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# Distribution of $\alpha$ -synuclein in the spinal cord and dorsal root ganglia in an autopsy cohort of elderly persons

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## Abstract

**Background:** Lewy body-related  $\alpha$ -synucleinopathy (LBAS, the abnormal accumulation of pathologic  $\alpha$ -synuclein) is found in the central and peripheral nervous systems, including the spinal cord, dorsal root ganglia, and sympathetic ganglia, of Parkinson's disease patients. However, few studies have focused on the distribution of LBAS in the spinal cord, primary sensory neurons, and preganglionic sympathetic nerves.

**Results:** We analyzed 265 consecutive subjects with LBAS who underwent autopsy at a general geriatric hospital. LBAS in the spinal cord was significantly associated with that in the lower brainstem regions that are directly connected to the spinal cord (i.e., the medullary reticular formation and locus ceruleus), but it was not associated with the olfactory bulb-amygdala system, which is not directly connected to the spinal cord, suggesting that the lower brainstem is a key structure regarding the spread of LBAS to the spinal cord. In the primary sensory neurons, most subjects with LBAS in the dorsal root ganglia had LBAS in the dorsal root, and all subjects with LBAS in the dorsal root also had LBAS in the dorsal horn, suggesting that LBAS spreads retrogradely from the axonal terminals of the dorsal horn to the somata of the dorsal root ganglia via the dorsal root. In the preganglionic sympathetic nerves, the LBAS in the sympathetic ganglia preceded that in the nucleus of the intermediolateral column of the thoracic cord, suggesting that LBAS spreads retrogradely through the preganglionic sympathetic nerves.

**Conclusions:** LBAS in the spinal cord was associated with the lower regions of the brainstem, but not with the olfactory bulb or amygdala. LBAS may spread centrifugally along the primary sensory neurons, whereas it may spread centripetally along the preganglionic sympathetic nerves.

## Background

Lewy bodies, neurites and dots are aggregates composed mainly of  $\alpha$ -synuclein and are pathological hallmarks of Lewy body diseases, which include Parkinson's disease and dementia with Lewy bodies [1]. In patients with Lewy body disease, these aggregates are found not only in the brain, but also in the spinal cord, sympathetic ganglia, and cardiac plexuses [2–4]. Recent studies have shown that pathologic  $\alpha$ -synuclein may propagate from one cell to another and spread to different interconnected brain regions [5–15].

Lewy body-related  $\alpha$ -synucleinopathy (LBAS, the abnormal accumulation of pathologic  $\alpha$ -synuclein) in the brain spreads in a sequential, predictable fashion, that is, it spreads rostrally from the brainstem in a process described by the Braak hypothesis [16, 17]. In addition to this brainstem-ascending pathway, an alternative pathway, the olfactory bulb-amygdala pathway, has been reported to be present in up to 30 % of cases of Lewy body disease [18, 19].

LBAS in the spinal cord may begin to develop in specific regions such as the dorsal horn, intermediolateral column of the thoracic cord (Th-IML), and intermediolateral column of the sacral cord (Sa-IML) [20]. So far, studies investigating LBAS in the spinal cord have only examined subjects in which the spread of LBAS was

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following the brainstem-ascending pathway of LBAS and not the olfactory bulb-amygdala pathway [20–22].

Anatomical studies have revealed that, unlike the olfactory bulb and the amygdala, the lower brainstem nuclei, including the medullary reticular formation, locus ceruleus, and raphe nucleus, are directly connected to the spinal gray matter via projecting neurons [23–26]. Little is known about whether the spread of LBAS to the spinal cord is different in patients in whom the LBAS is spreading following the brainstem-ascending pathway compared with those in which it is spreading following the olfactory bulb-amygdala pathway. Similarly, the distribution of LBAS in the primary sensory neurons, whose cell bodies are located within the dorsal root ganglia (DRG) and axons extend into the dorsal horn via the dorsal root, is yet to be fully examined.

The sympathetic ganglia, which are innervated by the nucleus of the Th-IML, are known to be where LBAS manifests in the early phase of the disease process [3]. However the association between LBAS in the sympathetic ganglia and that in the Th-IML is yet to be fully elucidated.

According to the hypothesis described by Braak and colleagues [16], the dorsal motor nucleus of the vagus is one of the first regions in which LBAS develops, suggesting that the pathological process of Lewy body diseases may begin in the gut. The preganglionic parasympathetic nerves that innervate the gut are comprised of axons whose cell bodies are located within the nucleus of the Sa-IML; however, it is not yet fully understood whether the Sa-IML is also one of the primary sites in the spinal cord where LBAS begins to develop.

In the present study, we investigated the distribution of LBAS in the spinal cord, primary sensory neurons, and preganglionic sympathetic nerves in a consecutive autopsy series of elderly subjects.

## Materials and methods

### Subject selection

Samples of brain and spinal cord were collected from 796 consecutive full autopsies of elderly persons conducted in our institution between January 2003 and July 2013. Paraffin sections of the lumbosacral cord, thoracic cord, medulla, pons, midbrain, olfactory bulb, amygdala, hippocampus, cervicothoracic level of the sympathetic ganglia, heart, esophagus, and brachial skin were prepared and subjected to immunohistochemical analyses. Of the 796 subjects examined, 265 had LBAS in at least one of the anatomical regions examined.

