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α -Synuclein pathology accumulates in sacral spinal visceral sensory pathways

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Abstract

Urinary urgency and frequency are common in α -synucleinopathies such as Parkinson's Disease, Lewy Body Dementia and Multiple System Atrophy. These symptoms cannot be managed with dopamine therapy, and their underlying pathophysiology is unclear. We show that in individuals with Parkinson's Disease, Lewy Body Dementia or Multiple System Atrophy α -synuclein pathology accumulates in the lateral collateral pathway, a region of the sacral spinal dorsal horn important for the relay of pelvic visceral afferents. Deposition of α -synuclein in this region may contribute to impaired micturition and/or constipation in Parkinson's Disease and other α -synucleinopathies.

Introduction

There is increasing recognition that α -synuclein pathology may contribute to a wide range of non-motor symptoms in Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB). Impaired bladder control, causing nocturia, urgency and frequency, as well as constipation are common complaints in patients with PD¹. To date, mainly spinal regions specialized in *motor* control of micturition and constipation have been examined for the presence of α -synuclein, i.e. the sacral parasympathetic nucleus innervating the smooth muscles of the bladder and distal colon and Onuf's nucleus innervating the striated sphincters^{2–6}. While α -synuclein pathology has been reported in the spinal dorsal horn in PD, DLB, incidental

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Lewy Body Disease (ILB), and multiple system atrophy (MSA) ^{2–7}, it is not known whether α -synuclein pathology accumulates in sacral visceral sensory afferent pathways.

The sacral spinal cord contains a unique region that is located lateral to the dorsal horn which receives visceral sensory afferents from the bladder and distal colon via the pelvic nerve (Fig 1A). This lateral collateral pathway ^{8,9} is neurochemically distinct from other parts of the dorsal horn ^{9,10}, and is well conserved among mammals ^{8,11}. As this area contains not only primary afferents, but also secondary afferent projection neurons (Fig 1B), we will refer to it as the lateral collateral region (LCR). In this study we assess the distribution of α -synuclein immunoreactivity in the LCR from older adults with and without a clinical diagnosis of an α -synucleinopathy.

Material and Methods

We obtained post-mortem tissue from autopsy cases from the Rush Alzheimer's Disease Center, Chicago (n=8; 4% paraformaldehyde fixed) and Beth Israel Deaconess Medical Center (BIDMC; n=4; 10% formalin fixed). This study was considered exempt from review by the Institutional Review Board of BIDMC.

Cortical regions and the substantia nigra were processed for routine histology as described previously ¹². Three subjects (Table 1) had clinically and pathologically confirmed PD or DLB with moderate to severe neuronal loss and Lewy bodies in the substantia nigra, and two subjects had clinically and pathologically confirmed MSA. Four control subjects had no clinical neurological diagnosis, and three had a primary clinical and pathological diagnosis of Alzheimer's Disease (AD; per NIA-Reagan criteria ¹³). In 9 cases, we cut brainstem, thoracic and lumbosacral segments into 12 series of 60 μ m sections, and counterstained 1 series with cresyl-violet. We prepared additional series for immunohistochemistry by treatment with 1% sodium borohydride (in phosphate buffered saline (PBS; 0.1M pH7.4), at room temperature (RT), 30min), followed by 1% H₂O₂ in PBS (RT, 30min). Following a 2 hour pre-incubation in 10% non-fat dry milk (NFDM) in PBS with Triton-X 0.3% (PBT), we transferred tissue to a solution containing the primary antibody.

One series was stained for α -synuclein with monoclonal antibody LB509 which recognizes amino acids 115–122 of human α -synuclein ¹⁴ (1:500, Invitrogen Cat# 180215; in PBT and 5% NFDM; 3 nights at 4oC). In selected cases, we stained a series for choline acetyl transferase (ChAT) to visualize preganglionic parasympathetic neurons (ChAT: 1:100; AB144P, EMD Millipore, Billerica, MA; specificity determined in human tissue by pre-adsorption ¹⁵). Vasoactive intestinal polypeptide (VIP) stains a subset of pelvic visceral afferent fibers and an antibody raised against VIP was used to demarcate the LCR (1:30,000; Immunostar, 20077; raised against VIP coupled to bovine thyroglobulin; Immunostar, Hudson, WI; pre-adsorption with VIP (Sigma) blocked staining in human sacral cord tissue). These latter incubations required antigen retrieval (heat bath at 90oC for 20 minutes in citrate buffer pH10.0) in addition to pretreatment steps. Tissue was then processed using a standard immunohistochemistry protocol ¹⁶.

