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RESEARCH ARTICLE

The Brainstem Pathologies of Parkinson's Disease and Dementia with Lewy Bodies

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Keywords
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Abstract

Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are among the human synucleinopathies, which show alpha-synuclein immunoreactive neuronal and/or glial aggregations and progressive neuronal loss in selected brain regions (eg, substantia nigra, ventral tegmental area, pedunculopontine nucleus). Despite several studies about brainstem pathologies in PD and DLB, there is currently no detailed information available regarding the presence of alpha-synuclein immunoreactive inclusions (i) in the cranial nerve, precerebellar, vestibular and oculomotor brainstem nuclei and (ii) in brainstem fiber tracts and oligodendroctyes. Therefore, we analyzed the inclusion pathologies in the brainstem nuclei (Lewy bodies, LB; Lewy neurites, LN; coiled bodies, CB) and fiber tracts (LN, CB) of PD and DLB patients. As reported in previous studies, LB and LN were most prevalent in the substantia nigra, ventral tegmental area, pedunculopontine and raphe nuclei, periaqueductal gray, locus coeruleus, parabrachial nuclei, reticular formation, prepositus hypoglossal, dorsal motor vagal and solitary nuclei. Additionally we were able to demonstrate LB and LN in all cranial nerve nuclei, premotor oculomotor, precerebellar and vestibular brainstem nuclei, as well as LN in all brainstem fiber tracts. CB were present in nearly all brainstem nuclei and brainstem fiber tracts containing LB and/or LN. These findings can contribute to a large variety of less well-explained PD and DLB symptoms (eg, gait and postural instability, impaired balance and postural reflexes, falls, ingestive and oculomotor dysfunctions) and point to the occurrence of disturbances of intra-axonal transport processes and transneuronal spread of the underlying pathological processes of PD and DLB along anatomical pathways.

INTRODUCTION

Parkinson's disease (PD) and dementia with Lewy bodies (DLB) belong to the human synucleinopathies, which share (i) neuronal and/or glial aggregations of the pathologically altered neuronal alpha-synuclein protein normally interacting with presynaptic membranes and (ii) the progressive neurodegeneration of selected brain regions (2, 15, 33–35, 38, 45, 59). PD and DLB-related alpha-synuclein immunoreactive aggregates include: (i) Lewy bodies (LB) in neuronal perikarya (2, 6, 8, 15, 26, 31, 32, 35, 38, 44, 59, 63), (ii) Lewy neurites (LN) in neuronal processes (2, 6, 8, 15, 26, 31, 32, 34, 35, 38, 44, 59, 63) and (iii) coiled bodies (CB) in affected oligodendrocytes (35, 68).

With an estimated prevalence of 200–300/100 000, PD represents the second most common human neurodegenerative disease (29, 31, 40, 59, 61, 63). Along with the cardinal symptoms (ie, hypo-/bradykinesia, gait and postural instability, rigidity, resting tremor, unilateral onset, profound response to L-dopa therapy) (11, 12, 19, 31, 40, 49, 65, 73, 74), PD patients also may suffer from cerebellar, vestibular, oculomotor dysfunctions, dysphagia, restless legs, sensory dysfunctions, autonomic disorders and depression. Approximately one-half of PD patients can also develop a dementing syndrome (ie, Parkinson's disease with dementia, PDD) (11, 19, 24, 28, 34, 39, 40, 43, 45, 70, 74).

With an estimated prevalence of 100/100 000, DLB also represents a frequent human synucleinopathy and is the second most

common dementing neurodegenerative syndrome in the elderly after Alzheimer's disease. Core neuropsychiatric symptoms of DLB are progressively impaired and fluctuating cognition and consciousness, as well as recurrent visual hallucinations in association with a PD-like extrapyramidal syndrome (24, 34, 35, 44, 45, 69, 70). Additional DLB symptoms may include: frequent falls and syncopes, autonomic dysfunctions, rapid eye movement (REM) sleep behavior disorders, impairments of executive dysfunctions, further visual system dysfunctions, delusions and depression, as well as increased neuroleptics and decreased L-dopa sensitivity (24, 44, 45, 69, 70). Clinically DLB is considered when dementia is predominant, manifests as an early disease symptom and precedes the PD-like somatomotor dysfunctions by at least one year (43, 44, 69, 70).

The progressive loss of dopaminergic neurons in the compact part of the substantia nigra and the widespread occurrence of alpha-synuclein immunoreactive LB and LN in the central and peripheral nervous system are currently considered as the neuropathological hallmarks of PD (6, 8, 15, 19, 24, 28, 29, 32, 34, 35, 38, 39, 49, 74). The predictable chronological sequence on disease spread with increasing brain pathology severity and ascending progression represents the base for the suggested staging system of the PD-related brain pathology. According to this staging system, neuronal LB and LN are first seen in the dorsal motor vagal nucleus and intermediate reticular zone of the medulla oblongata. From there, the pathology takes an ascending course, reaches select nuclei in the pontomedullary junction (ie, great raphe nucleus and gigantocellular reticular nucleus) and pons (locus coeruleus), then affects the midbrain with the dopaminergic substantia nigra, the basal forebrain, amygdala, thalamus and hypothalamus and ultimately involves the entire cerebral allo- and neocortex (6, 8, 12, 28, 34, 35, 39).

The neuropathological hallmarks of DLB likewise comprise loss of dopaminergic nigral neurons and widespread occurrence of alpha-synuclein immunoreactive LB and LN in the brain (6, 8, 24, 31–35, 39, 44). Although the severity and distribution of the nigral pathology show some differences between PD/PDD and DLB patients, the morphology and the ascending spread of the pathological processes from the medulla oblongata to the cerebral cortex apparently do not differ significantly between PD/PDD and DLB (31, 34, 35, 44).

As none of the previous PD and DLB brainstem studies (5, 6, 8, 15, 16, 21, 24, 27, 29, 30, 34, 35, 46, 48, 50, 60, 61, 75) provided detailed information on the presence of alpha-synuclein immunoreactive LB, LN and CB in the cranial nerve, precerebellar, vestibular and oculomotor brainstem nuclei, as well as in the brainstem fiber tracts and in the oligodendroctyes, we analyzed the extent and distribution of the alpha-synuclein burden in the brainstem nuclei and fiber tracts of clinically diagnosed and neuropathologically confirmed PD, PDD and DLB patients.

PATIENTS AND METHODS

Patients

We examined the brainstem of eleven individuals with clinically advanced synucleinopathies (five PD, one PDD and five DLB patients: one female, ten males; age at death: 77.0 ± 10.67 years) (Table 1), as well as the brainstem of five control individuals (two females, three males; age at death: 61.8 ± 12.8 years). This study was performed in accordance with the guidelines of and approved by the Ethics Committee of the Faculty of Medicine at the Goethe University of Frankfurt/Main.

All eleven patients presented with parkinsonism and/or dementing symptoms. Patients who suffered from parkinsonian features for at least 1 year prior to displaying dementing symptoms were diagnosed as PDD and patients with a dementing syndrome that manifested at least 1 year before motor parkinsonian symptoms were diagnosed as DLB (19, 24, 44, 45, 69, 70) (Table 1).

Diagnostic neuropathological examination of the brains of the synucleinopathy patients was performed on thin tissue sections by an experienced neuropathologist (W. den Dunnen) and included (i) assessment of neuronal loss and alpha-synuclein inclusion pathologies at acknowledged brain predilection sites, as well as (ii) a neuropathological synopsis of the routine neuropathological findings and their final categorization according to the Braak *et al* PD staging system and the McKeith neuropathological DLB criteria (6, 8, 34, 35, 44). These neuropathological DLB criteria define brainstem predominant, limbic or diffuse neocortical pathology in a semi-quantitative manner (34, 35, 44) (Table 1).

Tissue processing

Subsequent to the fixation of the brains in a 4% buffered solution of formaldehyde, the brainstem of all PD, PDD and DLB patients were dissected at the level of the inferior colliculus, embedded in polyethylene glycol (PEG 1000, Merck, Darmstadt, Germany) and cut into complete sets of 100 μm thick horizontal serial sections (7, 62).

Table 1. Overview of the synucleinopathy patients examined.
Abbreviations: bDLB = brainstem predominant type of DLB; DLB = dementia with Lewy bodies; dDLB = diffuse neocortical type of DLB; PD = Parkinson's disease; PDD = Parkinson's disease with dementia; F = female; M = male.

Patient	1	2	3	4	5	6	7	8	9	10	11
Age at death	62	76	81	90	95	76	60	69	76	78	84
Gender	M	M	M	M	M	F	M	M	M	M	M
Clinical diagnosis	PD	PD	PD	PD	PD	PDD	DLB	DLB	DLB	DLB	DLB
Post-mortem delay	9 h	<24 h	<24 h	<24 h	<24 h	3 h	<24 h	<24 h	<24 h	<24 h	<24 h
Neuropathological staging	PD 6	PD 6	PD 5	PD 4	PD 6	PD 6	bDLB	dDLB	dDLB	dDLB	dDLB

Age at death, gender, clinical diagnosis, post-mortem delay and results of post-mortem neuropathological staging (PD 1–6: neuropathological Braak *et al* Parkinson's disease stages 1 to 6) (6, 8, 34, 35, 44).