Next, paraffin sections of the cervical cord, lumbosacral level of the DRG, and sites stipulated in the third report of the Dementia with Lewy Bodies Consortium [27] were analyzed as described below. C8, Th8, L2, L5,

S1, and S2 were evaluated as representative levels of the spinal cord.

Neuropathological diagnoses were based on the third report of the Dementia with Lewy Bodies Consortium [27], our institute's original criteria [28], Braak staging of  $\beta$ -amyloid and neurofibrillary tangle formation [29], Consortium to Establish a Registry for Alzheimer's Disease [30], Thal phase evaluation [31], and argyrophilic grain staging [32].

The relatives' informed consent was obtained from all subjects. Our Brain Bank for Aging Research was approved by the ethics committee of Tokyo Metropolitan Geriatric Hospital (No. 16).

The following aspects were analyzed in this study:

- (A) Presence of LBAS in the spinal cord versus that in the brain ( $n = 265$ ).
- (B) Presence of LBAS in the spinal cord versus that in the (both  $n = 265$ ).
- (C) Presence of LBAS in the S2 dorsal horn versus that in specific regions of the brainstem (medullary reticular formation or locus ceruleus;  $n = 261, 260$ , respectively) or that in the amygdala ( $n = 261$ ). The S2 dorsal horn was selected as the representative site of the spinal cord because lower level of spinal cord usually contains the greatest amount of LBAS deposits [3, 20].
- (D) Distribution of LBAS in the primary sensory neurons at the S2, S1, L5, and L2 levels ( $n = 261, 46, 261, \text{ and } 225$ , respectively).
- (E) Presence of LBAS in the Th-IML versus that in the sympathetic ganglia ( $n = 222$ ).
- (F) Comparison of LBAS density in four different regions of the spinal cord: dorsal horn (Rexed lamina I–VI,  $n = 256$ ), ventral horn (Rexed lamina VIII–IX,  $n = 258$ ), intermediate zone (Rexed lamina VII and X,  $n = 258$ ), and large motor neurons in the ventral horn ( $n = 255$ ). Identification of large motor neurons was based on morphological features, including large, multipolar cell bodies containing large Nissl bodies.
- (G) Confirmation of the presence of LBAS in the Sa-IML ( $n = 261$ ).

### Histology

At the time of autopsy, half of the brain was preserved at  $-80^\circ\text{C}$ , and the other half was fixed in 20 % neutral-buffered formalin (WAKO, Osaka, Japan) for 7–13 days and then sectioned. Representative areas of the brain and spinal cord were embedded in paraffin and cut serially into 6- $\mu\text{m}$ -thick sections. For autopsies conducted prior to April 2011, spinal cords were fixed by using 20 % formalin (226 of 265 subjects with LBAS), whereas

spinal cords sampled from April 2011 (39 of 265 subjects with LBAS) and peripheral organs were fixed in 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 48 h. Sections were pretreated with 98 % formic acid for 5 min. For immunohistochemical analysis, we used a phosphorylation dependent anti- $\alpha$ -synuclein monoclonal antibody (pSyn#64; donated by Dr. Iwatsubo, University of Tokyo), which recognizes Ser129. Sections were processed with a Ventana Discovery automatic immunostainer (Roche, Basel, Switzerland). The diameters of the  $\alpha$ -synuclein immunoreactive structures were measured visually under a light microscope (Nikon ECLIPSE 80i) with the operator blinded to the clinical and pathological diagnoses.

### Semi-quantitative analysis of LBAS

The degree (density score) of LBAS in the spinal cord, dorsal root, DRG, and sympathetic ganglia was semi-quantitatively graded under a low-power microscopic field ( $\times 10$ ) as follows: 0, absent; 1, slight (only thin neurites or small dots less than 12.5  $\mu$ m in diameter); 2, mild (1–2 intraneuronal cytoplasmic bodies [ICBs] or neurites or dots greater than 12.5  $\mu$ m in diameter [larger neurites]); 3, moderate (3–5 ICBs or larger neurites); 4, severe (6 or more ICBs or larger neurites). Larger neurites or dots with a diameter greater than 12.5  $\mu$ m were quantitatively considered to be equivalent to ICBs.

The degree (density score) of LBAS in the brain was based on the third report of the Dementia with Lewy Bodies Consortium [27] as follows: 0, absent; 1, only neurites; 2, 1–3 ICBs; 3, more than 4 ICBs; 4, numerous ICBs filled with extensive immunoreactivity in the neuropil.

### Statistical analysis

Statistical analyzes were performed with the PASW Statistics 18 software (SPSS Japan, Inc.). For multiple comparisons, the Friedman's chi-square test was used, followed by the Wilcoxon *t*-test with Bonferroni correction as a post-hoc analysis. Correlations between regions were assessed with Spearman's correlation analysis. The McNemar test was used to test the difference between paired proportions.  $P < 0.05$  was considered statistically significant.

## Results

### LBAS in the spinal cord versus in the brain

An additional file shows the clinicopathological characteristics of the 265 subjects who had LBAS in at least one of the sampled anatomical regions [see Additional file 1: Table S1].

