

## Where Does Parkinson Disease Pathology Begin in the Brain?

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**Abstract.** The substantia nigra is not the induction site in the brain of the neurodegenerative process underlying Parkinson disease (PD). Instead, the results of this semi-quantitative study of 30 autopsy cases with incidental Lewy body pathology indicate that PD in the brain commences with the formation of the very first immunoreactive Lewy neurites and Lewy bodies in non-catecholaminergic neurons of the dorsal glossopharyngeus-vagus complex, in projection neurons of the intermediate reticular zone, and in specific nerve cell types of the gain setting system (coeruleus-subcoeruleus complex, caudal raphe nuclei, gigantocellular reticular nucleus), olfactory bulb, olfactory tract, and/or anterior olfactory nucleus in the absence of nigral involvement. The topographical parcellation of the nuclear grays described here is based upon known architectonic analyses of the human brainstem and takes into consideration the pigmentation properties of a few highly susceptible nerve cell types involved in PD. In this sample and in all 58 age- and gender-matched controls, Lewy bodies and Lewy neurites do not occur in any of the known prosencephalic predilection sites (i.e. hippocampal formation, temporal mesocortex, preneocortical cingulate areas, amygdala, basal nucleus of Meynert, interstitial nucleus of the diagonal band of Broca, hypothalamic tuberomammillary nucleus).

**Key Words:**  $\alpha$ -synuclein; Anterior olfactory nucleus; Extranigral pathology; Lewy body/neurite; Lower brainstem; Parkinson disease; Pathoanatomy.

### INTRODUCTION

In spite of the fact that Parkinson disease (PD) is the most widespread neurodegenerative movement disorder of the aging human nervous system, the clinical diagnosis—particularly the differential diagnosis with respect to related disorders—is not unproblematic and requires postmortem verification (1–6). In the course of PD, susceptible regions and vulnerable nerve cell populations become progressively impaired owing to the extensive presence of Lewy neurites (LNs) and Lewy bodies (LBs) (7–10). The abnormal inclusions consisting of nearly dissolvable aggregates within cellular processes and cell somata of involved nerve cells contain  $\alpha$ -synuclein ( $\alpha$ -SN), proteolytic stress proteins, such as ubiquitin and sometimes  $\alpha$ B-crystallin, as well as phosphorylated neurofilaments (11–18).

Although it is probable that the deterioration of the somatomotor system is attributable not only to nigrostriatal damage but also to detrimental events within other nerve cell populations and systems of the brain (19, 20), no definitive explanation exists for the apparent selective vulnerability (pathoklisis) on the part of some classes of neurons in the brain to PD (21–23).

Increasing awareness of PD as a multiple system neurodegenerative disorder has grown out of research demonstrating the existence of considerable extranigral pathology (24–33). The question, of course, arises whether any of these extranigral sites are affected before the substantia nigra. Some of the literature calls attention to the presence of inclusion bodies in the dorsal motor nucleus of the glossopharyngeal and vagal nerves (dorsal IX/X motor nucleus), but these studies either do not address the question of when this nuclear gray becomes involved in the disease process or which types of nerve cells there are preferentially affected (6, 9, 34, 35). Clinical experience, on the other hand, points to hyposmia as an early phenomenon in PD (36–39). The present paper, therefore, is intended to describe the locations of PD-related lesions seen in the lower brainstem and in anterior olfactory structures (bulb, tract, anterior olfactory nucleus) prior to involvement of the substantia nigra: The key brainstem lesions occur at predisposed sites, above all in the dorsal IX/X motor nucleus and dorsal pigmented nucleus of the glossopharyngeal and vagal nerves, the intermediate reticular zone, and the coeruleus-subcoeruleus complex of the gain setting system (25, 40–42).

### MATERIALS AND METHODS

#### Incidental Cases

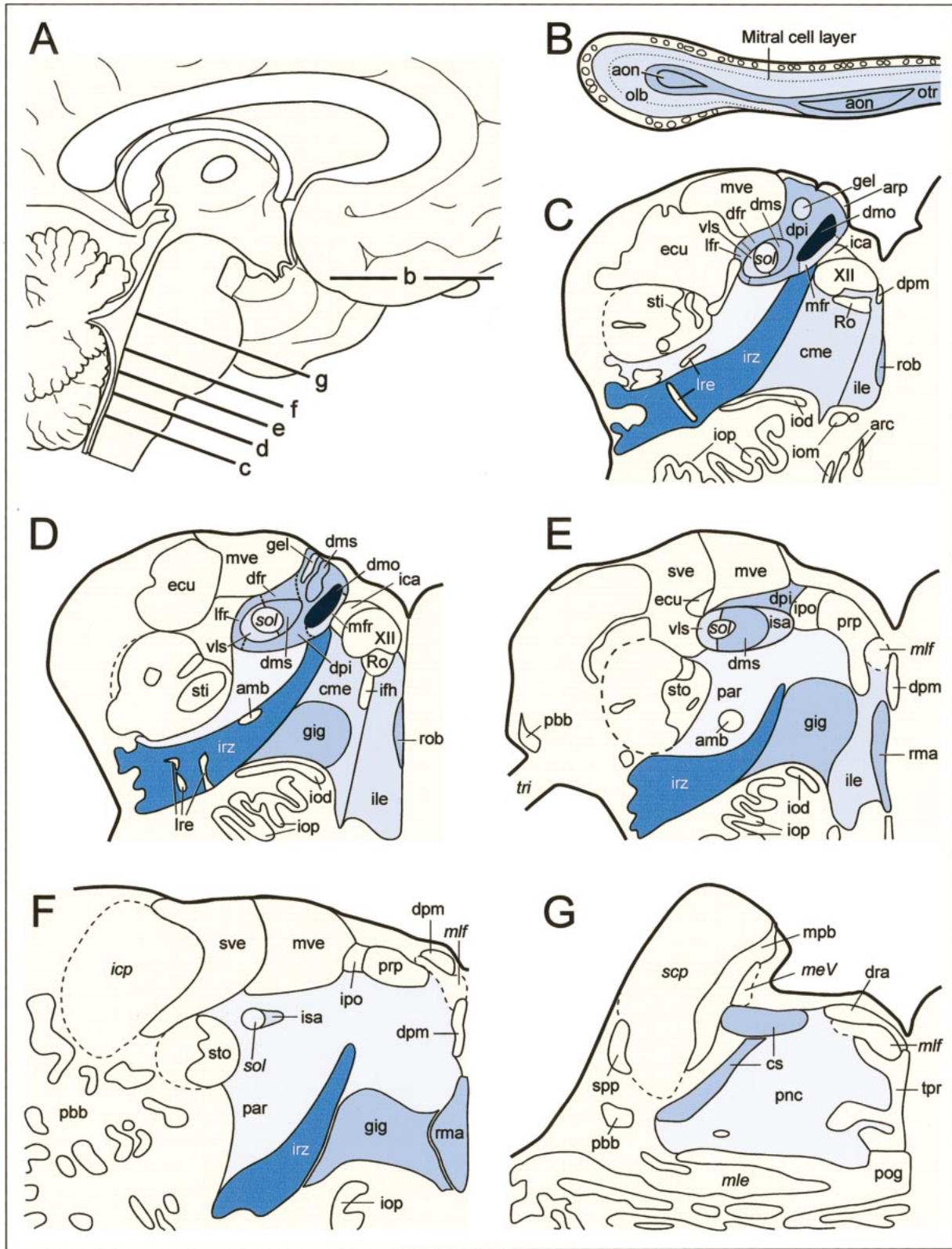
Brains from 413 autopsies were obtained from 2 institutes for pathology and 2 institutes for forensic medicine in the Rhein-Main area and screened to determine if the dorsal IX/X motor nucleus contained incidental LBs and/or LNs. Because clinical onset of idiopathic PD is seldom before the age of 40 and the largest risk group lies between the late-50s and early-70s (43), the cases included in this screening pool ranged from 40 yr and above, with no upper limit. Criteria for exclusion

