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Staging of brain pathology related to sporadic Parkinson's disease

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Abstract

Sporadic Parkinson's disease involves multiple neuronal systems and results from changes developing in a few susceptible types of nerve cells. Essential for neuropathological diagnosis are α-synuclein-immunopositive Lewy neurites and Lewy bodies. The pathological process targets specific induction sites: lesions initially occur in the dorsal motor nucleus of the glossopharyngeal and vagal nerves and anterior olfactory nucleus. Thereafter, less vulnerable nuclear grays and cortical areas gradually become affected. The disease process in the brain stem pursues an ascending course with little interindividual variation. The pathology in the anterior olfactory nucleus makes fewer incursions into related areas than that developing in the brain stem. Cortical involvement ensues, beginning with the anteromedial temporal mesocortex. From there, the neocortex succumbs, commencing with high order sensory association and prefrontal areas. First order sensory association/premotor areas and primary sensory/motor fields then follow suit. This study traces the course of the pathology in incidental and symptomatic Parkinson cases proposing a staging procedure based upon the readily recognizable topographical extent of the lesions.

Keywords: Parkinson's disease; Staging procedure; α-synuclein; Lewy bodies; Lewy neurites; Limbic system; Motor system

1. Introduction

Sporadic Parkinson's disease (PD) is a progressive degenerative illness of the human nervous system that manifests itself clinically after the pathology already has reached an advanced stage [31,32,51]. A prerequisite for the postmortem diagnosis of both the presymptomatic and symptomatic phases of the pathological process underlying PD is evidence of specific inclusion bodies, which develop as spindle- or thread-like Lewy neurites (LNs) in cellular processes, and in the form of globular Lewy bodies (LBs) in neuronal perikarya [33,53,54,60]. In sporadic PD, only a few specific types of nerve cells are prone to develop the lesions. A major component of LNs and LBs is an aggregated form of the normally presynaptic protein α-synuclein. It is still unknown why this hydrophilic protein leaves its binding sites within synaptic boutons and, together with other components such as phosphorylated neurofilaments and ubiquitin, a heat shock protein required for the non-lysosomal ATP-dependent breakdown of abnormal proteins, gradually transforms into virtually insoluble LNs or LBs [1,2,15,25,29,41,49,67,70].

Damage to specific subnuclei of the substantia nigra, pars compacta, with severe obliteration of their neuromelanin-laden projection neurons, frequently is considered to be the most important hallmark of PD [20,31,38,39,43]. The nigral damage, however, always is accompanied by extensive extranigral pathology, including that in the dorsal motor nucleus of the glossopharyngeal and vagal nerves (i.e. dorsal IX/X motor nucleus) and adjoining intermediate reticular zone, in some subnuclei of the reticular formation and the raphe system, the coeruleus–subcoeruleus complex, the magnocellular nuclei of the basal forebrain, and many subnuclei of the thalamus and amygdala. Cases with severe damage usually show lesions reaching the neocortex [10,13,22,23,50,53,63].

The question arises as to whether the pathology evolves simultaneously at all of these nigral and extranigral induction sites or whether the various sites differ in their susceptibilities to develop the disease-related alterations and, accordingly, follow a coherent sequence. The present study, therefore, intentionally includes a spectrum of cases exhibiting LNs and LBs in a specific subset of neuronal

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types and predilection sites, which are known to be involved in clinical PD cases. In doing so, we assume it to be correct that nonsymptomatic and symptomatic cases can be ordered in such a manner that cases exhibiting the mildest pathology represent the starting point and those most heavily involved the terminus of a disease spectrum, with a tendency toward increasing severity on the part of the overall pathology (Table 2). According to this assumption, the neuronal damage does not develop randomly but, rather, follows a predetermined sequence marked by characteristic changes in topographical extent. The present study is aimed at working out a neuropathological staging procedure based upon the topography of these changes. It is not our intent here to correlate the proposed neuropathological stages with clinical symptoms. Furthermore, we would like to emphasize that the study sample does not include cases clinically diagnosed as diffuse LB disease. Likewise, we did not detail study cases which were neuropathologically classified as fully-developed Alzheimer's disease (AD) with co-occurring LBs and LNs in prosencephalic areas. It remains to be seen whether deviations from the proposed staging scheme exist in cases of advanced AD with LBs or in cases of clinically assessed diffuse LB disease.

2. Materials and methods

Three groups of cases were studied. The first group consisted of brains obtained at autopsy from 41 individuals with clinical diagnoses of PD (19 females, 22 males, aged 75.7 ± 7.2 years, Table 2). The clinical protocols of these cases noted the predominance of either tremor or rigidity combined with hypokinesia and postural instability. The brain tissue exhibited nigral LBs and severe loss of nigral neuromelanin-laden neurons [16,30,37,62] (Fig. 2).

The second group included autopsy brains from 69 individuals. In most of these cases, the clinical records made no reference to PD-associated symptoms. A few cases with severe pathology were misdiagnosed or did not receive a clinical diagnosis (Table 2). All 69 cases showed the presence of LNs and/or LBs in a subset of neuronal types at the aforementioned predilection sites (35 females, 34 males, aged 76.1 ± 7.9 years). Cases with comparably mild alterations are referred to here as incidental cases. They were detected by screening 413 autopsy cases sent to the Institute from several general hospitals. Cases from specialized clinics for neurological or psychiatric diseases were excluded from the screening procedure [59]. None of the incidental cases found in the material was secondarily excluded.

The third group, which included 58 age- and gender-matched cases (25 females, 33 males, aged 75.9 ± 8.2 years, data from individual cases not shown), was used for comparison. The previous medical histories of these cases did not include a record of neurological or psychiatric disease. None of these cases contained LB/LNs in the dorsal IX/X motor nucleus. The same sets of tissue blocks were taken and the same staining procedures performed as those applied to the incidental cases, chiefly to exclude the possibility that cases develop the first LNs/LBs at induction sites other than those described in this study.

