Alpha-Synuclein Pathology and Axonal Degeneration of the Peripheral Motor Nerves Innervating Pharyngeal Muscles in Parkinson Disease

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Abstract

Parkinson disease (PD) is a neurodegenerative disease primarily characterized by cardinal motor manifestations and CNS pathology. Current drug therapies can often stabilize these cardinal motor symptoms, and attention has shifted to the other motor and nonmotor symptoms of PD that are resistant to drug therapy. Dysphagia in PD is perhaps the most important drug-resistant symptom because it leads to aspiration and pneumonia, the leading cause of death. Here, we present direct evidence for degeneration of the pharyngeal motor nerves in PD. We examined the cervical vagal nerve (cranial nerve X), pharyngeal branch of nerve X, and pharyngeal plexus innervating the pharyngeal muscles in 14 postmortem specimens, that is, from 10 patients with PD and 4 age-matched control subjects. Synucleinopathy in the pharyngeal nerves was detected using an immunohistochemical method for phosphorylated α-synuclein. Alpha-synuclein aggregates were revealed in nerve X and the pharyngeal branch of nerve X, and immunoreactive intramuscular nerve twigs and axon terminals within the neuromuscular junctions were identified in all of the PD patients but in none of the controls. These findings indicate that the motor nervous system of the pharynx is involved in the pathologic process of PD. Notably, PD patients who have had dysphagia had a higher density of α-synuclein aggregates in the pharyngeal nerves than those without dysphagia. These findings indicate that motor involvement of the pharynx in PD is one of the factors leading to oropharyngeal dysphagia commonly seen in PD patients.

Key Words: Alpha-synuclein aggregates, Dysphagia, Intramuscular nerve twigs, Lewy bodies, Lewy neurites, Motor nerve, Parkinson disease, Pharyngeal constrictor muscles, Pharyngeal plexus, Upper esophageal sphincter, Vagus nerve.

INTRODUCTION

Parkinson disease (PD) is a neurodegenerative disorder affecting the human central, peripheral, and enteric nervous systems. In addition to degeneration of the nigrostriatal dopaminergic pathway, a variety of neuronal systems are involved in PD that cause multiple neuromediator dysfunctions that account for the complex patterns of functional deficits (1). In the upper aerodigestive tract, the pharynx, larynx, and tongue play important roles in swallowing, airway protection, phonation, and speech articulation. Bulbar dysfunction characterized by dysphagia, sialorrhea, hypophonia, and dysarthria is frequently observed in patients with PD and can be equally or even more disabling than the cardinal features. Approximately 50% to 80% of patients with PD develop dysphagia (2–8), and 60% to 90% of PD patients exhibit speech and voice disorders (9, 10). The pathogenetic mechanisms involved in the upper airway disorders in PD are unclear.

The histopathologic hallmark of PD is the presence of fibrillar aggregates of α-synuclein called Lewy bodies (LBs) and Lewy neurites (LNs). Alpha-synuclein, a 140-α-amino acid and a 14-kDa protein, is a major component of the LBs and LNs in PD (11). It is expressed in the central and peripheral nervous systems (12, 13) and is involved in neurodegenerative diseases, including PD (14, 15). Lewy pathology has been identified in the autonomic nervous system in PD, including the cardiac plexus (16–20), enteric nervous system of the alimentary tract (21–23), and skin (24–28). Although α-synuclein histopathology has been found in peripheral autonomic and sensory nervous systems, little is known about its existence in peripheral motor nerves in PD.

Recently, we demonstrated histologic and histochemical alterations in the pharyngeal muscles of patients with PD.
Specifically, pharyngeal muscles in PD exhibited pathologic changes, including the presence of denervated and atrophied fibers, fiber-type grouping, and fast-to-slow myosin heavy-chain transformation (29). In addition, the pharyngeal muscle atrophy (29) and vocal fold atrophy (10, 30, 31) observed in patients with PD indirectly indicate motor impairment in PD. Motor innervation of the pharyngeal and laryngeal muscles is provided by cranial nerve X. Nerve X gives off several motor branches, including the pharyngeal branch of nerve X (Ph-X), recurrent laryngeal nerve, and superior laryngeal nerve (SLN), which innervate the pharyngeal, laryngeal, and some palatal muscles. The pharyngeal constrictor (PC) and cricopharyngeal (CP) muscles receive their motor innervation from the pharyngeal plexus formed from the Ph-X, glossopharyngeal nerve, and sympathetic nerve (32–36), whereas the laryngeal muscles are innervated by the recurrent laryngeal nerve and the external branch of the SLN. The Ph-X and laryngeal nerves are derived from cervical nerve X (37–41). Morphologic and immunohistochemical alterations in the pharyngeal muscle fibers in PD suggest that the motor nervous system in the pharynx may be affected by neuropathologic processes occurring in PD (29).

