schindlbeck et al., investigated somatosensory symptoms in de novo PD patients, and found that although somatosensory symptoms were present in 67% of study participants, there was not neurographic evidence of peripheral nerve damage, strongly indicating that in the early phase of the disease sensory changes are likely *not due to peripheral sensory neuropathy* (23).

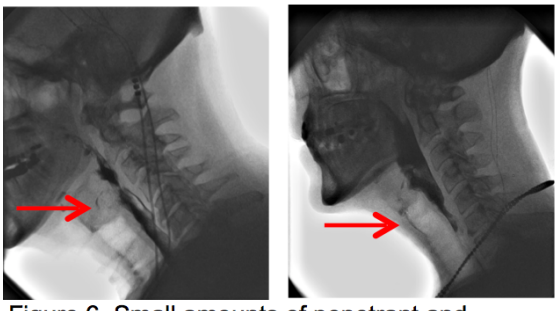

Figure 6. Small amounts of penetrant and aspirate material in 2 PD patients.

The neural mechanisms mediating swallowing and cough include a brainstem-mediated pathway as well as a supramedullary pathway (24). Studies have identified activity in multiple cortical areas during swallowing, voluntary cough and reflex cough to capsaicin (e.g., (19, 20, 25). This suggests that in cases where the intensity of a stimulus is insufficient to trigger the brainstem-mediated responses, the accurate perception of respiratory sensations is critically important for the generation of airway protective behaviors. When people with PD experience aspiration, it is typically small amounts of aspirate material that enter the larynx (Figure 6), and the strength of this stimulus is not necessarily adequate to trigger the brainstem-mediated reflex cough response. Therefore, the supra-medullary sensory processing of the airway stimulus is requisite to producing a cough response to eject the aspirate material. Experimentally, cough-inducing stimuli, such as low concentrations of capsaicin, can be used to study the cough response to varying intensities of the stimulus (i.e., those strong stimuli that would presumably activate the brainstem-mediated pathway) and those weak stimuli that likely depend on supra-medullary sensory process to modulate the cough response.

The sensory evoked potential (SEP) is a neural correlate of sensory perception that can be elicited across multiple sensory modalities, for example audition (26) somatosensation (27). For the airway, the SEP can be produced by a mechanical stimulus such as an inspiratory occlusion (28, 29) , which is similar to applying the inspiratory loads in terms of serving as a general respiratory stimulus. The resulting waveform consists of multiple component peaks (Figure 7) that reflect both discriminative and affective sensory processing (30, 31). Specifically, the early peak 'P1' is related to discriminative processing, the 'N1' is related to both discriminative and affective processing, and the later P2, and N2 peaks are related to higher order cognitive processing (32). This technique can also be used to examine sensory filtering, or gating, which is defined as the brain's ability to filter redundant sensory information (28, 32).

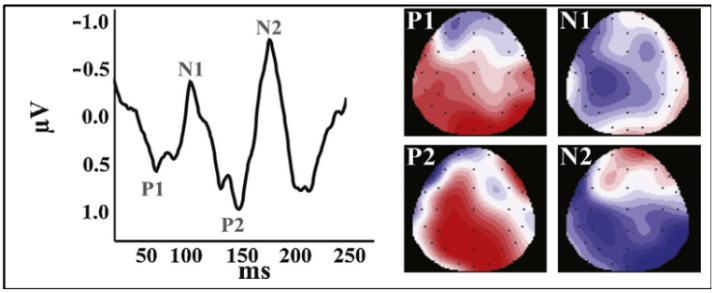

Figure 7. Airway sensory evoked potential peaks including P1, N1, P2, and N2 from a PD participant. The red and blue areas on the right indicate the locations of electrodes recorded the peaks (Pitts et al., 2016).