In one case (#10) with clinically and pathologically confirmed PD in which the sacral cord tissue had been embedded in paraffin, 20 μ m sections were cut and stained for α -synuclein with a monoclonal antibody raised against human α -synuclein 124–134, phosphorylated at Ser129 (1:20,000; 015-25191, clone pSyn#64; Wako Chemicals USA Inc., Richmond, VA)^{12, 17}. This protocol yielded a distribution of α -synuclein similar to cases 8 and 9. Tissue from cases #11 and #12 with a diagnosis of MSA was processed similarly to case #10. Both α -synuclein antibodies stained Lewy bodies in cases with clinical PD and no staining was observed in cases without a clinical neurological diagnosis of an α -synucleinopathy.

After mounting, dehydration, and clearing, we coverslipped slides with Permount. We examined and photographed slides using a Zeiss Axioplan, and assessed density of α -synuclein staining semi-quantitatively (+ light, ++ moderate, +++ high), and qualitatively as granular (G), neuritic (LN), or classical Lewy Body (LB) staining. In the MSA cases, we assessed the distribution of glial cytoplasmic inclusions (GCI) and neuronal nuclear or cytoplasmic inclusions (NI). Photomicrographs were adjusted for brightness and contrast using Adobe Photoshop CS6 and labels were applied with Adobe Illustrator CS6.

Results

The lateral collateral region

We used VIP immunostaining^{9, 10} and Nissl stained sections to demarcate the LCR (Fig 1C, Fig 2A–F) in sacral spinal segments S1–5. At level S1, the LCR was limited to the lateral part of the superficial dorsal horn. At S2 it ran along the lateral surface of the dorsal horn, and at S3 it merged with the sacral parasympathetic nucleus, which is mainly present at levels S3–4 as shown with ChAT immunostaining (Fig 2 B and D). Two clusters were recognized in the sacral parasympathetic nucleus: a dorsal cluster that contains preganglionic neurons presumably innervating the distal colon (Fig 2D), and a ventrolateral cluster thought to innervate the bladder¹¹. At level S5, only a single cluster could be distinguished.

Alpha-synuclein pathology

In the 3 cases with PD and LBD, α -synuclein immunoreactivity in the sacral spinal cord stood out in the LCR (Figure 2G–J). LBs were mainly present in the dorsal horn and LCR (Fig 1D; Fig 2K–O). Some neurons in the LCR and the ventral horn were diffusely filled with α -synuclein (Fig 1D; Fig 2K, L, N). Single or multiple LBs (Fig 2L) were present in some of these neurons in the LCR. Scattered LBs, but no neurons diffusely filled with α -synuclein were seen in the region of the sacral parasympathetic nucleus (compare Fig 2E with 2 I–J).

The distribution of LNs was more widespread than that of LBs, including the LCR, Onuf's nucleus, and area X (the region of the spinal cord around the central canal). At levels S2–3 (Fig 2R) but not L3–S1 (Fig 2P), many LNs crossed the midline ventral to the central canal. LNs in the white matter of S1, were most prominent ventrally in the medial funiculus (Fig

2Q), in a pattern resembling labeled axonal tracts (Fig 1B). More rostrally, at L1–2, they occupied a position in the ventrolateral funiculus.

In both cases with MSA, phosphorylated α -synuclein was found as glial cytoplasmic inclusions throughout the white and gray matter of the sacral and lumbar cord (Figure 2 S, T). In addition, the LCR contained an occasional neuron with a cytoplasmic inclusion (Figure 2 U) and multiple neurons with granular phosphorylated α -synuclein (Figure 2 V). In the MSA case with prominent autonomic features (Table 1 #12), dense staining of phosphorylated α -synuclein in neurites in the LCR made the LCR stand out, similar to the PD cases (Figure 2 S, W).

Two cases with a clinical and pathological diagnosis of AD had sparse LBs and LNs in the sacral spinal cord (cases 5 and 6, Table 1). All three AD cases had supraspinal Lewy body pathology, of which 2 cases intermediate likelihood and 1 low likelihood Dementia with Lewy Bodies based on CDLB criteria¹⁸ (Table 1). In one control case (case 2), dense granular α -synuclein immunoreactivity was present in the neuropil of the LCR and Onuf's nucleus, but we did not detect LBs, LNs or diffusely filled neurons. In the remaining 4 cases (one with AD) we did not find spinal LBs or LNs (Table 1).