The severity of co-occurring AD-related pathology was classified according to a procedure permitting differentiation of stages I–VI in the development of neurofibrillary changes and stages A–C in the evolution of β-amyloid deposits (Table 2) [6,48].

The brains were fixed by immersion in a 4% aqueous solution of formaldehyde. The 33 cases (Table 2, indicated by "4" under "mat") were processed in the following manner: the brain stems were severed at the border between the pontine tegmentum and mesencephalic tegmentum. The hemispheres then were divided midsagittally, and one hemisphere from each case was embedded in polyethylene glycol (PEG 1000) [7] and sectioned perpendicular to the intercommissural (Forel's) axis into uninterrupted series of 100 μm thick free-floating sections. The brain stems were similarly processed but cut perpendicular to the brain stem (Meynert's) axis.

The incidental cases with LNs and LBs were processed differently. The brain stems of some were severed from the hemispheres at the latitude of the mamillary bodies, thus including the substantia nigra in its entirety. These brain stems were processed as described earlier, and additional blocks of brain tissue were dissected from one of the hemispheres, usually cut in the frontal plane. In each case, the blocks included (1) a portion of the magnocellular nuclei of the basal forebrain (usually part of nucleus of the diagonal band) as well as adjoining portions of the amygdala, (2) uncal portions of the hippocampal formation, the entorhinal region, the anteromedial temporal mesocortex together with the adjoining neocortex usually extending up to the first temporal convolution, (3) the hippocampal formation at the level of the lateral geniculate body, (4) an expanse of tissue from the agranular to granular insular cortex, (5) the anterior cingulate proneocortex and adjoining frontal neocortex, and (6) the olfactory bulb, tract, and/or anterior olfactory nucleus.

The brain stems of all of the other cases (marked by "1" under "mat" in Table 2) were cut into slices of about 3 mm thickness. Selected sections from a subset of these slices included (1) the dorsal IX/X motor nucleus and adjoining intermediate reticular zone, (2) the gigantocellular reticular nucleus and nucleus raphes magnus, (3) the coeruleus–subcoeruleus complex, and (4) the posterior subnuclei of the substantia nigra. These brain stem blocks were supplemented by the full number of the above-listed prosencephalic blocks, which were removed from one of the hemispheres. The free-floating 100 μm thick sections were processed using various staining methods.

For topographical orientation, sections were stained for lipofuscin pigment (aldehyde-fuchsin) as well as for Nissl material (Darrow red). Aldehyde-fuchsin staining was employed because pigmentation properties can be utilized to

distinguish the different nerve cell types in the brain of the human adult [4].

PD-related lesions were visualized by means of immunoreactions for α-synuclein. The sections were pretreated according to a standard protocol designed to inhibit endogenous peroxidase and prevent nonspecific binding. This was followed by standard pretreatment with formic acid prior to incubation for 18 h in the affinity-purified α-synuclein antiserum (AFshp) at a dilution of 1:2000–4000 [63]. The antiserum was generated by W.P. Gai (Flinders Medical Centre, Australia) in sheep using a peptide corresponding to the amino acid residues 116–131 of the human α-synuclein [34]. Subsequent to processing with biotinylated secondary antibodies (anti-mouse IgG for 2 h), reactions were visualized with the ABC complex (Vectastain) and diaminobenzidine (Sigma). Omission of the primary antiserum resulted in nonstaining. Inasmuch as the α-synuclein antibody labels both normal and abnormal forms of the protein, moderately immunopositive punctae correspond to the normal protein in synaptic boutons, whereas intensely immunoreactive material represents the pathological aggregates.

Immunostained sections were partially counterstained for lipofuscin deposits and Nissl material to permit recognition of immunopositive material in particular types of nerve cells or to confirm the location of the immunopositive material within specific architectonic units. The severity of the PD-related inclusion body pathology was assessed semiquantitatively (− = absent or not discernible, + = slight, ++ = moderate, +++ = severe), although the staging procedure proposed here does not require evaluation of lesional density (Table 2).

Other sections were silver-stained using advanced methods exploiting physical development of nucleation sites. The Campbell–Switzer silver-pyridine technique was applied for detection of LBs and LNs as well as AD-associated amyloid deposits and neuromelanin [3,65]. The Gallyas silver-iodide method was used to visualize AD-related neurofibrillary pathology [7]. All of the sections were cleared and mounted in a synthetic resin (Permount, Fisher).

The χ^2-test modified by Cochran was used to determine whether a proposed stage in the evolution of the PD-related inclusion body pathology differs from a preceding one by virtue of a significant shift, between stages, in the frequency with which given brain structures, regarded here as characteristically affected at that stage, are involved [17].

3. Results

The brains of all of the incidental cases and of individuals with clinically overt sporadic PD display the presence of α-synuclein-immunoreactive LNs and LBs while at the same time being free of intracytoplasmic inclusions related to non-PD α-synucleinopathies [35,41]. The concomitant AD-related pathology lies within the expected range of the respective age groups [9].

LBs usually are present as spherical or reniform, weakly acidophilic inclusion bodies with smooth surfaces, varying in shape and size. Single LBs or groups of them typically are located within the deposits of lipofuscin or neuromelanin granules of the involved nerve cells and normally are not seen among the patches of Nissl substance. At times, ill-defined and weakly immunopositive “pale bodies” [18,40,49] appear between the pigment deposit and the cell nucleus or adjacent to a LB (Fig. 1a, b and f). Thick LNs are club- or corkscrew-shaped (Fig. 1c and f). Others are short and stubby or long and thread-like (Fig. 1d). LNs can attain a varicose appearance, and both the thread-like and more voluminous types of LNs tend to bifurcate repeatedly, often terminating in teardrop-like enlargements (Fig. 1e).