When LBAS was present in the spinal cord, all subjects had LBAS in the brain ( $n = 173$ ) (Table 1). No

**Table 1** Number of subjects with LBAS in the spinal cord or brain, or both

		LBAS in brain	
		(+)	(-)
LBAS in spinal cord	(+)	173	0
	(-)	77	15

LBAS Lewy body-related  $\alpha$ -synucleinopathy

(+) subjects with LBAS

(-) subjects without LBAS

subject had LBAS in the spinal cord without LBAS in the brain ( $P < 0.001$ ).

### LBAS in the spinal cord versus in the brainstem or olfactory bulb plus amygdala

Subjects with LBAS in the brainstem also had LBAS in the spinal cord (87 % [174/200],  $P < 0.001$ ) (Table 2). All subjects with LBAS in the spinal cord also had LBAS in the brainstem. Subjects lacking LBAS in the brainstem invariably failed to show LBAS in the spinal cord (Table 2).

Subjects with LBAS in the olfactory bulb plus amygdala ( $n = 159$ ) had LBAS in the spinal cord (68.5 % [159/232],  $P = 0.009$ ) (Table 3), and all of those 159 subjects also had LBAS in the brainstem.

Of the 16 subjects with LBAS in the brainstem but neither in the olfactory bulb nor amygdala, 12 (75 %) had LBAS in the spinal cord. In contrast, subjects with LBAS in the olfactory bulb and amygdala but not in the brainstem ( $n = 51$ ) invariably failed to show LBAS in the spinal cord.

### LBAS density score in the S2 dorsal horn versus in the medullary reticular formation, locus ceruleus, or amygdala

There was a significant correlation ( $P < 0.001$ ) between LBAS density score for the S2 dorsal horn and that for the medullary reticular formation or locus ceruleus in the lower brainstem region (Spearman's rank correlation coefficient ( $r_s$ ), 0.867 and 0.825, respectively; Fig. 1a, b).

Spearman's rank correlation coefficient of the comparison between the LBAS density scores for the S2 dorsal horn and the amygdala ( $r_s = 0.602$ ) was lower than that for the

**Table 2** Number of subjects with LBAS in the spinal cord or brainstem, or both

		LBAS in spinal cord	
		(+)	(-)
LBAS in brainstem	(+)	174	26
	(-)	0	65

LBAS Lewy body-related  $\alpha$ -synucleinopathy

(+) subjects with LBAS

(-) subjects without LBAS

**Table 3** Number of subjects with LBAS in the spinal cord or olfactory bulb plus amygdala

		LBAS in spinal cord	
		(+)	(-)
LBAS in olfactory bulb plus amygdala	(+)	159	73
	(-)	15	18

LBAS Lewy body-related  $\alpha$ -synucleinopathy

(+) subjects with LBAS

(-) subjects without LBAS

comparison between the LBAS density scores for the S2 dorsal horn and the medullary reticular formation ( $r_s = 0.867$ ) or locus ceruleus ( $r_s = 0.825$ ) (Fig. 1c). Most subjects with LBAS in the medullary reticular formation or locus ceruleus (Fig. 1c, open circles) had LBAS in the S2 dorsal horn. In contrast, subjects lacking LBAS in the medullary reticular formation and locus ceruleus (Fig. 1c, closed triangles) failed to show LBAS in the S2 dorsal horn.

#### Histology of LBAS in the primary sensory neurons

LBAS in the dorsal horn (Fig. 2a) and dorsal root (Fig. 2g) was mainly composed of Lewy neurites. LBAS in the DRG showed Lewy neurites (Fig. 2b), intracytoplasmic, diffuse, granular immunoreactivity (pre-inclusion) (Fig. 2c), ICBs (Fig. 2d, e), or Lewy bodies (Fig. 2f).

#### Distribution of LBAS in the primary sensory neurons

Table 4 shows the distribution of LBAS in the primary sensory neurons. Subjects with LBAS in the DRG consistently had LBAS in both the dorsal root and dorsal horn at all lumbosacral levels (e.g., 100 %; 43/43 and 43/43, respectively, in S2). Similarly, all subjects with LBAS in the dorsal root also had LBAS in the dorsal horn (100 %; 90/90, 20/20, 77/77, and 55/55 in S2, S1, L5, and L2, respectively). In contrast, no LBAS was observed in either the DRG or dorsal root when the dorsal horn was not involved (e.g., 0 %; 0/110 and 0/110 in the DRG and dorsal root, respectively, in S2). LBAS was not observed in the DRG when the dorsal root was not involved (e.g., 0 %; 0/171 in S2), except for in one subject (L2; 1/170).

#### LBAS in the sympathetic ganglia and Th-IML

There was a strong correlation between LBAS density score in the sympathetic ganglia and that in the Th-IML ( $r_s = 0.879$ ,  $P < 0.001$ , Fig. 3). When LBAS was present in the Th-IML, all subjects had LBAS in the sympathetic ganglia (Fig. 3).

Of 222 subjects whom both the sympathetic ganglia and Th-IML were sampled, 12 (5.4 %) had LBAS in the sympathetic ganglia, even in the absence of LBAS in the whole brain. Of these 12 subjects, three had LBAS in the Th-IML.

Of these three subjects, one had LBAS only in the Th-IML and not in any other region of the spinal cord.