Recent studies have demonstrated synucleinopathy in the cervical nerve X fibers in PD (23, 42–44). Because 80% to 90% of the fibers in nerve X are afferent fibers (45), it remains unknown whether the α-synuclein–positive fibers identified in nerve X are motor in nature. In addition, little is known about whether Lewy pathology exists in the intramuscular nerve twigs (INTs) and axon terminals within neuromuscular junctions (NMJs) in PD.

The purpose of this study was to test the hypothesis that the myofiber atrophy observed in PD pharyngeal muscles is caused by neurodegeneration of the pharyngeal motor nerves. To test this hypothesis, we examined pharyngeal motor nerves in PD, including the cervical nerve X trunk, the pharyngeal plexus formed mainly by the Ph-X, INTs, and axon terminals within NMJs to detect degenerated motor nerve fibers using immunohistochemistry for phosphorylated α-synuclein (psyn).

**MATERIALS AND METHODS**

**Patients**

Histochemical and immunohistochemical studies were performed on neural tissues obtained from 10 deceased patients with clinically diagnosed and neuropathologically confirmed PD and 4 age-matched controls. The PD pharynges were provided by the Brain and Body Donation Program at Banner Sun Health Research Institute, which is part of Banner Health, a regional nonprofit health care provider centered in metropolitan Phoenix, Ariz (46). Banner Sun Health Research Institute and Mayo Clinic Arizona are the principal institutional members of the Arizona Parkinson’s Disease Consortium, which conducts a longitudinal clinicopathologic study of PD and normal aging subjects with annual examinations from entry until death and autopsy. The healthy autopsied pharynges were obtained from Banner Sun Health Research Institute (n = 2) and the Department of Pathology at Hackensack University Medical Center (n = 2), Hackensack, NJ.

**Clinical and Neuropathologic Assessments**

Detailed clinical and neuropathologic data for each case were provided by the Arizona PD Consortium (Table 1). Patients received standardized neuropathologic examinations. Clinical assessments were performed by experienced movement

### TABLE 1. Demographic and Clinical Characteristics of Patients With PD and Control Subjects

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age at Death, years</th>
<th>Age at PD Onset, years</th>
<th>PD Duration, years</th>
<th>H&amp;Y Stages</th>
<th>Motor UPDRS</th>
<th>Cause of Death</th>
<th>UPDRS Months Before Death</th>
<th>PMI, hours</th>
<th>Dysphagia</th>
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<tbody>
<tr>
<td>PD 1</td>
<td>M</td>
<td>75</td>
<td>55</td>
<td>20</td>
<td>2</td>
<td>17</td>
<td>es-PD</td>
<td>21</td>
<td>69</td>
<td>Yes</td>
</tr>
<tr>
<td>PD 2</td>
<td>M</td>
<td>73</td>
<td>62</td>
<td>11</td>
<td>3</td>
<td>18</td>
<td>es-PD</td>
<td>26</td>
<td>26</td>
<td>No</td>
</tr>
<tr>
<td>PD 3</td>
<td>M</td>
<td>78</td>
<td>59</td>
<td>19</td>
<td>4</td>
<td>51</td>
<td>CAD</td>
<td>8</td>
<td>76</td>
<td>Yes</td>
</tr>
<tr>
<td>PD 4</td>
<td>F</td>
<td>84</td>
<td>64</td>
<td>20</td>
<td>3</td>
<td>29</td>
<td>CPF</td>
<td>10</td>
<td>48</td>
<td>No</td>
</tr>
<tr>
<td>PD 5</td>
<td>M</td>
<td>80</td>
<td>69</td>
<td>11</td>
<td>4</td>
<td>53</td>
<td>c-PD</td>
<td>11</td>
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<tr>
<td>PD 6</td>
<td>M</td>
<td>81</td>
<td>70</td>
<td>11</td>
<td>4</td>
<td>43</td>
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<td>F</td>
<td>79</td>
<td>68</td>
<td>11</td>
<td>4</td>
<td>47</td>
<td>Pneu</td>
<td>9</td>
<td>16</td>
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<tr>
<td>PD 8</td>
<td>M</td>
<td>75</td>
<td>45</td>
<td>30</td>
<td>4</td>
<td>66</td>
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<td>1</td>
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<td>PD 9</td>
<td>M</td>
<td>80</td>
<td>63</td>
<td>17</td>
<td>5</td>
<td>40</td>
<td>CPF</td>
<td>5</td>
<td>23</td>
<td>No</td>
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<tr>
<td>PD 10</td>
<td>M</td>
<td>79</td>
<td>56</td>
<td>23</td>
<td>2</td>
<td>28</td>
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<td>9</td>
<td>34</td>
<td>Yes</td>
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<tr>
<td><strong>Mean (range)</strong></td>
<td></td>
<td><strong>78 (73–84)</strong></td>
<td><strong>61 (45–70)</strong></td>
<td><strong>17 (11–30)</strong></td>
<td><strong>3.5 (2–5)</strong></td>
<td><strong>39 (17–66)</strong></td>
<td><strong>12.0 (1–26)</strong></td>
<td><strong>41 (16–76)</strong></td>
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</table>