Preliminary studies: *Reduced discriminative and affective processing of airway mechanical stimuli in PD:* We have recently reported the results of our study investigating the processing of an air-puff stimulus to the posterior pharyngeal wall in people with PD. Participants were 13 people with PD, and 7 age- and sex-matched healthy older adults (controls). In both the PD and control participants we were able to successfully record SEP component peaks (Figure 7). People with PD exhibit changes to

	P1		N1		P2		N2	
	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value
PD	0.59 (0.41)	***.05***	0.70 (0.55)	1.0	0.38 (0.35)	0.69	0.27 (0.24)	***.02***
Controls	1.26 (0.91)		0.62 (0.31)		0.34 (0.30)		0.88 (0.68)	

Table 4. Means and standard deviations (SD) for the airway SEP (Pitts et al., 2016)

both P1 (discriminative) and N2 (affective) peaks as compared to healthy older adults (Table 4). Specifically, people with PD over-filter the P1 and N2 peaks, indicating that there is sensory information that is prohibited from reaching the level of conscious perception and processing. A limitation of this study was that the apparatus for delivering the air puff stimulus was uncomfortable for most participants, and this may have increased attention to the stimulus, which is known to modulate the N1, P2 and N2 peaks (33). Nonetheless, this preliminary study lends support our hypothesis that discriminative and affective processing and stimulus filtering are affected in PD, however it remains unknown whether/how attention affected these results, or whether they relate to changes in the airway protective functions of cough and/or swallowing.

5. Specific Aims:

The specific aims for this project are:

Aim 1: To determine the relationships between blunted airway somatosensation, reflex cough sensitivity and effectiveness, and swallowing function in people with PD.

Hypothesis 1: We predict that blunted airway somatosensation will appear earlier (years 0 – 5 post-diagnosis) in the disease process than either cough or swallowing dysfunction, and that by 6-10 years post-diagnosis, blunted respiratory somatosensation will correlate with reduced reflexive cough sensitivity and effectiveness, and decline in swallow function and safety.

Specific aim 2: To determine whether measures of discriminative and affective processing of general airway sensation is/are associated with deficits in reflex cough and swallowing function in people with PD.

Hypothesis 2: People with PD will exhibit reduced discriminative processing components of airway stimuli. With disease progression, measures of affective processing components will decline and this will directly correlate with diminished reflex cough sensitivity and swallowing function.

Specific aim 3: To determine whether over-filtering of airway stimuli is associated with swallowing or cough deficits in people with PD.

Hypothesis 3: Over-filtering of airway stimuli will be associated with deficits in reflex cough and swallowing function in people with PD.

Aim 4: Determine the relationships between observable motor aspects of swallowing, cough, and cough sensitivity over a 3-year time period in people with PD.

Hypothesis 4: Swallowing safety and physiology in people with PD will exhibit significant differences over 3 time points, spaced 10 – 18 months apart. For people within 9 years of PD diagnosis, reduced reflex cough sensitivity will not be a sensitive and/or specific indicator of swallowing dysfunction. However, for those with a greater disease duration (10+ years), reflex cough sensitivity will be a sensitive and specific biomarker of swallowing dysfunction.

6. Research Plan:

Protocol 1 (Aims 1-3)

This prospective experimental study will include male and female adults with PD, as well as a control group consisting of healthy adults. We aim to recruit 30 participants in each group. We will recruit PD participants from the patient population at UF Fixel Institute for Neurological Diseases. Healthy control participants will be recruited from the community population, including spouses and caregivers of PD participants, as well as HealthStreet. All participants will provide written consent before enrollment into the study, which will be approved by Institutional Review Board (IRB) at the University of Florida.

Inclusion criteria:

1.) Between the ages of 45 and 85 years
2.) Diagnosis of PD, Hoehn and Yahr stages I – IV, by a fellowship trained neurologist arriving at the diagnosis of PD by applying strict UK brain bank criteria (PD participants only)

Exclusion criteria:

1. Neurological disorders other than PD (i.e., stroke, multiple sclerosis, etc.)
2. Difficulty complying due to neuropsychological dysfunction (i.e., severe depression)
3. Allergy to capsaicin or hot peppers
4. History of cancer in the head, neck, or lungs (not including skin cancer on the head/neck that did not require major surgical resection significantly altering anatomy or radiation to head/neck tissue).
5. History of smoking in the past 5 years
6. Any neurological disorder including PD (Healthy control group only)

Experimental design and procedures: This prospective experimental study will be completed in one or two study visit(s) taking between 1 and 3 hours each. Testing will be conducted in a quiet clinical research space. Verbal and written informed consent will be obtained by qualified members of the investigative team. Following informed consent, all participants will complete a health history questionnaire to assess inclusion/exclusion criteria (Appendix 1). As well, the consenting investigator will review the electronic medical record to ensure inclusion/exclusion criteria are met.