#### Intersegmental comparison of LBAS density in the spinal cord

Comparing the density score of LBAS in the dorsal horn among four segments (cervical, thoracic, lumbar, and sacral) of the spinal cord, we found a caudo-rostral gradient in which the greatest LBAS density score was at the sacral level followed by the lumbar, thoracic, and cervical levels (Fig. 4a). Similar results were obtained in the ventral horn (Fig. 4b) and intermediate zone (Fig. 4c).

#### LBAS in large motor neurons

In the ventral horn, not only Lewy neurites, but also LBAS in the cell soma of the large motor neurons was observed (Fig. 2h–j). In symptomatic subjects (Parkinson's disease and dementia with Lewy bodies) ( $n = 55$ ) only, the frequency of ICBs in the large motor neurons was 27.3, 21.8, 23.6 and 63.6 % in C8, Th8, L5, and S2, respectively (Additional file 2: Figure S1).

#### LBAS in the Sa-IML

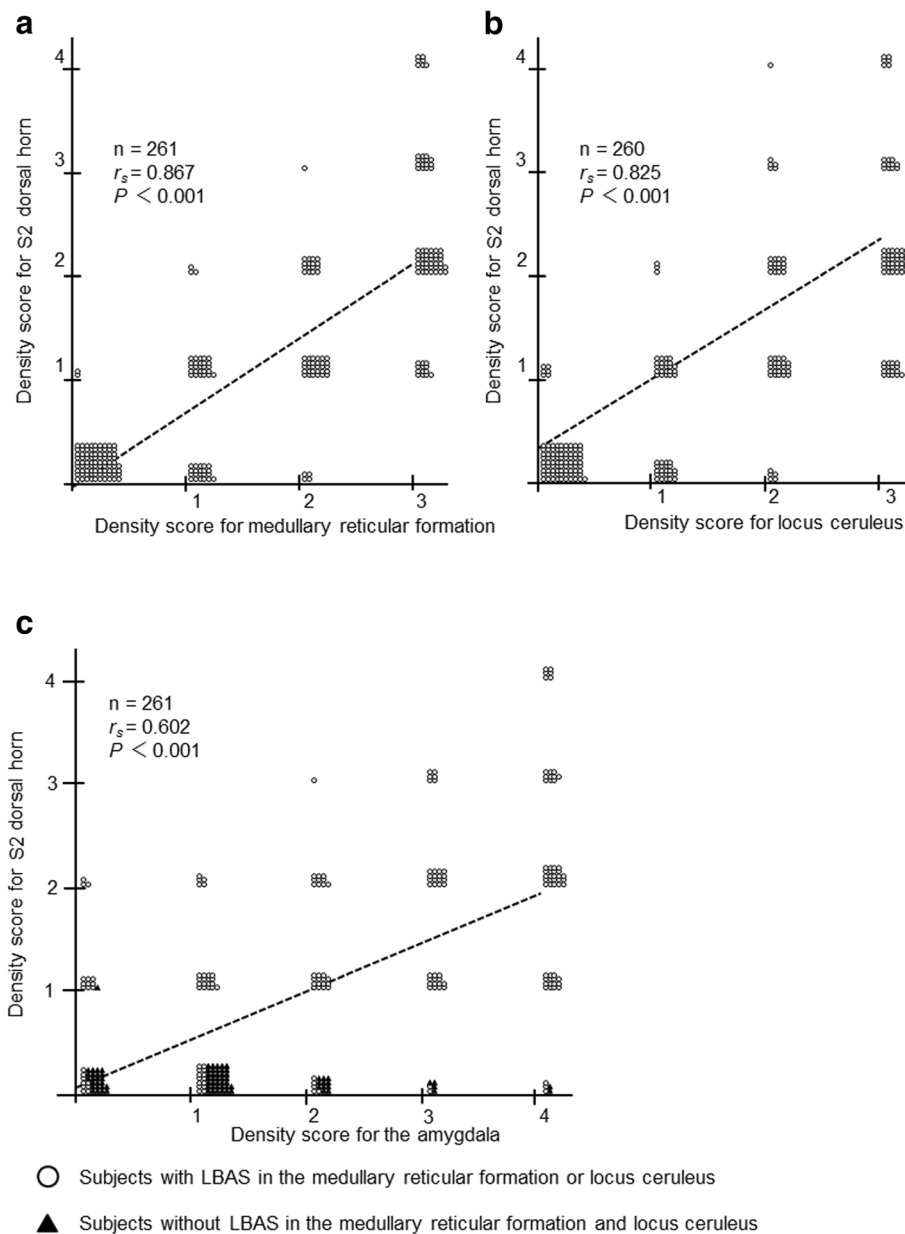
Of 261 subjects examined, no subject had LBAS that was limited to the Sa-IML. Those with LBAS in the Sa-IML also had LBAS in other sites of the spinal cord.

#### Discussion

In the present study, we examined the distribution of LBAS in the spinal cord, primary sensory neurons, and preganglionic sympathetic nerves in a consecutive series of elderly autopsy subjects. Our results suggest that 1) the presence of LBAS in specific regions of the lower brainstem (i.e., the medullary reticular formation and locus ceruleus) is strongly correlated with the presence of LBAS in the spinal cord, whereas the presence of LBAS in the spinal cord is independent of the presence of LBAS in the olfactory bulb and amygdala; 2) LBAS propagates retrogradely from the dorsal horn to the DRG through the dorsal root; 3) LBAS propagates retrogradely from the sympathetic ganglia to the Th-IML; and 4) LBAS in the spinal cord predominantly accumulates at the caudal level.