One set of horizontal serial sections of all brainstems was stained with aldehyde-fuchsin and Darrow red according to the pigment-Nissl method to highlight neuronal lipofuscin granules and Nissl material. These sections were used for neuroanatomical orientation and assessment of neuronal loss in the PD, PDD and DLB patients (7, 54–58). A second set of serial sections of all cases was immunolabeled with an antibody against the pathological form of the alpha-synuclein protein to visualize the neuronal and oligodendroglial inclusion pathologies (6, 8, 38, 59). After antigen retrieval with formic acid for 3 minutes at room temperature, the sections were incubated overnight at 4°C with the syn-1 antibody (1:2000, BD Biosciences, San Jose, CA, USA). Following incubation with the biotinylated secondary antibody, positive immunostainings were visualized with the AB complex and diaminobenzidine. Images were acquired using a Zeiss Axioplan Microscope with a Zeiss Axiocam and Axiovision 4-9 software (Zeiss, Jena, Germany). Figures were arranged using the Photoshop CS3 software.

Assessment of brainstem pathologies

The presence and extent of the alpha-synuclein immunoreactive inclusion pathology in nerve cells (ie, LB, LN), oligodendrocytes (ie, CB) and brainstem fiber tracts (ie, LN, CB) of the patients and the control individuals was semi-quantitatively assessed and scored as follows: inclusion pathology not detectable even after a time period of 3 minutes of careful microscopic investigation of a given brainstem nucleus or fiber tract (−), rare inclusions detectable only after a time period of approximately 3 minutes of microscopic examination (+), well-developed inclusion pathology decorating the outlines of affected brainstem nucleus or fiber tract rapidly detectable by microscopic examination (++), immunoreactive inclusion pathology already detectable with the naked eye in histological sections, completely filling brainstem nuclei or fiber tracts, only requiring light microscopical examination for confirmation and differentiation (+++) (Tables 2–5). The neuropathological assessments of the immunoreactive inclusion pathologies in brainstem greys were performed independently by J. Mahlke and U. Rüb and in brainstem fiber tracts by K. Seidel and U. Rüb. The second ratings of the neuronal and oligodendroglial alpha-synuclein immunoreactive pathologies were performed blinded to the results of the first rating. Pathoanatomical examination of neuronal loss in the PD, PDD and DLB brainstems was performed twice by U. Rüb and its extent was scored as not discernible (–), marked (+) or severe (++).

Statistical analysis

We calculated *Cohen's weighted kappa coefficient* K_w (BiAS for Windows, version 9.14, Epsilon, Darmstadt, Germany) to statistically estimate the consistency, reliability and reproducibility (ie, intrarater and interrater reliabilities) of the ordinal-scaled judgments of neuronal loss and of the presence and frequency of LB, LN and CB in the brainstem of the patients. In the event of disagreements of the successive assessments made independently by two of the investigators, the definite decision was made during a final consensus meeting of all investigators involved in this study.

Kendall's rank correlation coefficient tau (τ) (BiAS for Windows, version 9.14, Epsilon) was applied to describe the correlation between the frequency of alpha-synuclein immunoreactive (i) LB and LN, (ii) LB and CB (iii) LN and CB (Tables 2–5). We used the two-tailed test version to verify the statistical significance of τ non-parametrically. In the event that a given brainstem nucleus or brainstem fiber tract could only be examined in less than five PD or DLB patients, the findings of these examinations were excluded from statistical analysis.

RESULTS

Neuropathological classification

Neuropathological classification of the brains of the eleven synucleinopathy patients revealed one patient with Braak *et al* PD stage 4 pathology, one patient with Braak *et al* PD stage 5 pathology, four patients with Braak *et al* PD stage 6 pathology, one patient with brainstem predominant and four patients with diffuse neocortical type of DLB (6, 8, 34, 35, 44) (Table 1). All of the five control individuals were classified as PD stage 0.

Neuronal and oligodendroglial inclusion pathologies in the brainstem

In contrast to the control individuals studied, all of our synucleinopathy patients presented with a widespread affection of brainstem nuclei by LB, LN and CB and of brainstem fiber tracts by LN and CB (Figures 1–5; Tables 2–5). LB appeared as sharply contoured, spherical, ovoid, plum or paddle-shaped cytoplasmic inclusions (Figure 1A,C,D,G). Depending on the affected nerve cell, the size of LB may vary between 5 and 35 μm. LN were either slender or swollen, short or long, serpentine or chaplet-like, elongated, flagelliform, spiral or club-shaped (Figures 1A,C, 2B,G, 3B and 4B–D). LN in brainstem fiber tracts were filamentous, slender and long, and were combined to interrupted bundles of parallel oriented fiber-like structures, which followed the entire course of the affected fiber tracts (Figures 1B, 2D, 4A and 5). CB were considerably smaller and more difficult to detect than LBs and LNs, surrounded the lucent nucleus and were twisted in the small cytoplasmic rim of affected oligodendrocytes (Figures 1B–H and 5C–F). The distribution and extent of the neuronal and oligodendroglial pathologies showed no group differences between the PD and DLB patients and did not depend on the neuropathological stage of their overall brain pathologies (Tables 2–5).

As in previous PD and DLB brainstem studies, LB and LN were most prevalent in the dopaminergic nuclei of the ventral tegmental area and the compact part of the substantia nigra, in the cholinergic pedunculopontine and serotonergic raphe nuclei, in the periaqueductal gray, noradrenergic locus coeruleus, parabrachial nuclei, reticular formation, prepositus hypoglossal, dorsal motor vagal, as well as in the solitary nuclei of the PD and DLB patients (Figures 1A,C,G,H, 2B,E,F, 3E, 4 and 5B) (Tables 2–4). However, we also observed a more or less consistent affection of all cranial nerve nuclei (Tables 2–4), premotor oculomotor (Tables 2 and 3) and precerebellar brainstem nuclei (Tables 2 and 3), as well as vestibular nuclei (Tables 2 and 3) in our PD and DLB patients (Figures 2A–D,F,H and 3A–D,F).

Table 2. Alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in nuclei of the medulla oblongata of patients with PD or DLB.
Abbreviations: CB = coiled bodies; DLB = dementia with Lewy bodies; PD = Parkinson's disease; PDD = Parkinson's disease with dementia; LB = Lewy bodies; LN = Lewy neurites.

		1	2	3	4	5	6	7	8	9	10	11
Gracile nucleus	LN	–	–	–	+	–	–	–	–	–	+	–
	LB	+	–	+	+	+	+	+	+	+	+	+
	CB	–	–	–	–	–	–	–	–	–	+	–
Cuneate nucleus	LN	+	+	+	+	+	+	+	+	+	+	+
	LB	+	+	+	+	+	+	+	+	+	+	+
	CB	–		–	–	–	–	–	–	–	+	–
External cuneate nucleus	LN	–	+	+	–	+	–	–	–	–	+	+
	LB	–	+	+	–	+	+	+	+	+	+	+
	CB	–	–	–	–	–	–	–	–	–	–	–
Medial reticular nucleus	LN	++	+	+	++	++	+	++	++	+	++	++
	LB	++	+	+	++	++	++	++	++	+	++	++
	CB	++	+	+	++	++	+	++	++	+	++	++
Spinal vestibular nucleus	LN	+	++	+	+	+	+	+	+	+	++	+
	LB	+	++	+	+	+	+	+	+	+	++	+
	CB	+	++	+	+	+	+	+	+	+	++	+
Solitary nuclei	LN	++	++	++	+	++	++	++	++	++	++	+++
	LB	++	++	++	++	++	++	++	++	++	++	+++
	CB	++	++	++	+	++	++	+	+	+	+	++
Hypoglossal nucleus	LN	+	+	+	++	++	+	++	+	+	+	+
	LB	+	+	+	+	++	++	++	+	+	+	+
	CB	+	+	+	+	++	++	++	+	+	+	+
Lateral reticular nucleus	LN	+	++	++	++	++	+	+	++	+	++	+
	LB	+	++	++	+	++	+	+	++	+	++	+
	CB	+	++	++	++	+	+	+	++	+	++	+
Dorsal motor vagal nucleus	LN	+++	+++	+++	+++	++	++	++	+++	++	+++	++
	LB	+++	+++	+++	+++	++	++	+++	+++	++	+++	++
	CB	++	++	++	++	+	+	+++	++	++	+++	++
Intermediate reticular zone	LN	+++	++	+++	+++	+++	++	+++	+++	++	++	++
	LB	+++	++	+++	+++	+++	++	+++	+++	++	++	++
	CB	+++	++	+++	+++	+++	++	+++	+++	++	++	++
Inferior olive	LN	++	+	++	+	+	+	+	+	–	++	+
	LB	++	+	++	+	+	+	+	+	–	++	+
	CB	++	+	++	+	+	+	+	+	–	++	+
Arcuate nucleus	LN	+	+	+	+++	+++	+	+	+	+	++	+
	LB	+	+	+	++	+	+	+	+	+	++	+
	CB	+	+	+	++	++	+	+	+	+	++	+

Distribution and extent of the alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in nuclei of the medulla oblongata of patients with PD, PDD and DLB (cases 1 to 5: PD patients; case 6: PDD patient; cases 7–11 DLB patients).