Discussion

Our results show that in cases with a primary diagnosis of PD and LBD there is prominent accumulation of α -synuclein pathology in the sacral dorsal roots, Lissauer's tract and especially the LCR, structures that receive pelvic primary afferents from the bladder and distal colon. Furthermore, the distribution pattern of α -synuclein staining in the LCR and lumbosacral white matter show striking similarities to that of secondary afferent neurons and their axons as determined with classical tracing in animals (Fig 1B)¹⁹. The distribution pattern of α -synuclein pathology in the current study is in line with earlier studies which reported LBs in the sacral parasympathetic nucleus² or α -synuclein pathology in lamina I of the sacral cord and sacral parasympathetic nucleus^{3, 6}. The recognition that sacral cord α -synuclein pathology involves the LCR, which is anatomically and neurochemically distinct from the dorsal horn proper and the sacral parasympathetic nucleus, suggests that α -synuclein pathology accumulates not only in regions which control motor aspects of micturition, but also in peripheral and central visceral *afferent* pathways that mediate this function. Thus, there are several possible mechanisms through which the accumulation and spread of α -synuclein pathology in the spinal cord may contribute to bladder dysfunction and constipation in PD.

Urinary symptoms may affect up to 70% of individuals with PD¹. These include nocturia, urgency, and frequency, and are accompanied by detrusor overactivity. Dopaminergic treatment does not improve these symptoms¹. Our findings raise the question whether pathology in peripheral and central visceral afferent pathways that control the micturition reflex may account for some of these complaints. This would include pelvic primary afferent fibers and secondary afferent neurons that project from the sacral cord to the ventrolateral periaqueductal gray matter (PAG)^{16, 19}. The PAG is important for bladder filling as shown by functional imaging studies in humans^{20, 21} and projects to Barrington's nucleus²².

Barrington's nucleus, which does not receive dense innervation from the sacral spinal cord²² then in turn controls the detrusor muscle via descending projections to the sacral intermediolateral cell column. The accumulation of α -synuclein pathology in the afferent pelvic-PAG loop raises the question whether this may contribute to disinhibition or overactivation of Barrington's nucleus, resulting in detrusor overactivity.

Urinary frequency and incontinence is a common, often early symptom in MSA^{7, 23}. Based upon the above findings in the more common α -synucleinopathies, the question is whether in addition to pathology in Onuf's nucleus and the area of the pontine micturition center^{7, 24}, pathology in the LCR may contribute to urinary symptoms in MSA.

Phosphorylated α -synuclein in the form of labeled neurites was remarkably distinct in the LCR of the MSA case with prominent autonomic features, which included urinary incontinence. In both MSA cases, pathological, diffuse cytoplasmic phosphorylated α -synuclein, a common feature in MSA²⁵, was found in numerous medium sized neurons in the LCR. Widespread GCIs were found in line with prior reports²⁶. Larger studies will be necessary to assess how the clinical features in the various subtypes of MSA correlate with the severity and type of pathology in the LCR.

The current post-mortem findings may also be important for understanding the pathologic basis for constipation in PD. The pelvic nerve innervates the distal one third of the colon and rectum and these afferents share the same territory as bladder inputs in the sacral dorsal horn²⁷. However, reflex pathways for bladder control versus defecation differ^{27, 28}.

Colonic emptying relies on the myenteric plexus, which is also affected in PD, and a spinal reflex loop²⁷. Weakening of the afferent portion of this spinal reflex loop may contribute to constipation and is in line with the idea that a sensory deficit contributes to inhibition of the defecation reflex in the elderly²⁹.

We observed α -synuclein pathology in the sacral spinal cord of 2 out of 3 AD dementia cases. It is possible that this milder α -synuclein pathology contributes to micturition difficulties and constipation in older adults who do not meet diagnostic criteria for PD. Lewy body pathology was also found in brainstem and limbic regions in all 3 AD dementia cases and in cortical regions in 2 out of 3 cases. Altogether, these data show that pathological α -synuclein may be present throughout the CNS in older adults without a clinical diagnosis of PD. These data suggest that systematic studies of Lewy Body pathology in the brain, brainstem and spinal cord in older adults with and without a clinical diagnosis of PD are needed to delineate the clinical spectrum of α -synuclein pathology.