#### Association between LBAS in the spinal cord and that in the brainstem or amygdala

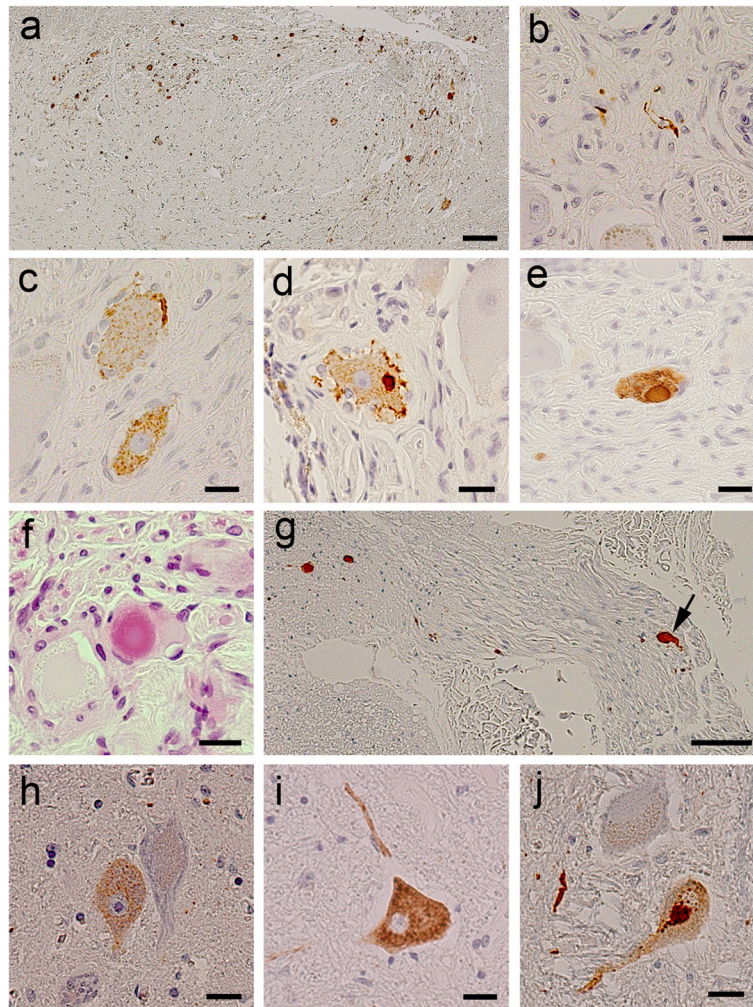
Here we found that LBAS develops first in the brain before developing in the spinal cord, which is consistent with the findings of a previous study [20]. We found that LBAS in the spinal cord was associated with LBAS in the medullary reticular formation and locus ceruleus. Previous studies have revealed the existence of direct connectivity via projecting neurons between the spinal cord and the medullary reticular formation and locus



**Fig. 1** Correlation between LBAS density in specific regions of the brain and in the S2 dorsal horn. Correlation between LBAS density in the medullary reticular formation (**a**), locus ceruleus (**b**), or amygdala (**c**) and in the S2 dorsal horn. Each symbol represents one subject. A density score of 1 or more indicates the deposition of LBAS, with increasing density score indicating greater deposition. Correlation was assessed by calculating Spearman's rank correlation coefficient. **c** Most subjects with LBAS in the medullary reticular formation or locus ceruleus (open circles) had LBAS in the S2 dorsal horn (S2 dorsal horn density score  $\geq 1$ ). Even when the amygdala showed a higher degree of LBAS (density score 3 or 4), subjects who lacked LBAS in the medullary reticular formation and locus ceruleus (closed triangles) failed to show LBAS in the S2 dorsal horn (S2 dorsal horn density score = 0). LBAS, Lewy body-related  $\alpha$ -synucleinopathy

ceruleus, but not the amygdala [23–26, 33, 34]. Our findings support the hypothesis of Del Tredici and Braak in which LBAS in the spinal cord descends from the supraspinal medullary reticular formation, locus ceruleus, and raphe nucleus via projecting neurons (Fig. 5a) [20]. In addition, we revealed a novel finding

that LBAS in the spinal cord is not correlated with LBAS in the olfactory bulb and amygdala. When LBAS follows the olfactory bulb–amygdala pathway and develops downward through the spinal cord, the lower brainstem is likely a key structure, implying that in Lewy body disease that is following the olfactory bulb–



**Fig. 2** Photomicrographs of paraffin-embedded sections of spinal cord and primary sensory neurons. **a** Sacral dorsal horn of a subject with Lewy bodies containing numerous LBAS deposits. **b–e** Lewy neurites (**b**), neuronal cytoplasmic diffuse granular appearance (**c**), and intraneuronal cytoplasmic bodies (**d, e**) in the dorsal root ganglia of subjects with dementia with Lewy bodies. **f** Lewy body in the dorsal root ganglia of a subject with Parkinson's disease. **g** Proximal portion of the cervical dorsal root showing a swollen neurite (arrow). **h–j** LBAS in large motor neurons of the ventral horn of subjects with dementia with Lewy bodies. Neuronal cytoplasmic diffuse granular appearance (**h, i**) and intraneuronal cytoplasmic body (**j**). Phosphorylated  $\alpha$ -synuclein immunohistochemical staining (**a–e, g–j**) or hematoxylin and eosin (**f**). scale bar = 100  $\mu$ m for (**a, g**); 20  $\mu$ m for (**b–f, h–j**). LBAS, Lewy body-related  $\alpha$ -synucleinopathy

amygdala pathway, LBAS primarily develops in the amygdala and then gradually spreads to the lower brainstem and finally the spinal cord.

