

Parkinson's disease: lesions in dorsal horn layer I, involvement of parasympathetic and sympathetic pre- and postganglionic neurons

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Received: 11 December 2006 / Revised: 3 January 2007 / Accepted: 3 January 2007 / Published online: 9 February 2007
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Abstract Clinical signs frequently recognized in early phases of sporadic Parkinson's disease (PD) may include autonomic dysfunctions and the experience of pain. Early disease-related lesions that may account for these symptoms are presently unknown or incompletely known. In this study, immunocytochemistry for α -synuclein was used to investigate the first relay stations of the pain system as well as parasympathetic and sympathetic pre- and postganglionic nerve cells in the lower brainstem, spinal cord, and coeliac ganglion in 100 μ m polyethylene glycol embedded sections from six autopsy individuals, whose brains were staged for PD-associated synucleinopathy. Immunoreactive inclusions were found for the first time in spinal cord lamina I neurons. Lower portions of the spinal cord downwards of the fourth thoracic segment appeared to be predominantly affected, whereas the spinal trigeminal nucleus was virtually intact. Additional involvement was seen in parasympathetic preganglionic projection

neurons of the vagal nerve, in sympathetic preganglionic neurons of the spinal cord, and in postganglionic neurons of the coeliac ganglion. The known interconnectivities between all of these components offer a possible explanation for their particular vulnerability. Lamina I neurons (pain system) directly project upon sympathetic relay centers, and these, in turn, exert influence on the parasympathetic regulation of the enteric nervous system. This constellation indicates that physical contacts between vulnerable regions play a key role in the pathogenesis of PD.

Keywords Lamina I · Lewy neurites/bodies · Pain · Parkinson's disease · Spinal cord

Introduction

Specific dysfunctions of the somatomotor system (i.e., hypokinesia, muscular rigidity, and resting tremor) usually dominate the clinical picture of sporadic Parkinson's disease (PD) [38, 75]. These complaints, however, the foremost ones in textbook cases, should not distract from less conspicuous symptoms that also emerge during the disease course [2, 3, 20, 70, 69]. Hyposmia is among the non-motor signs that develop early, often preceding motor symptoms by years [29, 30, 48, 49, 85, 88]. Olfactory impairment can be accompanied by autonomic dysfunction [1, 25, 44, 73, 84] as well as the experience of pain [19, 28, 33, 41, 45, 89, 90, 108].

The PD-associated pathological process is linked to the intraneuronal formation of inclusion bodies, chiefly consisting of α -synuclein-aggregations and appearing as Lewy neurites (LNs) in neuronal processes or as

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punctate structures, pale bodies, or Lewy bodies (LBs) in the somata of involved neurons [16, 31, 43, 61, 69, 77, 94]. Many affected cells survive for an as yet unknown period of time before they prematurely die. In all probability, it is the functional impairment and subsequent loss of these neurons that induces and sustains the clinical picture of PD.

Only projection neurons with a long and sparsely myelinated or unmyelinated axon become involved in PD, while short-axoned cells or neurons with a thickly myelinated axon resist the pathology [11, 12]. Vulnerable nerve cell types occur in peripheral, enteric, and central portions of the nervous system (PNS/ENS/CNS) [16, 17, 57, 98, 99]. The disease-related lesions do not develop in all aged individuals and, as such, their presence is required for the post-mortem diagnosis of PD [16, 26, 35, 36, 82, 100]. The inclusion body pathology, however, occurs not only in symptomatic PD cases, but also in “non-symptomatic” individuals (i.e., those who did not manifest the classical symptoms of PD) [32, 36, 39, 40, 60, 66, 67, 71, 86, 100, 105, 111, 112].

The present study is aimed at exploring the relevant anatomical structures within the human nervous system, whose involvement could be the cause of the appearance of painful sensations amidst autonomic dysfunction. The pathological process that underlies PD has long been known to involve autonomic control centers [56, 103–107]. Interestingly, these centers receive essential sensory information from the pain system [21]. Thus, the pathological involvement of the autonomic and pain systems will be discussed in the context of known anatomical interconnectivities [5, 23, 46, 55, 74, 101, 109, 110].

Materials and methods

Following autopsy and gross examination by one of the co-authors (RAI de V), the brain and complete spinal cords of six individuals and one control (JRB) examined in this study were fixed by immersion in a 4% aqueous solution of formaldehyde (Table 1). A set of nine tissue blocks was cut out of each of these brains. The blocks were embedded in polyethylene glycol (PEG) [65, 92] and cut at 100 μm according to a previously published procedure for staging PD-related lesions [13].

