

Multi-organ distribution of phosphorylated α -synuclein histopathology in subjects with Lewy body disorders

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Abstract A sensitive immunohistochemical method for phosphorylated α -synuclein was used to stain sets of sections of spinal cord and tissue from 41 different sites in the bodies of 92 subjects, including 23 normal elderly, 7 with incidental Lewy body disease (ILBD), 17 with Parkinson's disease (PD), 9 with dementia with Lewy bodies (DLB), 19 with Alzheimer's disease with Lewy bodies (ADLB) and 17 with Alzheimer's disease with no Lewy bodies (ADNLB). The relative densities and frequencies of occurrence of phosphorylated α -synuclein histopathology (PASH) were tabulated and correlated with diagnostic category. The greatest densities and frequencies of PASH occurred in the spinal cord, followed by the paraspinal sympathetic ganglia, the vagus nerve, the gastrointestinal tract and endocrine organs. The frequency of PASH within other organs and tissue types was much lower. Spinal cord and peripheral PASH was most common in subjects with PD

and DLB, where it appears likely that it is universally widespread. Subjects with ILBD had lesser densities of PASH within all regions, but had frequent involvement of the spinal cord and paraspinal sympathetic ganglia, with less-frequent involvement of end-organs. Subjects with ADLB had infrequent involvement of the spinal cord and paraspinal sympathetic ganglia with rare involvement of end-organs. Within the gastrointestinal tract, there was a rostrocaudal gradient of decreasing PASH frequency and density, with the lower esophagus and submandibular gland having the greatest involvement and the colon and rectum the lowest.

Keywords Parkinson's disease · Parkinsonism · Dementia with Lewy bodies · Alzheimer's disease · Incidental Lewy bodies · α -Synuclein · Spinal cord · Sympathetic nervous system · Peripheral nervous system · Autonomic nervous system · Enteric nervous system · Submandibular gland · Esophagus · Adrenal gland · Heart · Stomach · Gastrointestinal system

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Introduction

The topographical distribution and density of Lewy bodies and their associated abnormal neurites are much greater than formerly appreciated [8, 28, 31, 33, 37, 44, 46, 61, 64, 70, 71, 77, 82]. Furthermore, it is also now more clearly apparent that Lewy body pathology frequently extends to the spinal cord and peripheral nervous system [12, 14, 17–19, 29, 40, 48, 81]. Despite these recent achievements, there has not yet been published a wide survey of the distribution of Lewy-type histopathological changes in the peripheral nervous system. A sensitive immunohistochemical method for phosphorylated α -synuclein [8, 10]

was used to stain sets of spinal cord and peripheral nervous system sections from 92 subjects that had previously received neuropathological diagnoses and Lewy body central nervous system (CNS) staging. The densities and frequencies of occurrence of phosphorylated α -synuclein histopathology (PASH) in multiple regions of spinal cord, sympathetic ganglia and tissue from the major organ systems were tabulated and correlated with diagnostic category. The results are presented below.

Materials and methods

Human subjects

Deceased human subjects were autopsied at Sun Health Research Institute (SHRI), a division of the not-for-profit health care provider Banner Health, located in the Sun Cities retirement communities of northwest metropolitan Phoenix, Arizona. All subjects had volunteered for the SHRI Brain and Body Donation Program (BBDP) [5, 9]. The majority of BBDP subjects are clinically characterized at SHRI with annual standardized test batteries that include functional, neuropsychological and neuromotor components, including the Mini Mental State Examination (MMSE) and Unified Parkinson's Disease Rating Scale (UPDRS). In addition, private medical records are requisitioned and reviewed for each subject and the postmortem Dementia Questionnaire [32] or an adaptation of the Clinical Dementia Rating Scale (CDR) are administered to subject contacts to help determine the presence or absence of dementia for those subjects lacking standardized ante-mortem evaluations. Subjects sign consent that has been approved by the Banner Health Institutional Review Board.

Subjects were chosen by searching the BBDP database for all cases that had received a whole-body autopsy, a completed neuropathologist's examination and a neuropathologic diagnosis of a Lewy body disorder, including Parkinson's disease (PD), dementia with Lewy bodies (DLB), incidental Lewy body disease (ILBD) and Alzheimer's disease with Lewy bodies (ADLB). Comparison groups were composed of subjects without evidence of dementia or parkinsonism (normal elderly subjects) and subjects with AD but no Lewy body pathology (ADNLB).

Subjects received standardized neuropathological examinations. Specific clinicopathologic diagnostic criteria were used for AD [1], PD [36], and DLB [58]. For both AD and DLB, cases received the diagnosis if they were classified as "intermediate" or "high" probabilities in their respective classification schemes. Cases with PASH, but not meeting these diagnostic criteria were designated as either ILBD, if they had no clinical history of parkinsonism or dementia, or ADLB if they had dementia, Alzheimer's

disease and Lewy bodies in any brain region, but failed to meet clinicopathologic criteria for DLB or PD.

Gross and microscopic neuropathologic assessments were made by a single observer (TGB) without knowledge of the clinical history or clinical diagnosis; subsequently the clinical history was reviewed with the neurologists (CHA, JNC, HAS, MNS) in order to make an appropriate clinicopathologic diagnosis.

Histologic methods

Diagnostic histologic methods were performed on standard blocks of tissue that were fixed in 3.75% neutral-buffered formaldehyde and then either dehydrated and embedded in paraffin or cryoprotected and cut on a freezing, sliding microtome. Each case was first staged according to the Unified Staging System for Lewy body disorders with a standard set of brain sections stained with an immunohistochemical method for phosphorylated α -synuclein as previously described [8]. The Unified Staging System is a modification of the scheme first devised by the Dementia with Lewy Bodies Consortium [8, 58, 59].

Paraffin-embedded sections from multiple body sites (Table 1) were stained in an identical fashion as the brain sections, using a polyclonal antiserum raised against an α -synuclein peptide fragment phosphorylated at serine 129, after epitope exposure with proteinase K [34]. The process leading to the choice of immunohistochemical method, as well as details of the method, has been described in a previous publication [10]. In each body region, the density of α -synuclein-immunoreactive perikaryal neuronal cytoplasmic inclusions as well as puncta and neurites was scored, at the site of highest density, by a single observer (TGB) without knowledge of diagnosis, as none, sparse, moderate, frequent and very frequent, using the templates provided by the Dementia with Lewy Bodies Consortium [58]. The total number of body sites examined varied between subjects as an initially broad sampling scheme was progressively reduced to those regions showing a greater likelihood to have positive staining. To evaluate the relative frequency of immunoreactivity in the different body regions, paraffin sections on a single stained slide from each body site listed in Table 1 were used. Following this analysis, selected regions of interest were further evaluated with up to five additional paraffin sections and/or 80 μ m thick formalin-fixed, frozen sections.