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**Fig. 1.** A: Median view of a mediosagittal section through the human brain and brainstem for orientation. Line b indicates the position of anterior olfactory structures in (B). Lines c–g indicate the planes of the following frontal sections: line c plane of section shown in (C), line d plane of section shown in (D), etc. B: Horizontal section through the olfactory bulb (olb) and olfactory tract (otr), including portions of the anterior olfactory nucleus (aon). C–G: Schematized sections cut perpendicular to Meynert’s brainstem axis showing the distribution pattern of the PD-related pathology in the lower brainstem. The frequency

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were a known history of psychiatric or neurological disorder, including PD (44), and/or the presence of Alzheimer disease (AD)-related neurofibrillary pathology beyond NFT stage III (45, 46). The initial diagnosis (i.e. sighting of PD-related inclusion bodies) in sections through the dorsal IX/X motor nucleus and the intermediate reticular zone (Fig. 1A, C) was confirmed by at least 2 additional co-authors. The resulting 30 positive cases (15 females, 15 males; mean age  $75.4 \pm 8.2$  yr, Table 1) were supplemented in each instance by 3 additional blocks from the lower brainstem containing 1) the gigantocellular reticular nucleus and caudal raphe nuclei, 2) the coeruleus-subcoeruleus complex, and 3) the posterior nuclei of the substantia nigra, together with 6 blocks of brain tissue dissected from 1 hemisphere. These blocks included the following known PD predilection sites: 1) a portion of the magnocellular nuclei of the basal forebrain (either medial septal nucleus, interstitial nucleus of the diagonal band, or basal nucleus of Meynert); 2) the tuberomammillary nucleus, amygdala, uncus portions of the hippocampal formation, the entorhinal region, the anteromedial temporal mesocortex plus adjoining neocortex—usually extending up to the first temporal convolution; 3) the hippocampal formation at the level of the lateral geniculate body; 4) a segment from the agranular to granular insular cortex; 5) a portion from the anterior cingulate proneocortex and adjoining frontal neocortex; and 6) whenever available, one or both olfactory bulbs and olfactory stalks, including portions of the olfactory tract and anterior olfactory nucleus. All of this material was examined for the presence or absence of LBs and LNs.

### Controls

The same number of blocks processed in the same manner as the 21 incidental cases mentioned in the following paragraph from 58 age- and gender-matched control brains free of PD-related pathology (25 females, 33 males; mean age  $75.9 \pm 8.2$  yr; data from individual cases not shown) were examined for control and comparison to minimize the chances that any of the above-mentioned sites might have developed LNs and LBs in the absence of lesions within the dorsal IX/X motor nucleus.

### Tissue Preparation

Following fixation in a 4% aqueous formaldehyde solution, the tissue was processed as follows: The brainstems from 21 of the incidental cases and all controls were severed between the pontine tegmentum and mesencephalic tegmentum, the hemispheres midsagittally divided, and the 10 blocks listed above from each case were embedded in polyethylene glycol (PEG 1000, Merck). For the aforementioned 21 cases (see asterisk in Table 1, cases 1, 2, 5–7, 18) were processed differently: The brainstems were severed from the hemispheres at the latitude of the mammillary bodies so as to include the substantia nigra in its entirety and cut into uninterrupted series of 100- $\mu$ m-thick sections. Every tenth section was processed further so that sections were spaced 1 mm apart. In 1 case (Table 1, case 29), serial hemisphere sections and the 3 medullary blocks were prepared, and, finally, in 2 cases (Table 1, cases 21, 22), serial sections through one of the hemispheres and the brainstem were prepared. The upper brainstem blocks and the hemispheres were microtomed perpendicular to the intercommissural (Forel's) axis. The lower brainstems were similarly processed but sectioned perpendicular to the brainstem (Meynert's) axis. A thickness of 100  $\mu$ m was chosen to optimize the optical superposition of biological structures (47).

### Immunocytochemistry and Staining

Free-floating 100- $\mu$ m-thick sections from incidental cases and controls underwent the same staining procedures. The PD-related pathology was visualized by means of immunoreactions for  $\alpha$ -SN enhanced by formic acid pre-treatment (48). All sections were pre-treated according to a standard protocol designed to inhibit endogenous peroxidase and prevent non-specific binding. This was followed by incubation for 18 hours (h) in the affinity-purified  $\alpha$ -SN antiserum (Afshp) at a dilution of 1: 2,000–4,000. This antiserum was generated by W.P. Gai (Flinders Medical Centre, Bedford Park, Australia) in sheep using a peptide corresponding to the amino acid residues 116–131 of

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with which a given nuclear gray in this sample of 30 incidental cases is involved is indicated by increasing degrees of blue shading: 1–3 cases (pale blue), 4–9 cases (light blue), 10–17 cases (medium blue), 18–27 cases (deep blue), 28–30 cases (dark blue). Abbreviations: XII = motor nucleus of the hypoglossal nerve; amb = ambiguus nucleus; aon = anterior olfactory nucleus; arc = arcuate nucleus; arp = area postrema; cme = central medullary nucleus; cs = coeruleus-subcoeruleus complex, dfr = dorsal fringe of the dorsal IX/X pigmented nucleus; dmo = dorsal motor nucleus of the glossopharyngeal and vagal nerves; dms = dorsomedial solitary nucleus; dpi = dorsal pigmented nucleus of the glossopharyngeal and vagal nerves, including also dfr, lfr, mfr; dpm = dorsal paramedian nucleus; dra = nucleus raphes dorsalis; ecu = external cuneate nucleus; gel = gelatinous nucleus; gig = gigantocellular reticular nucleus; ica = intercalated nucleus; icp = inferior cerebellar peduncle; ifh = interfascicular hypoglossal nucleus; ile = intralemniscal nucleus; io = inferior olive; iod = inferior olive, dorsal accessory nucleus; iom = inferior olive, medial accessory nucleus; iop = inferior olive, principal nucleus; ipo = interpositus nucleus; irz = intermediate reticular zone; isa = inferior salivatory nucleus; lfr = lateral fringe of the dorsal IX/X pigmented nucleus; lre = lateral reticular nucleus; meV = mesencephalic trigeminal tract; mfr = medial fringe of the dorsal IX/X pigmented nucleus; mle = medial lemniscus; mlf = medial longitudinal fascicle; mpb = medial parabrachial nucleus; mve = medial vestibular nucleus; par = parvocellular reticular nucleus; pbb = pontobulbar body; pnc = central pontine nucleus; pog = pontine gray; prp = prepositus hypoglossal nucleus; rma = nucleus raphes magnus; Ro = nucleus of Roller; rob = nucleus raphes obscurus; rpa = nucleus raphes pallidus; scp = superior cerebellar peduncle; sn = substantia nigra; sol = solitary tract, including the interstitial nucleus of the solitary tract; spp = subpeduncular pigmented nucleus; sti = spinal trigeminal nucleus, interpolar portion; sto = spinal trigeminal nucleus, oral portion; sve = spinal vestibular nucleus; tpr = tegmentopontine reticular nucleus (nucleus of Bechterew); tri = trigeminal nerve; vls = ventrolateral solitary nucleus.