#### LBAS in primary sensory neurons and preganglionic sympathetic nerves

The present study is the first to evaluate how LBAS spreads in primary sensory neurons. The dorsal root is composed of axons derived from the DRG. LBAS primarily develops in the dorsal horn and then spreads to the DRG through the dorsal root (i.e., central-to-peripheral spreading) (Fig. 5b).

Regarding the preganglionic sympathetic nerves, the sympathetic ganglia may be one of the starting points for LBAS development, and LBAS may propagate from the sympathetic ganglia to the Th-IML (i.e., peripheral-to-central spreading) (Fig. 5c).

Our results suggest that LBAS spreads retrogradely through the primary sensory neurons and preganglionic sympathetic nerves, which is plausible because the axonal development of LBAS precedes perikaryal aggregation [35–39]. Indeed, LBAS has already been shown to spread retrogradely in cardiac postganglionic sympathetic nerves (Fig. 5s) [2]. Recent *in vivo* studies have revealed that

**Table 4** Distribution of LBAS in primary sensory neurons

	S2 (n = 261)			S1 (n = 46)			L5 (n = 261)			L2 (n = 225)			
	DRG	Dorsal	Dorsal	DRG	Dorsal	Dorsal	DRG	Dorsal	Dorsal	DRG	Dorsal	Dorsal	
	(+)	root (+)	horn (+)	(+)	root (+)	horn (+)	(+)	root (+)	horn (+)	(+)	root (+)	horn (+)	
DRG	(+)	-	43/43 (100)	43/43 (100)	-	12/12 (100)	12/12 (100)	-	45/45 (100)	45/45 (100)	-	34/35 (97.1)	35/35 (100)
	(-)	-	47/218 (21.6)	108/218 (49.5)	-	8/34 (23.5)	15/34 (44.1)	-	32/216 (14.8)	102/216 (47.2)	-	21/190 (11.1)	83/190 (43.7)
Dorsal root	(+)	43/90 (47.8)	-	90/90 (100)	12/20 (60)	-	20/20 (100)	45/77 (58.4)	-	77/77 (100)	34/55 (61.8)	-	55/55 (100)
	(-)	0/171 (0)	-	61/171 (35.7)	0/26 (0)	-	7/26 (26.9)	0/184 (0)	-	70/184 (38)	1/170 (0.6)	-	63/170 (37.1)
Dorsal horn	(+)	43/151 (28.5)	90/151 (59.6)	-	12/27 (44.4)	20/27 (74)	-	45/147 (30.6)	77/147 (52.4)	-	35/118 (29.7)	55/118 (46.6)	-
	(-)	0/110 (0)	0/110 (0)	-	0/19 (0)	0/19 (0)	-	0/114 (0)	0/114 (0)	-	0/107 (0)	0/107 (0)	-

Percentage of cases is given in parentheses

DRG Dorsal root ganglion, LBAS Lewy body-related  $\alpha$ -synucleinopathy

(+) subjects with LBAS

(-) subjects without LBAS

LBAS also spreads retrogradely along the vagus nerve from the gut [40], and along the dopaminergic neurons in the nigrostriatal system [41]. In the olfactory nervous system, LBAS spreads both anterogradely (Fig. 5¶) and retrogradely from the periphery of the olfactory bulb to

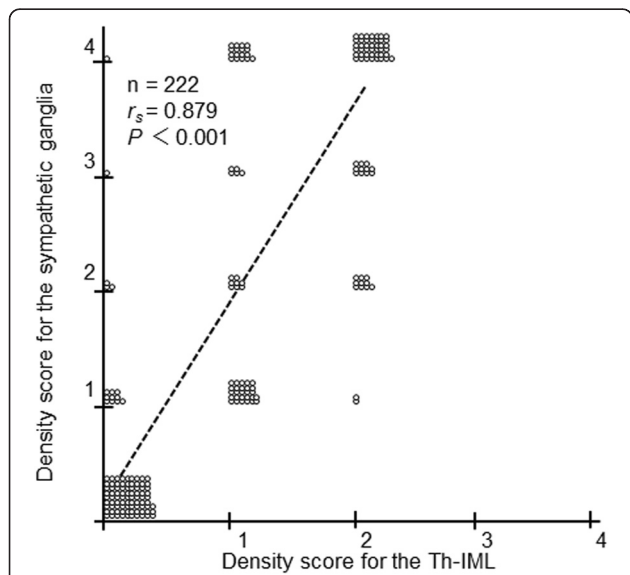
interconnected regions [16, 18, 33, 42]. Whether retrograde spread alone explains the Lewy body disease process needs to be further examined.