The severity of the PD-related pathology varies among cases and ranges from a single LN (+) in the dorsal IX/X motor nucleus to extremely high densities of inclusion bodies (+++) at multiple sites, including the cerebral cortex (Table 2). The disease-related destruction focuses upon distinct neuronal types within particular nuclear grays as well as specific cortical areas and layers. The susceptible nerve cell types show systematic differences with respect to the degree to which they are inclined to develop LNs/LBs (Fig. 2).

The most mildly affected cases usually display lesions in the dorsal IX/X motor nucleus and/or intermediate reticular zone and, frequently, in the anterior olfactory nucleus [58] (Table 2). There is no evidence in our material for involvement of the cerebral cortex in the absence of lesions in the brain stem (Tables 1 and 2). The brain stem lesions in mildly involved cases consistently remain confined to the medulla oblongata and pontine tegmentum (Table 2, cases 1–34; Table 1, stages 1–2). Cases with moderate involvement (Table 2, cases 35–82; Table 1, stages 3–4) exhibit additional lesions in select mesencephalic and prosencephalic nuclei, and all cases with severe pathology (most of them with clinically diagnosed PD, Table 2, cases 83–110; Table 1, stages 5–6) show additional deterioration of the neocortex.

The data summarized in Tables 1 and 2 indicate that the brain pathology begins in the dorsal IX/X motor nucleus and/or adjoining intermediate reticular zone, from there reaching additional lower brain stem nuclei while assuming an essentially upward course and eventually extending into the cerebral cortex (Fig. 4).

3.1. Stages 1 and 2: involvement virtually confined to the medulla oblongata

The least affected brains display PD-related inclusion bodies only within the spindle-shaped projection neurons of the dorsal IX/X motor nucleus and, in some instances, in projection cells of the intermediate reticular zone (stage 1: Fig. 1i) [23a]. The pathology thus is confined to only a small stripe of gray matter extending from the ala cinerea underneath the ependymal lining of the fourth ventricle to the ventrolateral surface of the lower brain stem between