| Control 1 | F   | 74                  | –                      | –                 | –          | –          | MSF           | –                       | 24          | No       |
| Control 2 | M   | 80                  | –                      | –                 | –          | –          | PC            | –                       | 73          | No       |
| Control 3 | M   | 70                  | –                      | –                 | –          | –          | Stroke        | –                       | 26          | No       |
| Control 4 | F   | 70                  | –                      | –                 | –          | –          | CPF           | –                       | 30          | No       |

| **Mean (range)** |     | **73.5 (70–80)** |                     |                   |           |            |               |                         | 38 (24–73) |          |

**Notes:** CAD, coronary artery disease; c-PD, complications of PD; CPF, cardiopulmonary failure; es-PD, end stage of PD; F, female; H&Y, Hoehn-Yahr Clinical Rating Scale (score range, 1–5); M, male; MNP, malignant neoplasm of prostate; MSF, multisystem failure; PC, pancreatic cancer; PD, Parkinson disease; PMI, postmortem interval; Pneu, pneumonia; UPDRS, Unified Parkinson Disease Rating Scale.
disorder specialists (Charles H. Adler, John N. Caviness, Holly A. Shill, Johan E. Samanta), who rated the clinical severity of PD using the Hoehn and Yahr Scale (47) and disability and motor impairment using the Unified Parkinson’s Disease Rating Scale (UPDRS) (48). Specific clinicopathologic diagnostic criteria for PD were used (49). Dysphagia was assessed subjectively using item 7 of the UPDRS Part II Scale (i.e. swallowing score [0–4]: 0 [normal], 1 [rare choking], 2 [occasional choking], 3 [requires soft food], and 4 [requires nasogastric tube or percutaneous endoscopic gastrostomy feeding]). Gross and microscopic neuropathologic assessments were made by a single neuropathologist (Thomas G. Beach), who provided a detailed report on the findings for each patient with PD.

**Tissue Sampling and Preparation**

The mean postmortem interval between death and tissue preparation was 41 hours (range, 16–76 hours) for PD specimens (Table 1) and 38 hours (range, 24–73 hours) for controls; this postmortem interval does not hamper reliable histochemical analysis of autopsied tissues when the body has been stored in a refrigerated area (50, 51).

The neural structures studied included cervical nerve X, Ph-X, cervical superior sympathetic ganglion (SSG), and cervical sympathetic trunk. First, a 3-cm-long segment of the cervical nerve X trunk on each side was sampled immediately after it gave off Ph-X and SLN. The removed nerve X trunk was then divided into 4 subsegments (~7 mm in length each). One nerve segment was prepared for psyn immunohistochemistry for this study, and the remaining nerve segments were prepared for other purposes. Second, 1 cm of the Ph-X was sampled immediately after it is divided from nerve X. The removed Ph-X was then divided into 2 subsegments (~5 mm in length each), one of which was prepared for psyn immunohistochemistry. Finally, the entire SSG and a segment of sympathetic trunk (~7 mm in length each) were also harvested to detect α-synuclein pathology because both components gave off sympathetic nerve fibers to join the pharyngeal plexus (Fig. 1). The removed nerve and SSG samples were fixed with 10% neutral buffered formalin overnight, embedded in paraffin, and sectioned (4 μm) longitudinally or transversely.

After nerve samples were removed, the motor zone in the PC and CP muscles, where the pharyngeal plexus is located, was outlined and sampled as shown in Figure 1. The muscle samples were frozen in isopentane cooled by dry ice, sectioned longitudinally (60 μm thick) on a cryostat (Reichert-Jung 1800, Mannheim, Germany) at −25°C, and stored at −80°C until staining was performed. The muscle sections were immunostained to identify α-synuclein–immunoreactive INTs and axon terminals.