The majority of the nuclei of the *medulla oblongata* of our PD and DLB patients (Figures 1A,C,D, 2A–D and 5B; Table 2) were consistently affected by LB and LN, whereas the external cuneate and gracile nuclei were involved only in some of the synucleinopathy patients studied. With the exception of the external cuneate, cuneate and gracile nuclei, all medullary nuclei regularly exhibited CB (Figure 1C,D; Table 2).

In the *pons* (Figures 2E–H and 3; Table 3) and *midbrain* (Figures 1G and 4; Table 4), LBs and LNs were consistently present in all nuclei of the PD and DLB patients studied. CB occurred in some instances less frequently, but were present in all nuclei of the pons (Figure 1F,H; Table 3) and midbrain (Figure 1G,H; Table 4) harboring LB and LN.

All *brainstem fiber tracts* of our PD and DLB patients exhibited LN as well as CB (Figures 1B and 5; Table 5).

Neuronal loss in the brainstem

In addition to the alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathologies, our PD and DLB patients showed a marked to severe neuronal loss in the dopaminergic nuclei of the ventral tegmental area and the compact part of the substantia nigra, the serotonergic dorsal raphe nucleus, the cholinergic compact part of the pedunculopontine nucleus, the noradrenergic locus coeruleus and the dorsal motor vagal nucleus (data not shown).

Statistical analysis

There was a significant correlation between the occurrence of (i) LB and LN in brainstem nuclei (PD: τ values for all midbrain,

Table 3. Alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in nuclei of the pons of patients with PD or DLB. Abbreviations: CB = coiled bodies; DLB = dementia with Lewy bodies; PD = Parkinson's disease; PDD = Parkinson's disease with dementia; LB = Lewy bodies; LN = Lewy neurites).

		1	2	3	4	5	6	7	8	9	10	11
Prepositus hypoglossal nucleus	LN	+++	+++	++	+++	++	+++	+++	++	+++	++	++
	LB	+++	+++	++	+++	++	+++	+++	++	+++	++	++
	CB	++	++	+	++	+	++	++	+	++	+	+
Dorsal paragigantocellular reticular nucleus	LN	+	++	+	+	++	++	++	+	+	++	++
	LB	+	++	+	+	++	++	++	+	+	++	++
	CB	+	++	+	+	++	++	++	+	+	++	++
Gigantocellular reticular nucleus	LN	++	+++	+++	++	+++	+++	+++	++	++	+++	+++
	LB	++	+++	+++	++	+++	+++	+++	++	++	+++	+++
	CB	+	++	++	+	++	++	++	++	++	+++	+++
Great raphe nucleus	LN	++	+++	++	+++	+++	+++	+++	++	++	+++	+++
	LB	++	+++	++	+++	+++	+++	+++	++	++	+++	+++
	CB	++	+++	++	+++	+++	+++	+++	++	++	+++	+++
Pontine nuclei	LN	+	++	+	+	+	+	+	+	+	+	++
	LB	+	++	+	+	+	+	+	+	+	+	++
	CB	+	++	+	+	+	+	+	+	+	+	++
Medial vestibular nucleus	LN	++	+	++	+	++	+	++	+	++	++	++
	LB	++	+	++	++	++	++	++	+	++	++	++
	CB	++	+	++	++	++	++	++	+	++	++	++
Superior olive	LN	+	+	+	+	++	+	++	++	+	+	+
	LB	+	+	+	+	++	+	++	++	+	+	+
	CB	+	+	+	+	++	+	++	++	+	+	+
Facial nucleus	LN	+	+	+	++	++	+	+	++	+	++	+
	LB	+	+	+	++	++	+	+	++	+	++	+
	CB	+	+	+	++	++	+	+	++	+	++	+
Abducens nucleus	LN	++	+	+	+	+	+	+	+	+	++	+
	LB	++	+	+	+	+	+	+	+	+	++	+
	CB	++	+	+	+	+	+	+	+	+	++	+
Parvocellular reticular nucleus	LN	++	++	+	+++	++	++	++	+	+	++	++
	LB	++	++	+	+++	++	++	++	+	+	++	++
	CB	++	++	+	+++	++	+	++	+	+	++	++
Lateral vestibular nucleus	LN	+	+	+	+	++	++	+	+	+	++	++
	LB	+	+	+	++	++	++	+	+	+	++	++
	CB	+	+	+	++	++	+	+	+	+	++	+
Raphe interpositus nucleus	LN	+	+	+	+	++	++	+	++	+	+	+
	LB	+	+	+	+	++	++	+	++	+	+	+
	CB	+	+	+	+	++	++	+	++	+	+	+
Area of the excitatory burst neurons for horizontal saccades	LN	+	++	++	+	++	++	+	++	+	++	++
	LB	+	++	++	+	++	++	+	++	+	++	++
	CB	+	++	++	+	++	++	+	++	+	++	++
Principal trigeminal nucleus	LN	+	+	+	++	+	+	+	++	+	+	+
	LB	+	+	+	++	+	+	+	++	+	+	+
	CB	+	+	+	++	+	+	+	++	+	+	+
Motor trigeminal nucleus	LN	+	+	++	+	+	++	+	++	+	+	+
	LB	+	+	++	+	+	++	+	++	+	+	+
	CB	+	+	++	+	+	++	+	++	+	+	+
Superior vestibular nucleus	LN	+	++	++	++	+	++	+	++	++	++	+
	LB	+	++	++	++	+	++	+	++	++	++	+
	CB	+	++	++	++	+	++	+	++	++	++	+
Pontine reticular formation, caudal subnucleus	LN	+	+	+	++	+	++	++	++	+	++	++
	LB	+	+	+	++	+	++	++	++	+	++	++
	CB	+	+	+	++	+	++	++	+	+	++	++
Reticulotegmental nucleus of the pons	LN	++	++	++	++	+++	++	+	++	++	++	+++
	LB	++	++	++	++	+++	++	+	++	++	++	++
	CB	+	+	+	+	++	+	+	++	++	++	++
Central raphe nucleus	LN	+++	++	++	++	++	+++	+++	++	++	++	++
	LB	+++	++	++	++	++	+++	+++	++	++	++	++
	CB	++	+	+	+	+	++	+++	++	++	++	++
Pontine reticular formation, oral subnucleus	LN	++	++	++	+	++	++	++	++	+	++	++
	LB	++	++	++	+	++	++	++	++	+	++	++
	CB	++	++	++	+	++	++	++	++	+	++	++
Lateral parabrachial nucleus	LN	+	+	++	++	+	+	+	+	+	++	+
	LB	+	+	++	++	+	+	+	+	+	++	+
	CB	+	+	++	++	+	+	+	+	+	++	+
Medial parabrachial nucleus	LN	++	++	++	++	++	+	++	+	+	++	++
	LB	++	++	++	++	++	+	++	+	+	++	++
	CB	++	++	++	++	++	+	++	+	+	++	++
Locus coeruleus	LN	+++	+++	++	+++	++	++	+++	+++	+++	++	+++
	LB	+++	+++	++	+++	++	++	+++	+++	+++	++	+++
	CB	++	++	+	++	+	+	++	++	++	+	++

Distribution and extent of the alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in nuclei of the pons of patients with PD, PDD and DLB (cases 1 to 5: PD patients; case 6: PDD patient; cases 7–11 DLB patients).

Table 4. Alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in midbrain nuclei of patients with PD or DLB. Abbreviations: CB = coiled bodies; DLB = dementia with Lewy bodies; PD = Parkinson's disease; PDD = Parkinson's disease with dementia LB = Lewy bodies; LN = Lewy neurites; nd = not determined.