TABLE 1  
Incidental Parkinson Disease Cases

Case	Age	Gender	NFT stage	A $\beta$ -amyloid
1	54	f	I	0
2	58	m	I	0
3*	61	m	II	0
4*	67	m	II	A
5	69	m	II	0
6	69	f	III	0
7	70	f	I	A
8*	70	f	II	B
9*	71	f	II	B
10*	72	m	I	0
11*	75	f	III	A
12*	75	f	II	A
13*	75	m	I	0
14*	75	m	I	0
15*	76	m	III	C
16*	77	f	I	0
17*	77	m	II	A
18	77	m	II	0
19*	78	f	II	C
20*	79	f	II	B
21	80	f	I	0
22	81	m	I	0
23*	81	m	III	A
24*	83	f	I	B
25*	85	f	I	0
26*	85	m	I	B
27*	85	f	II	B
28*	85	f	II	0
29	85	m	I	0
30*	86	m	I	B

Age, gender (f = female, m = male), NFT stage (Braak stages I-VI in the development of Alzheimer-related neurofibrillary pathology), A $\beta$ -amyloid (Braak stages A-C of cortical Alzheimer-related  $\beta$ -amyloid deposition, 0 = no A $\beta$ -amyloid deposits). Asterisk refers to material available (9 tissue blocks containing subcortical and cortical PD predilection sites, see Incidental Cases under Materials and Methods). The location and degree of the inclusion-body pathology (LNs and LBs) are neither gender- nor age-dependent.

the human  $\alpha$ -SN. The specificity of the antibody to  $\alpha$ -SN has been described previously (49). Subsequent to processing with biotinylated secondary antibodies (anti-mouse IgG for 2 h), immunoreactions were visualized with the ABC complex (Vectastain, Vector Labs, Burlingame, CA) and 3,3'-diaminobenzidine-tetra-HCl/H<sub>2</sub>O<sub>2</sub> (DAB; D7679 Sigma, St. Louis, MO). Omission of the primary antiserum resulted in non-staining. Under these conditions, the  $\alpha$ -SN immunoreactivity of pathological structures was relatively stable. There was no difference either in the staining pattern or properties of  $\alpha$ -SN immunoreactions within the 24-h time span of postmortem intervals in human autopsy material fixed by immersion in 4% formaldehyde solutions.

The  $\alpha$ -SN antibody labels both normal and abnormal forms of the protein, whereby faintly immunoreactive dots seen even in control cases correspond to the normal  $\alpha$ -SN in synaptic

boutons, and intense immunolabeling signals the presence of pathological  $\alpha$ -SN aggregates (LBs and LNs). Since ubiquitin is known to co-stain co-occurring small globose NFTs and Pick bodies in brain tissue, antibodies against ubiquitin were not included (3).

For topographical orientation, immunostained sections were partially counter-stained for lipofuscin pigment (aldehyde-fuchsin) and Nissl material (Darrow red). Aldehyde-fuchsin staining was chosen to highlight the pigmentation properties of the different nerve cell populations within the adult human brain (50). Because, in our experience, it can be difficult to detect the presence of intraneuronal LBs and LNs in neuromelanin-containing neurons, sections through the dorsal IX/X motor nucleus, intermediate reticular zone, coeruleus-subcoeruleus complex, and substantia nigra were stained using the SG Substrate Kit (SK 4700; Vector) to highlight the PD-related lesions in dark blue. Additional sections were silver-stained with advanced methods that exploit physical development of nucleation sites: The Gallyas silver iodide technique was employed to visualize AD-associated neurofibrillary tangles and neuropil threads (47), and use of the Campbell-Switzer silver pyridine method facilitated detection not only of LNs/LBs but also of AD-related  $\beta$ -amyloid deposits as well as neuromelanin (51). Finally, all of the sections were cleared and mounted in a synthetic resin (Permount, Fisher).

The presence of the PD-related pathology was assessed according to a semi-quantitative rating scale: -, none/not discernible; +, slight; ++, moderate; +++, severe; and n.e., not evaluated. The topographical limits and pigmentoarchitectonics of the nuclei under consideration are, for the most part, those recommended by Olszewski and Baxter (52), Braak (53, 54), Saper and Petito (55), Martin et al (56), and Huang and Paxinos (57) (Fig. 1).

## RESULTS

All the incidental cases presented with  $\alpha$ -SN-immunopositive LNs and/or LBs and were free of other inclusion bodies related to non-PD  $\alpha$ -synucleinopathies (3, 15). LNs clearly predominated over LBs in the available material, and the severity of the incidental lesions was predominantly slight to moderate (+ or ++ on a scale of +++) , varying little from one case to another with one exception (Table 2, case 5). In Table 2, the data are organized by topographical area into 6 blocks of columns, moving rostrally from left to right.

Apart from the lesions seen in the olfactory bulb (olb), olfactory tract (otr), and anterior olfactory nucleus (aon), the pathology in all but 4 cases (cases 20, 27, 29, 30) was confined to nuclear grays below the level of the substantia nigra (sn) (Table 2).

None of the melanin-containing nerve cells in the dorsal IX/X nuclear complex or adjoining intermediate reticular zone (A1/A2 catecholaminergic groups) were affected by the pathological process. The first melanized nerve cells in the lower brainstem to develop LNs and LBs (apparently, in that sequence as well) were those

populating the coeruleus-subcoeruleus complex (cs) (Fig. 3F).

Of the 58 control brains, none displayed  $\alpha$ -SN-immunoreactive LNs or LBs in any portion of the PD-related subcortical or cortical predilection sites investigated. Co-occurring AD-related pathology was relatively minimal (NFT stages I–III), thereby remaining within the anticipated span of the respective age groups for that disorder (58).

#### Lesions in the Dorsal Glossopharyngeus-Vagus Complex and Intermediate Reticular Zone

In addition to the dorsal IX/X motor nucleus (dmo), the dorsal IX/X complex encompasses the visceromotor inferior salivatory nucleus (isa), small-celled viscerosensible nuclei of the solitary tract (ventrolateral solitary nucleus [vls], dorsomedial solitary nucleus [dms], interstitial nucleus of the solitary tract [sol]), the gelatinous nucleus (gel), the area postrema (arp), and the dorsal IX/X pigmented nucleus (dpi) (Fig. 1C–E). The latter includes, in part, 3 fringe areas with ill-defined borders (Fig. 2A). A distinctive feature of the dorsal pigmented IX/X nucleus as a whole in cell-stained sections is the manner in which it fills the spaces between the other nuclear grays of the dorsal IX/X complex like the mortar applied in bricklaying (Fig. 1C, D) (59). The intermediate reticular zone (irz) in frontal sections adjoins the dorsal IX/X complex dorsally and emanates ventrolaterally through the medulla oblongata almost to its lateral surface (Fig. 1C). It contains a single motor areal, the ambiguous nucleus (amb), and adjoins the gigantocellular reticular nucleus (gig) laterally (Fig. 1D).

The dmo of all 30 incidental cases contained some degree of PD-related  $\alpha$ -SN-immunoreactive inclusion pathology (Table 2; Fig. 2A, E, F). In areas with fewer lesions (+), it also tended to be asymmetrical and did not readily catch the eye because it may have consisted only of a single LN (Fig. 2F). As the lesions increased, however, the asymmetry became less pronounced, as seen in cases 5, 9, 23, and 27 (Table 2).