The experimental protocol will consist of 1.) Review of electronic medical record to determine participant disease status 2.) Baseline pulmonary function measures 3.) Baseline depression and apathy scales, 4.) Inspiratory resistive load presentation 5.) Reflex cough assessment 6.) fluoroscopic swallow study 7.) Measures of event-related sensory evoked potential (SEP) to an airway stimulus.

Participant disease status:
A qualified member of the investigative team will review the electronic medical record and record the following information for each PD participant: disease severity (Unified Parkinson's Disease Rating Scale, Hoehn & Yahr score), medications, levodopa equivalent dose, and surgical status (specifically presence, target site, and laterality of deep brain stimulator(s)).

***Baseline pulmonary function measures*:**
We will analyze pulmonary function in all participants. The forced expired volume in the first second (FEV1) of a forced vital capacity (FVC) maneuver will be measured for each participant using a digital spirometer (Spirovision 3+m, Futuremed; or Koko spirometry, nspire health). Maximum inspiratory and expiratory pressure (PiMax; P_EMax) will be measured with a manometer (MicroRPM, Micromedical Inc.). We will use standard procedures, as put forth by the American Thoracic Society, for measuring FVC, FEV1, the ratio of FEV1/FVC, and PiMax/ P_EMax.

Baseline depression and apathy:
Participants will complete 2 validated scales, the Beck Depression Index-II (BDI-II) and Starkstein Apathy Scale (AS) to determine baseline depression and apathy scores.

***Inspiratory resistive load presentation*:**
Participants will rate perceived difficulty of breathing in response to various resistive loads applied to single inspirations during tidal breathing in order to assess general respiratory somatosensation. The participant will be seated in a chair, separated from the investigator and experimental apparatus. The participant will be instructed to "relax and breathe" through a facemask connected to a non-rebreathing valve (Hans Rudolph, 2700 series) in line with a differential pressure transducer. The inspiratory port of the valve will be connected to the resistive loading manifold. The manifold consists of 5 differential resistors ranging from 2.5 – 40 cmH2O/lps of resistive pressure, separated by stoppered ports, as well as a no load condition. The load is applied by removing the stop for an entire inspiratory breath, and then replacing the port to continue resting tidal breathing. The pressure transducer will provide measures of mouth pressure and airflow, which will be digitized (PowerLab, ADInstruments) and recorded to a desk top computer using LabChart software (ADInstruments). Following each loaded breath, the participant will provide an estimate of the perceived difficulty of breathing using a modified Borg scale.

Participants will be familiarized with the loads in a practice session prior to initiating the experimental protocol. The resistive loads will be applied in a randomized block design, with each loaded breath separated by at least three unloaded breaths. Two blocks will be completed with each load presented between 3 and 5 times within each block. Therefore, there will be a total of between 15 and 25 loaded breaths (3-5 loads x 5 presentations) per block. Following the loaded breath, and one random non-loaded control breath, the participant will be asked to estimate the perceived difficulty of breathing on the Borg scale.

Reflex Cough Assessment Procedures:
Participants will be outfitted with a facemask covering the nose and mouth. The facemask will be coupled to a pneumotachograph, differential pressure transducer, and have a side port with a one-way inspiratory valve for nebulizer connection. The nebulizer will be connected to a dosimeter that delivers aerosolized solution during inspiration with a delivery duration of 2 seconds. The cough airflow signal will be digitized (Power Lab Data Acquisition System) and recorded (LabChart 7; ADInstruments, Inc.) to a laptop computer.

Participants will be seated for an initial 30 seconds of quiet breathing in order to acclimate to the facemask. Participants will then complete a capsaicin challenge with three randomized blocks of

0, 50, 100, 200, and 500 μM capsaicin dissolved in a vehicle solution. Participants will be given the instruction "cough if you need to" prior to capsaicin delivery. The solution will be administered automatically upon detection of an inspired breath and there will be a minimum of one minute between each trial. Participants will be provided water to drink between trials. Following each capsaicin trial, participants will rate their urge-to-cough on a modified Borg Rating Scale measured 0-10, where 0 is no urge, and 10 is maximal urge. Our research group has extensive experience utilizing this methodology to induce cough (e.g., (1, 34-36)). We also hold an FDA IND # 76866 for the use of capsaicin in the study of cough reflex sensitivity and motor pattern.