Alzheimer's disease histopathology was staged and graded on 40 μ m thick sections stained with the Gallyas method for neurofibrillary tangles and the Campbell-Switzer and thioflavine-S methods for senile plaques [15]. Braak's neurofibrillary tangle stages and CERAD neuritic plaque densities were assigned as described [16, 62].

Table 1 Body sites, organs and tissue types investigated with an immunohistochemical method for phosphorylated α -synuclein histopathology

Body region	Sites investigated within region
Spinal cord	Cervical (C4–5), thoracic (T6–7), lumbar (L3–4) and sacral (S1–5) spinal cord
Sympathetic ganglia	Middle cervical ganglia, middle ganglia of thoracic chain
Vagus nerve	Alongside the common carotid artery at the level of the larynx
Sciatic nerve	Overlying the external iliac artery in the pelvic cavity
Gastrointestinal system	Upper third and lower third of esophagus, stomach (body), duodenum, jejunum, ileum, transverse colon, sigmoid colon, rectum, submandibular gland, liver, pancreas (head), gallbladder
Respiratory system	Larynx, primary bronchus, lung
Endocrine system	Adrenal gland, thyroid gland, parathyroid gland, ovary, testis
Cardiovascular system	Thoracic and abdominal aorta, left and right ventricle and epicardium of heart at apex
Genitourinary tract	Kidney, urinary bladder, uterus, vagina
Musculoskeletal system	Rib (bone and associated muscle and soft tissue), psoas muscle, diaphragm
Skin	Abdominal skin, scalp
Miscellaneous	Spleen, lymph nodes (parabronchial), small bowel mesentery, breast

Statistical analysis

Statistical analyses consisted of, for comparing group means, analysis of variance (ANOVA), or, for non-parametric data, Kruskal–Wallis ANOVA. Proportional measures were compared using χ^2 tests.

Results

Ninety-two subjects had a full autopsy as well as neuropathological diagnoses within the targeted groups. All data from the study are given within the supplementary online table, for brevity some of the data are grouped for summary here. Descriptive measures of these groups are given in Tables 2, 3 and 4. The subjects were all of advanced age (Table 2) and the group means differed significantly

($P < 0.0001$), with the youngest group (PD) having a mean age of 79.3 while the oldest group (ILBD) mean was 86.7. As with all subjects of this advanced age, all had at least some neurofibrillary tangles in the brain [13] and even the elderly control group had a mean Braak stage of 2.6. All diagnostic groups had more male subjects and this was pronounced in the PD and DLB groups. The median postmortem intervals were uniformly short, ranging from 2.7 to 5.9 h and the group means were not significantly different.

The distribution of PASH within the brain is summarized in Table 3 using the Unified Staging System for Lewy Body Disorders and the mean densities of PASH within the ten scored brain regions are given in Table 4. An estimate of the aggregate CNS load of microscopic disease was also determined, using the sum of the mean PASH density scores in all ten evaluated brain regions (Table 4).

Table 2 General characteristics of the study subjects, by neuropathologic diagnosis, age, gender, postmortem interval (PMI), Braak neurofibrillary stage and CERAD neuritic plaque (NP) density

Diagnosis (<i>N</i>)	Age ^a	Gender (% male)	PMI (h)	Braak stage ^a	CERAD NP density ^a
Normal (23)	81.0 (14.1)	56.5	3.9 (2.5)	2.6 (1.3)	1.0 (1.3)
ILBD (7)	86.7 (8.5)	57.1	3.8 (2.9)	3.0 (1.0)	1.3 (1.4)
PD (17)	79.3 (7.5)	76.5	4.9 (4.1)	2.5 (1.1)	0.8 (1.2)
DLB (9)	83.2 (9.5)	67	2.7 (0.4)	4.4 (0.7)	3.0 (0.0)
ADLB (19)	84.0 (5.0)	53.0	4.3 (3.8)	5.2 (1.0)	2.7 (0.7)
ADNLB (17)	84 (5.9)	53.0	5.9 (10.2)	4.4 (1.2)	2.8 (0.4)

Means and standard deviations (SD) are given

Subjects did not differ significantly in terms of gender distribution or PMI but differed significantly in terms of age, Braak stage and CERAD neuritic plaque density

^a Group means were significantly different ($P < 0.0001$)

Table 3 Classification of subjects with phosphorylated α -synuclein histopathology by the Unified Staging System for Lewy body disorders

Diagnosis	Olfactory bulb only (I)	Brainstem predominant (IIa)	Limbic predominant (IIb)	Brainstem and limbic (III)	Neocortical (IV)
ILBD ^a	0	1 (17%)	2 (33%)	3 (50%)	0
PD ^a	0	2 (12.5%)	0	10 (62.5%)	4 (25%)
DLB	0	0	0	1 (11%)	8 (89%)
ADLB	2 (10%)	3 (16%)	11 (58%)	3 (16%)	0

Number and percentage of subjects in each stage are given

^a One subject was not classifiable due to missing brain regions needed for staging

Table 4 Mean (SD) of phosphorylated α -synuclein histopathology regional brain density scores for all subjects by diagnostic classification, with an aggregate total brain load (last row) given by the sum of the mean regional density scores

Brain region	ILBD	PD	DLB	ADLB
Olfactory bulb	2.5 (1.0)	2.6 (0.9)	3.6 (1.1)	3.0 (0.9)
Medulla	1.6 (1.6)	3.2 (0.7)	3.4 (0.7)	1.0 (1.2)
Pons	1.1 (1.5)	3.0 (0.9)	3.1 (1.0)	0.3 (0.6)
Midbrain	0.4 (0.8)	3.0 (0.9)	2.9 (1.5)	0.2 (0.7)
Amygdala	2.5 (1.6)	3.1 (0.9)	3.9 (0.3)	2.4 (1.7)
Transentorhinal	0.8 (1.8)	2.3 (1.1)	3.6 (0.7)	2.4 (1.8)
Cingulate cortex	0.3 (0.8)	1.8 (1.0)	2.9 (1.2)	0.3 (0.4)
Mid. temp. gyrus	0.3 (0.5)	1.2 (1.1)	2.6 (0.9)	0.2 (0.4)
Mid. front. gyrus	0.3 (0.5)	1.1 (0.9)	1.7 (1.1)	0.05 (0.2)
Inf. par. lobule	0.3 (0.5)	1.0 (1.0)	1.8 (1.0)	0.05 (0.2)
Sum of mean scores	10.1	22.3	29.5	9.9

More than 80% of subjects with PD and DLB were in the two highest stages, involving either brainstem through limbic regions (Stage III) or brainstem through neocortical regions (Stage IV). Most subjects with ILBD were in the brainstem-predominant (IIa) or limbic-predominant (IIb) stages while the most frequent stage for ADLB subjects was limbic predominant (IIb). Subjects with ILBD and ADLB had similar overall low aggregate PASH load scores while subjects with PD and DLB had overall high aggregate PASH load scores.