In addition, 15 tissue blocks were taken from every second cervical, thoracic, lumbar, and sacral segment of the spinal cord. These blocks were embedded in PEG and cut at 100 μm to facilitate visualization of pathological axonal inclusions over considerable distances. The coeliac ganglion and respective spinal ganglia were also removed for assessment of PD-related lesions.

Immunoreactions for α -synuclein were performed on free-floating sections to visualize all forms of PD-related intraneuronal inclusions. Following pre-treatment with H_2O_2 and bovine serum albumin to inhibit endogenous peroxidase and to prevent non-specific binding, and subsequent additional pre-treatment with formic acid to facilitate the immunoreaction, incubation with an antibody against α -synuclein took place for 12 h (Syn-1; 1:2,000, Transduction Laboratories). After processing with a biotinylated secondary antibody (anti-mouse IgG for 2 h), immunoreactions were visualized with the avidin–biotin complex (Vectastain) and diaminobenzidine (Sigma). Omission of the primary antiserum resulted in non-staining.

Table 1 Clinical and neuropathological data from the cases studied

Case	Gender	Age	PD stage	dmX	sn	mes	Neo assoc	Neo prim	NFT stage	A β stage	Clinical diagnosis
1	m	64	3	+	+	–	–	–	I	A	PD
2	m	76	4	+	+	+	–	–	II	A	PD
3	m	76	5	+	+	+	+	–	II	C	PD
4	m	77	5	+	+	+	+	–	II	B	PD
5	f	71	6	+	+	+	+	+	III	C	PD
6	f	86	6	+	+	+	+	+	III	C	PD
7	f	70	0	–	–	–	–	–	I	0	Cardiac failure

Cases from which both the brain and complete spinal cord were examined (cases 1–6: 2 females, 3 males; mean age 75 years, with clinically diagnosed and neuropathologically confirmed Parkinson’s disease (PD); case 7: control individual). It has been proposed that the PD-related inclusion body pathology in the brain progresses in six stages [13]. In stage 1, lesions always appear in the dorsal motor nucleus of the vagal nerve (*dmX*). Stage 2 cases show additional pathology in selected nuclei of the lower brainstem. The substantia nigra (*sn*) becomes involved in stage 3. Involvement of the cerebral cortex follows in stage 4, beginning with the temporal mesocortex (*mes*). From there, the neocortical association fields (*neo assoc*) become affected in stage 5, followed by the primary fields of the neocortex (*neo prim*) in the final stage 6. The control case contained no Lewy body pathology. The same sets of tissue blocks were taken from the control and the same immunostaining procedures were performed as those applied to the PD cases. The distribution and severity of concomitant Alzheimer’s disease-related pathology was assessed in the brain using a staging procedure for neurofibrillary lesions (*NFT stage*) and A β -amyloid protein deposition (*A β stage*) [9]. *f*, Female, *m* male

Subsequent sections (100 μm) from all blocks were oxidized with performic acid and stained with aldehydefuchsin and Darrow red for lipofuscin granules and Nissl material for overview purposes.

Sections immunostained against α -synuclein were used to assess the stage of PD-related brain pathology [13]. The degree of co-occurring Alzheimer's disease-related pathology was classified according to a procedure for differentiation of stages I–VI in the development of neurofibrillary changes and stages A–C in the evolution of β -amyloid deposits (Table 1) [9, 18].

The immunolabelled material was assessed with a Vanox AHB53 Olympus microscope and micrographs were taken with a digital camera using the analysis[®] Soft Imaging System (Münster, Germany).

Results

Characteristic inclusion bodies immunoreactive for α -synuclein occur in vulnerable nerve cell types of the lower brainstem and spinal cord of all clinically diagnosed and neuropathologically confirmed PD cases (Table 1, PD stage 3 and higher). Involvement of the spinal cord alone in the absence of PD-related pathology at other nervous system sites is not found in any of the material examined. Immunoreactions do not show any α -synuclein aggregations in the control case (Table 1).

Tissue sections of unconventional thickness (100 μm) facilitate visualization and assessment of widely dispersed or subtle lesions in lower PD stages by the superimposition of large numbers of biological structures [10, 15, 17]. Moreover, thread-like inclusions occurring in individual axons can be followed for relatively long distances. In all areas of the material studied, LNs predominate over LBs and other somatic inclusions.