In the dmo, LNs (Fig. 2B, F, G) prevailed numerically over the smooth-surfaced spherical LBs in cell somata, at least initially (Fig. 2A). LBs were not detectable in the extracellular space (60, 61). The diameters of the most slender LNs hardly exceeded those of healthy axons. As seen previously (62), some of the cases examined (Table 2, cases 9, 10, 12, 15, 18, 21, 23, and 27) had fine but very intensely  $\alpha$ -SN-immunoreactive LNs within intramedullary axons of the vagus nerve, and these were traceable from the dmo to the ventrolateral surface of the medulla.

The inferior salivatory nucleus (isa) is the rostral continuation of the dmo (Fig. 1F). In contrast to the dense network of lesions often indicating the position of the isa

in the brains of clinically diagnosed PD-patients at autopsy, isolated LNs and LBs were present in 5 of our cases (Table 2; Fig. 2C).

The small, mostly spindle-shaped nerve cells that harbor sparse amounts of fine lipofuscin granules and populate the subnuclei immediately surrounding the solitary tract were slightly (+) affected: the dorsomedial solitary nucleus (dms) 14 times, the ventrolateral solitary nucleus (vls) in 4 instances (Table 2; Fig. 1C–E). Once again, LNs outnumbered LBs and were more conspicuous than the latter. Remarkably, 3 cases displayed LNs (+) within the interstitial nucleus of the solitary tract (sol) (Table 2; Fig. 1C).

Somewhat more frequently involved than the ventrolateral solitary nucleus was the gelatinous nucleus (gel) (Table 2; Figs. 1C, 2A). A few LNs (+) appeared within cellular processes of some of the mostly pale-staining nerve cells residing there in 6 cases. LBs were absent or not detectable. Although the gel is reported to include some norepinephrine-synthesizing neurons of the A2 group (63, 64), none occurred within this nuclear gray in our material.

Lesions were present in the intermediate reticular zone (irz) of 19 cases (Table 2; Fig. 2H–K). LBs and preliminary forms of LBs were confined solely to the spindle-shaped and triangular projection neurons containing lipofuscin granules. These cells are easily identified because their orientation along their longitudinal axes is always parallel to the downward sweeping course of this nuclear gray. The few sparsely scattered and somewhat smaller nerve cells with inter-individually variable quantities of neuromelanin (A1 catecholaminergic cell group) did not display the pathological material (55, 57, 63).

Among the heterogeneous, for the most part small-celled, neuronal types comprising the dorsal IX/X pigmented nucleus (dpi), the most conspicuous constituents are the melanized neurons that correspond to those of the A2 group (55, 63). The chief hallmark of these medium-sized melanoneurons, which are ovoid or pear-shaped and somewhat smaller than their A6/A7-counterparts in the coeruleus-subcoeruleus complex, is that they are not uniformly compactly arranged. Rather, they tend to form localized and somewhat loosely associated islands of cells, so-called “fringe areas” (lateral fringe pigmented nucleus [lfr], dorsal fringe pigmented nucleus [dfr], medial fringe pigmented nucleus [mfr]) (Fig. 2A). None of the fringe area melanoneurons contained LBs. By contrast, 18 cases displayed PD-related pathology within the few, but large, strongly lipofuscin-staining nerve cells and/or other types of smaller neurons populating the dpi (Fig. 2D). Notably, 2 or more fringe area subnuclei were involved in 11 of the 18 cases (Table 2).

Nuclei in this sample of 30 cases that remained untouched by the lesions were the commissural nucleus (not shown), the motor nucleus of the hypoglossal nerve (34)

TABLE 2

	Dorsal glossopharyngeus-vagus complex										Interm. reticular zone	
	dmo	isa	sol	dms	vls	gel	lfr	dfr	mfr	dpi*	irz	amb
1	++	-	-	-	-	-	-	-	-	-	-	-
2	+	-	-	+	-	-	-	+	-	+	+	-
3	++	+	-	+	-	-	-	+	-	-	++	-
4	++	n.e.	-	-	-	-	+	+	+	+	+	-
5	+++	++	+	+	+	-	+	++	+	+	++	-
6	+	-	-	-	-	-	-	-	-	-	-	-
7	++	+	-	-	-	-	-	-	-	-	-	-
8	+	n.e.	-	-	-	-	+	+	-	+	++	-
9	+++	-	-	+	-	+	+	+	-	+	++	-
10	++	-	-	+	-	-	-	-	-	-	-	-
11	++	-	-	+	-	-	+	-	+	-	++	-
12	++	-	+	+	-	+	+	-	-	-	+	-
13	++	-	-	-	-	-	-	-	-	-	-	-
14	++	-	+	+	-	-	+	+	-	+	+	-
15	+	++	-	-	-	-	-	-	-	-	-	-
16	+	-	-	-	-	-	-	-	-	-	-	-
17	+	-	-	+	+	-	-	-	-	-	+	-
18	++	-	-	-	-	-	+	-	-	-	++	-
19	+	-	-	-	-	-	-	-	-	-	-	-
20	++	+	-	+	-	-	+	+	+	+	+	-
21	+	-	-	-	-	-	-	+	-	+	+	-
22	+	-	-	-	-	-	+	-	-	-	+	-
23	+++	-	-	+	-	+	+	++	+	++	++	-
24	++	n.e.	-	+	-	-	-	-	-	-	+	-
25	+	-	-	-	-	-	-	-	-	-	-	-
26	+	-	-	-	-	-	-	-	-	-	-	-
27	+++	-	-	+	-	+	+	+	+	+	-	-
28	++	-	-	-	-	-	+	-	-	-	++	-
29	++	-	-	+	+	+	++	++	+	++	++	-
30	+	n.e.	-	-	+	+	+	+	-	+	+	-

The presence of LNs and LBs in the lower brainstem and anterior olfactory structures was evaluated semi-quantitatively as follows: -, none/not discernible, +, slight, ++, moderate, +++, severe, n.e., not evaluated.

Abbreviations: dmo = dorsal motor nucleus of the glossopharyngeus-vagus complex (dorsal IX/X motor nucleus); isa = inferior salivatory nucleus; sol = solitary tract, including the interstitial nucleus of the solitary tract; dms = dorsomedial solitary nucleus; vls = ventrolateral solitary nucleus; lfr = lateral fringe pigmented subnucleus; dfr = dorsal fringe pigmented subnucleus; mfr = medial fringe pigmented subnucleus; dpi\* = pigmented nucleus of the glossopharyngeus-vagus complex (dorsal IX/X pigmented nucleus), here used to designate the remaining, i.e. non-fringe, areas within the reaches of the dorsal IX/X pigmented nucleus; gel = gelatinosus nucleus; irz = intermediate reticular zone; amb = ambiguus nucleus; gig = gigantocellular reticular nucleus; par = parvocellular reticular nucleus; cme = central medullary nucleus; ile = intralemniscal nucleus; rma = nucleus raphes magnus; rob = nucleus raphes obscurus; rpa = nucleus raphes pallidus; cs = coeruleus-subcoeruleus complex; sn = substantia nigra, pars compacta; aon = anterior olfactory nucleus; olb = olfactory bulb; otr = olfactory tract. The designation n.e. under the final 3 columns occurs frequently because, according to available protocols, one or both bulbi olfactorii had been damaged at some point during the autopsy and were no longer available for study.