Figure 1 shows photomicrographs of the immunohistochemical staining for α -synuclein in the spinal cord, sympathetic ganglia and vagus nerve. Generally, sections with positive staining contained fibers, puncta and neuronal perikaryal staining (a, c, e, f, h) but occasionally sections or regions contained just fibers (i) or just perikaryal staining. The perikaryal staining was either diffusely distributed in the cytoplasm (e) or condensed into defined inclusions (h), only a subset of which resembled classical Lewy bodies. Within the vagus and peripheral nerves, and generally within nerves seen elsewhere in other body regions, positive staining was only in the form of nerve fibers (i). Within the spinal cord, positively-stained structures were found in

all gray matter regions, including posterior horn (b, c), anterior horn (d, e), intermediolateral region (a) and adjacent to the central canal (f). Although not formally analyzed here, the most frequent regions affected appeared to be the thoracolumbar intermediolateral horn and the base of the posterior horn of the sacral cord.

Figure 2 shows photomicrographs of the immunohistochemical staining for α -synuclein in multiple bodily organs and tissue types. As described for the spinal cord and sympathetic ganglia above, sections with positive staining contained either fibers, puncta or neuronal (ganglion cell) perikaryal staining, but ganglion cell staining was much less frequently seen, with the great majority of immunoreactive elements being fibers and puncta. A much more detailed morphologic delineation was seen in 80- μ m thick sections of submandibular gland and lower esophagus (Fig. 3), where extensive fiber networks and moderately frequent ganglion cells were often visualized, especially in subjects with PD or DLB. Generally, immunoreactive structures in the gastrointestinal tract were concentrated in the myenteric plexus of Auerbach and the submucosal plexus of Meissner. In the submandibular gland and pancreas, positively stained elements tended to be concentrated in nerve bundles within the connective tissue stroma. In the submandibular gland, 80- μ m section staining occasionally showed nerve fibers apparently investing arterioles (Fig. 3d). Adrenal gland staining was exclusively seen in the medulla and in nerve bundles in the surrounding fatty tissue, with no staining seen in the adrenal cortex.

The regional distribution of PASH, as evaluated in single microscopic slides from each body site, is shown in Table 5 and Fig. 4. No immunoreactive elements were present in any subject that had previously been classified, on the basis of the brain examination, as being free of PASH. Subjects with DLB and PD had similar profiles (Fig. 4b, c) and will be discussed together. In both the groups, the most frequently affected body region was the spinal cord, with 25 of 26 subjects involved, followed by the sympathetic ganglia (19/24), the vagus nerve (15/21), the gastrointestinal system (16/26), the sciatic nerve (10/22) and endocrine system (5/14), with other organ systems and tissues following at generally much lower frequencies.

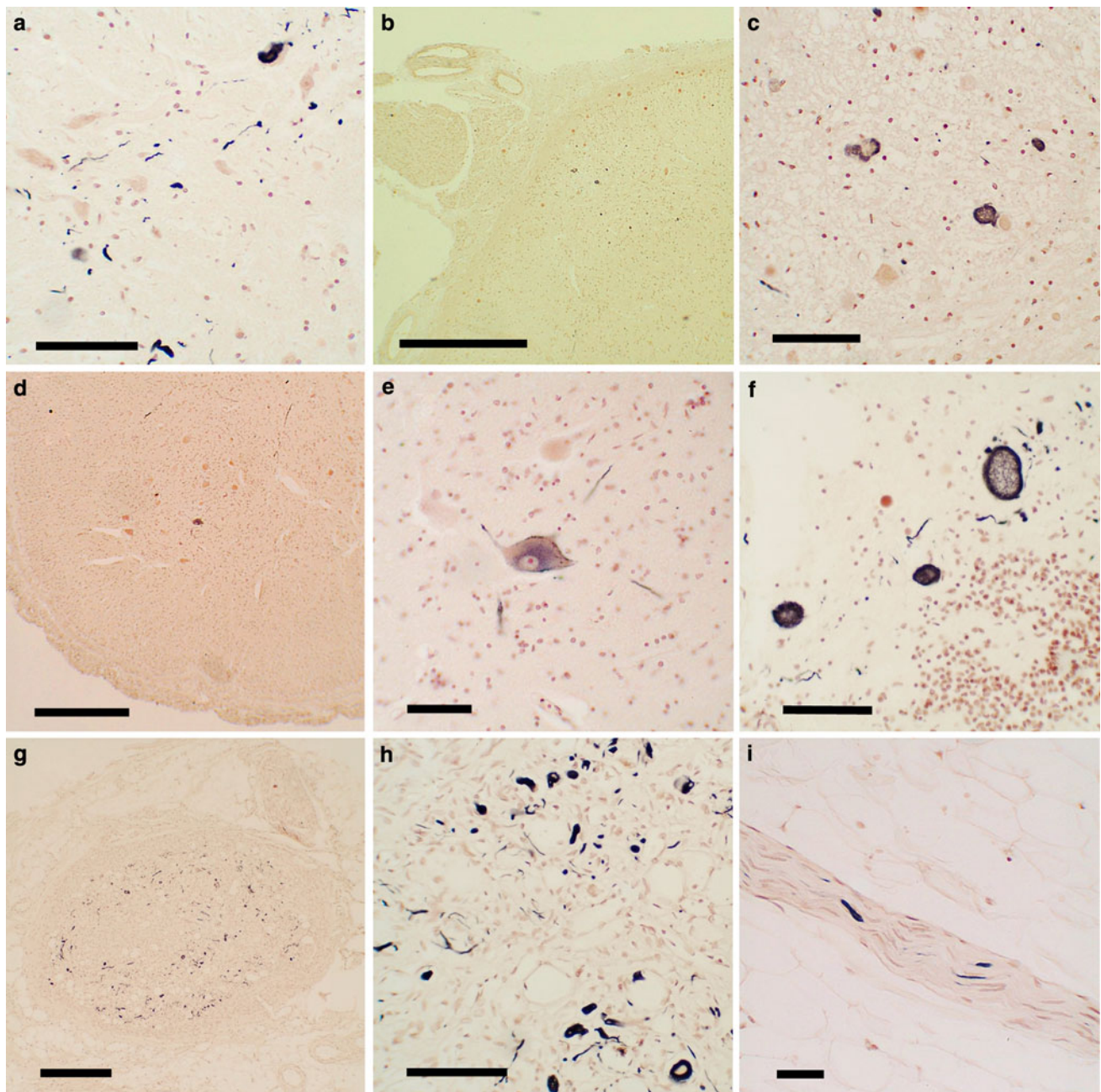
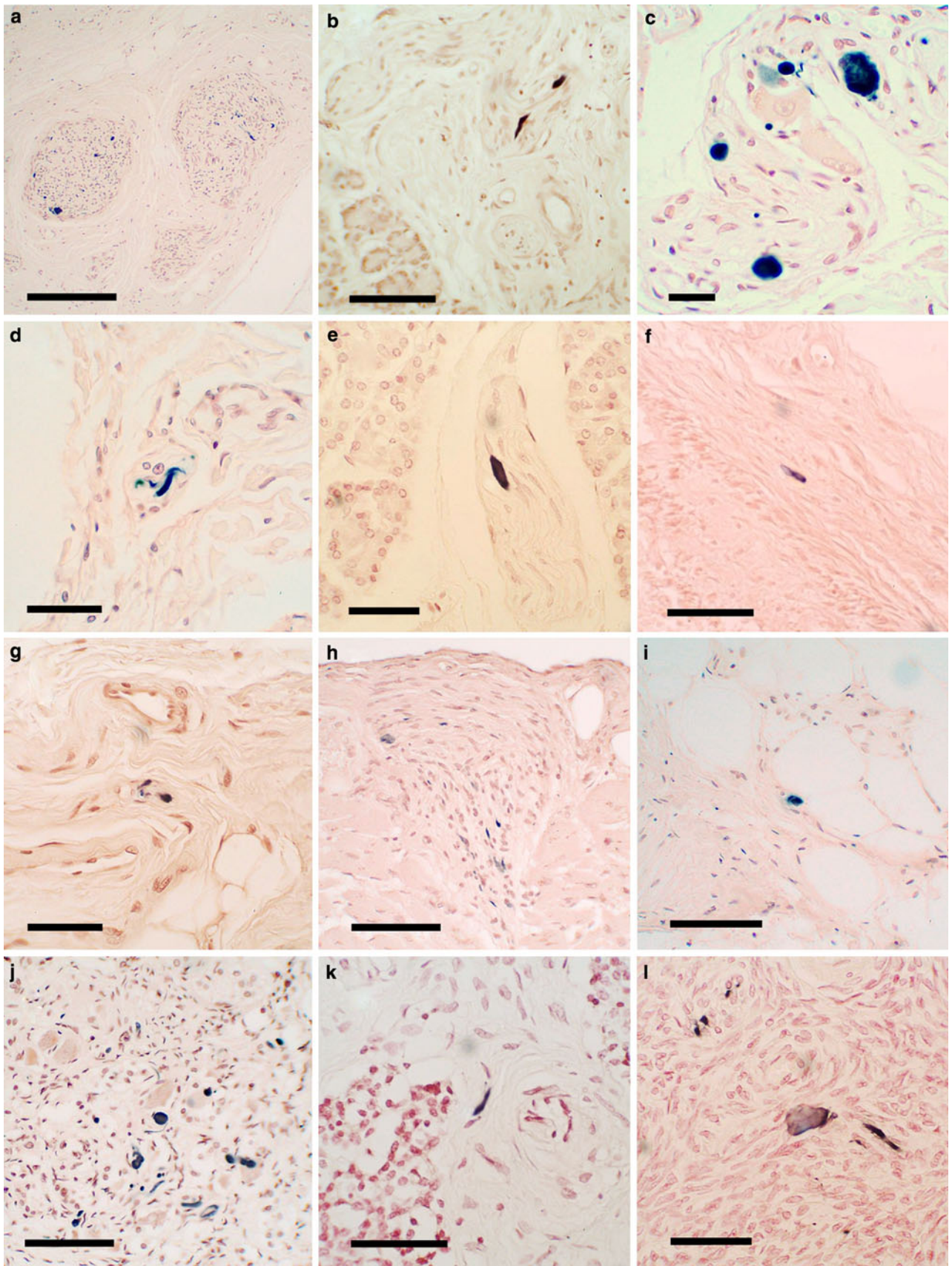