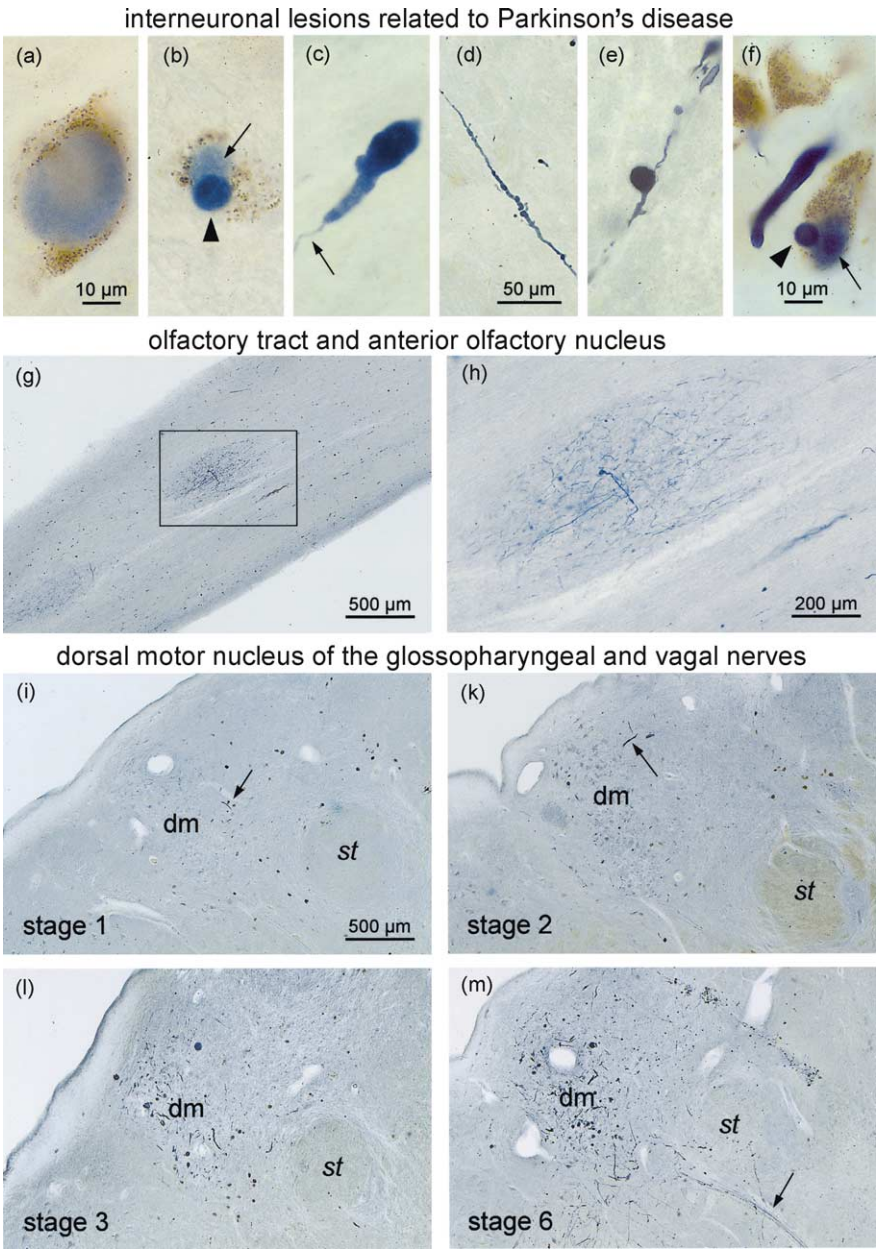


Fig. 1. PD-related brain lesions. Intraneuronal lesions in the form of (a) a large pale body (case 37), (b and f) a combination of a pale body (arrows) and a small LB (arrowheads) in melanized projection cells of the substantia nigra (cases 50 and 63). (c) A thread-like LN (arrow) terminating in a club-shaped enlargement (case 88), (d) a filiform LN decorated with small spine-like appendages (case 88) with or (e) a large globular swelling (case 88), as well as (f) coarse plump LNs (case 63). Bar in (a) is valid for (b and c), bar in (d) is also valid for (e). The olfactory tract contains islands of the anterior olfactory nucleus (g, h). One of these islands, framed in (g), is shown at higher magnification in (h). Typically, the anterior olfactory nucleus develops a dense net of thin LNs dotted with numerous small LBs (case 76). The PD-related brain stem pathology begins in the dorsal IX/X motor nucleus (i–m). (i) Initially, a few LNs appear in this nuclear gray (arrow, stage 1, case 7). (k) With further disease progression, the number of LNs increases (arrow, stage 2, case 32) followed by (l) the appearance of many LBs (stage 3, case 44). (m) The severity of the lesions increases until the end stage of the disease (stage 6, case 109). Note the axonal LNs (arrow). List of abbreviations: dm, dorsal IX/X motor nucleus; *st*, solitary tract. Bar in (i) is also valid for (k–m). α-synuclein immunoreactions, 100 µm thick PEG-sections.

the dorsolateral sulcus and the upper boundary of the olive. Typically, the first observable changes are LNs (Fig. 1i and k). In more advanced cases, LNs initially outnumber the small and inconspicuous LBs. In the brain of the human adult, the vulnerable visceromotor projection neurons of the dorsal IX/X motor nucleus contain large amounts of lipofuscin granules. The numerous nerve cells containing neuromelanin and scattered throughout the predilection zone outlined above (dark spots in Fig. 1i) do not show PD-related alterations at stage 1. Likewise, the small neurons

Table 1
Stages in the evolution of PD-related pathology

Stage 1 $N = 21$; medulla oblongata	Lesions in the dorsal IX/X motor nucleus and/or intermediate reticular zone
Stage 2 $N = 13$; medulla oblongata and pontine tegmentum	Pathology of stage 1 plus lesions in caudal raphe nuclei, gigantocellular reticular nucleus, and coeruleus–subcoeruleus complex
Stage 3 $N = 24$; midbrain	Pathology of stage 2 plus midbrain lesions, in particular in the pars compacta of the substantia nigra
Stage 4 $N = 24$; basal prosencephalon and mesocortex	Pathology of stage 3 plus prosencephalic lesions. Cortical involvement is confined to the temporal mesocortex (transentorhinal region) and allocortex (CA2-plexus). The neocortex is unaffected
Stage 5 $N = 17$; neocortex	Pathology of stage 4 plus lesions in high order sensory association areas of the neocortex and prefrontal neocortex
Stage 6 $N = 11$; neocortex	Pathology of stage 5 plus lesions in first order sensory association areas of the neocortex and premotor areas, occasionally mild changes in primary sensory areas and the primary motor field

of the subnuclei surrounding the solitary tract as well as those of the area postrema and the subnucleus gelatinosus remain minimally involved.

A more accentuated affection of both the dorsal IX/X motor nucleus and the intermediate reticular zone is observed at stage 2 (Fig. 1k). Its key feature, however, is the first appearance of LNs and LBs in the lipofuscin-laden projection neurons of the caudal raphe nuclei (nucleus raphes magnus, obscurus, pallidus) and the reticular formation ($P < 0.01$). The gigantocellular reticular nucleus bears the brunt of the pathology developing within the reticular formation [13]. The projection neurons of the coeruleus–subcoeruleus complex are the first neuromelanin-laden nerve cells in the brain to develop LNs and LBs [23a] (Fig. 2a). Once again, LNs antedate the appearance of LBs. Small nerve cells found within the coeruleus–subcoeruleus complex that display features different from those of the neuromelanin-laden neurons remain exempt from the formation of LNs and LBs. Most notably, nuclear grays of the mesencephalon, in particular the substantia nigra, remain uninvolved during stages 1 and 2 (Tables 1 and 2).

3.2. Stages 3 and 4: involvement chiefly confined to the lower and upper brain stem in the absence of cortical lesions (stage 3) or with initial affection of the anteromedial temporal mesocortex (stage 4)

The severity of the previously described lesions gradually increases during the next stages (Figs. 1l, 2b and c). Melanin-laden nerve cells of the dorsal IX/X motor nucleus and of the intermediate zone begin to develop LBs in stages 3 or 4. The principal characteristics of stage 3, however, are the affection of a subset of melano-neurons in the substantia nigra ($P < 0.01$; Fig. 2d–f) and the involvement of lipofuscin-laden projection neurons in the magnocellular nuclei of the basal forebrain ($P < 0.01$; Fig. 2k), both of which occur in the absence of lesions in non-olfactory cortical areas (Table 2). Notably, there is no indication of macroscopically detectable depigmentation of the substantia nigra at stage 3, and the damage there initially is restricted to melanized projection neurons of the posterolateral and posteromedial subnuclei [5]. The very considerable number of long LNs within the reaches of the vulnerable nigral subnuclei precede the development of LBs in melano-neurons, whereas nonmelanized nerve cells (small local circuit neurons and ectopic cells of the reticular portion) within the area of destruction refrain from developing LNs/LBs. The melanized neurons usually develop LBs within the boundaries of their neuromelanin accumulations, and, not infrequently, a group of small LBs occurs within a single perikaryon.

Extranigral melano-neurons in the mesencephalic tegmentum still display no signs of the inclusion body pathology at stage 3. Other mesencephalic predilection sites, such as the compact portion of the pedunculopontine tegmental nucleus, commence developing a network of lengthy LNs. The same holds true for the magnocellular nuclei of the basal forebrain (medial septal nucleus, interstitial nucleus of the diagonal band, basal nucleus of Meynert, Fig. 2k). At the same time, the hypothalamic tuberomamillary nucleus accumulates large numbers of globular inclusion bodies.