According to the dual-hit hypothesis proposed by Hawkes and by other groups^{30, 31}, and in line with observations that α -synuclein is indeed transported in anterograde and retrograde directions³² α -synuclein may enter the CNS via an anterograde route involving the olfactory bulb and via a retrograde route from the gastrointestinal tract via the vagus nerve to the caudal brainstem, e.g.³³. The distribution pattern of α -synuclein pathology in sacral afferent circuitries shown in this report suggests that there may be an additional portal of entry into the CNS through anterograde, transsynaptic transmission via bladder and/or distal colon primary afferents. To reconstruct a possible temporal and spatial course of spread, a larger number of samples are needed.

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Figure 1.

Schematic drawings of the sacral spinal cord in cat (A, B) and human (C, D).

A and B: Location of the lateral collateral region (LCR; yellow) in relation to the sacral parasympathetic nucleus (SPN; blue), Onuf's nucleus (ON; purple), somatic motoneurons (mn; gray) and retrogradely labeled neurons that project to the periaqueductal gray (PAG; magenta) and their axons (in cat, B). C: Location of the LCR (yellow) in relation to the sacral parasympathetic nucleus (SPN; blue), Onuf's nucleus (purple), and somatic motoneurons (mn; gray) in human sacral spinal cord. D: Schematic representation of Lewy Bodies (gray dots) and α -synuclein filled neurons (black dots) in the human sacral cord (case 9). A and B are adaptations from VanderHorst et al., 1996¹⁹.

Figure 2.

Photomicrographs of the human sacral spinal cord. *Location of the LCR:* Nissl stained (A and D; case 9), ChAT stained (B and E; case 9) and VIP stained sections (C and F; case 4) marking the lateral collateral pathway (LCR) in relation to the dorsal horn and sacral parasympathetic nucleus (SPN). Note that the LCR can be clearly distinguished from the dorsal horn at level S2 (A and B), rostral to the location of preganglionic ChAT-immunoreactive neurons in the SPN (D and E).

Distribution of α -synuclein in the sacral cord: G-R: α -synuclein immunoreactive LBs and LNs in the sacral cord of case 9 and case 10 (K) with PD. Low power photomicrographs show an abundance of immunoreactivity in the LCR at levels S2-S4 and in Onuf's nucleus (H-J), whereas labeling is less dense rostrally in S1 (G).

K and L: In addition to LBs and LNs, diffusely filled neurons were present especially in and near the LCR, many of which contained one (large arrows in K and L) or multiple (small arrow in L) LBs. These were also seen using an antibody against phosphorylated α -synuclein (K, magenta reaction product).

M: Large numbers of LBs and LNs in the dorsal root (arrow) at level S2 and LBs in Lissauer's tract (arrowheads).

N, O: Higher magnifications of boxed LCR regions in panels H and I, showing dense α -synuclein immunoreactivity including LBs (arrowheads), LNs and diffusely filled neurons (arrows) in the LCR at level S2 and S3. P: Only a few LNs were seen ventral to the central canal at level S1 which has a relatively low density of LBs and LNs (see G). Q: LNs in the white matter of S1 are abundant in the ventral part of the medial funiculus. R: Higher magnification of the boxed area around the central canal in I showing that at level S3, numerous LNs were present ventral to the central canal. The presence of fibers ventral to the central canal at caudal sacral levels and the distribution pattern in the sacral white matter resembles the pattern seen following conventional tracing of PAG-projecting neurons in animal models (compare Fig 1B).

S - W: In case 12 with MSA characterized by autonomic dysfunction, phosphorylated α -synuclein (magenta reaction product) is present in the sacral dorsal horn (S). The LCR contained numerous labeled neurites (arrowheads in W). Glial inclusions are found throughout the white and gray matter of the sacral cord (T). In addition, the LCR contains occasional neuronal cytoplasmic inclusions (arrowhead in U) and neuronal nuclear inclusions (arrowhead in V) as well as neurons with granular cytoplasmic labeling (asterisks in S; U, V).

Bars in A-E, G-J= 750 μ m, F=200 μ m, K and M=50 μ m, L=20 μ m, N-R= 185 μ m, S=100 μ m, T-W= 15 μ m.

LCR: lateral collateral region; dh: dorsal horn; mn: somatic motoneurons; SPN: sacral parasympathetic nucleus; On: Onuf's nucleus; dr: dorsal root; LT: Lissauer's tract; wm: white matter; vh: ventral horn; cc: central canal.