Fig. 1 Photomicrographs of slides of immunohistochemical staining for phosphorylated α -synuclein histopathology (*PASH*) in paraffin sections of the spinal cord, sympathetic ganglia and vagus nerve. Positive staining is *black*, the counterstain is neutral red. **a** The intermediolateral horn of the thoracic spinal cord of a subject with PD, showing immunoreactive fibers, puncta and perikaryal cytoplasmic inclusions. Calibration bar 80 μ m. **b, c** Low and higher magnification images of the posterior root entry into the sacral spinal cord of a subject with DLB, showing immunoreactive neuronal perikaryal staining. Calibration bar in **b** 0.3 mm; in **c** 80 μ m. **d, e** Low and higher magnification images of the anterior horn of the

sacral spinal cord in a subject with PD, showing diffuse cytoplasmic perikaryal immunoreactivity of a large motoneuron. Calibration bar in **d** 0.5 mm; in **e** 60 μ m. **f** Sacral spinal cord adjacent to the central canal of a subject with PD, showing immunoreactive swollen degenerating neurons or neurites. Calibration bar 80 μ m. **g, h** Low and higher magnification images of a middle cervical sympathetic ganglion of a subject with DLB, showing frequent immunoreactive dystrophic neurites, puncta and neuronal perikaryal cytoplasmic inclusions. Calibration bar in **g** 0.1 mm; calibration bar in **h** 100 μ m. **i** Small branch of the vagus nerve in a subject with PD, showing several immunoreactive nerve fibers. Calibration bar 60 μ m

No positive staining was observed in the abdominal skin in any of the 14 subjects with DLB or PD. Examination of additional sets of immunostained serial paraffin and 80- μ m

sections of submandibular gland and esophagus from DLB and PD subjects showed positive staining in 22 of 23 subjects for which the extra sections were stained.



◀ **Fig. 2** Photomicrographs of immunohistochemical staining for phosphorylated α -synuclein in paraffin sections of bodily organs and tissue. Positive staining is *black*, the counterstain is neutral red. **a, b** Low and higher magnification images of the submandibular gland in two subjects with PD, showing immunoreactive nerve fibers within the stroma of the gland. *Calibration bar* in **a** 0.2 mm; in **b** 100 μ m. **c** The submucosa of the lower esophagus of a subject with PD, showing immunoreactive puncta, fibers and perikaryal cytoplasmic inclusions in ganglion cells. **d** Immunostaining of fibers in the duodenal submucosa of a subjects with DLB. *Calibration bar* 20 μ m. **e** A single immunoreactive fiber in the stroma of the pancreas from a subject with PD. *Calibration bar* 40 μ m. **f** A single immunoreactive fiber in the submucosa of a primary bronchus of a subject with PD. *Calibration bar* 40 μ m. **g** A few immunoreactive fibers in the submucosa of the larynx of a subject with PD. *Calibration bar* 20 μ m. **h** Several immunoreactive fibers in an epicardial nerve twig entering the myocardium in a subject with DLB. *Calibration bar* 100 μ m. **i** A single immunoreactive fiber in the intermyenteric plexus of the urinary bladder of a subject with DLB. *Calibration bar* 20 μ m. **j** Frequent immunoreactive fibers, puncta as well as cells with diffusely stained perikaryal cytoplasm in the adrenal medulla of a subject with PD. *Calibration bar* 100 μ m. **k** A single immunoreactive fiber in the stroma of the parathyroid gland of a subject with PD. *Calibration bar* 10 μ m. **l** The ovary of a woman with PD showing diffuse perikaryal immunostaining of a neuron-like cell with adjacent immunoreactive fibers and puncta. *Calibration bar* 10 μ m