**Caudo-rostral gradient in the spinal cord**

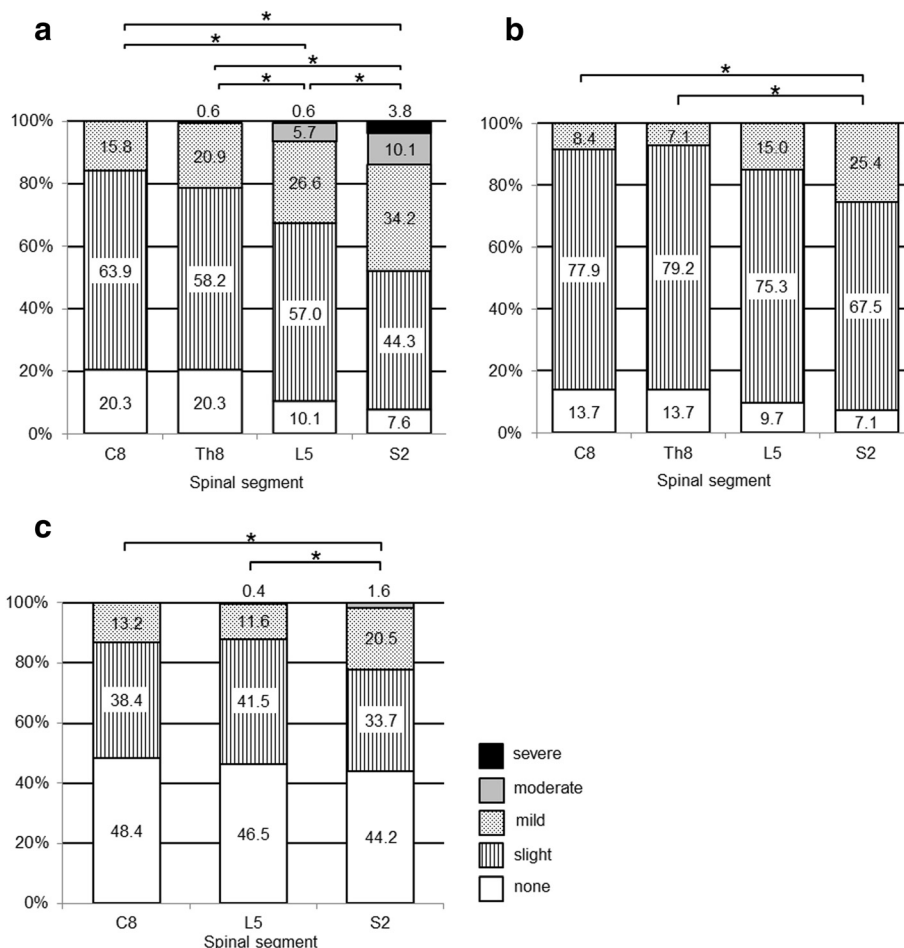
The amount of LBAS in the spinal gray matter showed a caudo-rostral gradient, which is consistent with previous descriptions [3, 20, 22]. The precise reason for this gradient is unclear, but may well be ascribed to 1) a selective caudo-rostral vulnerability to pathologic  $\alpha$ -synuclein accumulation, or 2) the sacral segment having a higher amount of endogenous  $\alpha$ -synuclein. Lewy neurites may play a direct role in peripheral nerve damage [43–45]. Parkinson’s disease patients often suffer from limb pain, especially in the lower limbs, which is localized to the anatomical territory of a peripheral nerve or nerve root [46–49]. The development of LBAS in primary sensory neurons and the development of the caudo-rostral gradient may be associated with sensory disorders in Parkinson’s disease patients.

**LBAS in large motor neurons**

LBAS in the large motor neurons preferentially accumulated at the lower level of the spinal cord. Recent studies indicate the subclinical denervation especially in the lower limbs in Parkinson’s disease [50, 51]. It remains unclear whether LBAS propagates anterogradely or retrogradely in large motor neurons. One experimental study has shown retrograde spread of LBAS into the spinal cord via the peripheral nerves in transgenic mice



**Fig. 3** Correlation between the LBAS density for the Th-IML and that for the sympathetic ganglia. Each symbol represents one subject. A density score of 1 or more indicates the deposition of LBAS, with increasing density score indicating greater deposition. Subjects who had LBAS in the Th-IML (density score for the Th-IML  $\geq 1$ ) invariably showed LBAS in the sympathetic ganglia (density score for the sympathetic ganglia  $\geq 1$ ). Correlation was assessed by calculating Spearman’s rank correlation coefficient. LBAS, Lewy body-related  $\alpha$ -synucleinopathy



**Fig. 4** Comparison of LBAS density at different levels of the spinal cord. The degree of LBAS among four different segments (cervical, thoracic, lumbar, and sacral) in three different regions (dorsal horn, ventral horn, and intermediate zone) of the spinal gray matter showed a caudo-rostral gradient in which the greatest was at the sacral level followed by the lumbar, thoracic, and cervical levels. **a** Dorsal horn (Rexed lamina I–VI,  $n = 256$ ). **b** Ventral horn (Rexed lamina VIII–IX,  $n = 258$ ). **c** Intermediate zone (Rexed lamina VII and X,  $n = 258$ ). \*  $P < 0.01$

who received intramuscular injection of pathogenic  $\alpha$ -synuclein [52].