Voluntary cough procedure:
Voluntary cough will be assessed using the same equipment used for reflex cough testing, without the nebulizer attached to the side port (side port is capped off). The participant will be asked to produce a sequential voluntary cough three times into the facemask attached to the pneumotachograph, and to maintain a comfortable seated position for all cough testing. Each cough trial will be separated by approximately 1 minute of quiet breathing. Participants will be given the following instructions for cough production: "When you are ready, cough as if something has gone down the wrong pipe."

Cough outcome variable: The concentration of capsaicin that elicits the 2-cough response (C2) will be recorded and serve as our measure of cough reflex sensitivity. Additionally, airflow measurements will be derived from the reflex and voluntary cough waveforms. Measurements will include compression phase duration (CPD), peak expiratory flow rate (PEFR), and peak expiratory flow rise time (PEFRT) and cough volume acceleration (CVA= PEFR / PEFRT).

Urge-to-cough (UtC): Following each capsaicin trial participants will rate their urge-to-cough (UtC) on a modified Borg rating scale, where 0 is no UtC, and 10 is maximal UtC. The UtC provides information regarding participants' perceived magnitude of the stimulus.

Fluoroscopic swallow study:
Swallowing function and physiology will be assessed using high-resolution digital fluoroscopy at a pulse rate of 30 frames per second, digitally stored for retrieval and offline analysis. Participants will be seated in the lateral viewing plane. Three trials each of the following swallowing tasks will be performed: Thin hold of 10 – 20mL liquid barium administered via cup, 5-10mL of pudding-thick barium administered via spoon, and 3-oz thin liquid sequential swallow administered by cup. Should aspiration be observed on the thin liquid swallows, we will omit the third trial and administer the pudding. Should aspiration occur with the pudding bolus, we will omit the remaining boluses (bail-out criteria). This protocol was selected because it mirrors our clinical protocol, and thus it would be possible to reduce participant burden and radiation exposure by replacing a clinical study with a research study, or vice-versa.

Our primary swallowing outcome measure will be score on the penetration-aspiration scale (PAS) measured from the videofluoroscopic evaluations of swallowing. Our secondary swallowing outcome measures will provide metrics of swallow physiology including swallow reaction time (SRT; H1 to B1), duration of laryngeal vestibule closure (dLVC; LVC to LVO),

duration to laryngeal vestibule closure (LVC) from hyoid burst (H1) (dtLVC_H1), duration to LVC from bolus head in pharynx (B1) (dtLVC_B1) and pharyngeal transit time (PTT; B1 to B2).

SEP Procedures:
Participants will be asked to refrain from strenuous physical activity, large meals and caffeine for at least four hours prior to participating. The participant will be seated in a private, sound/electrical shielded experimental room, with his or her back, neck and head comfortably supported. The participant will be monitored with a video camera. The electroencephalogram (EEG) will be collected using a 64-channel Electrical Geodesics Inc. (EGI; Eugene, Oregon, USA) net and amplifier system (amplification 20 K, nominal bandpass 0.10–100 Hz). Netstation software will be used for continuous recording of EEG data.

The 64-channel EGI electrode net will be placed on the head of the participant. Each participant will be instructed to breathe normally through a mouthpiece connected to a non-rebreathing valve. The inspiratory port will be connected to an airflow sensing pneumotachograph and inspiratory occlusion valve that obstructs the inspiratory airflow. Airflow, mouth and occlusion valve pressures will be sensed with differential pressure transducers and signal conditioners. Paired, identical inspiratory occlusions will be presented by silently inflating the occlusion valve approximately 50ms after the onset of inspiration, and will last for 150 ms. Each occlusion pair will be separated by a 500 ms interstimulus interval. The occluded breaths will be separated by at least 2 unoccluded breaths. The total time required for the presentation of the occlusions will be 700ms after the onset of inspiration, which is within the normal 1.5 second inspiratory duration. Up to 100 occlusion pairs will be delivered in each experimental trial. The latency from stimulus delivery and amplitude for the P1, N1, P2 and N2 peaks will be recorded from both the first (S1) and second (S2) occlusion.