Subjects with ILBD and ADLB had much lower frequencies of positive staining in body regions (Fig. 4a, d). In subjects with ADLB, positive staining was limited entirely to the spinal cord and sympathetic ganglia, with only 1/18 and 2/18 subjects affected, respectively. Subjects with ILBD showed higher frequencies of positive staining than ADLB subjects in the spinal cord, sympathetic ganglia, vagus nerve and gastrointestinal tract while all other areas, as with the ADLB cases, showed no positive staining. When several microscopic slides from each spinal cord subdivision were stained, 5/6 of the ILBD cases had PASH present while on single-slide analysis, only 1 of 6 subjects had been positive. For the ADLB subjects for which serial paraffin and 80- μ m sections of submandibular gland and esophagus were examined, 3/15 were found to be positive, whereas examination of single paraffin sections had found no positively stained structures. Figure 4e depicts the regional staining distribution, derived from analysis of a single slide per region, when data from all subjects were combined. This shows generally the same pattern as for PD and DLB subjects.

Table 6 and Fig. 5a and b show the regional distribution and density of PASH within paraffin sections on single slides of the major subdivisions of the spinal cord as well as cervical and thoracic sympathetic ganglia, for all subjects combined. The frequency of positive staining between different cord regions is similar, with the cervical cord being the least-often affected (Fig. 5a). In terms of the density scores, the thoracic and sacral cord regions have higher mean scores than the cervical and lumbar regions.

Cervical sympathetic ganglia had the highest frequency and mean density scores of any region.

Table 7 and Fig. 5c and d show the regional distribution and density of PASH within major sites within the gastrointestinal (GI) system. There is a marked trend for a diminishing rostrocaudal gradient. The submandibular gland and lower esophagus have the highest frequency of PASH, followed by the stomach, small bowel regions, large bowel regions and rectum. The data shown for submandibular gland and esophagus do not include, to provide an unbiased comparison between GI regions, results from the serial paraffin or 80- μ m sections. As mentioned previously, when staining from these extra sections was considered, a higher percentage of subjects were graded as positive. A striking finding was the complete absence of positive staining in upper esophagus and therefore all data for esophagus shown are derived from analysis of the lower esophagus. Although not quantitatively assessed here, it was apparent that PASH was more frequently present in the myenteric plexus than in the submucosal plexus.

Discussion

The results of this investigation support prior studies [12, 14, 17–19, 29, 40, 48, 81] that have indicated that PASH is widespread throughout the spinal cord and peripheral nervous system of subjects with PD. The present study adds to this body of knowledge by providing data for other Lewy body disorders including DLB, ILBD and ADLB, by surveying many more sites than had previously been investigated, and using large enough sample sizes to provide preliminary estimates of the relative frequency and density of α -synuclein histopathology at these locations. In addition, the use of a method that is specific and sensitive for phosphorylated α -synuclein, which is detected only in pathological structures [34] effectively eliminates the ambiguity of staining results derived using antibodies that recognize normal α -synuclein.

For spinal cord and sympathetic ganglia, subjects with PD as well as DLB invariably have PASH in the regions of the cord containing preganglionic autonomic neurons as well as within sympathetic ganglia. Because these neurons project widely throughout the body, it is highly probable that, for subjects with PD and DLB, that α -synuclein histopathology is also very widely present within the end-organ targets of the autonomic nervous system, although a full test of this hypothesis will require multiple-section examination of each of the many sites [49]. This study has found that the relative frequency of PASH in end-organs was generally much lower than in the spinal cord or sympathetic ganglia, but when sampling was expanded