In order to assign cases to stage 3, it is important that neither components of the anteromedial temporal mesocortex (i.e. the periallocortical transentorhinal region and the proneocortical ectorhinal region) nor any areas of the mature neocortex be affected (Fig. 3e). By contrast, the cortical and subcortical regions joined to the anterior olfactory nucleus generally show mild involvement. The plexus of long LNs extending throughout the second sector of the Ammon's horn [26,27] generally begins developing at stage 3 (Fig. 3a).

At stage 4, marked devastation of the melano-neurons in the vulnerable subnuclei of the substantia nigra is seen (Fig. 2g). Comparison with control cases reveals a significant loss of neurons, especially in the posterior regions of the pars compacta. In some instances, the local depigmentation

Table 2
Key features in the evolution of PD-related pathology

case	mat	*A*	G	nfp	aβ	PD	clin	ol	dm	rm	co	sn	db	CA2	mc	hc	fc
1	1	78	M	V	C	1	–	–	+	–	–	–	–	–	–	–	–
2	2	54	F	I	0	1	–	–	+	–	–	–	–	–	–	–	–
3	1	77	F	I	0	1	–	n.e.	+	–	–	–	–	–	–	–	–
4	1	81	M	III	B	1	–	–	+	–	–	–	–	–	–	–	–
5	1	85	F	I	0	1	–	–	+	–	–	–	–	–	–	–	–
6	1	61	M	II	0	1	–	–	+	–	–	–	–	–	–	–	–
7	2	58	M	I	0	1	–	–	+	–	–	–	–	–	–	–	–
8	2	69	M	II	0	1	–	+	+	–	–	–	–	–	–	–	–
9	1	89	F	III	C	1	–	+	+	–	–	+	–	–	–	–	–
10	1	70	F	II	B	1	–	–	+	–	–	–	–	–	–	–	–
11	1	86	M	I	B	1	–	–	+	–	–	–	–	–	–	–	–
12	1	72	M	I	0	1	–	+	+	–	–	–	–	–	–	–	–
13	4	81	M	I	0	1	–	–	+	–	–	–	–	–	–	–	–
14	2	70	F	I	A	1	–	–	+	–	–	–	–	–	–	–	–
15	1	75	M	I	0	1	–	–	+	–	–	–	–	–	–	–	–
16	1	77	M	II	A	1	–	+	+	–	–	–	–	–	–	–	–
17	1	78	F	II	C	1	–	++	+	–	–	–	–	–	–	–	–
18	1	85	M	I	B	1	–	++	+	–	–	–	–	–	–	–	–
19	1	76	M	III	C	1	–	+	++	–	–	n.e.	–	–	–	–	–
20	1	75	F	II	A	1	–	–	++	–	–	–	–	–	–	–	–
21	4	80	F	I	0	1	–	+	++	–	–	–	–	–	–	–	–
22	1	77	M	II	A	2	–	++	+	+	+	–	–	–	–	–	–
23	2	69	F	III	0	2	–	–	+	+	+	–	–	–	–	–	–
24	1	85	F	II	0	2	–	++	+	+	+	–	–	–	–	–	–
25	1	75	F	III	A	2	–	++	+	+	+	–	–	–	–	–	–
26	1	74	F	III	0	2	–	n.e.	+	+	+	n.e.	–	–	–	–	–
27	1	83	F	I	B	2	–	++	+	+	+	–	–	–	–	–	–
28	1	85	F	II	B	2	–	+	++	+	–	–	–	–	–	–	–
29	3	85	M	I	0	2	–	–	++	+	+	–	–	–	–	–	–
30	1	67	M	II	A	2	–	++	++	+	+	–	–	–	–	–	–
31	1	71	F	II	B	2	–	n.e.	++	+	+	–	–	–	–	–	–
32	1	79	F	II	B	2	–	+	++	+	+	–	–	–	–	–	–
33	2	77	M	II	0	2	–	+	++	+	+	–	–	–	–	–	–
34	1	81	M	III	A	2	–	++	++	+	+	–	–	–	–	–	–
35	1	94	F	III	A	3	–	+++	++	++	++	+	+	–	–	–	–
36	1	77	M	0	A	3	–	++	++	+	+	+	+	+	–	–	–
37	1	83	F	II	B	3	–	n.e.	++	+	+	+	+	–	–	–	–
38	1	77	M	I	A	3	–	n.e.	++	+	+	+	+	–	–	–	–
39	1	64	F	II	0	3	–	++	+	++	+	+	+	–	–	–	–
40	1	80	F	III	B	3	–	+++	+	+	+	+	n.e.	–	–	–	–
41	4	86	F	III	A	3	–	++	+	+	+	+	+	–	–	–	–
42	1	73	F	III	B	3	PD	++	++	n.e.	n.e.	+	+	–	–	–	–
43	1	71	F	I	B	3	–	++	+	++	+	+	+	–	–	–	–
44	1	83	M	II	0	3	–	++	+++	++	+	+	+	–	–	–	–
45	1	71	F	III	B	3	–	++	++	++	+	+	+	–	–	–	–
46	1	83	M	I	0	3	–	+++	+	n.e.	+	++	n.e.	–	–	–	–
47	1	77	M	I	B	3	–	++	+	+	+	+	+	–	–	–	–
48	1	71	F	I	B	3	–	++	+++	++	++	+	+	–	–	–	–
49	1	71	M	II	A	3	–	+++	+++	++	+++	+	+	–	–	–	–
50	1	80	M	II	B	3	–	+++	++	++	++	+	++	–	–	–	–
51	1	73	F	II	B	3	–	++	+	++	+	+	+	–	–	–	–
52	1	88	M	0	A	3	–	++	++	++	++	+++	++	–	–	–	–
53	1	78	M	III	0	3	–	++	++	++	+++	+	n.e.	–	–	–	–
54	1	78	M	I	0	3	–	n.e.	++	++	++	+	++	+	–	–	–
55	1	72	M	II	0	3	PD	++	+	+	++	+++	++	+	–	–	–
56	1	73	M	I	A	3	–	+++	+	++	++	+	+++	+	–	–	–
57	1	75	M	I	0	3	–	+++	+++	++	++	+++	+++	+	–	–	–
58	4	75	M	II	0	3	PDV	++	+++	++	++	++	+	+++	–	–	–
59	1	72	M	II	0	4	–	++	++	+	++	+++	++	+	+	–	–
60	4	68	F	I	B	4	PD	++	+++	++	++	+++	n.e.	+	+	–	–
61	1	80	M	I	0	4	–	+++	+++	++	+++	++	+	+	+	–	–
62	1	78	F	II	0	4	–	+++	+++	++	+++	++	+	+	+	–	–