(XII in Fig. 1C, D), the prepositus hypoglossal nucleus (prp) (Fig. 1E, F), the ambiguus nucleus (amb), and the subnuclei of the inferior olive (Fig. 1D, E). Furthermore, no LNs and LBs were observable in this material within the area postrema (arp) situated near the opening of the central canal beneath the ependymal lining and comprised of numerous tiny, plump lipofuscin-containing cells (Figs. 1C, 2E).

No free-lying neuromelanin or neurolipofuscin was detectable within the reaches of either the dorsal IX/X complex or intermediate reticular zone.

#### Lesions in the Reticular Formation, Caudal Raphe Group, and Coeruleus-Subcoeruleus Complex

PD-related  $\alpha$ -SN-immunoreactive inclusions were present in 11 cases within one or more of the 4 medullary reticular nuclei examined (gigantocellular reticular nucleus [gig], intralemniscal nucleus [ile], central medullary nucleus [cme], parvocellular reticular nucleus [par]) (Table 2; Fig. 1C-F). Among the nuclear grays composing the core of the human brainstem, the gig, situated at the pontomedullary junction, predominates (Fig. 1F). LBs

TABLE 2 (Extended)

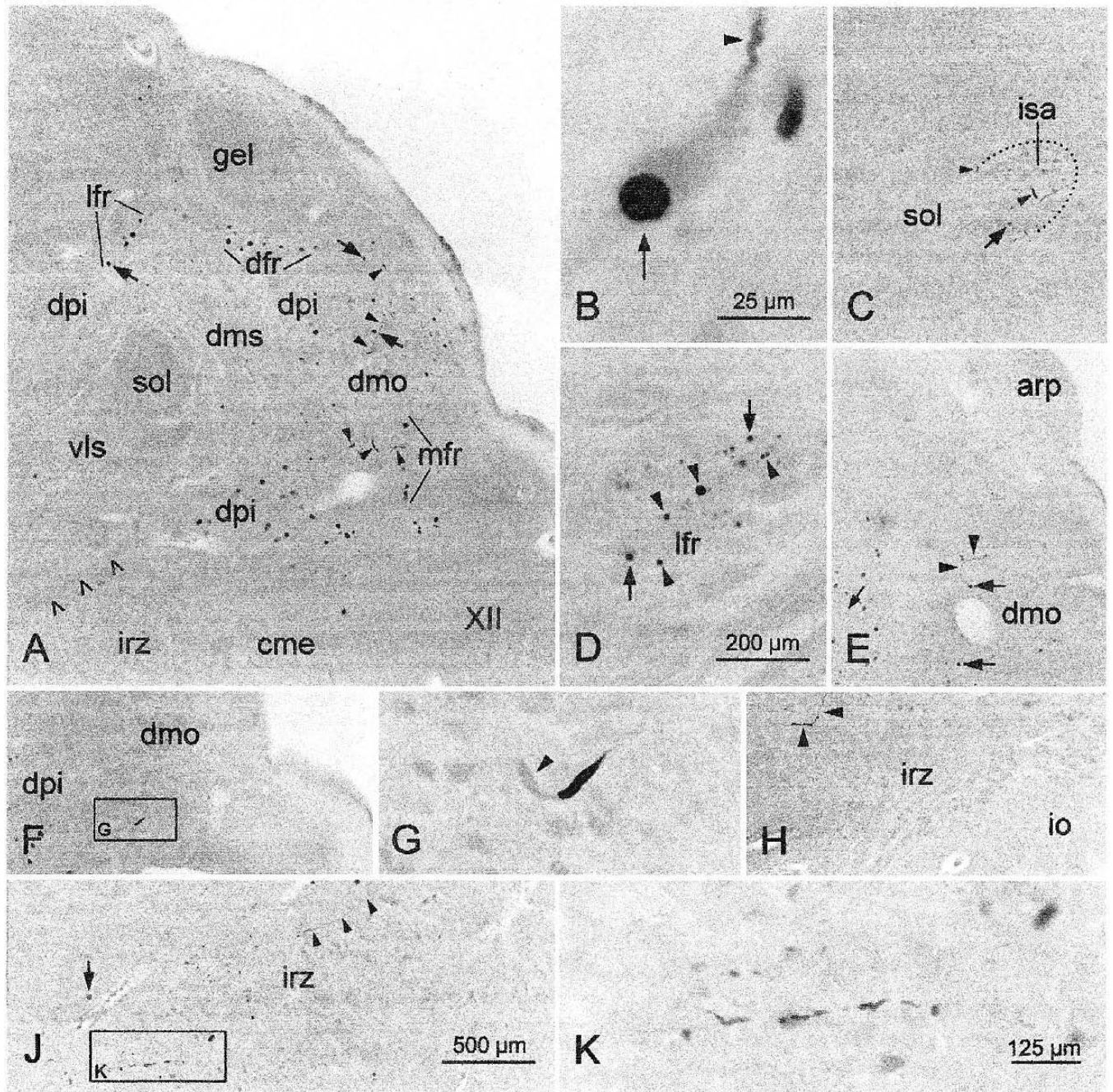
Pontomedullary reticular formation				Caudal raphe group and coeruleus-subcoeruleus complex				Anterior olfactory structures				
gig	par	cme	ile	rma	rob	rpa	cs	sn	aon	olb	otr	
-	-	-	-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	-	-	-	
++	-	-	-	++	++	-	+	-	++	n.e.	++	
-	-	-	-	-	-	-	+	-	-	-	-	
++	++	++	+	++	++	-	++	-	+	+	++	
-	-	-	-	+	-	-	+	-	-	n.e.	-	
-	-	-	-	-	-	-	-	-	-	n.e.	n.e.	
n.e.	-	-	+	-	-	-	-	-	++	++	++	
+	-	-	-	+	-	-	+	-	n.e.	n.e.	n.e.	
-	-	-	-	-	-	-	n.e.	-	-	-	+	
+	-	-	+	++	+	-	+	-	++	++	++	
-	-	-	-	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	-	-	-	
++	-	+	+	++	+	-	++	-	n.e.	n.e.	n.e.	
-	-	-	-	-	+	-	+	n.e.	+	n.e.	+	
-	-	-	-	-	-	-	n.e.	-	-	n.e.	n.e.	
-	-	-	-	-	-	-	-	-	++	n.e.	++	
+	-	-	-	++	+	+	++	-	+	n.e.	+	
-	-	-	-	-	-	-	+	-	++	++	++	
+	-	-	-	+	+	-	+	+	-	++	++	
-	-	-	-	-	-	-	+	-	-	n.e.	+	
-	-	-	-	+	-	-	+	-	-	-	-	
+	-	-	+	-	++	-	+	-	++	++	++	
-	-	-	-	-	+	-	+	-	-	+	+	
-	-	-	-	-	-	-	-	-	n.e.	n.e.	-	
-	-	-	-	-	-	-	-	-	-	++	++	
-	-	-	-	-	-	-	-	+	n.e.	++	++	
+	-	+	-	+	-	-	-	-	++	-	++	
+	+	++	++	+	++	-	++	+	n.e.	-	-	
-	-	-	-	-	-	-	+	+	-	-	-	

occurred within this gray's imposingly large lipofuscin-laden projection neurons (Fig. 3C), as opposed to the other 2 less strongly lipofuscin-containing populations of nerve cells residing there, including a mixed class of smaller neurons (52, 56).