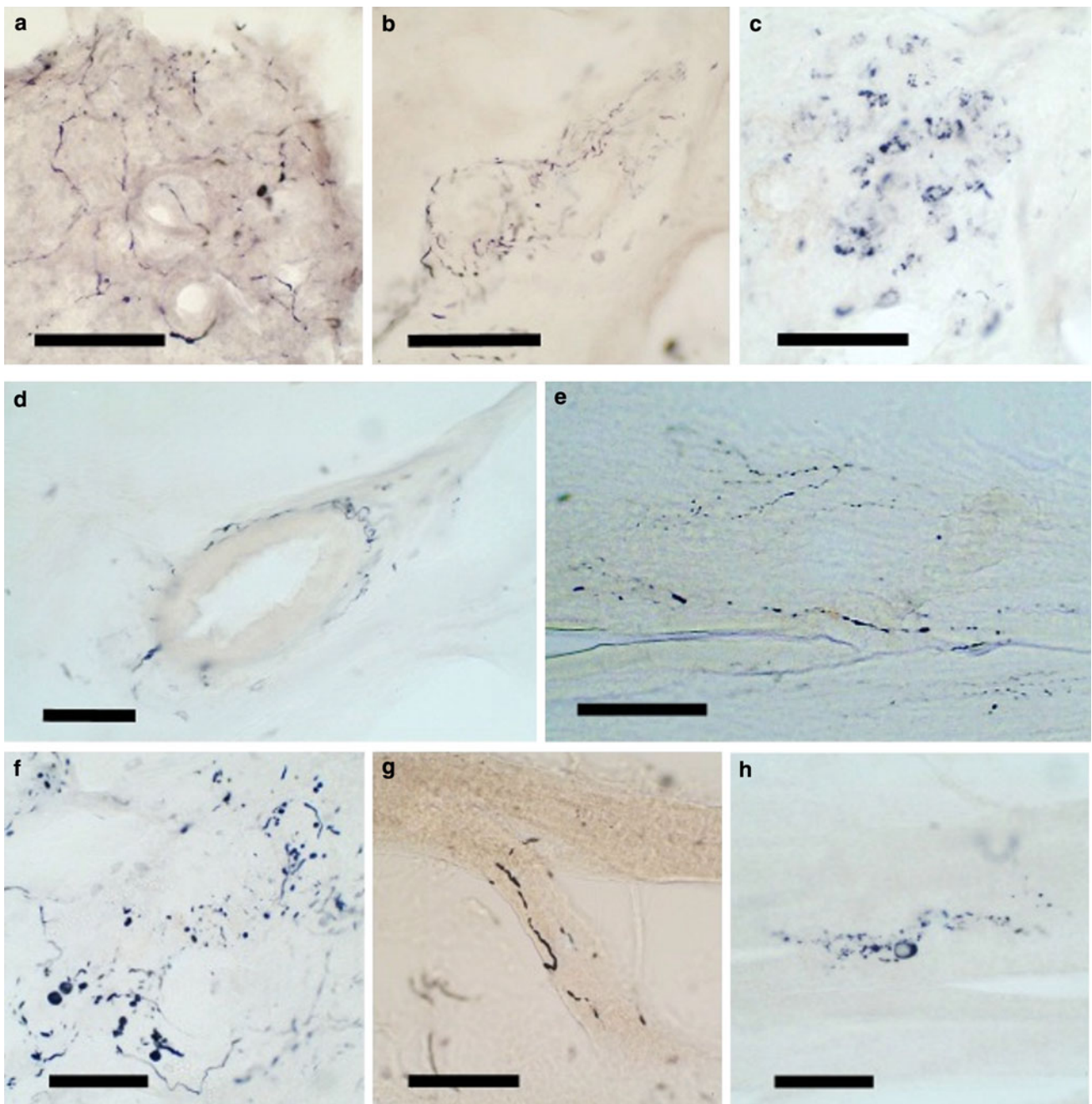


Fig. 3 Photomicrographs of immunohistochemical staining for phosphorylated α -synuclein in 80- μ m thick frozen sections of formalin-fixed, cryoprotected tissue blocks of submandibular gland and lower esophagus. Positive staining is *black*. There is no counterstain. Submandibular gland from subjects with DLB (**a–c**) and PD (**d**) showing frequent immunoreactive fibers in the gland parenchyma (**a**),

stroma (**b**) and around a small artery (**d**), while frequent immunoreactive puncta are seen in **c**. Lower esophagus from subjects with PD (**e, g, h**) and DLB (**f**) showing immunoreactive nerve fibers in **e**, nerve fibers and puncta in **f**, a thickened nerve fiber adherent to a bundle of smooth muscle fibers in **g** and a ganglion cell with diffuse perikaryal cytoplasmic staining in **h**

considerably through the use of multiple paraffin or 80- μ m sections, such as was done for the submandibular gland and esophagus, the frequency of positive staining was increased.

For subjects with ILBD, PASH has a more limited distribution, being more likely to be confined to the spinal

cord, sympathetic ganglia, vagus nerve and a subset of end-organs. Given the wide projections of the involved spinal preganglionic and sympathetic ganglion neurons, a wide but sparse involvement of the peripheral nervous system is likely to exist in ILBD, although again a more definitive investigation with multiple-section analysis will be

Table 5 Regional frequency of phosphorylated α -synuclein histopathology in single slides of different body regions, with subjects grouped by neuropathological diagnosis

Dx	SpCd	Sym	Vagus	Sciat	GI	Resp	Endo	Cardio	GU	MSK	Skin
ILBD	1/6 ^a	3/6	2/7	1/6	1/7	0/4	1/4	0/6	0/4	N/A	0/2
PD	16/17	12/15	11/15	8/16	11/17 ^b	1/8	2/9	0/9	1/8	0/6	0/8
DLB	9/9	7/9	4/6	2/8	5/9 ^c	1/4	3/5	1/4	2/5	0/3	0/3
ADLB	1/19	2/15	1/15	0/17	1/19 ^d	0/10	0/11	0/12	0/10	0/7	0/9
All	27/51	24/45	18/43	11/47	18/52	2/26	6/29	1/31	3/29	0/16	0/22

See Table 1 for listing of individual sites sampled and Fig. 4 for graphic representation; see supplementary online table for frequency of PASH within individual sites

Dx diagnosis, SpCd spinal cord, Sym sympathetic ganglia, Vagus vagus nerve, Sciat sciatic nerve, GI gastrointestinal system, Resp respiratory tract, Endo endocrine system, Cardio cardiovascular, GU genitourinary tract, MSK musculoskeletal

^a 6/7 when multiple slides of paraffin-embedded spinal cord were examined

^b 14/15 when multiple slides of paraffin-embedded and 80- μ m frozen sections of esophagus and submandibular gland were examined

^c 8/8 when multiple slides of paraffin-embedded and 80- μ m frozen sections of esophagus and submandibular gland were examined

^d 3/15 when multiple slides of paraffin-embedded and 80- μ m frozen sections of esophagus and submandibular gland were examined

required to confirm this. Subjects with ADLB have a very restricted frequency and distribution of PASH, with very sparse involvement outside the spinal cord and sympathetic ganglia, detectable only when multiple sections are examined.

Other groups have reported higher frequencies for PASH in selected body regions including skin, adrenal medulla, urinary bladder and cardiac epicardium [35, 43, 45, 61]. Each of these earlier studies stained multiple slides from each site, however, probably accounting for most of the differences from the present work, in which for most of these regions only a single slide was stained. Differing sites of sampling may also have been responsible for differing results, for example Iwanaga et al. [45] sampled the heart from around the coronary arteries while in the present study the cardiac apex was sampled. Differences in staining methods may also have contributed to the different findings. Minguez-Castellano et al. [61] used an antibody against normal (unphosphorylated) α -synuclein while in the present study the antibody was specific for α -synuclein phosphorylated at serine 129, which is found only in pathological α -synuclein deposits [34]. Most other groups have used formic acid pretreatment for epitope exposure while the present study used proteinase K pretreatment [10, 41]. Proteinase-K would theoretically destroy normal α -synuclein and thus further eliminate non-specific (non-pathological) staining.

A critical question has been whether or not α -synuclein histopathology begins in the brain or within elements of the peripheral nervous system [20, 38, 55]. The stimulus for this intriguing hypothesis has come largely from clinical studies of PD that have found a wide range of non-motor signs and symptoms that accompany the disease [4, 78]. Many of these non-motor accompaniments are related to dysfunction of the peripheral autonomic system. These

may occur early in the motor progression and there is suggestive evidence that some may even occur in the premotor prologue [2, 3, 47, 66, 74]. The description of Lewy bodies within the sympathetic and parasympathetic ganglia, adrenal medulla and GI tract within autopsied subjects with PD [17, 21, 29, 35, 39, 50, 75] has shown that peripheral nervous system α -synuclein histopathology is certainly present but there has been insufficient data regarding the findings in prodromal phases of disease. Autopsy studies of relatively small numbers of subjects with ILBD have demonstrated a high prevalence of α -synuclein histopathology within the spinal cord, sympathetic ganglia, adrenal medulla and upper GI tract [12, 17, 21, 48, 61], consistent with accumulating reports of premotor autonomic dysfunction in PD [78], but only two subjects, out of more than a thousand examined in recent studies, have had α -synuclein histopathology in the spinal cord or peripheral nervous system in the absence of brain involvement. Fumimura et al. [35] reported one case out of 783 with adrenal medulla as the only site with α -synuclein histopathology, but the olfactory bulb was not examined. Miki et al. [60] reported a single subject with α -synuclein histopathology restricted to the heart and stellate ganglion; in this case, the olfactory bulb was examined. The present work is in general agreement with these prior studies as, of the 40 subjects without brain and olfactory bulb PASH, none had PASH within the spinal cord or peripheral nervous system sites sampled. However, owing to the relatively small sample size and single-section analysis at many sites, it cannot be excluded that α -synuclein pathology may rarely begin in the peripheral nervous system prior to CNS involvement.