Table 1 (*Continued*)

case	mat	*A*	G	nfp	aβ	PD	clin	ol	dm	rm	co	sn	db	CA2	mc	hc	fc
63	4	90	F	III	0	4	–	+++	+++	+++	++	++	+	+	+	–	–
64	4	85	M	II	0	4	PDIV	++	+++	+++	+++	+++	++	+	+	–	–
65	4	86	F	II	A	4	PDV	++	+++	++	+++	+++	++	+	+	–	–
66	1	87	M	III	B	4	PD	n.e.	+++	++++	+++	+++	+++	+	+	–	–
67	1	78	M	II	A	4	–	+++	+++	+++	+++	+++	++	+	+	–	–
68	1	75	F	I	0	4	–	+++	+++	+++	+++	+++	++	+	+	–	–
69	4	86	M	II	B	4	PD	++	+++	++	++	++	+++	+	+	–	–
70	1	78	F	II	B	4	–	+++	+++	+++	+++	+++	++	+	+	–	–
71	3	75	F	II	C	4	PDIV	++	n.e.	n.e.	n.e.	+++	++	++	+	–	–
72	4	64	F	I	B	4	PDIV	+	++	++	+++	++	++	+	+	–	–
73	4	68	M	I	B	4	PDV	+++	+++	++	+++	+++	+	++	+	–	–
74	4	76	M	II	A	4	PDV	n.e.	+++	+++	+++	+++	++	+	+	–	–
75	4	76	M	II	0	4	PD	+++	+++	+++	+++	+++	+++	+	+	–	–
76	1	68	F	I	0	4	–	+++	+++	+++	+++	++	+++	+	+	–	–
77	4	69	F	I	A	4	PDIV	n.e.	++	++	+++	+++	++	+	+	–	–
78	1	56	F	I	A	4	–	++	+++	+++	+++	++	+++	+	+	–	–
79	4	84	M	II	0	4	PD	++	+++	++	+++	+++	++	+	+	–	–
80	1	78	F	II	B	4	–	+++	+++	+++	+++	+++	+++	+	++	–	–
81	4	68	M	III	C	4	PDIV	+++	+++	+++	+++	+++	+++	++	++	–	–
82	4	82	F	IV	B	4	PDV	n.e.	+++	++	+++	+++	+++	++	+	–	–
83	1	65	M	II	0	5	PD	++	n.e.	++	+++	+++	+++	+++	++	+	–
84	1	64	F	II	A	5	PD	n.e.	n.e.	n.e.	+++	+++	+++	+	++	+	–
85	1	81	M	III	A	5	PD	n.e.	n.e.	++	+++	+++	++	+	++	+	–
86	4	68	F	I	B	5	PDV	+++	+++	++	+++	+++	+++	++	++	+	–
87	4	81	M	II	0	5	PDIII	+++	+++	++	+++	+++	+++	+	++	+	–
88	3	76	M	I	A	5	–	++	+++	++	+++	++	++	+	++	+	–
89	4	80	M	II	A	5	PDV	++	+++	+++	+++	+++	+++	++	++	+	–
90	2	61	M	II	A	5	n.e.	+++	++	++	+++	+++	n.e.	+	++	+	–
91	4	82	M	III	A	5	PDIV	n.e.	+++	+++	+++	+++	+++	+	++	+	–
92	3	78	F	II	C	5	PDIV	+++	n.e.	n.e.	n.e.	+++	+++	+	++	+	–
93	3	79	M	II	C	5	PDIV	+++	n.e.	n.e.	n.e.	+++	+++	+	++	+	–
94	4	77	M	II	B	5	PDV	+++	+++	++	+++	+++	+++	+++	++	+	–
95	4	62	F	I	B	5	PDV	+++	+++	++	++	+++	+++	+++	++	+	–
96	4	61	M	I	0	5	PDIII	+++	+++	++	+++	+++	+++	+	++	+	–
97	4	80	F	II	C	5	PDV	+++	+++	++	+++	+++	+++	+	++	+	–
98	4	68	F	II	B	5	PDIII	+++	+++	+++	+++	+++	+++	+++	++	+	–
99	4	71	F	II	B	5	PDV	+++	+++	++	+++	+++	++	++	++	+	–
100	4	86	f	III	C	6	PDV	+++	+++	+++	+++	+++	+++	++	+++	++	+
101	4	76	M	III	C	6	PDV	+++	+++	++	n.e.	+++	+++	+++	+++	++	+
102	1	77	M	V	C	6	PD	n.e.	+++	++	n.e.	+++	+++	++	+++	++	+
103	2	84	F	III	B	6	PD	+++	++	++	+++	+++	n.e.	+++	+++	++	+
104	3	82	F	III	B	6	PDV	+++	n.e.	n.e.	n.e.	+++	+++	+++	+++	++	+
105	1	83	F	III	C	6	AD	n.e.	+++	n.e.	+++	+++	+++	n.e.	+++	++	+
106	4	77	M	II	B	6	PDIV	+++	+++	++	+++	+++	+++	+++	+++	++	+
107	4	78	F	II	C	6	PDIII	+++	++	+++	++	+++	+++	+	+++	++	+
108	4	76	M	I	B	6	PDIII	+++	+++	+++	++	+++	+++	++	+++	++	+
109	1	68	F	III	C	6	–	n.e.	+++	+++	+++	+++	+++	+++	+++	++	+
110	4	77	F	II	A	6	PDV	+++	++	+++	+++	+++	+++	+++	++	++	+