Thirteen cases were affected by PD-related alterations in one or more of the serotonergic subnuclei comprising the caudal raphe group (nucleus raphes magnus [rma], nucleus raphes obscurus [rob], nucleus raphes pallidus [rpa]) (Table 2; Fig. 1C–F, rpa not shown in Fig. 1C, D). Riding mostly on or around the midline, the lesions were confined exclusively to the clusters of medium-sized, triangular lipofuscin-laden projection neurons within the first 2 nuclei (Fig. 3A). Other cells, with far fewer, tightly packed and coarse lipofuscin granules, that also have been described within the caudal raphe group, remained uninvolved (52, 53). The distribution of the lesions tended to be bilaterally symmetrical. The strongly lipofuscin-containing cells of the rpa were minimally affected (+) only once (Table 2, case 18). Further rostrally, the rma is replaced by the reticulotegmental nucleus of the pons (tpr or nucleus of Bechterew, Fig. 1G),

but no  $\alpha$ -SN-immunoreactive inclusion bodies were seen there yet in this sample.

Beginning at the level of the decussation of the IVth cranial nerve, the coeruleus-subcoeruleus complex (cs) extends caudally to the level of the VIIth cranial nerve (Figs. 1F, 3D). Within both nuclear grays, LNs and an occasional LB were observable in 17 of the 30 cases examined (Table 2; Fig. 3E). LBs did not develop in the somata of the small, non-melanized cells strewn throughout the complex (25). Rather, it was the melanoneurons of the A6/A7 groups (55) located at the lateral fringe of the pontine tegmentum near the aqueduct that did so. The darkly melanin-pigmented neurons of the locus coeruleus appeared as closely bunched accumulations of cells, whereas those comprising the subcoeruleus nucleus were scattered. Occasionally, preliminary or weak forms of  $\alpha$ -SN-immunopositive LBs could be seen emerging in clusters within the aggregations of neuromelanin granules in the larger projection neurons (Fig. 3F). Notably, the large, spherical-shaped neurons comprising the mesencephalic root of the trigeminal nerve were free of PD-related material.



**Fig. 2.** A–G: Incidental Parkinson disease (PD) pathology in the dorsal glossopharyngeus-vagus complex (dorsal IX/X complex) and (H–K) intermediate reticular zone.  $\alpha$ -synuclein ( $\alpha$ -SN) immunoreactions. Lesions are highlighted by the SG Substrate Kit (SK 4700; Vector) in 100- $\mu$ m-thick PEG sections. A: Overview of the dorsal IX/X complex (case 12, Table 2): The very earliest lower brainstem lesions do not develop in the substantia nigra but in cellular processes (arrowheads indicate LNs) and cell somata (arrows indicate LBs) within the lipofuscin-containing projection neurons of the dorsal IX/X motor nucleus (dmo) and fringe areas of the dorsal IX/X pigmented nucleus (here, lateral fringe subnucleus, lfr). LNs within intramedullary axons of the vagus nerve (open arrowheads) can be traced, without interruption, from the dmo to the ventrolateral surface of the medulla. B: Large visceromotor neuron (case 27) in the dmo containing an intensely  $\alpha$ -SN-immunoreactive LN (arrowhead) as well as LB (arrow). In this sample of 30 incidental cases, the dmo is always affected, even when other nuclear grays are devoid of lesions (Table 2). Incidental LNs/LBs are not “occasional” or “haphazard” lesions accompanying the normal process of aging. Rather, they are inherently pathological entities and, even if subclinical, always represent the harbingers of PD. C: The inferior salivatory nucleus (isa, case 15) constitutes the rostral continuation of the dmo and displays isolated LNs (arrowheads) and/or LBs (arrow) in 5 instances (Table 2). D:  $\alpha$ -SN-immunopositive inclusion bodies (arrowheads) in the lfr of the dorsal IX/X pigmented nucleus (dpi). In this case (5), all 3 of the fringe subnuclei are affected (Table 2). The fringe area melanoneurons (arrows) appear to be less sedentary than the other neuronal types located within the reaches of the dpi and remaining nuclei of the dorsal IX/X complex. Occasionally, a few of these melanized nerve cells “stray” into cell groups belonging to nuclear grays that have more



LN or LBs were not detectable within the reaches of the pedunculopontine nucleus and parabrachial nuclei (not shown) in this sample, although both are known predilection sites in PD.

#### Lesions in the Substantia Nigra

Single, isolated LNs appeared in 4 cases (Table 2, cases 20, 27, 29, 30) amidst the long, finger-like clusters of heavily melanin-pigmented projection neurons (A9 group) in the posterolateral subnucleus of the pars compacta (sn) (54, 55). No LBs were present and none were seen within the posteromedial, posterosuperior, or magnocellular subnuclei of the pars compacta. The anteromedial, anterointermediate, and anterolateral subnuclei were free of LNs as well as LBs. Three of the 4 individuals were 85 yr of age at death (Table 1). In the material at our disposal here, no macroscopically discernible depigmentation and no microscopically visible neuromelanin depletion or neuronal loss was evident. The melanoneurons (A10 group) of the paranigral nucleus, parabrachial pigmented nucleus, and perirubral formation (54, 55) still were free of lesions.

#### Lesions in the Olfactory Bulb, Olfactory Tract, and Anterior Olfactory Nucleus

In 16 cases, one or more anterior olfactory structures (olfactory bulb [olb], olfactory tract [otr], anterior olfactory nucleus [aon]) displayed PD-related pathology (Table 2; Figs. 1A, B, 3G, H). Elongated rather than thick-caliber LNs surpassed small LBs numerically. The lesions occurred in layers V–VI of the olb, which is characterized by a concentric eight-layered lamination pattern (65, 66), as well as in the otr and cellular islands of the aon harbored by the otr. Exceptionally dense accumulations of LNs interspersed by LBs could be seen in the aon, which contained 2 types of neurons: both presented with LBs and both contained lipofuscin granules (28, 65–67). Where portions of above-mentioned anterior olfactory entities from both hemispheres were intact (cases 14, 18), the lesions were bilateral in 1 case (Table 2, case 18) (68). In 1 case (case 26), the olb and otr were the only sites sustaining damage in addition to the dmo (Table 2). In no instance were anterior olfactory structures affected

to the exclusion of all other nuclear grays examined (Table 2). The olfactory tubercle, piriform cortex, and periamygdaloid cortex did not contain LNs and/or LBs (66).