		1	2	3	4	5	6	7	8	9	10	11
Interpeduncular nucleus	LN	+	+	+	+	++	++	nd	++	+	+	+
	LB	+	+	+	+	++	++	nd	++	+	+	+
	CB	+	+	+	+	++	++	nd	++	+	+	+
Substantia nigra, compact part	LN	++	++	++	++	+++	++	nd	++	++	++	+
	LB	++	+++	++	++	+++	++	nd	++	++	++	+
	CB	++	++	++	++	+++	++	nd	++	++	++	+
Ventral tegmental area	LN	++	++	++	++	++	+++	nd	++	++	++	+++
	LB	++	++	++	++	++	+++	nd	++	++	++	+++
	CB	++	++	++	++	++	+++	nd	++	++	++	+++
Periaqueductal gray	LN	++	++	++	+++	++	+++	nd	+	++	++	++
	LB	++	++	++	+	++	+++	nd	+	++	++	++
	CB	++	++	++	+	++	+++	nd	+	++	++	++
Dorsal raphe nucleus, supratrochlear part	LN	++	++	+++	++	++	++	+	++	++	++	++
	LB	++	++	+++	++	++	++	+	++	++	++	++
	CB	++	++	+++	++	++	++	+	++	++	++	++
Trochlear nucleus	LN	nd	nd	+	+	++	++	nd	+	+	+	nd
	LB	nd	nd	+	+	++	++	nd	+	+	+	nd
	CB	nd	nd	+	+	++	++	nd	+	+	+	nd
Pedunculopontine nucleus, compact part	LN	++	nd	++	++++	+++	+++	nd	++	+++	++	nd
	LB	++	nd	++	++	+++	+++	nd	++	+++	++	nd
	CB	++	nd	++	++	+++	+++	nd	++	+++	++	Nd
Inferior colliculus	LN	+	+	+	+	++	+	nd	+	+	+	++
	LB	+	+	+	+	++	+	nd	+	+	+	++
	CB	+	+	+	+	++	+	nd	+	+	+	++

Distribution and extent of the alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in midbrain nuclei of patients with PD, PDD and DLB (cases 1 to 5: PD patients; case 6: PDD patient; cases 7–11 DLB patients).

pontine and medullary nuclei 1.00; $P < 0.0023$; DLB: τ values for all midbrain, pontine and medullary nuclei 1.00; $P < 0.02$), (ii) LB and CB in brainstem nuclei (PD: τ values for all midbrain, pontine and medullary nuclei 1.00; $P < 0.0023$; DLB: τ values for all midbrain, pontine and medullary nuclei 1.00; $P < 0.02$), (iii) of LN and CB in brainstem nuclei (PD: τ values for all midbrain, pontine and medullary nuclei 1.00; $P < 0.0023$; DLB: τ values for all midbrain, pontine and medullary nuclei 1.00; $P < 0.02$) and (iv) CB and LN in brainstem fiber tracts (PD: τ values for all fiber tracts 1.00, $P < 0.0023$; DLB: τ values for all fiber tracts 1.00; $P < 0.02$).

Calculation of the weighted kappa coefficient revealed the following interrater reliabilities for our semi-quantitative assessments: LB in brainstem nuclei ($K_w = 0.84$; $P < 0.0001$), LN in brainstem nuclei ($K_w = 0.83$; $P < 0.0001$), CB in brainstem nuclei ($K_w = 0.75$; $P < 0.0001$), LN in brainstem fiber tracts ($K_w = 0.92$; $P < 0.0001$), CB in brainstem fiber tracts ($K_w = 0.86$; $P < 0.0001$).

DISCUSSION

In the past four decades, numerous studies have been performed on the pathological brainstem involvement in PD and DLB patients (5, 6, 8, 15, 16, 21, 25, 27–30, 34, 35, 46, 48, 50, 60, 61, 75). The present study confirms the findings of these previous studies (ie, widespread alpha-synuclein immunoreactive neuronal inclusion pathology and neuronal loss in select brainstem nuclei) and reinforces that it is not possible to reliably distinguish the neuropathological features of PD and DLB patients in the advanced clinical stages by means of histological and immunocytochemical post-mortem examination (10, 15). Moreover, our study also revealed that neuronal loss as well as the types, location, distribution and extent of the alpha-synuclein immunoreactive inclusion pathologies commonly present in the brainstem of PD and DLB patients resemble those observed in the brainstem of the familial PD form caused by an autosomal dominantly inherited A30P mutation in the alpha-synuclein gene. The results thus emphasize that PD, DLB and familial PD caused by the A30P mutation are closely related clinically as well as neuropathologically (61).

As novel results, our study for the first time demonstrates the consistent alpha-synuclein immunoreactive inclusion pathologies in the cranial nerve, vestibular, precerebellar and premotor oculomotor nuclei, as well as in brainstem fiber tracts of PD and DLB patients. The finding that the types, location, distribution patterns and the extent of the brainstem pathologies are highly similar in PD patients with Braak *et al* stage 4, 5 or 6 brain pathologies and do not differ substantially between the brainstem and diffuse DLB types suggests that the occurrence of the brainstem pathology is not a late event during the disease processes of PD and DLB. For PD, concepts have been proposed with regard to the origin and development of brainstem pathologies (6, 8, 13, 26, 28, 31, 66). As such concepts are currently missing for DLB patients, further

Table 5. Alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in brainstem fiber tracts of patients with PD or DLB. Abbreviations: CB = coiled bodies; DLB = dementia with Lewy bodies; PD = Parkinson's disease; PDD = Parkinson's disease with dementia; LN = Lewy neurites; nd = not determined.

		1	2	3	4	5	6	7	8	9	10	11
Cuneate fascicle	LN	+	+	+	+	+	+	+	++	+	+	+
	CB	+	+	+	+	+	+	+	++	+	+	+
Gracile fascicle	LN	+	+	+	+	+	+	+	++	+	+	+
	CB	+	+	+	+	+	+	+	++	+	+	+
Ventral spinocerebellar tract	LN	+	+	+	+	++	+	+	+	++	+	++
	CB	+	+	+	+	++	+	+	+	++	+	++
Dorsal spinocerebellar tract	LN	+	+	+	+	++	+	+	+	++	+	+
	CB	+	+	+	+	++	+	+	+	++	+	+
Hypoglossal nerve	LN	+	+	+	++	++	+	+	++	+	+	+
	CB	+	+	+	++	++	+	+	++	+	+	+
Vagal nerve	LN	++	++	++	++	++	++	+	++	++	++	++
	CB	+	++	++	++	++	++	+	++	++	++	++
Solitary tract	LN	+	+	++	++	+	++	+	++	+–	++	++
	CB	+	+	++	++	+	++	+	++	+	++	++
Pyramidal tract	LN	+	+	++	+	+	+	+	++	+	+	+
	CB	+	+	++	+	+	+	+	++	+	+	+
External arcuate fibres	LN	+	+	++	+	+	+	+	+	+	++	+
	CB	+	+	++	+	+	+	+	+	+	++	+
Olivocerebellar fibres	LN	+	+	++	++	++	+	++	+	+	+	+
	CB	+	+	++	++	++	+	++	+	+	+	+
Inferior cerebellar peduncle	LN	+	+	+	+	++	+	+	++	+	+	+
	CB	+	+	+	+	++	+	+	++	+	+	+
Vestibulocochlear nerve	LN	+	+	+	+	++	++	+	++	+	+	+
	CB	+	+	+	+	++	++	+	++	+	+	+
Spinal trigeminal tract	LN	+	+	++	+	+	+	+	++	+	+	+
	CB	+	+	++	+	+	+	+	++	+	+	+
Trapezoid body	LN	+	+	+	+	++	+	+	++	+	+	+
	CB	+	+	+	+	++	+	+	++	+	+	+
Facial nerve	LN	++	+	+	+	++	+	+	+	+	+	++
	CB	+	+	+	+	++	+	+	+	+	+	++
Abducens nerve	LN	+	+	+	++	++	+	+	++	+	+	+
	CB	+	+	+	++	++	+	+	++	+	+	+
Trigeminal nerve	LN	+	+	++	+	+	+	+	++	+	+	+
	CB	+	+	++	+	+	+	+	++	+	+	+
Medial cerebellar peduncle	LN	+	+	++	+	+	+	+	+	+	++	+
	CB	+	+	++	+	+	+	+	+	+	++	+
Pontocerebellar fibres	LN	+	+	+	+	+	++	+	+	+	++	+
	CB	+	+	+	+	+	++	+	+	+	++	+
Superior cerebellar peduncle	LN	+	+	++	+	+	+	+	+	+	++	++
	CB	+	+	++	+	+	+	+	+	+	++	++
Medial longitudinal fascicle	LN	++	++	++	+	++	++	++	+	+	++	+
	CB	++	++	++	+	++	++	++	+	+	++	+
Medial lemniscus	LN	++	+	++	+	+	+	+	+	+	++	+
	CB	++	+	++	+	+	+	+	+	+	++	+
Lateral lemniscus	LN	+	+	+	+	++	+	+	+	+	+	++
	CB	+	+	+	+	++	+	+	+	+	+	++
Trochlear nerve	LN	+	+	+	+	++	+	nd	+	+	+	nd
	CB	+	+	+	+	++	+	nd	+	+	+	nd
Oculomotor nerve	LN	nd	+	+	+	++	++	nd	+	nd	++	+
	CB	nd	+	+	+	++	+	nd	+	nd	+	+

Distribution and extent of the alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in brainstem fiber tracts of patients with PD, PDD and DLB (cases 1 to 5: PD patients; case 6: PDD patient; cases 7–11: DLB patients).