### LBAS in Sa-IML

Given the early development of gastrointestinal abnormalities in Lewy body disease [53–56], we hypothesized that the Sa-IML would be one of the initial sites where LBAS develops through the preganglionic parasympathetic nerves from pelvic organs such as the descending colon or rectum (Fig. 5d) [15, 57]. However, we did not find any subject harboring LBAS exclusively in the Sa-IML. One reason for this may be that the S2 level we examined contains fewer neurons than the S3–5 levels, which reduced the potential for detecting LBAS in the Sa-IML. Further, investigation of both the pelvic organs and Sa-IML will be important to clarify whether an alternative pattern of LBAS propagation, in which LBAS originates in the Sa-IML before spreading to the entire spinal cord, exists or not.

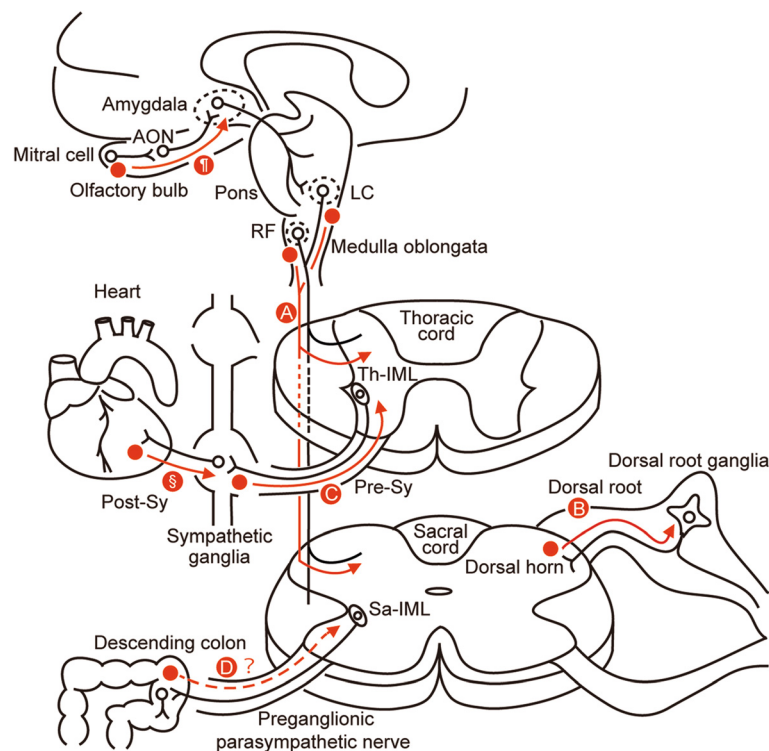
### Future direction

The findings of the present neuropathological examination should be interpreted with caution because these results do not mean that LBAS in the spinal cord always originates from outside the spinal cord. Some LBAS in the spinal cord may be derived from single or multiple sites within the spinal cord. Furthermore, the study cohort included only elderly subjects and therefore the results may not be generalizable to younger populations. Our neuropathological assessment did not allow us to see the actual process of LBAS propagation within a neuron; therefore, clinicopathological studies are required to establish a causal relationship between LBAS and peripheral nerve disorders in Lewy body diseases.

### Conclusion

LBAS in the spinal cord was associated with the lower regions of the brainstem but was not associated with the olfactory bulb and amygdala. LBAS may spread centrifugally





**Fig. 5** Schematic summarizing the propagation of  $\alpha$ -synuclein. Red circles represent pathologic  $\alpha$ -synuclein accumulation. Proposed pathway of the spread of LBAS from the RF or LC to the spinal gray matter (A), in the primary sensory neurons (B), in the preganglionic sympathetic nerves (C), and in the preganglionic parasympathetic nerves (D). AON, anterior olfactory nucleus; LC, locus ceruleus; Post-Sy, postganglionic sympathetic nerve; Pre-Sy, preganglionic sympathetic nerve; RF, medullary reticular formation; Sa-IML, nucleus of intermediolateral column of the sacral cord; Th-IML, nucleus of intermediolateral column of the thoracic cord. Pathway of the spread of LBAS in the olfactory nervous system (I) [16, 18], and in cardiac postganglionic sympathetic nerves (S) [2]

along the primary sensory neurons, whereas it may spread centripetally along the preganglionic sympathetic nerves. Further study of the progressive spread of LBAS in connected neuronal systems will assist in clarifying the precise progression of the Lewy body diseases.

### Availability of supporting data

The data sets supporting the results of this article are included within the article and its additional files.

### Additional files

**Additional file 1: Table S1.** Clinicopathological characteristics of 265 subjects with LBAS. (XLSX 12 kb)

**Additional file 2: Figure S1.** Intraneuronal cytoplasmic bodies in the large motor neurons. (PDF 93 kb)

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

HS carried out the immunohistochemical analyzes in this study. HS and MT participated in the design of the study. HS, HH, SI, YN, AU, AN and YS participated in the neuropathological assessments. HM and SM supervised

the whole project. HS and MT wrote the manuscript. All authors read and approved the final manuscript.

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