Pain system lesions

All of the PD cases listed in Table 1 show immunoreactive pathological changes in layer I of the spinal cord dorsal horn (Fig. 1), whereas the similarly composed layer I of the spinal trigeminal nucleus in the lower brainstem is nearly uninvolved. Nerve cells of spinal ganglia that provide input to lamina I neurons are not affected.

A network of LNs appears in the cervical segments of the spinal cord (Fig. 1a), gradually gains in density further caudally, and reaches its culmination in the lower thoracic, lumbar, and sacral segments (Fig. 1b, c). Figure 1b,d displays such full-blown pathology in

the 12th thoracic segment. A loosely woven net of intra-axonal inclusions can be seen in layer I and is accompanied by small globular and intensely immunoreactive inclusions (Fig. 1d).

Most of the various neuronal types in lamina I [109] contain no PD-related lesions. In a few types that do show the α -synuclein-positive material, however, the somata and neuronal processes are almost completely filled, so that they bear a close resemblance to successfully impregnated neurons in Golgi-silver-stained material (Fig. 1d–f). The diseased cells belong to the class of medium-sized multipolar lamina I projection neurons. In a few neurons of this same class, widely dispersed punctate aggregations, a single globular LB, or multiple LBs are visible. Pale bodies are absent in lamina I neurons in all six cases here.

Thin, varicose axons filled with aggregated α -synuclein extend from lamina I to the intermediate horn, where branches contact the autonomic nuclei with bouton-like enlargements on their terminal axon. Pathologically altered axons also occur in the commissures directly below the central canal. By contrast, lamina II (substantia gelatinosa) and the following layers appear virtually uninvolved (Fig. 1d).

Autonomic centers: preganglionic parasympathetic projection neurons

In all cases studied, the somatodendritic compartment and the axons of preganglionic parasympathetic projection neurons located in the dorsal motor nucleus of the vagal nerve contain α -synuclein immunoreactive inclusions [52]. Thread-like aggregates occur not only throughout the intramedullary course of these axons, but also in their extramedullary peripheral portions. Other nuclei of the dorsal vagal area, i.e., the nucleus gelatinosus, area postrema, and the small-celled nuclei surrounding the solitary tract, are minimally involved or normal in appearance [27]. The same holds true for the multipolar motor neurons of the ambiguous nucleus within the intermediate reticular zone [53]. The catecholaminergic melanoneurons in the dorsal vagal area (corresponding to the A1 group of the rat) and those in the intermediate reticular zone (group A2) remain intact in non-symptomatic cases and become involved relatively late in the course of PD [27].

Autonomic centers: preganglionic and postganglionic sympathetic projection neurons

Many medium-sized multipolar projection neurons in the intermediomedial and intermediolateral nuclei of the spinal cord show PD-related immunoreactivity