mat: material at disposal (1: a specific set of 10 different subcortical nuclei/cortical areas in blocks, 2: brain stem in serial sections spaced 1 mm apart and supplemented by respective blocks through portions of one hemisphere, 3: hemisphere in serial sections, spaced 1 mm apart and supplemented by respective blocks through the brain stem, 4: serial sections through both hemisphere and brain stem). *A*: age, G: gender, nfp: neurofibrillary pathology, stages I–VI, aβ: stages of cortical β–amyloid deposition A–C (0 = no AD-related lesions), PD: PD stages 1–6, clin: clinical diagnoses (−, no mention of PD symptoms in the clinical protocols, PD I–V: clinically diagnosed cases are indicated by PD and—if available—the Hoehn and Yahr stages I–V [47]). AD: Alzheimer's disease. The following 10 columns list the predisposed induction sites of the PD-related pathology. ol: olfactory bulb, tract and/or anterior olfactory nucleus, dm: dorsal IX/X motor nucleus, rm: nucleus raphes magnus and/or gigantocellular reticular nucleus, co: coeruleus–subcoeruleus complex, sn: posterior portion of substantia nigra [pars compacta], db: interstitial nucleus of the diagonal band and/or basal nucleus of Meynert, CA2: second sector of the Ammon's horn, mc: transentorhinal region and/or ectorhinal region [anteromedial temporal mesocortex], hc: high order sensory association areas and prefrontal areas of the neocortex, fc: first order sensory association areas and premotor areas and/or primary sensory and motor fields of the neocortex. The degree of pathology is assessed semiquantitatively and indicated by: −, absent or not discernible, +, slight; ++, moderate; +++, severe; n.e.: not evaluated.