A derivative of the “body-first” hypothesis has been the conjecture as to whether an exogenous pathogen might be the cause of disease and gain entry through peripheral

Fig. 4 Relative frequency of PASH by diagnostic group for phosphorylated α -synuclein in different body regions including spinal cord, sympathetic ganglia, vagus nerve, sciatic nerve and multiple organs and tissues. Relative frequency is the percentage of subjects that showed immunoreactive tissue elements of any kind (fibers, puncta, perikaryal diffuse staining, perikaryal cytoplasmic inclusions) in single slides from each of the sites evaluated. For list of individual sites within each body region or organ system, see Table 1 and supplementary online table. Frequency was investigated further for some sites (see text)

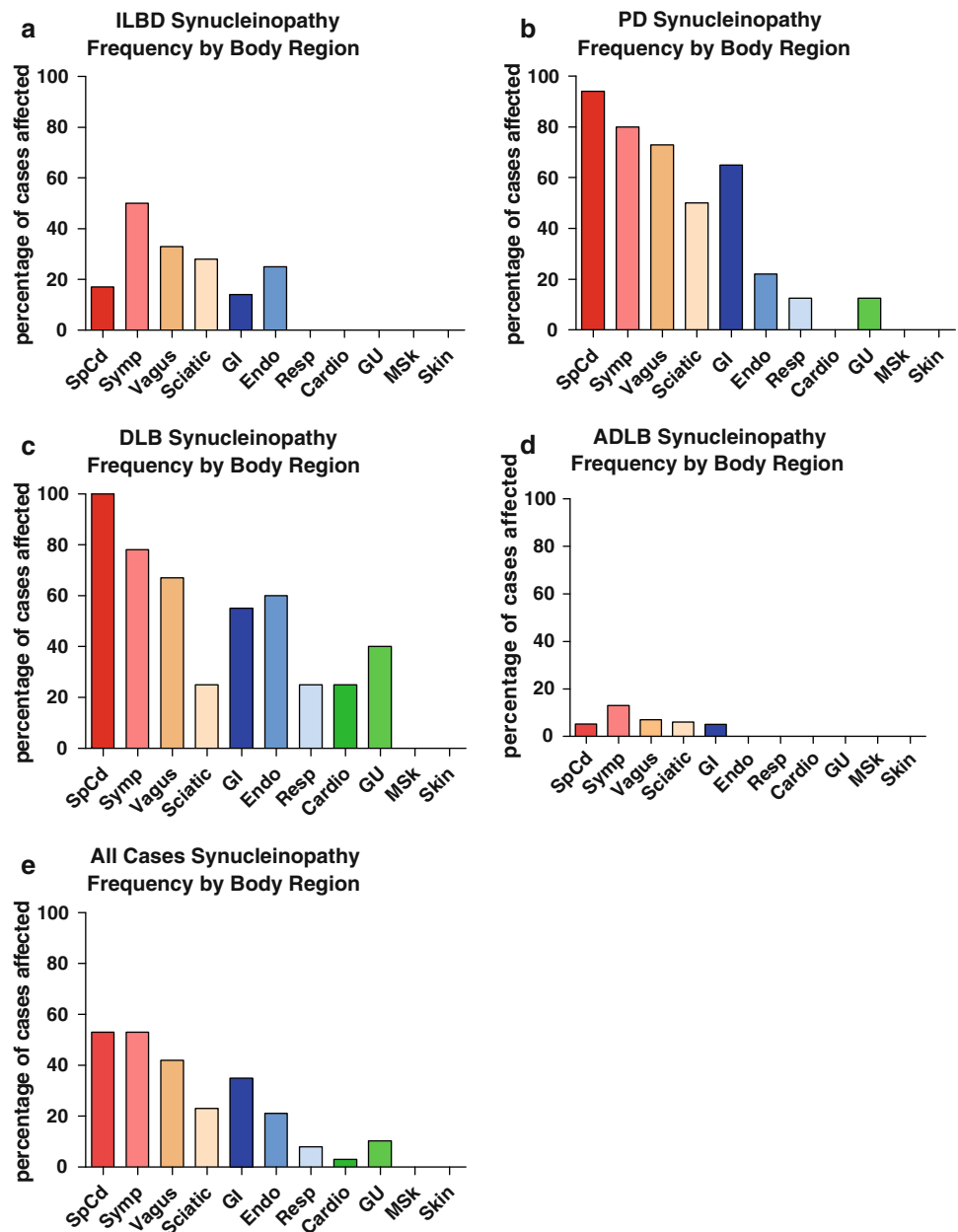


Table 6 Frequency and mean (SD) density of with phosphorylated α -synuclein histopathology in single slides from major spinal cord and sympathetic nervous system subdivisions (all cases considered together)

	Cervical	Thoracic	Lumbar	Sacral	CSymp	ThSymp
Frequency	23/49 (47%)	26/47 (55%)	25/49 (51%)	25/48 (52%)	14/20 (70%)	19/36 (53%)
Density score	0.7 (0.9)	1.2 (1.3)	0.9 (1.1)	1.4 (1.5)	2.1 (1.6)	1.5 (1.6)

See Fig. 5 for a graphic representation

CSymp cervical sympathetic, ThSymp thoracic sympathetic

nerve endings [20, 38], either through the olfactory epithelium or GI mucosa. The findings of the present study are not incompatible with a GI entry for PD, ILBD and DLB, but as subjects with ADLB have relatively rare or sparse

involvement of the caudal neuraxis this seems unlikely for that group. The universal and primal involvement of the olfactory bulb in all of the Lewy body disorders [8, 11] is highly compatible with the exogenous pathogen

Fig. 5 Relative frequency and density of PASH by diagnostic group in different spinal cord regions and sympathetic nervous system subdivisions. Relative frequency is the percentage of subjects that showed immunoreactive tissue elements of any kind (fibers, puncta, perikaryal diffuse staining, perikaryal cytoplasmic inclusions) in single slides taken from each of the sites evaluated. Density was calculated only for sites with immunoreactivity, negative or “zero” scores were not included