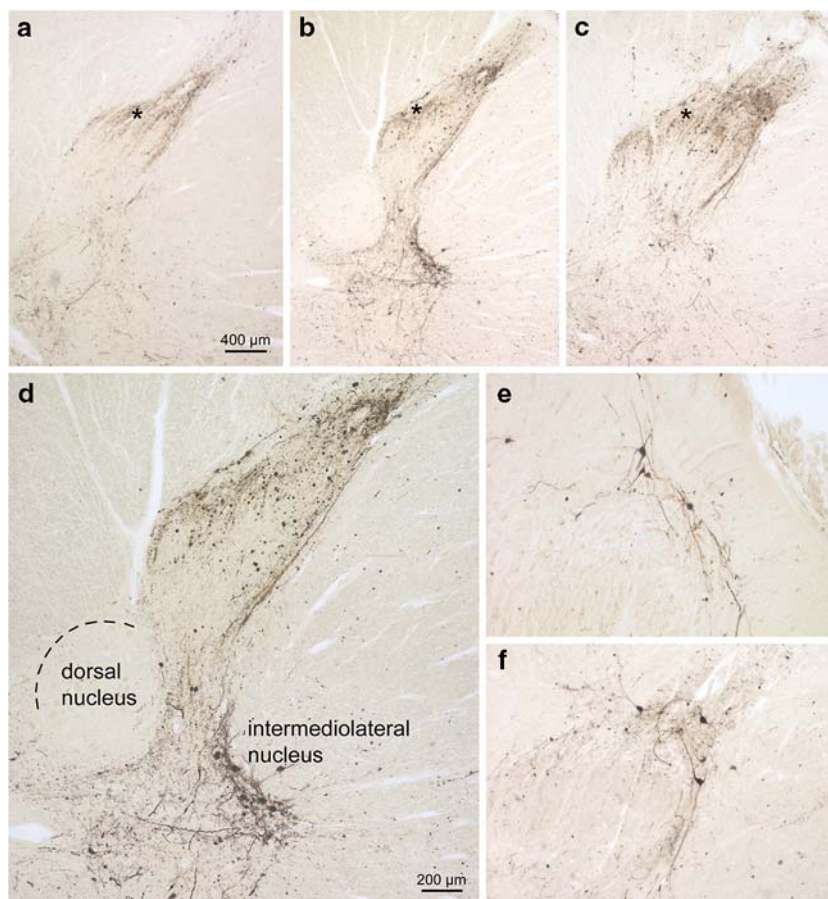


Fig. 1 PD-related involvement of the spinal cord. **a** Seventh cervical segment. **b** Twelfth thoracic segment. **c** Third lumbar segment. Note the increase caudally in the density of the LN network in lamina I (*asterisks*) and close to it. **d** Detailed micrograph of **b** showing involvement of the intermediolateral nucleus. Several multipolar preganglionic sympathetic relay neurons are filled with α -synuclein aggregates. Note that the dorsal nucleus (Clarke's column) is virtually uninvolved. **a–d** originate from a

76-year-old male at stage 4 (case 2). **e, f** Both sections show the multipolar relay neurons in lamina I as being exclusively affected. In contrast, the nerve cells in the subjacent layer II remain intact. **e** Originates from the seventh cervical spinal cord segment of case 6, **f** from the tenth thoracic spinal cord segment of case 3. Syn-1 (Transduction Laboratories) immunoreactions in 100 μ m polyethylene glycol-embedded tissue sections. *Scale bar in a* is valid for **b** and **c**. *Scale bar in d* applies to **e** and **f**

(Fig. 1d). The pathological material either occurs as punctate aggregations, fills the cell almost entirely, or it is compressed into globular LBs of varying size. Axons of these multipolar cells contain the same material. Peripherally located segments in the ventral root or in peripheral nerves also show axonal aggregates, but these are not readily identifiable as belonging to preganglionic sympathetic axons. Other nuclei in the immediate vicinity, including the dorsal nucleus (Clarke's column), show slight involvement or are free of α -synuclein inclusions.

A large proportion of the multipolar projection neurons located in the coeliac ganglion develop the same forms of PD-associated lesions as described above (Fig. 2). The coeliac ganglion harbors the postganglionic sympathetic projection neurons that influence the input into the enteric nervous system (Fig. 3).

Discussion

PD-related lesions are not confined to projection neurons of the dopaminergic system [16, 26, 57]. Indeed, based on the present state of knowledge, it appears that the involved regions of the nervous system are those efferents that control somatomotor and visceromotor functions, whereas sensory systems are largely spared—with two conspicuous exceptions: the olfactory system and lamina I of the spinal cord (Fig. 1). It is remarkable that only a few of the many neuronal types involved in the transmission of somatosensory input become affected in PD. The pathological process involves only those that directly contact sympathetic preganglionic neurons, a constellation that indicates that physical contacts between vulnerable regions play a key role in the pathogenesis of PD.