**Immunohistochemistry**

Nerve sections were deparaffinized in xylene and brought to distilled water through graded alcohols. The nerve and muscle sections were stained with an immunohistochemical method for psyn, as previously described (43, 44, 52–54). Briefly, the tissue sections were 1) pretreated with 1:100 protease K (Enzo Life Sciences, Farmingdale, NY) diluted in 0.1 mol/L PBS at 37°C for 30 minutes and washed 3 times in PBS; 2) immersed for 30 minutes in 1% H2O2 in 0.1 mol/L PBS with 0.3% Triton X-100 (PBS-TX) at pH 7.4 and washed 3 times in PBS-TX; 3) incubated at room temperature overnight in anti-psyn monoclonal antibody (psyn no. 64; Wako, Richmond, VA) at 1:1000 dilution in PBS-TX and washed 3 times in PBS-TX; 4) incubated with a secondary biotinylated antibody (anti-mouse immunoglobulin G diluted 1:1000 in PBS-TX; Vectastain, Vector Laboratories, Burlingame, CA) for 2 hours at room temperature and washed 3 times in PBS-TX; 5) treated for 30 minutes with avidin–biotin complex (Vector), with A and B components of the kit both at 1:100 dilution, and followed by 2 washes in PBS-TX and a last
Data Analysis

The frequencies of α-synuclein aggregates were compared between nerve X and Ph-X in PD patients with and without dysphagia. The influence of dysphagia on the density of α-synuclein lesions was evaluated in the PD group with 2-way repeated-measures analysis of variance. The main factors were the presence or absence of dysphagia (yes or no) and nerve type (nerve X and Ph-X). In addition, statistical analysis of the semiquantitative rates on α-synuclein lesions were compared between 4 experimental conditions (combination of nerve type and presence or absence of dysphagia) with the Kruskal-Wallis test, followed by post hoc analysis with Bonferroni corrected multiple comparisons. Statistical computations were performed using the Statistical Analysis System 9.2 (SAS, Inc., Cary, NC). Statistical significance was set at p < 0.05.

RESULTS

Demographic Features

There were 10 patients with PD (8 men and 2 women), with a mean age of 78 years (range, 73–84 years) (Table 1). All PD patients were white. The mean age at onset of PD was 61 years (range, 45–70 years), and the mean duration of PD at the time of death was 17 years (range, 11–30 years); 7 PD patients had developed dementia. The mean Hoehn and Yahr stage was 3.5: 2 patients were stage 2, 2 patients were stage 3, 5 patients were stage 4, and 1 patient was stage 5. The mean score for the motor UPDRS (UPDRS: Part III) was 39 points (range, 17–66). The mean interval between last neuro/movement examination and autopsy (UPDRS months before death) was 12 months (range, 1–26 months) for PD cases (Table 1). The 4 age-matched control subjects consisted of 2 men and 2 women with a mean age of 73.5 years (range, 70–80 years). The cause of death for each of the PD cases and normal control subjects was also given in Table 1.

Based on the UPDRS Part II Scale, dysphagia occurred in 5 of 10 PD patients (Table 1). In the 5 dysphagic patients, the swallowing score was rated as 1 in 2 cases (PD 1 and 6) and 2 in 3 cases (PD 3, 8, and 10). The mean PD duration in the dysphagia group was slightly longer than that in the nondon- dysphagia group, but there were no significant differences between dysphagia (PD 1, 3, 6, 8, and 10) and nondysphagia (PD 2, 4, 5, 7, and 9) groups with respect to mean PD duration (20.6 vs 14.0 years), mean age (77.6 vs 79.2 years), mean Hoehn and Yahr stage (3.2 vs 3.8), or mean score for the motor UPDRS (41.0 vs 37.4 points) (p > 0.05).

Neuropathology

All autopsied brains of the PD patients met neuropathologic criteria for PD. Microscopic examinations revealed that the substantia nigra and locus ceruleus showed moderate to marked depletion or loss of pigmented neurons, and there were several LBs in each region (data not shown). Immunohistochemical staining for psyn showed frequent immunoreactive LBs and LNs in the olfactory bulb, brainstem, amygdala, transentorhinal area, and cingulate gyrus, with variable densities in the 3 neocortical regions examined (temporal, frontal, and parietal). The major spinal cord subdivisions were examined in 7 cases; 6 of these had positive Lewy-type synucleinopathy in the spinal cord. Using the Unified Staging System for Lewy Body Disorders (54), 6 cases were classified as “neocortical stage” and 2 were “brainstem and limbic stage.” Seven of the PD cases also had dementia; of these, 5 cases also met consensus neuropathologic criteria for Alzheimer disease (56). One other case had dementia on the basis of progression of PD without concurrent Alzheimer disease.