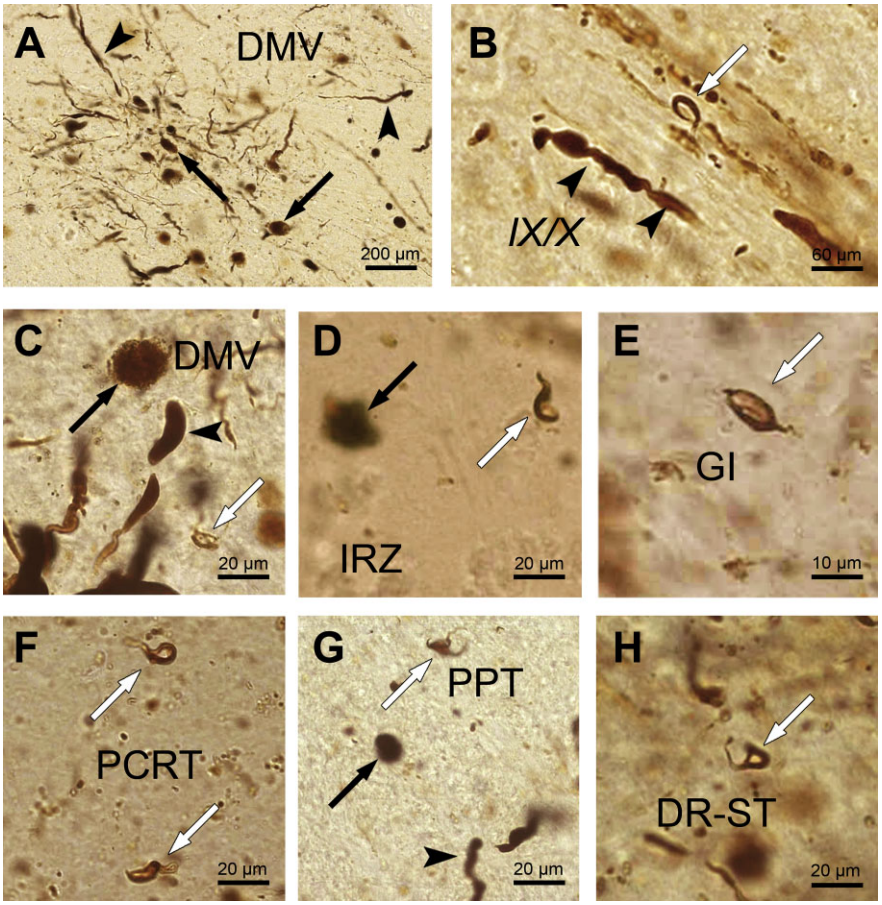

Figure 1. *Alpha-synuclein immunoreactive neuronal and oligodendroglial aggregation pathology in patients with Parkinson's disease (PD) or dementia with Lewy bodies (DLB).* **A.** Alpha-synuclein immunoreactive neuronal Lewy bodies (LB, black arrows) and Lewy neurites (LN, arrowheads) in the dorsal motor vagal nucleus (DMV) of a PD patient (case 1; Table 1). **B.** LN (arrowheads) and coiled body (CB, white arrow) in the vagal nerve (*IX/X*) of the same PD patient (case 1; Table 1). **C.** LB (black arrow), LN (arrowhead) and CB (white arrow) in the DMV of a PD with dementia patient (case 6; Table 1). **D.** LB (black arrow) and CB (white arrow) in the intermediate reticular zone (IRZ) of a Dementia with Lewy bodies (DLB) patient (case 10; Table 1). **E.** CB (white arrows) in the gigantocellular reticular nucleus (GI) of a PD patient (case 1; Table 1) and **F.** in the parvocellular reticular nucleus (PCRT) of a PD patient (case 3; Table 1). **G.** LB (black arrow), LN (arrowhead) and CB (white arrow) in the compact part of the pedunculopontine nucleus, compact part (PPT) of a DLB patient (case 10; Table 1). **H.** CB (white arrow) in the midbrain supratrochlear part of the dorsal raphe nucleus-supratrochlear part (DR-ST) of the same DLB patient (case 10; Table 1). (A–H: alpha-synuclein immunostaining; 100 µm PEG sections).

studies are required to disclose whether the brainstem pathologies of PD and DLB originate and ascend from identical brainstem locations.

In the present study, we also observed that alpha-synuclein immunoreactive oligodendroglial inclusions (ie, CB) in PD and DLB are not restricted to the seriously damaged dopaminergic substantia nigra as previously suggested (68), but represent a widespread and common feature of the brainstem neuropathology of PD and DLB patients, which apparently has been neglected or overlooked for approximately hundred years of research. Although there is currently no evidence available that CB represent a determining factor in the pathological processes of PD or DLB (17, 18), the consistent presence of CB in the brainstem nuclei and fiber tracts of PD and DLB patients indicate that PD and DLB do not represent purely neuronal synucleinopathies, but may be more closely related to the synucleinopathy multiple system atrophy (MSA) than previously thought (1, 33, 71, 72). This synucleinopathy is associated with a widespread occurrence of alpha-synuclein immunoreactive cytoplasmic CB in white and grey brain components, which are currently considered as a characteristic and primary neuropathological feature of MSA and accepted as a leading neuropathological diagnostic criterion for the predominantly oligodendroglial synucleinopathy MSA (1, 15, 17, 18, 26, 33, 38, 43, 71, 72).

The close statistical correlation between the presence of LB/LN and CB in the brainstem nuclei and fiber tracts of our PD and DLB patients suggests that the development of cytoplasmic neuronal and oligodendroglial protein aggregations is interlinked and may be causally related. This hypothesis needs to be corroborated in further studies which should focus on the following questions:

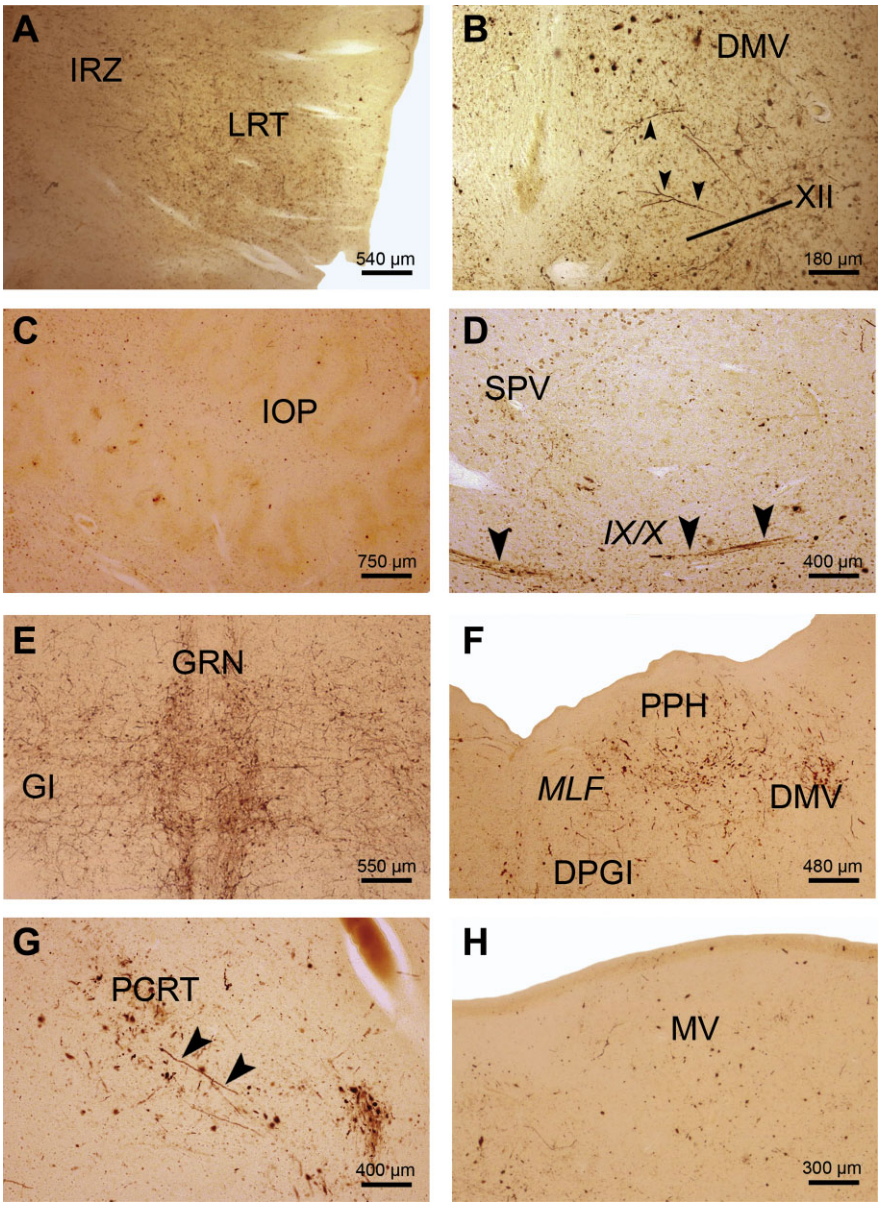

Figure 2. *Alpha-synuclein immunoreactive neuronal and oligodendroglial aggregation pathology in nuclei of the medulla oblongata and pontomedullary junction of patients with Parkinson's disease (PD) or dementia with Lewy bodies (DLB).* **A.** Severe alpha-synuclein aggregation pathology in the precerebellar lateral reticular nucleus (LRT) and conspicuously affected autonomic intermediate reticular zone (IRZ) of a DLB patient (case 10; Table 1). **B.** Markedly involved somatomotor hypoglossal (XII) and severely affected autonomic dorsal motor vagal nuclei (DMV) of the same DLB patient. Arrowheads point to Lewy neurites (LN; case 10; Table 1). **C.** Marked alpha-synuclein immunoreactive neuronal and oligodendroglial inclusions of the principal nucleus of the inferior olive (IOP) of a PD patient (case 3; Table 1). **D.** Alpha-synuclein immunopositive neuronal and oligodendroglial inclusions in the spinal trigeminal nucleus (SPV) and vagal nerve (*IX/X*) (arrowheads) of a DLB patient (case 10; Table 1). **E.** Severe alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathologies in the gigantocellular reticular (GI) and great raphe nuclei (GRN) of the brainstem gain setting system of a DLB patient (case 11; Table 1). **F.** Severe alpha-synuclein immunoreactive neuronal and oligodendroglial aggregation pathology of the oculomotor prepositus hypoglossal (PPH) and autonomic DMV, as well as markedly affected dorsal paragigantocellular reticular nucleus (DPGI) of a PD patient (case 4; Table 1). **G.** Prominent alpha-synuclein immunoreactive neuronal (Lewy bodies, LB, LN) and oligodendroglial (coiled bodies, CB) aggregations in the ingestive parvocellular reticular nucleus (PCRT) of a DLB patient. Arrowheads point to LN (case 11; Table 1). **H.** Markedly involved medial vestibular nucleus (MV) of a PD with dementia patient (case 6; Table 1). (A–H: alpha-synuclein immunostaining; 100 µm PEG sections). (Abbreviation: *MLF* = Medial longitudinal fascicle).

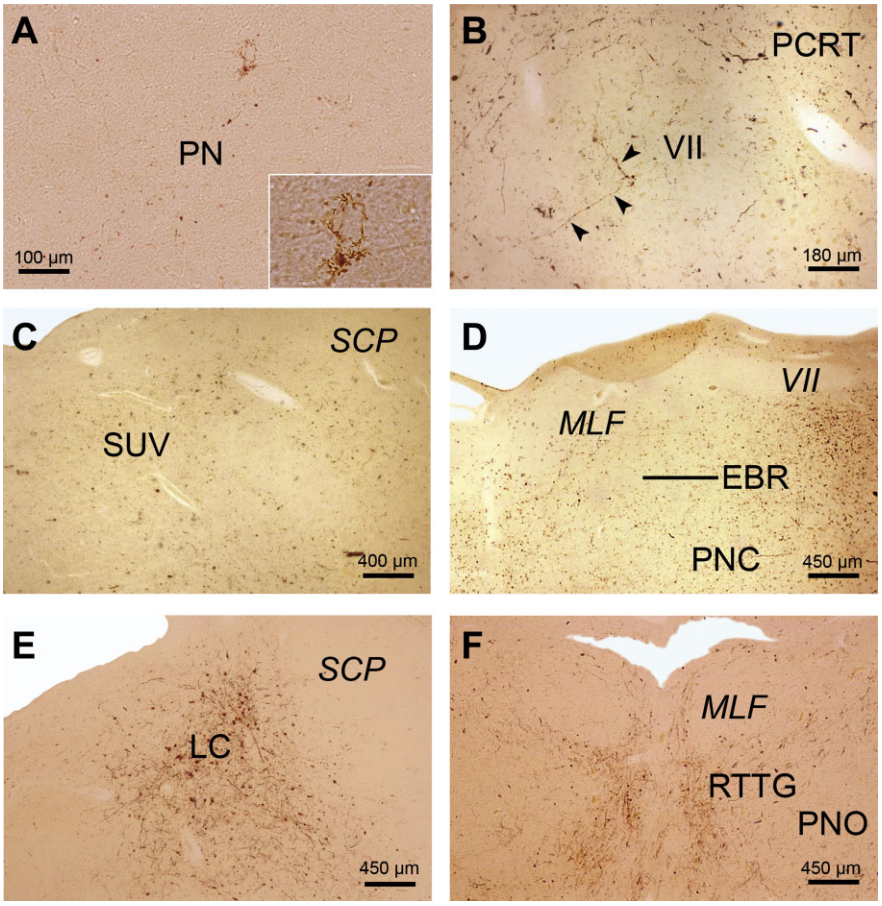

Figure 3. *Alpha-synuclein immunoreactive neuronal and oligodendroglial aggregation pathology in nuclei of the pons of patients with Parkinson's disease (PD) or dementia with Lewy bodies (DLB).* **A.** Alpha-synuclein immunoreactive Lewy bodies (LB), Lewy neurites (LN) and coiled bodies (CB) in the precerebellar pontine nuclei (PN) of a PD patient (case 1; Table 1). Inset shows alpha-synuclein immunoreactive PN neuron. **B.** Alpha-synuclein immunoreactive LB, LN and CB in the somatomotor facial nucleus (VII) and severely involved adjacent ingestive parvocellular reticular nucleus (PCRT) of a DLB patient (case 11; Table 1). **C.** Marked affection of the superior vestibular nucleus (SUV) of a PDD patient by the alpha-synuclein immunoreactive aggregation pathology (case 6; Table 1). Neuronal and oligodendroglial alpha-synuclein aggregates in **D.** the cell-poor premotor oculomotor area of the excitatory burst neurons for horizontal saccades (EBR) and **E.** the noradrenergic locus coeruleus (LC) of a PD patient (case 3; Table 1). **F.** The alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology marks the outlines of the precerebellar oculomotor reticulotegmental nucleus of the pons (RTTG) of a DLB patient (case 11; Table 1). (A–F alpha-synuclein immunostaining; 100 µm PEG sections). (Abbreviations: *MLF* = Medial longitudinal fascicle; PNC = Pontine reticular formation, caudal nucleus; PNO = Pontine reticular formation, oral nucleus; *SCP* = Superior cerebellar peduncle; *VII* = Facial nerve).

(i) Which are the mechanisms and pathways through which alpha-synuclein, a neuronal protein normally localized at presynaptic terminals and expressed in oligodendroctyes only at low levels or levels below detectability (31, 59, 71), gets access to and forms insoluble cytoplasmic aggregations in oligodendrocytes?
(ii) Do the alpha-synuclein immunoreactive aggregations in oligodendrocytes develop prior to, simultaneously with or subsequent to the cytoplasmic synuclein pathology of nerve cells in PD and DLB?
(iii) Do the alpha-synuclein immunoreactive oligodendroglial aggregations in PD and DLB follow a similar temporal and spatial evolutional pattern as the neuronal alpha-synuclein inclusions?
(iv) Is the occurrence of CB in PD and DLB associated with dysfunctions, structural damage, reduced viability and demise of the affected oligodendrocytes?
(v) Are the alpha-synuclein immunoreactive oligodendroglial inclusions accompanied by dysfunctions of the associated nerve cells (ie, impairments of saltatory conduction of action potentials, axonal transport mechanisms, maintenance and regeneration of axons, decrease and loss of myelinization) and do CB contribute to neuronal damage and demise and white matter loss in PD and DLB?