Table 1

Summary of Clinical and Post-Mortem Findings

α-synuclein pathology													
Diagnosis				Sacral cord			Thoracolumbar			Supraspinal			
Case	Age	Sex	Pathological	Clinical	Severity	Type	Location	Severity	Type	Location	Type	Location	
1	53	F		hepatic cirrhosis	-	-	-	-	-	-	-	-	
2	62	F		leukemia	+++	G	LT, lam I, LCR, On	+	G	LT/lam I	NA	NA	
3	84	M		multiple myeloma	-	-	-	-	-	-	+	G DMV/LC/SN	
4	86	F	AD-LL (NIA), Braak NFT-II	MCI	-	-	-	-	-	-	-	-	
5	77	F	AD-IL (NIA), Braak NFT-III, gross infarction, lipohyalinosis, DLB-IL (neocortical)	AD	+	LN, LB	LT, lam I, LCR	+	LN	LT/lam I	+	LN, LB DMV/LC/SN/MF/MT/EC/AC	
6	83	F	AD-HL (NIA), Braak NFT-V, gross infarction, lipohyalinosis, DLB-IL (neocortical)	AD	+	LN, LB	LT, lam I, LCR	+	LN	LT/lam I	+	LN, LB DMV/LC/SN/MF/MT/EC/Inf parietal/AC	
7	90+	F	AD-HL (NIA), Braak NFT-V, gross infarction, lipohyalinosis, DLB-LL (limbic)	AD	-	-	-	-	-	-	+	LN, LB DMV/LC/SN/EC/AC	
8	87	F	AD-IL (NIA), Braak NFT-III, DLB-HL (neocortical)	DLB	+++	G, LN, LB	LT, lam I, LCR, On, vh	++	G, LN, LB	LT/lam I/IML/vh	++	G, LN, LB DMV/LC/SN/MF/MT/EC/Inf parietal/AC	
9	89	F	AD-LL (NIA), Braak NFT-III, PD, lipohyalinosis	PD	+++	G, LN, LB	LT, lam I	++	G, LN, LB	LT/lam I/IML/vh	+++	G, LN, LB DMV/LC/SN/MF/MT/EC/Inf parietal/AC	

α-synuclein pathology														
Diagnosis					Sacral cord		Thoracolumbar					Supraspinal		
Case	Age	Sex	Pathological	Clinical	Severity	Type	Location	Severity	Type	Location	Type	Location	Type	Location
LCR, On, vh LCR, On, vh														
10	85	M	AD-IL (NIA), Braak NFT-III, PD, arteriolosclerosis, marcoinfarcts	PD	+++	LN, LB	LT, lam I, LCR, On, vh	++	LN, LB	LT/lam I/IML/vh	+++	LN, LB		DMV/LC/SN/EC
11	82	F	AD-LL (NIA), Braak NFT-IV, MSA	MSA (ataxia, parkinsonism, urinary incontinence), MCI	+	NI	gray matter, including LCR	+	NI	gray matter	++	LN, NI		basis pontis/IO/SN/dentate gyrus/EC/Am/NC
					++	GCI	gray and white matter	++	GCI	gray and white matter	++	GCI		medulla/basis pontis/midbrain/BG/AM/EC/NC
12	90+	F	AD-IL (NIA), Braak NFT-III, MSA	MSA (urinary incontinence, mild gait ataxia)	+++ +	LN NI	LCR gray matter, including LCR	+	LN NI	gray matter gray matter	++ +	LN NI		medulla/basis pontis/cerebellar white matter>midbrain/SN/BG
														IO/basis pontis/cerebellar white matter/midbrain/SN/BG/EC/MF
					++	GCI	gray and white matter	++	GCI	gray and white matter	++	GCI		medulla/basis pontis/cerebellar white matter/midbrain/SN/BG

α -Synuclein pathology was assessed as G: granular; LN: Lewy neurites, LB: Lewy Bodies; GCI: glial inclusions; NI: neuronal inclusions. IML: intermedialateral cell column; Lam I: Lamina I; LT: Lissauer's tract; LCR: lateral collateral region; On: Onuf's nucleus; vh: ventral horn. The density of the staining was scored as - (absent), + (low), ++ (moderate), +++ (high). MCI: minimal cognitive impairment; AD: Alzheimer's Disease; PD: Parkinson's Disease; ILB: incidental Lewy Bodies; DLB: Lewy Body Dementia; LL: low likelihood; IL: intermediate likelihood; HL: high likelihood; NFT: neurofibrillary tangles; DMV: dorsal motor nucleus of the vagus complex; LC: locus coeruleus; SN: substantia nigra; IO: inferior olive; MF: mid frontal gyrus; MT: med temporal gyrus; EC: entorhinal cortex; IP: inferior parietal cortex; AC: anterior cingulate gyrus; Am: amygdala; BG: basal ganglia; NC: neocortex.