Overview of Branching of the Cervical Nerve X and Formation of the Pharyngeal Plexus

Nerve X originates in the medulla oblongata and extends through the jugular foramen below the head to the neck, chest, and abdomen. It carries both afferent and efferent
fibers. The somatic motor fibers of nerve X, which arise from the cells of the nucleus ambiguus in the medulla, form the Ph-X and laryngeal nerves, which innervate pharyngeal, laryngeal, and some palatal muscles.

Figure 1 illustrates the branching and distribution patterns of the cervical nerve X and the nerve branches forming the pharyngeal plexus. Sihler stain, a whole-mount nerve staining technique, shows that on leaving the jugular foramen, nerve X gives off its Ph-X and then the internal and external branches of the SLN. The Ph-X arises from the upper part of the nodose ganglion of nerve X and passes across the internal carotid artery to the upper border of the middle PC, where it divides into numerous INTs. The PCs and CP sphincter receive their motor innervation from the pharyngeal plexus, which is formed mainly by the Ph-X and less by the pharyngeal branch of the glossopharyngeal nerve, external branch of the SLN, and sympathetic nerve fibers derived from the SSG. Thus, the somatic motor nerve fibers innervating the PC and CP muscles are carried by nerve X, are concentrated in the Ph-X, run in the nerve fascicles forming the pharyngeal plexus, and are distributed to the motor zone in the middle portion of the muscles where they give off axon terminals to innervate the NMJs. Therefore, identification of α-synuclein aggregates and degenerated axons in these neural structures would provide direct evidence of involvement of the peripheral motor nervous system controlling the pharynx in PD.

Phosphorylated α-Synuclein in Nerve X, Ph-X, INTs, and Axon Terminals Within NMJs

Immunostained longitudinal sections of the cervical nerve X, Ph-X, and INTs in PD cases are shown in Figure 2. Aggregated axonal α-synuclein inclusions were identified in both the

![Figure 2](http://jnen.oxfordjournals.org/)

**FIGURE 2.** Photomicrographs of the longitudinal sections of cervical nerve X trunk (A, D), Ph-X (B, E), and pharyngeal muscles (C, F) immunostained for psyn in a PD patient with dysphagia (A–C) and one without dysphagia (D–F), showing LNs in the nerves examined and immunoreactive INTs in the muscles. (A) A stained section of a cervical nerve X trunk from a PD patient with dysphagia (PD 3). There are numerous LNs (darker stained threads and dots) in nerve X. (B) A stained section of the Ph-X from the same patient. The Ph-X in this case also contains frequent LNs (lesion severity: moderate, ++). (C) A section of the inferior PC from the same PD. An INT running across the muscle fibers is horizontally sectioned. Several axons on the superior and inferior margins of the INT are positively immunostained for psyn (arrows), whereas the normal axons in the center of the INT are unstained. (D) Section of cervical nerve X trunk from a PD patient without dysphagia (PD 9), showing numerous LNs in nerve X. (E) In a section of the Ph-X from this PD patient, there are several LNs in the Ph-X (lesion severity: mild, +). (F) A stained section of CP sphincter from the same PD patient shows 3 immunoreactive axons in the muscle. Original magnification: (A–F) 200×.
cervical nerve X trunk (Fig. 2A, D) and Ph-X (Fig. 2B, E). Immunoreactive INTs (Figs. 2C, F; 3A–D) and axon terminals within NMJs (Fig. 3E–H) were revealed in the PD inferior PC and CP muscles.

There was a marked difference in the occurrence of α-synuclein pathology in the studied nerves and INTs between PD and control groups. For example, in the PD group, the most frequently affected structure was nerve X (10/10), followed by the Ph-X (8/9) and the INTs (7/10). No LNs were observed in nerve X or the Ph-X and no immunoreactive INTs were found in the muscles studied in the control subjects (data not shown).

On average, the cervical nerve X contained more α-synuclein aggregates than the Ph-X in PD. Variable lesion severity in the PD nerve X and Ph-X is summarized in Table 2. In the PD group, the severity of α-synuclein aggregates in nerve X was rated as mild (+) in 3 cases, moderate (++) in 4 cases, and severe (+++) in 3 cases. The Ph-X was obtained from 9 PD patients. The severity of α-synuclein lesions was rated as negative (no lesions) in 1 case, mild in 3 cases, and...
are located within the AChE-Ag–stained NMJ. Original magnification: 640.

that the preterminal axons (small arrows) innervate an NMJ (large arrow), and that the immunoreactive axon terminals as shown in

magnification: 640.

terminals.

terminals within NMJs.

lesions in 3 cases (PD 3, 8, and 10). In contrast, in the 5 PD

affected in 7 cases (7 of 10), whereas the Ph-X was moderately

affected in 5 cases (5 of 9). In PD, the density or severity of

synuclein lesions in nerve X was larger as compared with that

in the Ph-X (Table 2).