The widespread and consistent presence of alpha-synuclein immunoreactive axonal protein aggregates and oligodendroglial inclusions in brainstem fiber tracts of clinically diagnosed PD and DLB patients emphasizes that the fiber tract inclusion pathology is not restricted to the vagal nerve as frequently believed (6, 8). Considering their size, diameter, length and insolubility, the intra-axonal aggregations in the brainstem fiber tracts most likely lead to malfunctions or blockade of the anterograde and retrograde intra-axonal transport processes essential for the survival of nerve cells

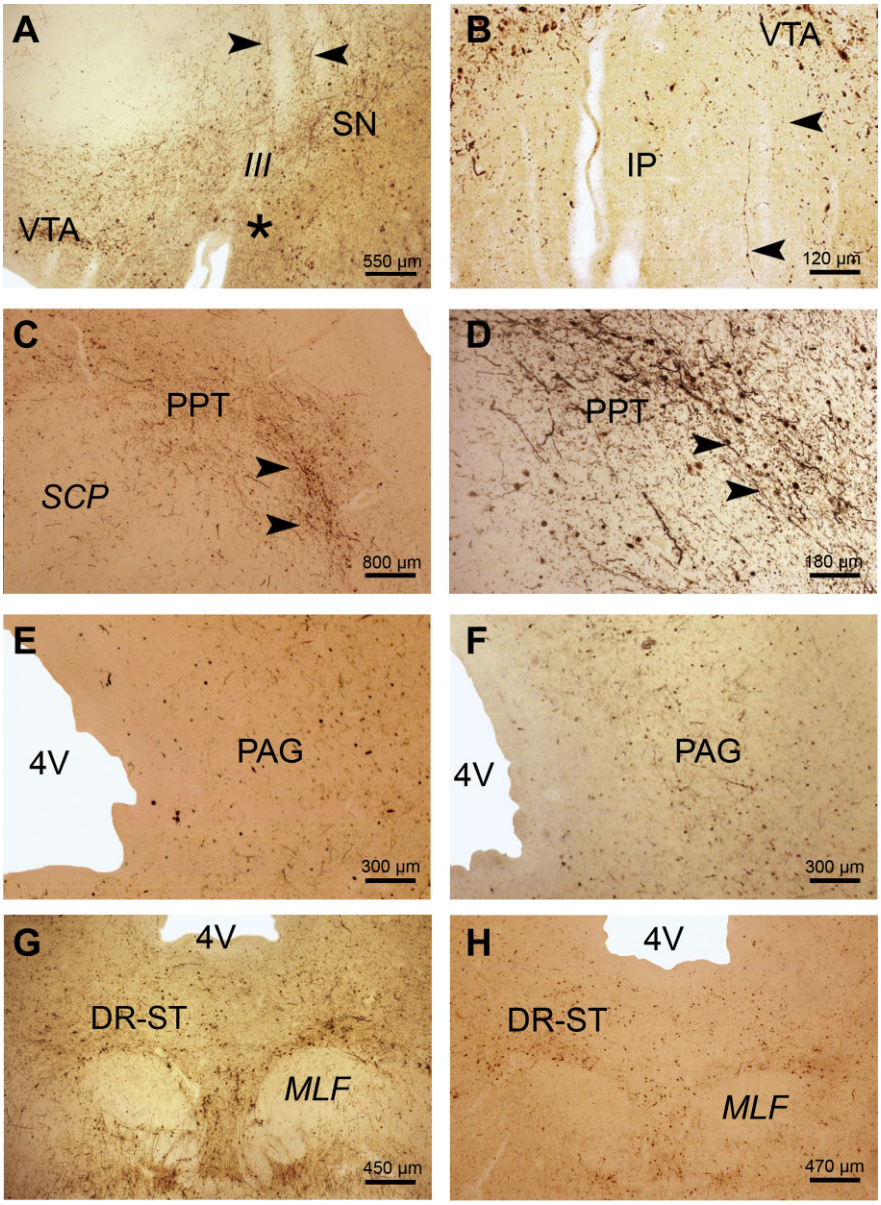

Figure 4. *Alpha-synuclein immunoreactive neuronal and oligodendroglial aggregation pathology in midbrain nuclei of patients with Parkinson's disease (PD) or dementia with Lewy bodies (DLB).* **A.** Alpha-synuclein immunoreactive neuronal (Lewy bodies, LB; Lewy neurites, LN) and oligodendroglial (coiled bodies, CB) aggregations in the dopaminergic substantia nigra (SN), nuclei of the ventral tegmental area (VTA) and oculomotor nerve (*III*) (arrowheads) of a Parkinson's disease with dementia (PDD) patient (case 6; Table 1). Note the substantial loss of melanin-containing dopaminergic nerve cells and the occurrence of extraneuronal neuromelanin deposits in the SN (asterisk). **B.** The midbrain interpeduncular nucleus (IP) adjacent to the severely affected VTA of the same PDD patient (case 6; Table 1). Arrowheads point to LN. Alpha-synuclein immunoreactive neuronal and oligodendroglial inclusion pathology in the cholinergic compact part of the pedunculopontine nucleus (PPT) of **C.** a PD patient (case 3; Table 1) and **D.** a DLB patient (case 10; Table 1). Arrowheads point to LN. Well-developed alpha-synuclein immunoreactive neuronal and oligodendroglial aggregations in the periaqueductal gray (PAG) of **E.** a PDD patient (case 6; Table 1) and **F.** a DLB patient (case 10; Table 1). Considerable amounts of LB, LN and CB in the midbrain supratrochlear part of the dorsal raphe nucleus (DR-ST) of **G.** a PDD patient (case 6; Table 1) and **H.** a DLB patient (case 10; Table 1). (A–H: alpha-synuclein immunostaining; 100 µm PEG sections). (Abbreviations: *MLF* = Medial longitudinal fascicle; *SCP* = Superior cerebellar peduncle; 4V = Fourth ventricle).

(14, 35, 53), may hamper a lot of vital functions depending on these axonal transport mechanisms (ie, transport of organelles, proteins, membrane components, synaptic vesicles and neurotransmitters) and eventually result in structural changes that contribute to the demise of involved nerve cells (14, 53). In order to understand the pathological cascades of intracellular events that lead to structural damage and demise of neurons in PD and DLB, we suggest (i) to further investigate the composition of the

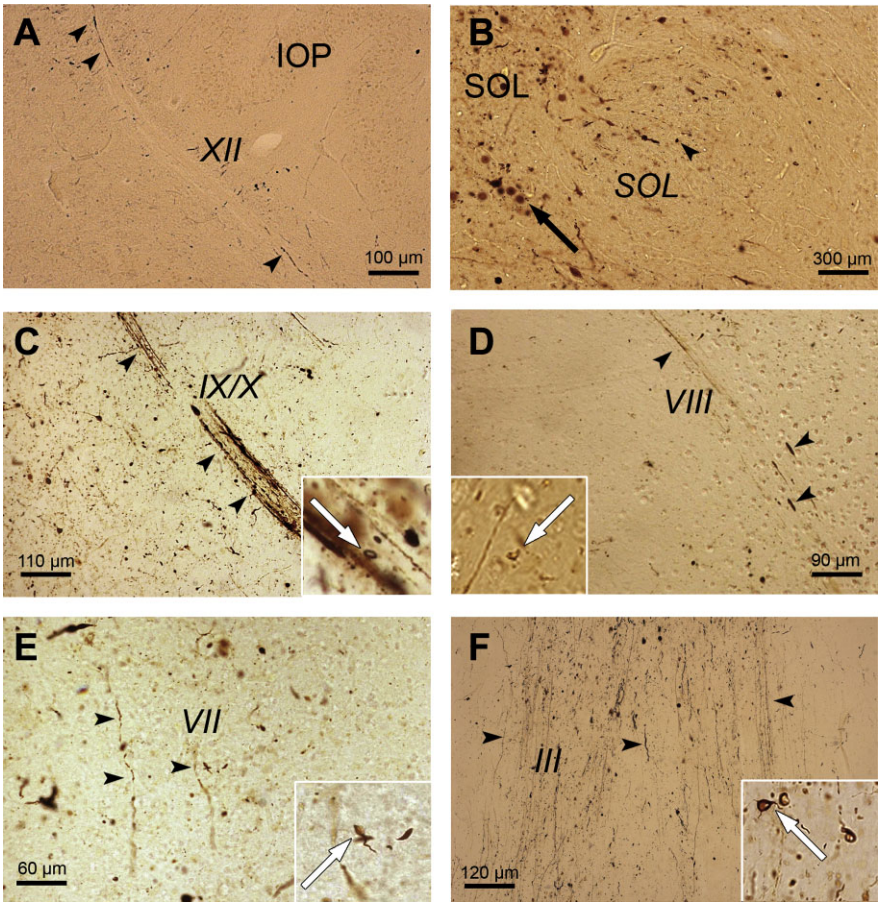

Figure 5. *Alpha-synuclein immunoreactive neuronal and oligodendroglial aggregation pathology in brainstem fiber tracts of patients with Parkinson's disease (PD) or dementia with Lewy bodies (DLB).* Marked alpha-synuclein immunoreactive neuronal (Lewy neurites, LN) and oligodendroglial (coiled bodies, CB) aggregations in **A.** the hypoglossal nerve (XII) of a DLB patient (arrowheads point to LN) (case 11; Table 1), **B.** the solitary tract (*SOL*) of a PD patient (arrowhead points to LN in the *SOL* and dark arrow points to a LB in the solitary nucleus = SOL) (case 3; Table 1), **C.** the vagal nerve (*IX/X*) of a PD patient (arrowheads point to LN and inset shows CB = white arrow) (case 6; Table 1), **D.** the vestibulocochlear nerve (*VIII*) of a PD patient (arrowheads point to LN and inset shows CB = white arrow) (case 3; Table 1), **E.** the facial nerve (*VII*) of a DLB patient (arrowheads point to LN and inset shows CB = white arrow) (case 11; Table 1), and **F.** the oculomotor nerve (*III*) of a PD with dementia patient (arrowheads point to LN and inset shows CB = white arrow) (case 6; Table 1). (A–F: alpha-synuclein immunostaining; 100 µm PEG sections). (Abbreviations: IOP = Inferior olive, principal subnucleus).

intra-axonal protein aggregates and the temporal and spatial features of their evolution in the brain and (ii) to determine their role for the structural damage and demise of nerve cells.