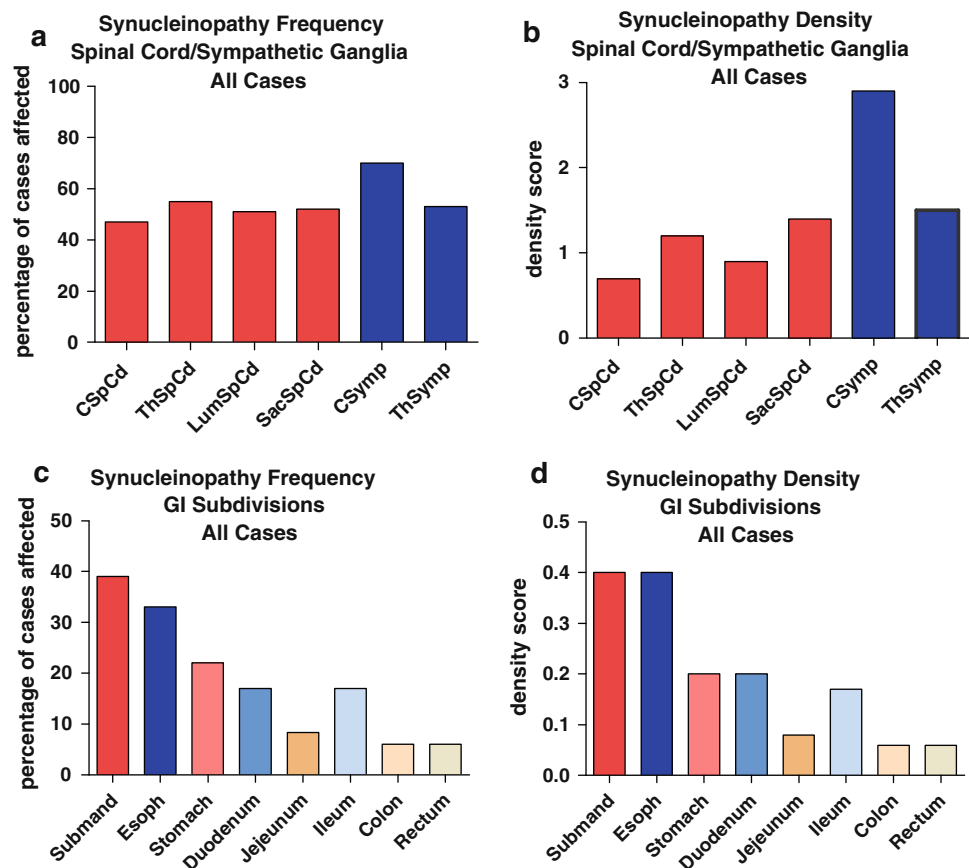


Table 7 Frequency of and mean density of with phosphorylated α -synuclein histopathology in single slides of subdivisions of the gastrointestinal system (all cases considered together)

	Submand	Esoph	Stomach	Duodenum	Jejunum	Ileum	Colon	Rectum
Frequency	11/37 (39%)	17/51 (33%)	7/31 (22%)	5/30 (17%)	2/24 (8.3%)	4/24 (17%)	3/50 (6%)	3/50 (6%)
Density score	0.4 (0.7)	0.4 (0.7)	0.2 (0.4)	0.2 (0.6)	0.08 (0.28)	0.17 (0.4)	0.06 (0.2)	0.06 (0.2)

See Fig. 5 for a graphic representation

Submandib submandibular gland, *esoph* lower esophagus

hypothesis. If an exogenous pathogen is involved, whether it be a virus, micro-organism or toxin, if it was able to induce aggregation of α -synuclein in exposed neurons, this change could then be propagated throughout the remainder of the PNS and CNS, generating the observed brain and spinal cord regional pattern of α -synuclein histopathology through trans-synaptic transmission. Recently, there have been reports of PD subjects that have developed Lewy bodies within non-host neurons transplanted into the striatum more than a decade earlier, supporting the possibility that α -synuclein histopathology might be acquired and passed along from neuron to neuron [51–53, 57]. In relation to this, experimental studies have recently shown that aggregated α -synuclein may be transferred between neurons by endocytosis [26, 27, 30].

The rostrocaudal gradient of PASH within the gastrointestinal system is an interesting finding of the present work and confirms a previous report by Wakabayashi et al. [81] who mapped Lewy bodies in the alimentary tract of seven PD subjects using classical stains. The reason for this rostrocaudal gradient may be of interest. It could be due to the known distribution of vagal innervation, which extends only as far as the proximal colon and which has been documented to be more heavily distributed to the lower esophagus and stomach than to the small bowel or proximal colon [25, 42]. If so, this would suggest that, for the gastrointestinal tract, α -synuclein histopathology within vagal efferents predominates over α -synuclein histopathology originating from sympathetic ganglia or from enteric neurons. However, as Lewy bodies and α -synuclein

histopathology have been demonstrated to occur within sympathetic ganglia as well as intrinsic neurons of the enteric nervous system [17, 54, 69, 80, 81] an alternative explanation is that either or both of these have a selective rostrocaudal vulnerability to α -synuclein histopathology. A notable exception to the rostrocaudal α -synuclein histopathology gradient is the upper esophagus, which on single-slide analysis was always negative for PASH. This may be due to the fact that while most of the vagal innervation of the GI tract is derived from neuronal cell bodies located in the dorsal motor nucleus of the vagus, the cell bodies giving rise to the vagal innervation of the upper esophagus arise in the nucleus ambiguus. The former develop α -synuclein histopathology but the latter do not [20].

Spinal cord involvement with α -synuclein histopathology has usually been found to be concentrated in the preganglionic parasympathetic cell columns of the thoracolumbar intermediolateral horn and its clinical expression has, therefore, been assumed to be mainly autonomic dysfunction, but there have also been reports localizing substantial α -synuclein histopathology to the dorsal horn, where this might conceivably be a cause of neuropathic pain, and to the ventral horn, where it may affect motor neuron function [12, 21, 48, 63, 79]. The findings of the present study support these earlier works, as PASH, although most densely and frequently seen within the intermediolateral horn, was also not uncommonly present within posterior and anterior horns. The large size of some affected anterior horn neurons is consistent with involvement of small numbers of α -motor neurons, a surprising finding because there are, to our knowledge, no prior reports of Lewy bodies or α -synuclein histopathology in either spinal cord or brainstem motor neurons. Several electrophysiological studies, however, have been consistent with mild spinal motor neuron disease in PD, with evidence of motor unit reinnervation and dropout [22–24, 72, 73].

To our knowledge, this is the first report of α -synuclein histopathology within many of the sites studied. Of these, the most interesting location was the submandibular gland, which, together with the lower esophagus, was the most frequent non-neural peripheral structure affected. Sialorrhea, or drooling, has long been recognized as a characteristic sign of PD. Rather than being due to overproduction of saliva, however, sialorrhea appears to be primarily due to decreased swallowing and hence oral accumulation, and that secretion of saliva is actually decreased in subjects with PD [6, 7, 32, 56, 65, 67, 68, 76]. As both sympathetic and parasympathetic innervation of salivary glands have stimulatory effects on saliva production, it is possible that α -synuclein histopathology within either or both of these could be responsible for the decreased production observed in PD. Of further and perhaps more practical interest is the accessibility of the submandibular

gland to biopsy, which could theoretically improve the low clinical diagnostic accuracy for early PD and DLB.

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