Parkinson disease patients with dysphagia had a higher
density of α-synuclein lesions in either nerve X or the Ph-X as
compared with those without dysphagia (Table 2). In the 5 PD
patients with dysphagia (PD 1, 3, 6, 8, and 10), nerve X
exhibited moderate lesions in 2 cases (PD 1 and 6) and severe
lesions in 3 cases (PD 3, 8, and 10). In contrast, in the 5 PD
patients without dysphagia (PD 2, 4, 5, 7, and 9), nerve X
disclosed mild lesions in 3 cases (PD 2, 5, and 7) and
moderate lesions in 2 cases (PD 4 and 9).

Two-way repeated-measures analysis of variance showed a
statistically significant effect of both factors and the
interaction between them. The density of α-synuclein lesions in the
dysphagia group was larger than that in the nondysphagia

The severity of α-synuclein–positive lesions in the cervical nerve X and Ph-X was rated according to the mean density of Lewy neurites and assessed semiquantitatively using an arbitrary grading system: −, no lesions; +, 1 to 20 lesions per field (mild); ++, 21 to 50 lesions per field (moderate); ++++, more than 50 lesions per field (severe). NA, not available.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Cervical Nerve X</th>
<th>Ph-X</th>
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<tbody>
<tr>
<td></td>
<td>Lewy Neurites (Range)</td>
<td>Severity of Lesions</td>
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<tr>
<td>1</td>
<td>26 (22–30)</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>13 (10–14)</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>60 (42–69)</td>
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</tr>
<tr>
<td>4</td>
<td>45 (33–56)</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>20 (14–26)</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>48 (35–57)</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>17 (12–24)</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>54 (39–62)</td>
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</tr>
<tr>
<td>9</td>
<td>40 (21–55)</td>
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<tr>
<td>10</td>
<td>55 (46–61)</td>
<td>+++</td>
</tr>
<tr>
<td>Mean (range)</td>
<td>38 (13–60)</td>
<td>17 (6–24)</td>
</tr>
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</table>

The severity of α-synuclein–positive lesions in the cervical nerve X and Ph-X was rated according to the mean density of Lewy neurites and assessed semiquantitatively using an arbitrary grading system: −, no lesions; +, 1 to 20 lesions per field (mild); ++, 21 to 50 lesions per field (moderate); ++++, more than 50 lesions per field (severe). NA, not available.

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affected in 7 cases (7 of 10), whereas the Ph-X was moderately
affected in 5 cases (5 of 9). In PD, the density or severity of
α-synuclein lesions in nerve X was larger as compared with that
in the Ph-X (Table 2).

Longitudinal sections of inferior PC and CP muscles
immunostained for psyn showed immunoreactive axons and INTs (Figs. 2C, F; 3A–D), preterminal axons and axon terminals
within NMJs (Fig. 3E–H). Some muscle sections immunostained
for psyn (Fig. 3H) were restained with AChE-Ag. Acetylcholinesterase and silver staining showed that the pre-
terminal axons innervated NMJs and that the immunoreactive
axon terminals were located within the NMJs (Fig. 3I), indicating that they were motor in nature. No immunoreactive
INTs or axon terminals were observed in control muscles (data
not shown).

α-Synuclein Aggregates in the SSG and Sympathetic Trunk

Cervical SSG and sympathetic trunk (SN) provide nerve
fibers to join the pharyngeal plexus that supplies the PC and CP
muscles (Fig. 1). Superior sympathetic ganglion and SN
obtained from 7 PD (PD 4–10) and 3 control subjects were
immunostained for psyn. All of the 7 PD SSG (Fig. 4A–C) and
SN (Fig. 4D–F) samples exhibited α-synuclein aggregates. The
SSG and SN were affected slightly in 2 cases (PD 4 and 7),
moderately in 2 cases (PD 5 and 6), and severely in 3 cases (PD