The highly stereotypical topographic propagation of the PD and DLB-related disease processes leads to a characteristic and interindividually consistent nuclei-specific, area-specific, lamina-specific and cell-type specific distribution pattern of the brain pathology (6, 8, 26, 31, 33–35, 66). The widespread presence of alpha-synuclein immunoreactive intra-axonal aggregates in brainstem fiber tracts of PD and DLB patients support the concept that the non-random disease processes of PD and DLB spread transneuronally along anatomical pathways interconnecting affected and still healthy brain components and thus mirror the anatomical connections of the sequentially affected brain components (2, 6, 8, 9, 13, 20, 26, 36, 59, 66). In view of the stereotypical topographic progression throughout the brain and the potential transneuronal spread of neuronal pathologies resulting in characteristic brain distribution patterns, PD and DLB are currently suspected to be among the so-called "prion-like" diseases (2, 9, 13, 20, 26, 31, 36, 59, 63, 66). The widespread intra-axonal alpha-synuclein immunoreactive aggregations demonstrated here may conform to the idea that PD and DLB in fact represent chronically progredient prion-like protein misfolding diseases. However, further studies are required to prove the prion-like nature of the alpha-synuclein protein aggregates and their connectivity-dependent spread.

Considering the functional significance of the brainstem fiber tracts associated with the cerebellum (ie, olivocerebellar, pontocerebellar and spinocerebellar tracts, vestibulocochlear nerve, cerebellar peduncles, dorsal column pathways), as well as of the precerebellar and vestibular brainstem nuclei affected by alpha-synuclein immunoreactive inclusion pathology in PD and

DLB (3, 7, 22, 23, 55, 56), our new brainstem findings may offer an explanation for some clinically identified PD and DLB symptoms (ie, tremor, gait and postural instability, impaired balance and postural reflexes, repeated falls) (11, 12, 44, 45, 65, 67, 74). Moreover, these new findings suggest that the progressive disease processes of PD and DLB may not come to an end with the appearance of alpha-synuclein immunoreactive inclusions in the cerebral neo- and allocortices as currently assumed (6, 8). On the contrary, the affection of the precerebellar and vestibular nuclei as well as the involvement of all brainstem fiber tracts associated with the cerebellum may suggest that the disease processes also reach the cerebellum where they affect its deep white matter, as well as its cells in the cortex and deep nuclei. As detailed postmortem studies on the cerebellum in PD and DLB are rare and revealed conflicting results (47, 52, 64, 74), detailed reinvestigations of the cerebellum in PD and DLB patients are now needed. These reinvestigations (i) may provide important new insights into the ill-defined pathophysiological role of the cerebellum in PD and DLB and its possible contribution to the motor symptoms refractory to the L-dopa substitution therapy (eg, tremor, gait and postural instability, impaired balance and postural reflexes, repeated falls) (11, 12, 44, 45, 65, 67, 74) and (ii) may lead to supplementation and extension of the neuropathological PD and DLB staging procedures by the introduction of terminal precerebellar and/or cerebellar phases (6, 8, 34, 35, 43).

The contribution of the brainstem pathology at the well-known brainstem predilection sites to the PD and DLB disease symptoms (ie, substantia nigra and ventral tegmental area, pedunculopontine nucleus, periaqueductal gray, locus coeruleus, raphe nuclei, gigantocellular reticular nucleus and intermediate reticular zone, dorsal motor vagal and solitary nuclei) (4–6, 8, 10, 15, 16, 21, 25, 27–29, 34, 35, 46, 48, 50, 60) has been extensively outlined and discussed in the literature available to us. Therefore, we confine our discussion on the possible functional consequences of the newly described pathology in the cranial nerve, premotor oculomotor, precerebellar and vestibular nuclei and associated brainstem fiber tracts.

The ingestive process in humans is complex and fined-tuned: it comprises an anticipatory phase, a preparatory stage and the lingual, pharyngeal and esophageal phases of swallowing, which with the exception of the anticipatory phase are under control of the lower brainstem ingestive network (42, 54). The preparatory phase is controlled by the principal, motor and spinal trigeminal nuclei, the facial and hypoglossal nuclei and includes an oral sensory evaluation and all subsequent processes that render foods and liquids swallowable (42, 54). Together with the ambiguus nucleus and distinct portions of the pontine and medullary brainstem reticular formation (ie, parvocellular reticular nucleus and intermediate reticular zone), these five lower brainstem nuclei are also involved in the lingual phase of swallowing, during which foods and liquids are transported to the oropharynx (42, 54). Their transfer through the pharynx is accomplished during the pharyngeal phase of swallowing under airway protection and guaranteed by the interplay of the facial, ambiguus and hypoglossal nuclei, as well as the coordinative action of the parvocellular reticular nucleus and intermediate reticular zone (42, 54). Finally, the esophageal phase of swallowing subserves peristaltic transport of foods or fluids to the stomach which is coordinated by the neuronal activity of the ambiguus and dorsal motor vagal nuclei (42, 54). Considering the well-understood role of the trigeminal, facial, dorsal motor vagal, hypoglossal and pontine parvocellular reticular nuclei, as well as of the medullary intermediate reticular zone in the ingestive process, the detrimental effects of the neuronal protein aggregation pathology on these ingestive brainstem nuclei most likely contributes significantly to dysfunctions of the preparatory stage, as well as the lingual, pharyngeal and esophageal phases of swallowing, which are observed predominantly in the late clinical phases of PD and DLB. Dysfunction of these processes may significantly contribute to malnutrition and weight loss in PD and DLB and often result in bronchopneumonia associated with the aspiration of foods and/or fluids. These symptoms respond only inconsistently to standard anti-parkinson agents (37, 51).

The superior, medial, lateral and spinal vestibular nuclei are crucial for the regulation of truncal and postural stability thereby contributing substantially to the prevention of falls (3, 22, 23, 55). Accordingly, the affection of these four vestibular brainstem nuclei can account for the occurrence of truncal and postural instability, as well as repeated falls of PD and DLB patients. These symptoms associated with the central vestibular system, likewise occur preferentially during the advanced clinical stages of PD and DLB and, if at all, only respond poorly to the L-dopa therapy (11, 12, 44, 45, 65, 67, 69, 70). In addition, the superior, medial and lateral vestibular nuclei also subserve control of the optokinetic nystagmus, while the medial and superior vestibular nuclei additionally represent important components of the neural circuits that generate the vestibulo-ocular reaction. The medial vestibular nucleus together with the adjacent prepositus hypoglossal nucleus is essential for horizontal gaze holding (41, 55, 57, 58). Considering their normal function, impairment of the neuronal activity of the nerve cells of these vestibular nuclei by the alpha-synuclein immunoreactive aggregates most likely contributes substantially to the dysfunction of the optokinetic nystagmus and vestibulo-ocular reaction in PD and DLB patients (41, 67).

The nuclei of the human premotor oculomotor network are integral components of the neural circuits subserving the generation of saccades, smooth pursuits, vergence, the vestibulo-ocular reflex and optokinetic nystagmus, fixation, as well as gaze holding (41, 57, 58). The reticulotegmental nucleus of the pons is crucial for the performance of accurate horizontal saccades and the generation of proper horizontal smooth pursuits (41, 57, 58). The superior, medial, lateral and spinal vestibular nuclei participate in oculomotor circuits. The pontine area of the excitatory burst neurons for horizontal saccades provides the premotor signals for the generation and plays a decisive role in the performance of horizontal saccades (41, 57, 58). The pontine raphe interpositus nucleus comprises morphologically unique saccadic omnipause neurons acting as a trigger for the initiation of saccades in all directions (41, 57, 58). The prepositus hypoglossal nucleus together with the neighboring medial vestibular nucleus participates in horizontal gaze holding (41, 57, 58). The dorsal paragigantocellular reticular nucleus harbors the inhibitory burst neurons for the generation of vertical saccades (41, 57, 58). Based on these neuroanatomical data, our neuropathological findings in the premotor oculomotor network of our PD and DLB patients now offer plausible explanations for the disturbed and slowed smooth pursuits, abnormal vestibulo-ocular reaction, initiation defects of saccades, their increased latency and slowing that

commonly show no or only little response to the L-dopa therapy (41, 67, 73). As the medial vestibular nucleus and the topographically and functionally intimately related prepositus hypoglossal nucleus play an important role for horizontal gaze holding, their consistent aggregation pathology suggests that horizontal gaze-evoked nystagmus may represent an additional common oculomotor sign of PD and DLB patients (41).

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