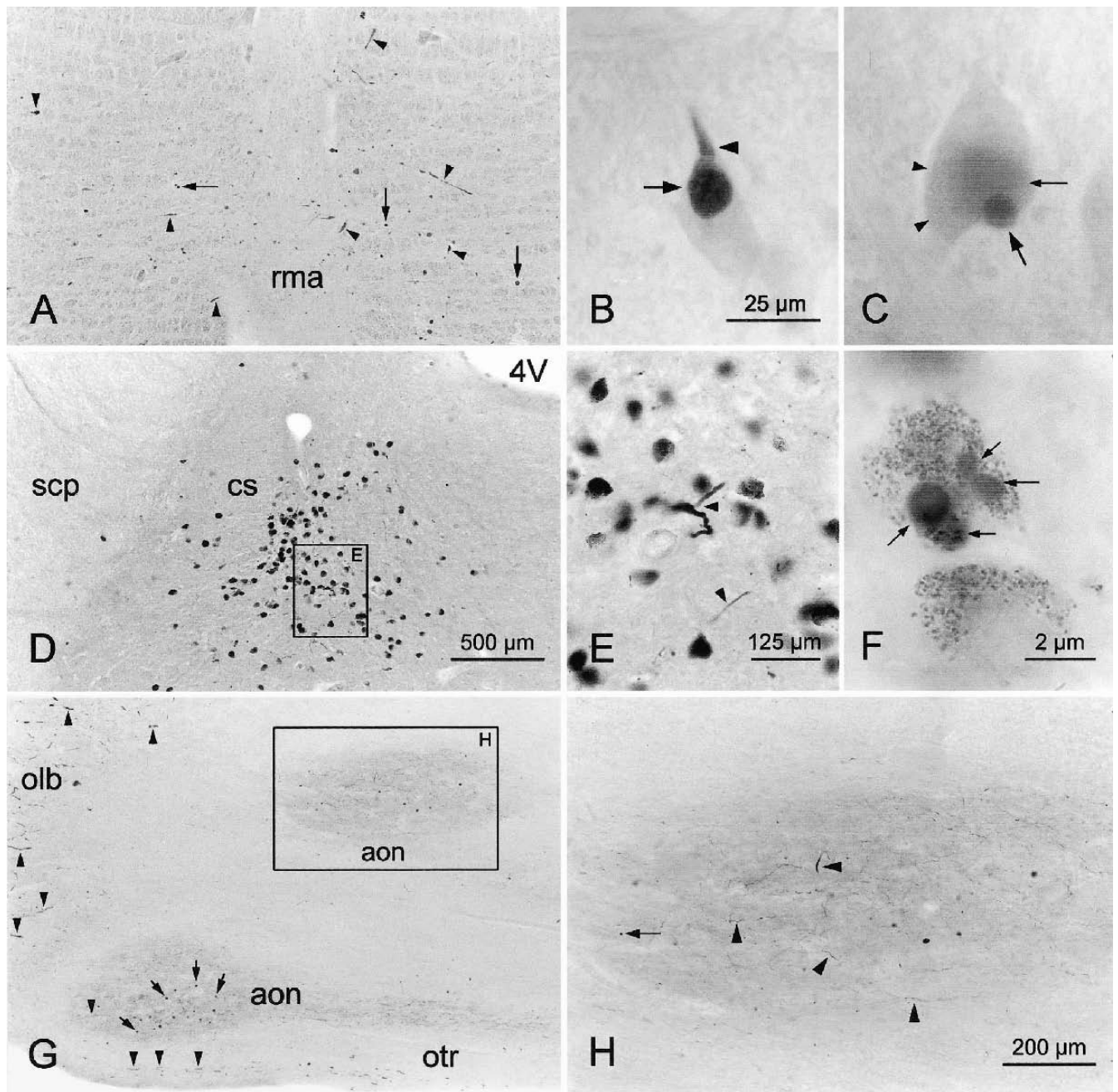
#### DISCUSSION

An important issue with respect to the pathogenesis of PD in the brain is not only how the pathological process begins but also where. Granted,  $\alpha$ -SN-immunoreactive inclusion bodies are known to occur in amyotrophic lateral sclerosis, Hallervorden-Spatz disease, and multiple system atrophy (69, 70). Nonetheless, PD is the only illness that always presents postmortem with LBs and LNs as the sole pathological markers in specific types of nerve cells at predisposed sites. In the other neurodegenerative disorders, the inclusion bodies are restricted to either glial cells or nerve cell types not involved in PD, such as oligodendrocytes in multiple system atrophy, astrocytes and Schwann cells in amyotrophic lateral sclerosis, and/or other neuronal populations at other predilection sites within the nervous system (e.g. nerve cells of the pons, inferior olives) (3, 15, 69, 71–73). As such, it can be said that the constellation of the key  $\alpha$ -SN-immunoreactive lesions seen in the 30 incidental cases studied here most closely resembles that encountered at the same sites in autopsy brains from clinically overt PD cases and not the configurations such as they exist in other diseases, the neuropathological difference between incidental and fully developed PD being a matter of degree and not of morphology (6, 74). This also implies, however, that “incidental” LNs/LBs are not “occasional” or “haphazard” lesions accompanying the normal process of aging. Instead, they are from the very outset inherently pathological entities and, even if subclinical, always represent the precursors of PD (44, 71, 75–77).

Based upon known pigmentoarchitectonics of the various types of nerve cells populating the lower brainstem nuclei described above, a picture emerges in which the very earliest LBs display a marked preference for specific classes of neurons at a recurrent pattern of induction sites, namely, 1) the lipofuscin-containing projection cells of the dorsal IX/X motor nucleus (dmo) and dorsal IX/X pigmented nucleus (dpi), intermediate reticular zone (irz), and 2) the melanoneurons of the coeruleus-subcoeruleus

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clearly delineated boundaries than the dpi, e.g. the gelatinosus or dorsal IX/X motor nuclei (55, 98). E: The small, pale-staining nerve cells of the area postrema (arp) are free of lesions in our material. In the dmo (case 21), arrows point to 2 LBs and arrowheads indicate an LN. A tiny LB (arrow) also is detectable in the immediately adjacent dpi. F, G: In the mildest incidental cases, the pathology in the dmo is asymmetrical and can consist of a single LN (framed in [F] and shown at higher magnification in [G]) within the cellular process of a visceromotor neuron (arrowhead). The only other sites sustaining damage is this instance (case 26) are the olfactory bulb (olb) and olfactory tract (otr) (Table 2). H: As in the dmo, the first detectable PD-related changes within the intermediate reticular zone (irz, case 17) often are confined to a very few LNs (arrowheads). J, K: The arrow indicates a LB in a non-melanized projection cell of the irz (case 28). LNs may be thread-like and elongated (arrowheads) in appearance but also distended and varicose (detail in [K] is framed in [J]). Whereas the irz is affected in 19 of the 30 cases studied, no LNs/LBs are observable in the ambiguous nucleus (amb, Table 2). Scale bar in (B) applies to (G). Scale bar in (J) also is valid for (A, C, E, F, and H).



**Fig. 3.** A–F: Parkinson disease pathology in the gain setting nuclei of the lower brainstem (gigantocellular reticular nucleus, caudal raphe group, coeruleus-subcoeruleus complex) and (G, H) anterior olfactory structures.  $\alpha$ -SN immunoreactions. Lesions were highlighted using the SG Substrate Kit (SK 4700; Vector) in 100- $\mu$ m-thick PEG sections. A: The distribution pattern of LBs (arrows) and LNs (arrowheads) in the nucleus raphes magnus (rma, case 9) is relatively symmetrical at and around the midline. A third of the incidental cases show lesions in this nuclear gray as well as in the gigantocellular reticular nucleus (gig, Table 2). B: Intraneuronal pathology in the form of a contiguous LB (arrow) and LN (arrowhead) in a medium-sized projection cell of the rma (case 26). C: A lipofuscin-laden (arrowheads) projection neuron of the gig (case 18) exhibits an LB (large arrow) accompanied by a pale body (small arrow). Together with the 3 caudal raphe nuclei (magnus, obscurus, pallidus) and coeruleus-subcoeruleus complex, the gig is responsible for modifying the level of excitability (i.e. gain) of brainstem and spinal motor and premotor neurons (25, 40). In addition to its gain setting influence upon motor and premotor neurons, the nucleus raphes magnus also is involved in the processing of nociceptive impulses (56). D, E: The first melanoneurons in the lower brainstem to develop the PD-related lesions are the projection cells of the coeruleus-subcoeruleus complex (cs, A6/A7 catecholaminergic group) and not those of the substantia nigra (A9 group), paranigral nucleus, parabrachial pigmented nucleus, perirubral formation (A10 group), intermediate reticular zone (A1 group), or dorsal IX/X pigmented nucleus (A2 group). Two swollen LNs and a thread-like one (arrowheads) in the cs are framed in (D) and shown in detail in (E) (case 14). Only 4 of the incidental cases examined here (cases 20, 27, 29–30, Table 2) display isolated LNs in the posterolateral subnucleus of the substantia nigra, pars compacta.

complex (cs) (A6/A7 groups). These are followed closely in frequency by the lipofuscin-containing nerve cells of the dorsomedial nucleus of the solitary tract (dms), gigantocellular reticular nucleus (gig), and caudal raphe group.