FIGURE 3. Longitudinal sections of inferior PC and CP sphincter immunostained with anti-phosphorylated α-synuclein immunohistochemistry in PD patients showing immunoreactive INTs and axon terminals within NMJs. (A–C) Inferior PC muscle from a PD patient with dysphagia (PD 8). In (A), there are immunoreactive axons (darkly stained threads). Original magnification: 100×. There are 3 obliquely cut intramuscular nerve branches (arrows) in (B). There are several α-synuclein–immunoreactive axons (darkly stained threads) in each of the nerve branches. Original magnification: 100×. (C) Higher magnification of (B) shows the profiles of the nerve branches and α-synuclein–positive and –negative (unstained) axons within the intramuscular nerve branches. Original magnification: 200×. (D–F) Inferior PC muscle from a PD patient without dysphagia (PD 9) showing α-synuclein–immunoreactive INTs and axon terminals within NMJs. (D) Low-power view shows immunoreactive INTs (arrows) containing α-synuclein–positive axons. Original magnification: 100×. (E) Higher power view shows α-synuclein–positive axon terminals within an NMJ (arrow). Original magnification: 200×. (F) Higher magnification of (E) shows the profiles of the immunoreactive axon terminals within the NMJ (arrow). Original magnification: 640×. (G–I) Sections of CP muscle from a PD patient with dysphagia (PD 10) showing immunoreactive axon terminals. (G) A CP with 2 α-synuclein–positive preterminal axons (small arrows) and their terminals within the NMJ (large arrow). Original magnification: 200×. (H) Close-up photograph of (G) shows the organization of the axon terminals within the NMJ. Note that 2 immunoreactive preterminal axons (small arrows) innervating an NMJ give off terminals within the NMJ (large arrow). Original magnification: 640×. (I) The muscle section shown in (H) was restained with acetylcholinesterase and silver stain (AChE-Ag). Note that the preterminal axons (small arrows) innervate an NMJ (large arrow), and that the immunoreactive axon terminals as shown in (H) are located within the AChE-Ag–stained NMJ. Original magnification: 640×.
FIGURE 4. Cross sections of the cervical SSG (A–C) and longitudinal sections of the cervical sympathetic nerve (D–F) from patients with PD immunostained for phosphorylated α-synuclein. (A) A stained section of SSG from a PD patient without dysphagia (PD 7). The SSG is slightly affected as it contains some α-synuclein aggregates. Original magnification: 200×. (B) A stained section of SSG from a PD patient without dysphagia (PD 5). The SSG is moderately affected because it exhibited abundant α-synuclein aggregates. Original magnification: 200×. (C) A stained section of SSG from a PD patient with dysphagia (PD 10). The SSG is severely affected because it contained numerous α-synuclein aggregates. Original magnification: 200×. (D) A stained longitudinal section of a cervical sympathetic trunk from the same PD patient as in (B) (PD 5). Note that there are abundant α-synuclein aggregates in the sympathetic nerve. Original magnification: 200×. (E) Low-power view of a stained longitudinal section of a cervical sympathetic trunk from the same PD patient as in (C) (PD 10). Note that this sympathetic nerve contained numerous α-synuclein aggregates. Also note that α-synuclein–immunoreactive axons are not randomly distributed throughout the nerve but are concentrated in two thirds of the nerve. Original magnification: 100×. (F) Higher power view of (E). Original magnification: 200×.

8, 9, and 10). No α-synuclein aggregates were identified in the SSG or SN in the control samples (data not shown).

DISCUSSION

To our knowledge, this is the first study to demonstrate α-synuclein pathology in the peripheral motor nerves innervating the pharynx in PD. We show that some nerve fibers of the pharyngeal plexus innervating the PCs and CP sphincter undergo degeneration in PD. Degenerative changes in the motor nerve fibers were documented with anti-psyn immunohistochemistry. We detected α-synuclein aggregates in cervical nerve X, Ph-X, cervical SSG, and sympathetic trunk in PD; found that there were psyn-immunoreactive INTs and axon terminals within the NMJs in the PD PC and CP muscles; and showed that some α-synuclein–immunoreactive axon terminals in the pharyngeal muscles were motor in nature because they were located within the NMJs and positively stained with the AChE-Ag method. Parkinson disease patients with dysphagia had a higher density of α-synuclein aggregates in the nerves studied as compared with those without dysphagia. Axonal degeneration of the pharyngeal motor nerves could, therefore, be responsible for denervation atrophy of the pharyngeal muscle fibers (29). Involvement of the peripheral motor nervous system controlling the pharynx in PD is most likely a major factor leading to the oropharyngeal dysphagia that is common in PD patients.

Disability in PD is mostly attributed to motor impairment (49, 57). Electrophysiologic abnormalities include decreased muscle activity (58), abnormal motor unit morphology (59), prolonged latencies and smaller motor nerve action potentials (60), and chronic partial denervation in the PD limb muscles
Motor unit number estimation is a unique electrophysiologic means that can provide a numeric estimate of the number of axons innervating a muscle. Caviness et al (65) demonstrated that mild motor neuron dropout with reinnervation occurs in PD. The detectable electrophysiologic changes suggest that motor degeneration is part of the pathologic processes occurring in PD. These observations gain support from immunohistochemical studies by Beach et al (44), who reported that occasional large anterior horn neurons as well as some fibers within the vagus and sciatic nerves in PD patients were immunoreactive for psyn.