Attend/ignore conditions: In order to understand and account for the effect of attention to the stimulus on the latency, amplitude, and gating of the airway SEP peaks, we will perform the SEP procedure under 2 conditions: attend and ignore. For the attend condition, participants will be instructed to pay attention to their breathing and press a button one time whenever they feel an obstructed breath. In the ignore trial, the subject will not be given any instructions to pay attention to their breathing, and will be asked to watch a series of neutral affective pictures (IAPS; (58)) and ignore their breathing.

SEP outcome measures*:* *a. SEP peaks:* The latency and amplitude of the SEP P1 and N2 peaks under the attend and ignore conditions are the primary outcome variables. Secondary outcome measures include the latency and amplitude of the other component peaks, including N1 and P2 peaks. *b. SEP Gating:* The ratio of the amplitude the peaks generated by the first and second paired occlusions will be calculated for each peak, within the attend and ignore conditions. The primary outcome variable will be the ratio of S2/S1 for the P1 and N2 peaks, and the S2/S1 ratio for N1 and P2 peaks will serve as secondary outcome variables.

Statistical Analysis

Aim 1: A multiple regression analysis will be conducted with the slope magnitude estimation as the outcome and disease duration, age, and sex as the independent variables in addition to their mutual interaction terms. Model selection technique will be adopted to select the optimal parsimonial model deemed for the data. We will also perform subgroup analysis to

compare the slope magnitude estimation between PD patients with PAS (penetration-aspiration scale) no more than 2 and PD patients with PAS greater than 2 by adjusting confounding factors of age, gender and BMI, etc. to demonstrate that reduced slope of magnitude estimation is present in PD patients regardless of PAS status. We will include other swallow and cough outcome variables in this model to determine relationships between dependent variables.

Aims 2 and 3: We will first fit a multiple regression model with P1 and N2 peak amplitude and gating ratios as the dependent variable and and condition (1 = attend and 2 = ignore) as independent variables by controlling confounding variables including age, gender, BMI, etc. In order to determine the relationship between P1 and N2 peaks and swallow and cough function, we will fit a second multiple regression model with P1 and N2 (amplitude and gating ratio), cough and swallow outcomes as the dependent variables and disease duration as the primary independent variable by controlling confounding variables including age, gender, BMI, etc. Also, model selection technique will be adopted to select the optimal model. Besides the primary outcomes of P1 and N2 peaks, other secondary outcomes such as N1 and P2 amplitudes and gating ratios will be analyzed.

Protocol 2 (aim 4)

Participants: This study will include men and women with PD, and we will recruit from those participating in protocol 1, in addition to the general PD population at UF Fixel Institute for Neurological diseases given protocol 2's larger required N. For participants who did complete protocol 1, the procedures will not be replicated in protocol 2 (therefore these participants would only complete the 2nd and 3rd protocol 2 visits). The inclusionary and exclusionary criteria will be identical to those of protocol 1, with the exception that this protocol will also include people with possible or probable progressive supranuclear palsy (PSP) or multiple systems atrophy (MSA).

Power Analysis: To test the hypothesis that the relationship between swallow and cough motor deficits, and reflex cough sensitivity varies as a function of disease duration in PD, we would compare the penetration/aspiration scale change from baseline with 10 to 18 months apart for PD patients. As shown in our pilot study data, the mean (SD) penetration-aspiration scale change from baseline for the first two 10 to 14 months period are 2.08 (1.97) and 2.82 (2.33), respectively. Based on it, to reach a 0.85 power by controlling the type I error rate to 0.05 for detecting this effect size with two-sided test, we would need 155 PD patients.

Procedures to be included from protocol 1 (See protocol 1 for complete description of these procedures)

Pulmonary function, depression and apathy, Reflex and voluntary cough procedures, Swallowing evaluation procedures will be completed in a manner identical to that described in the preceding paragraphs.

Procedures unique to protocol 2:
Longitudinal measurement of swallowing and cough function: Measures of swallowing and cough will be collected at 3 time points spaced 10 – 18 months apart.

For participants who were also in study protocol 1, those swallowing and cough data will be used for the first time-point in order to reduce participant burden.