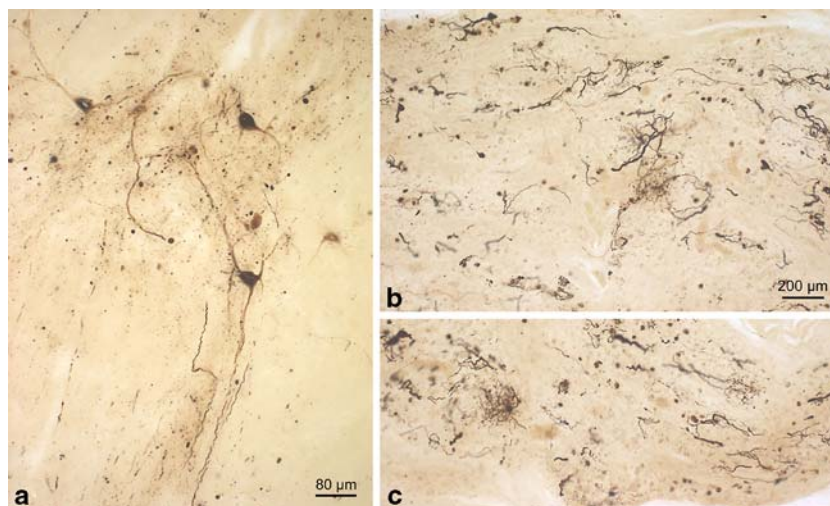
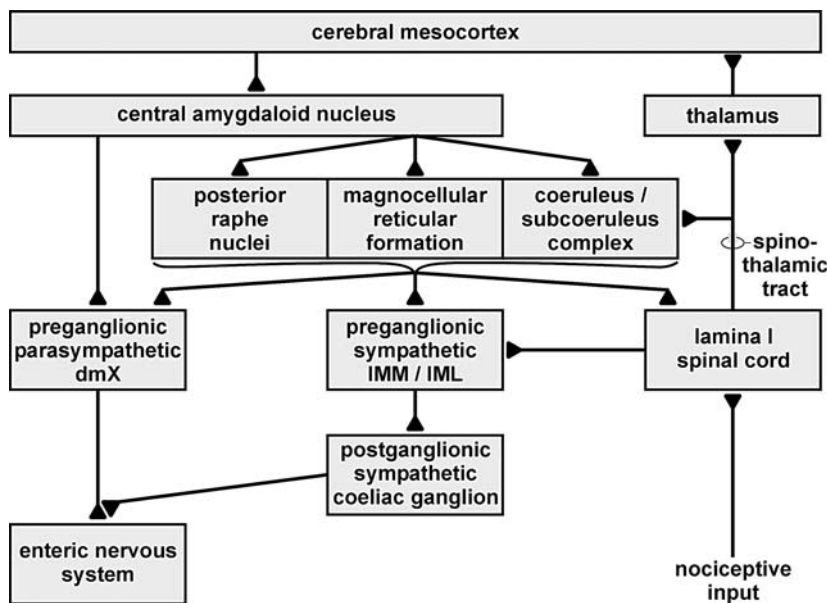


Fig. 2 a. PD-related lamina I pathology at the level of the tenth thoracic spinal cord segment (case 3). Again, the affection of the relatively large multipolar relay neurons in lamina I predominates. The α -synuclein aggregates almost completely fill the somatodendritic domain of these cells, thereby producing in unconventional thick sections an almost Golgi-like appearance.

b–c Coeliac ganglion of case 5 (71-year-old female). Lewy neurites and Lewy bodies are distributed throughout the entire ganglion. Syn-1 (Transduction Laboratories) immunoreactions in 100 μ m polyethylene glycol-embedded tissue sections. Scale bar in **b** is also valid for **c**

Fig. 3 Major connections between the enteric nervous system, the autonomic relay nuclei that influence it, and spinal cord lamina I. See Discussion. Abbreviations *dmX* dorsal motor vagal nucleus, *IMM/IML* intermediomedial and intermediolateral nuclei of the spinal cord



Though complaints of painful sensations are not infrequent, it is still unclear where pain belongs within the spectrum of symptoms characterizing PD [19, 28, 33, 41, 90, 108]. The present findings suggest that lamina I is regularly affected in the course of the PD-related pathological process. Moreover, lamina I neurons become involved not only in later, but also in early phases of PD (Table 1, case 1). Thus, in combination with other signs, including autonomic dysfunction, pain can be a precursor of PD and can also overlap with the onset of motor dysfunctions [19].

Any condition causing local irritations of lamina I may give rise to pain. In PD, only a proportion of lamina I neurons show lesions. Diseased nerve cells that survive, however, probably show functional impairments before they vanish from the tissue. Unfortunately, the physiological properties of the diseased neurons are presently not well characterized. Nonetheless, it can be assumed that the lamina I lesions in PD may provide a morphological counterpart to the experience of pain. The ultimate loss of lamina I neurons, then, theoretically should be accompanied by growing

insensitivity to pain signals. It is unclear why the lamina I pathology spares the spinal trigeminal nucleus while chiefly involving the lower portions of the spinal cord.

The knowledge of early involvement of lamina I neurons plus that of sympathetic pre- and postganglionic neurons could possibly be utilized for diagnostic purposes by examining the excitatory effects of noxious or thermal stimuli on sympathetic outflow (e.g., cold pressor test) [50].