Remarkably, the lesional distribution pattern seen in the dorsal IX/X complex and intermediate reticular zone in the majority of the incidental PD cases differs from that of the pathological process in AD. Whereas it is the melanized nerve cells (A1/A2 groups) at both of these sites that show the greatest proclivity initially for developing the AD-related cytoskeletal lesions (NFT/NT stages I–II) (59), by contrast, in PD it is the large, lipofuscin-laden visceromotor projection neurons that display the earliest LBs. In PD, the initial lesion within the dorsal IX/X complex and intermediate reticular zone is followed by the gradual appearance of inclusion bodies within the melanized nerve cells of the catecholaminergic A1/A2 groups. In AD, on the other hand, the lipofuscin-containing projection neurons in both regions display the first, albeit slight, neurofibrillary pathology at stages V–VI (59). It should also be emphasized that the coeruleus-subcoeruleus complex becomes involved early in both AD (NFT/NT stages I–II) and PD. Persons with PD and AD concomitantly, therefore, bear a double burden of lesions within specific neuronal populations of select medullary nuclear grays (1, 59).

In the present study, the affection of the A6/A7 melanin-laden nerve cells clearly not only exceeds but also precedes that of the substantia nigra (A9 group), thereby corroborating, at least in part, the findings of Perry and colleagues (44) as well as those of Lindboe and Hansen (78). In contradistinction to the former study (44), however, our results show that the coeruleus-subcoeruleus complex is not the lower brainstem nuclear gray most prone to develop LBs but one of several vulnerable targets there, with the dorsal IX/X motor nucleus being more susceptible than the coeruleus-subcoeruleus complex. Ultimately, with regard to the dorsal IX/X motor complex, Eadie (34) correctly observed that it is the non-melanized as opposed to melanized neurons that consistently sustain the major portion of the damage in PD, at least at first.

TABLE 3  
Age-specific Prevalence of Incidental Parkinson Disease Pathology in the Dorsal IX/X Motor Nucleus

Age	Number	Percent
40–49	0/15	0%
50–59	2/18	11.1%
60–69	4/115	3.5%
70–79	14/180	7.8%
80–89	10/73	13.7%
>90	0/12	0%

The number before the backslash indicates the number of incidental cases in each age category, the number after the backslash is the total number in each age category for all of the cases included in the original screening pool of 413. The age-specific prevalence of the PD-related lesions in the dorsal IX/X motor nucleus increases with age to 7.8% over 70 and 13.7% over 80 years of age. The total prevalence of incidental Lewy body pathology for the dorsal IX/X motor nucleus is 7.3% (30/413). The 30 incidental cases range in age from 54 to 86 years with a mean age of  $75.4 \pm 8.2$  (Table 1). For the substantia nigra, on the other hand, the total prevalence of incidental Lewy body pathology is not anticipated to exceed 4.54% (4/88).

The fact that the olfactory bulb, olfactory tract, and/or lipofuscin-containing nerve cells in the anterior olfactory nucleus of all cases are never the sole sites involved in the PD-related pathology would seem to preclude the anterior olfactory system as the sole induction site of PD in the brain (28).

PD pathology will continue to evade recognition if postmortem assessment relies too heavily on the status of the substantia nigra while leaving the dorsal IX/X complex, intermediate reticular zone, gain setting nuclei, and anterior olfactory structures under- or unevaluated. Our data strongly suggest that it is injudicious to equate incidental or preclinical PD with the absence of somatomotor symptoms and occurrence of LB pathology and/or neuronal or loss in the substantia nigra (Table 3, cases 44, 71, 79, 80).

Recent PET-based studies estimate a relatively short subclinical phase of between 3 and 7 yr duration for PD (81, 82), in the course of which it is not unreasonable to

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F: Multiple LBs emerge in clusters (arrows) within aggregations of neuromelanin granules of a projection cell in the cs (case 23). G, H: Lesions are found in anterior olfactory structures, i.e. the olfactory bulb (olb), tract (otr), and/or anterior olfactory nucleus (aon) of 16 cases (Table 2). Arrowheads in (G) point to LNs in the olb and LBs in the aon are indicated by arrows. A thicket of LNs (arrowheads) interspersed by LBs (arrow), framed in (G) and detailed at higher magnification in (H), is typical of the cellular islands of the aon embedded in the otr (case 19). The fact that no anterior olfactory structures are affected to the exclusion of all other subcortical or cortical sites argues against the anterior olfactory system's being the sole induction site of PD in the brain. Taken together, the lesional distribution pattern of the  $\alpha$ -SN-immunoreactive inclusion bodies studied light microscopically in 30 incidental cases here most closely resembles that seen at the same sites in autopsy brains from clinically manifest PD cases and not the lesional distribution patterns (i.e. different neuronal populations, different predilection sites) that exist in other non-PD  $\alpha$ -synucleinopathies. Scale bar in (B) is valid for (C). Scale bar in (D) applies to (A) and (G).

speculate that some manner of symptoms, whether somatomotor, autonomic, or olfactory in nature, might manifest themselves (36, 43, 68, 83). This, in turn, prompts the question why the “early lead” on the part of the inclusion bodies in lower brainstem nuclei (which clearly are antecedent to the initial lesions developing in substantia nigra) is not reflected electrophysiologically or clinically in the guise of incipient autonomic regulatory and/or early extranigral somatomotor derailments prior to measurable changes in striatal fluorodopa or [<sup>123</sup>I]β-CIT uptake (84, 85), particularly when the lesions within the dorsal IX/X complex and gain setting system begin while the nigrostriatal pathway still is intact (25). Does this imply the existence in PD not only of a differential susceptibility on the part of specific subsets of nerve cells and nuclear grays but also of subclinical phases of varying duration within the involved nuclei of a given functional neuronal system? If so, then perhaps the pathology (or degree of neuronal damage) accruing in the aforementioned autonomic and gain setting centers of the lower brainstem exacts its toll more gradually or imperceptibly than the lesions developing in the substantia nigra. Alternatively, currently available panels of pathophysiological screening tests for PD simply may be too insensitive to detect early signs of autonomic or motor disorders, whose pathoanatomical substrata and clinical manifestations are protean (86).

For the purpose of early clinical evaluation and effective patient management, diagnostic strategies based on the usual screening panels for nigrostriatal somatomotor symptoms associated with PD (87, 88) might be supplemented for persons at risk by tests designed to elicit some constellation of, for lack of a better term, the following “soft” PD signs: 1) symptoms related to dysautonomia (43, 89, 90); 2) signs indicating subtle disruptions within the gain setting nuclei of the lower brainstem (91–93); and 3) quantifiable deficits in olfactory acuity, differentiation, or memory of substances smelled (37, 39, 94–97). Currently, none of the above-mentioned avenues have been adequately explored, let alone exhausted. Given the results of this study, we anticipate that new, relatively simple and cost-effective, diagnostic tools could be developed that might assist clinicians in the early diagnosis and ongoing assessment of PD.

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