Motor disturbances of the upper aerodigestive tract such as oropharyngeal dysphagia and speech and voice disorders in PD are common. Indeed, there are approximately 8 million or more individuals in the world each year that have or will have swallowing and speech disorders associated with PD (7). Swallowing and speech problems in PD have been attributed to orofacial-laryngeal bradykinesia and rigidity (67). However, studies using simultaneous videoradiography and pharyngeal manometry found no correlation between overall muscle rigidity score and abnormal pharyngeal wall motion in PD patients (68). Importantly, pharmacologic and surgical interventions for PD-related swallowing, speech, and voice disorders are minimally effective. Whereas neuropharmacologic and brain stimulation have marked therapeutic effects on limb motor functions, their effects on speech and swallowing in PD are less impressive and, in some cases, adverse (7, 8, 69–72). These findings suggest that oropharyngeal dysphagia in PD may not be caused solely by a reduction in basal ganglia dopaminergic activity. Therefore, other neurotransmitter systems or nondopaminergic mechanisms may also be involved (6, 70, 73). Various neuronal systems are likely involved in PD (1), thus resulting in multiple neuromediator dysfunctions that account for the complex patterns of functional deficits (74).

We recently reported that pharyngeal muscles in PD exhibit pronounced pathologic changes suggestive of muscle denervation (29, 35). Decreased motor activity has been found in PD patients with dysphagia. Using pharyngeal manometry, Ali et al (68) found that PD patients with dysphagia had a higher hypopharyngeal intrabolus pressure and lower pharyngeal contraction pressures, suggesting decreased muscle function. Clinical and radiologic assessments also revealed that dysphagic PD patients had decreased tongue movements (75, 76) and pharyngeal peristalsis (70, 75), indicating an impaired motor function. Here, we provide evidence for involvement of the peripheral motor nervous system controlling the pharyngeal muscles. Evidently, structural and functional changes in pharyngeal muscles are caused at least in part by axonal degeneration of the pharyngeal motor plexus.

Motor dysfunction of the pharynx and larynx could be caused by degenerative alterations in the peripheral nervous system and/or neuropathologic lesions in motoneurons. Degenerated peripheral motor nerve fibers with Lewy neurites in both nerve X and Ph-X suggest that Lewy pathology could occur in their motoneurons. The nucleus ambiguus gives origin to the somatic motor fibers of nerve X, which innervate the striated muscles of the pharynx, larynx, and upper esophagus (77). Therefore, the lesions at both the peripheral and central levels could lead to swallowing and voice disorders in PD. However, motoneurons in the nucleus ambiguus with their myelinated axons have been reported to be free of Lewy bodies and Lewy neurites during the course of PD (78–80). These negative findings may be because the nucleus ambiguous in PD has not been systemically investigated. It is also possible that the pathologic process of PD may affect the pharyngeal plexus before the nucleus ambiguous becomes involved. Therefore, it remains unknown whether axonal degeneration of the pharyngeal motor nerves progresses from distal (peripheral) to proximal (central) versus from proximal to distal. We hypothesize that the nucleus ambiguous and hypoglossal nucleus could be affected by the pathologic process of PD, although several reports have specifically noted that these nuclei do not seem to be affected. Because we see involvement in the axons arising from these nuclei, we suspect that subtle involvement may have not been identified by previous investigators. Alternatively, it has been demonstrated that Lewy pathology occurs at an early stage of the disease in the peripheral autonomic nervous system, including the cardiac plexus (16–20) and enteric nervous system of the alimentary tract (21–23). Braak et al (22, 78) and Del Tredici et al (79) hypothesized that neuroactive substances or pathogenes may pass through the mucosa of the gastrointestinal tract to enter the CNS via peripheral autonomic nerve fibers. A similar neuropathologic pathway might be applicable to the upper aerodigestive tract, where pathogens may affect the pharyngeal plexus first and then progress gradually from peripheral nerves to the nucleus ambiguous. More work is needed to assess alpha-synuclein expression pathways in the upper aerodigestive tract. Data from the present study point to the need for further systematic investigation of the peripheral nervous system elements that control the upper aerodigestive tract, which plays important roles in swallowing, speech articulation, and voice production. Determination of the neural alterations in the oral, pharyngeal, and laryngeal structures will shed light on the elucidation of the neural mechanisms of swallowing, voice, and speech disorders in PD.

ACKNOWLEDGMENTS

The authors thank the Banner Sun Health Research Institute Brain and Body Donation Program and the Arizona Parkinson’s Disease Consortium for the provision of whole-mount tongue-pharynx-larynx specimens and associated clinical and neuropathologic data from PD patients.

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