Longitudinal measures of Participant disease status: Disease severity (unified Parkinson's disease rating scale and Hoehn & Yahr score), medications, levodopa equivalent dose, and surgical status (specifically deep brain surgery) will be recorded at each study visit. Pharmacologic and/or surgical therapies for PD may have known and unknown influences on swallowing and/or cough function (i.e., (43)) and will therefore be recorded for this study.

Swallow and cough outcome measures (described in detail in protocol 1):
1. Slope of UtC sensitivity
2. Cough sensitivity: Capsaicin concentration that elicits the 2-cough response (C2)
3. Cough motor (airflow) measures: CPD, PEFR, PEFRT, CVA
4. Swallowing performance measures: Penetration-aspiration scale scores (PAS)
5. Swallowing physiology measures: SRT, dtLVC_H1, dtLVC_B1, dLVC, PTT

Statistical analysis: In the statistical analysis, we would fit a linear mixed effects model to take into account the repeated measurement by regarding each subject as a cluster. Specifically, in the analysis, the repeatedly measured outcome, i.e. change of penetration-aspiration scale scores (PAS), C2, cough and swallowing physiology variables from baseline, will be fitted by adjusting for fixed effects terms including baseline PAS, age, sex, medications, levodopa equivalent dose, surgical status, and disease duration (2=third 10-18 month; 1=second 10-18 month; and 0=first 10-18 month) and the random effects term due to participants. Restricted resampling model selection (Zou et al. 2015) technique will be adopted to conduct the valid statistical inference for repeated measurement data and it is also robust to missing data which is particularly useful for this study. Expected outcomes: Results of this aim are expected to provide a clinical deliverable in the form of sensitive and specific predictor(s) of airway protection dysfunction that are more customized for patients with different PD durations. As well, because of the study's prospective longitudinal design, this study will also be the first to examine the natural history of cough function in people with PD over the proposed 3-year time period.

Data storage: All participant records will be maintained in HIPPA compliant storage. Paper copies of the ICF will be kept in a locked cabinet, in the locked research laboratory of the PI (D2-063 or DG-143; K. Hegland). Paper data collection forms will be kept in a separate, locked file cabinet, also in the research laboratory of the PI. Paper forms will not include participant names or dates; rather participants will be assigned a unique participant ID number that will be kept separate from the name. Electronic database files containing summary data for individual participants and compiled data for all participants will be saved according to participant number within a limited access, password protected folder on the PI's P-drive.

7. Possible Discomforts and Risks

Completion of the Beck Depression Index-II (BDI-II) and Starkstein Apathy Scale (AS) may make some participants feel uncomfortable or sad. We will provide participants whose scores fall outside the norm with contact information for UF psychiatry and psychology for further evaluation and care if desired.

Some participants may find breathing against a resistive load and/or through a mouthpiece to be effortful or difficult and may report feeling breathless. We will instruct the participants to "relax and breathe" as well as provide breaks to allow them to breath naturally without the mouthpiece in place throughout the tasks.

Delivery of capsaicin has been found to be safe, reliable, and reproducible in multiple research studies involving both healthy and disordered populations. Although risks associated with capsaicin are low, participants may report a 'tingling' or 'hot' sensation that passes quickly with the swallowing of water. Thus, water will be available throughout study procedures.

Completion of a fluoroscopic swallow study involves patient exposure to radiation. However, we will minimize the amount of radiation exposure according to standard clinical practice: thoroughly explaining the protocol to the patient prior to the study and engaging the fluoroscopy only when the patient is completing each swallowing task. In addition, our protocol allows for the substitution of a clinical fluoroscopic swallow study with a research study, or vice-versa, to reduce unnecessary participant burden and radiation exposure.

For the SEP procedure, the EEG net that will be placed on the patient's head will be soaked in an electrolyte solution prior to placement. As such, the patient's head and hair may become damp. We will place clean dry towels around the patient's shoulders to keep any excess moisture from seeping into their clothes and provide additional towels to dry off their hair following completion of the procedure.

8. Possible Benefits

For most participants, there are no direct benefits for participating in this study. However, completion of a fluoroscopic swallow study, if one has not yet been completed as a part of his/her normal clinical care, may identify signs and symptoms of dysphagia and/or aspiration, with subsequent referral for appropriate management.

9. Conflict of Interest

There are no real or potential conflicts associated with this project for any members of the investigative team.

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