Figure 3 summarizes the most important pathways between lamina I (pain system) and the autonomic centers of the lower brainstem and spinal cord. Unmyelinated and sparsely myelinated primary afferent A δ and C fibers transfer thermal and noxious stimuli from all parts of the body to the CNS. This input accumulates at the tip of layer I of the spinal and trigeminal dorsal horns, bifurcating into ascending and descending branches that form the myelin-poor Lissauer's tract (dorsolateral fasciculus) [37, 74, 80, 110]. These primary afferents, in turn, synapse almost exclusively with medium-sized multipolar projection neurons of lamina I. In addition, lamina I neurons receive modulatory (most probably anti-nociceptive) supraspinal input from the medullary raphe nuclei, reticular formation, coeruleus/subcoeruleus complex, and the hypothalamic paraventricular nucleus [95–97, 110]. These sources also generate descending projections to sympathetic preganglionic projection neurons located in the intermediomedial nucleus and intermediolateral nucleus of the spinal cord [76], as well as to the parasympathetic preganglionic nerve cells of the dorsal motor nucleus of the vagal nerve (Fig. 3).

Lamina I projection neurons generate axons that partially cross the midline and ascend in the ventrolateral funiculus as a component of the spinothalamic tract [110]. Notably, these axons provide a direct excitatory input to sympathetic preganglionic neurons of the spinal cord [5, 22–24, 34, 55]. Additional target sites of lamina I projections neurons are the noradrenergic neuromelanin-containing neurons within the dorsal vagal area (group A1) and adjacent intermediate reticular zone (group A2) with their ascending projections to the rostral ventrolateral medulla and hypothalamus. The rostral ventrolateral medulla, in turn, generates descending projections to both the dorsal horn (probably anti-nociceptive effects) and the sympathetic preganglionic neurons of the spinal cord [6, 7, 95, 96]. Noxious stimuli, therefore, have an appropriate excitatory effect on sympathetic outflow. They also exert an excitatory influence upon the coeruleus/subcoeruleus complex (A6, A7) [21, 47] (Fig. 3).

As further shown by Fig. 3, the predominant parasympathetic (vagal) input to the ENS [51] is modified by pre-

synaptic inhibition via sympathetic postganglionic axons from the coeliac ganglion. The ganglion is controlled by sympathetic preganglionic projection neurons of the spinal cord. Aggregations of α -synuclein have been described repeatedly in these sympathetic pathways [54, 103]. Here, our findings confirm notions of two recent studies that describe lesions in the coeliac ganglion and sympathetic preganglionic projection neurons even in presymptomatic patients [8, 64]. These pathological changes are consistent with clinical observations pointing to an early beginning of autonomic dysfunctions in PD [1, 3, 4, 42, 62, 63, 68, 72, 78, 79, 81, 84, 87, 91, 93].

It remains to be seen whether affection of the spinal cord occurs prior to that of the dorsal motor vagal nucleus and/or affection of the ENS plexuses. To answer this question, much larger numbers of spinal cords and brains need to be screened. However, given that all six cases with spinal cord involvement also displayed lesions in the lower brainstem, it may be that the spinal cord abnormalities in PD develop concurrently with, or directly after, the vagal parasympathetic neurons being affected at stage 1 [13].

The results of recent cross-sectional studies lend credence to the hypothesis that the PD-related pathological process begins at predisposed brain predilection sites, leaving a distinct lesional pattern in its wake [13, 14, 58, 59, 83, 102]. Taken altogether, the additional involvement of lamina I neurons, seen here for the first time in PD, and that of sympathetic pre- and postganglionic projection neurons, including the coeliac ganglion, can be viewed as a further argument for viewing PD as a multi-system disorder that selectively involves interconnected neuronal systems or nervous system centers and progresses caudo-rostrally via physical contacts in the form of axonal connections [14, 17]. There would be major objections to this concept, of course, were new evidence to become available showing that nuclei or nervous system centers become involved in PD that do not establish interconnections to affected sites (such as the superior olivary nucleus, superior colliculus, lateral geniculate body, or pallidum).

Acknowledgments This work was funded by a grant (Br 317/17-3) from the Deutsche Forschungsgemeinschaft (DFG). The contributing authors have no existing or pending conflicts of interest. Autopsies were performed in compliance with the ethics committee guidelines of all three participating institutions. We thank Mr. Mohamed Bouzrou (laboratory technical assistance) and Ms. Inge Szász-Jacobi (graphics) for technical support.

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