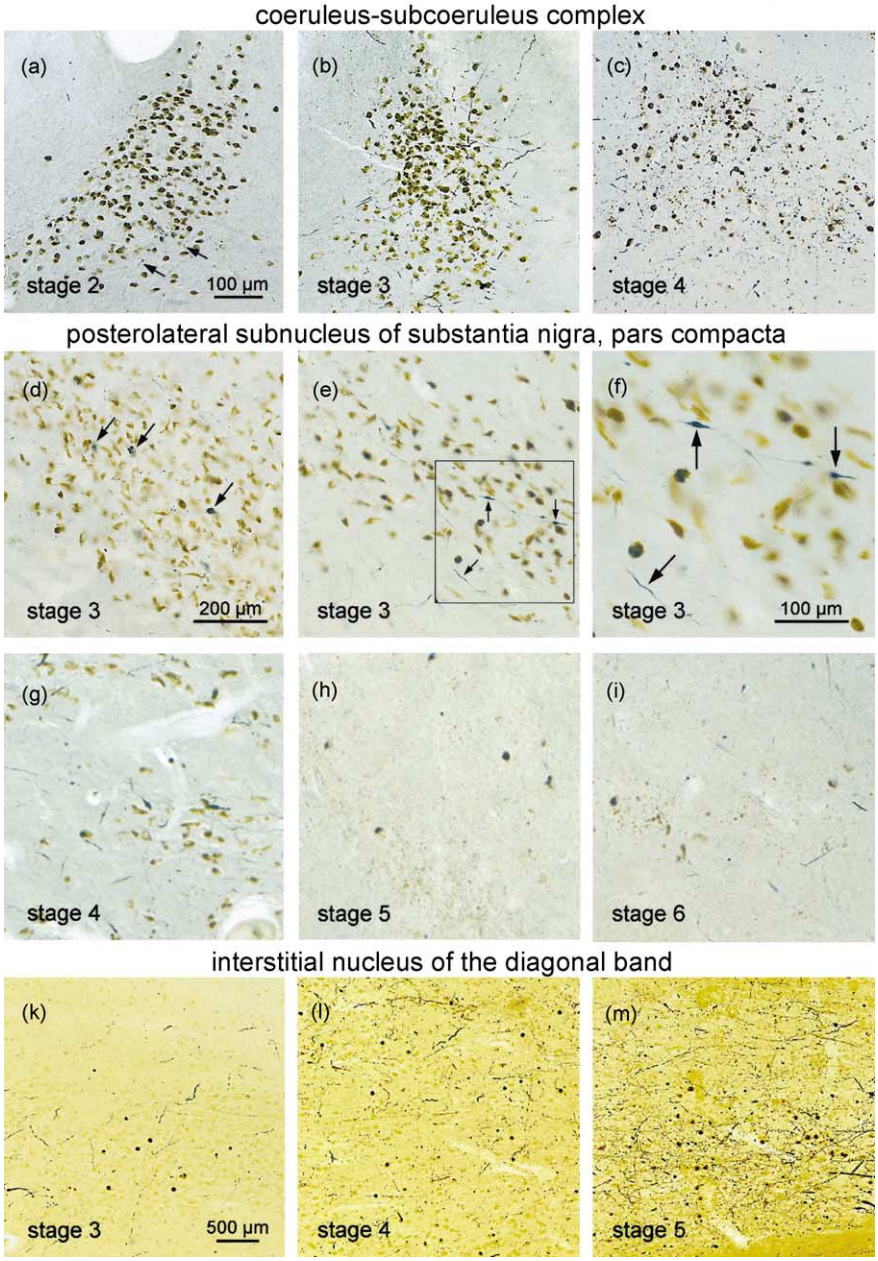


Fig. 2. Development of PD-related brain stem lesions. (a) The coeruleus–subcoeruleus complex is mildly involved at stage 2 (case 33, arrows point to LNs), (b) severely affected at stage 3 (case 56, note the increase in LNs), and (c) severely depleted of melanin-containing projection neurons at stage 4 (case 61). Bar in (a) is valid for (b and c). (d) The posterolateral and, thereafter, the posteromedial subnuclei of the substantia nigra, pars compacta, become first involved at stage 3 (case 56, arrows point to LBs in melanized neurons). Elongated LNs with spindle-shaped enlargements at irregular intervals (arrows) occur in the vulnerable subnuclei (case 53, the framed portion of (e) appears at higher magnification in (f)). (g) Loss of melanized nerve cells is severe at stage 4 (case 63). The vulnerable subnuclei are almost depleted of projection neurons at stages 5–6 (h and i). It is difficult to find surviving LB-containing nerve cells in these portions of the substantia nigra (cases 90 and 103). Bar in (d) is valid for (e) and (g–i). (k) Like the substantia nigra, the interstitial nucleus of the diagonal band begins to develop LNs and LBs at stage 3 (case 51). The density of both LNs and LBs increases in the following stages 4–5 (cases 67 and 89; l–m). Bar in (k) is valid for (l and m). α-synuclein immunoreactions, 100 µm thick PEG-sections.

is pronounced enough to be macroscopically recognizable. The net of nigral LNs appears to be slightly reduced in thickness and there are increased signs of pigment incontinence, i.e. local accumulations of extraneuronal neuromelanin granules. The susceptible subnuclei are severely depleted of melanized projection neurons (Fig. 2g).

The destructive process also encroaches at this stage upon neuromelanin-containing projection neurons of additional mesencephalic nuclear grays, such as the paranigral nucleus and the pigmented parabrachial nucleus. LBs and LNs likewise appear within the lipofuscin-laden projection neurons of the oral raphe nuclei (nucleus raphes linearis,

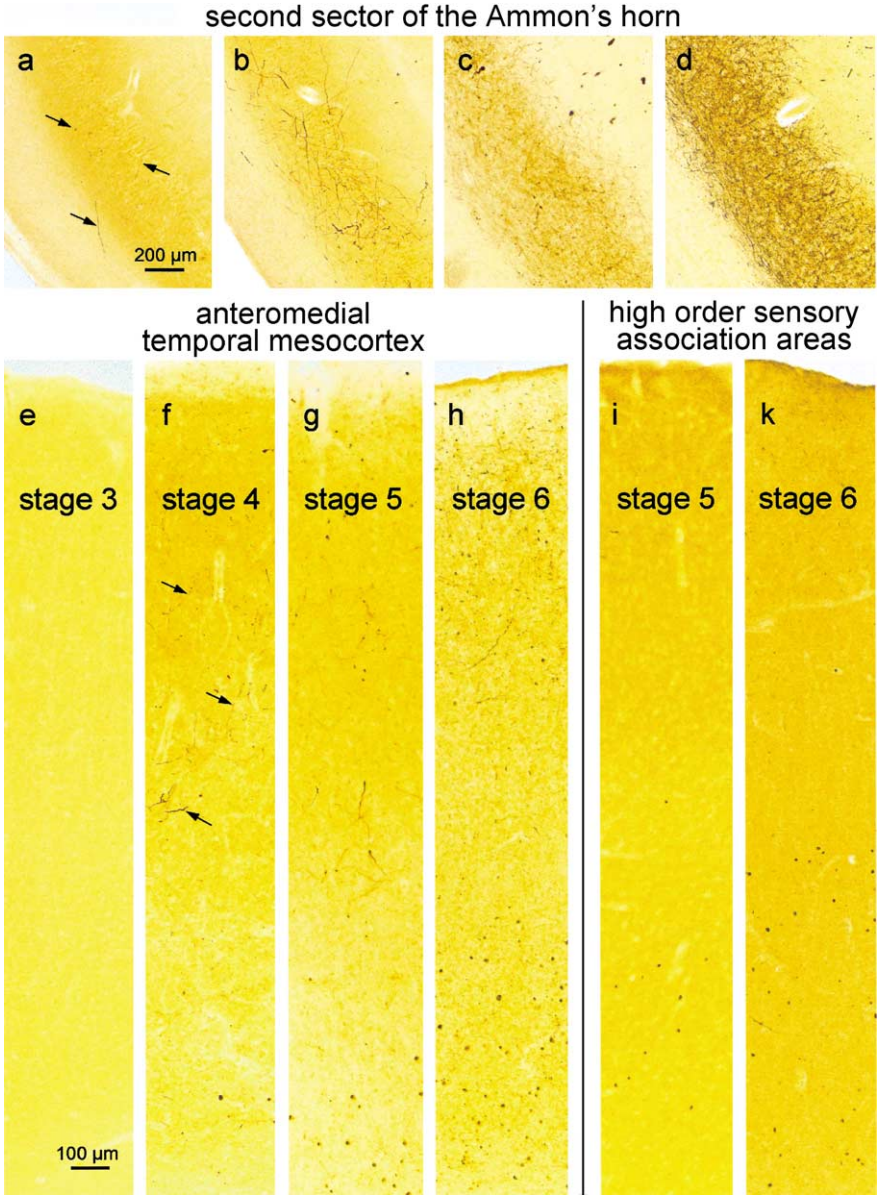
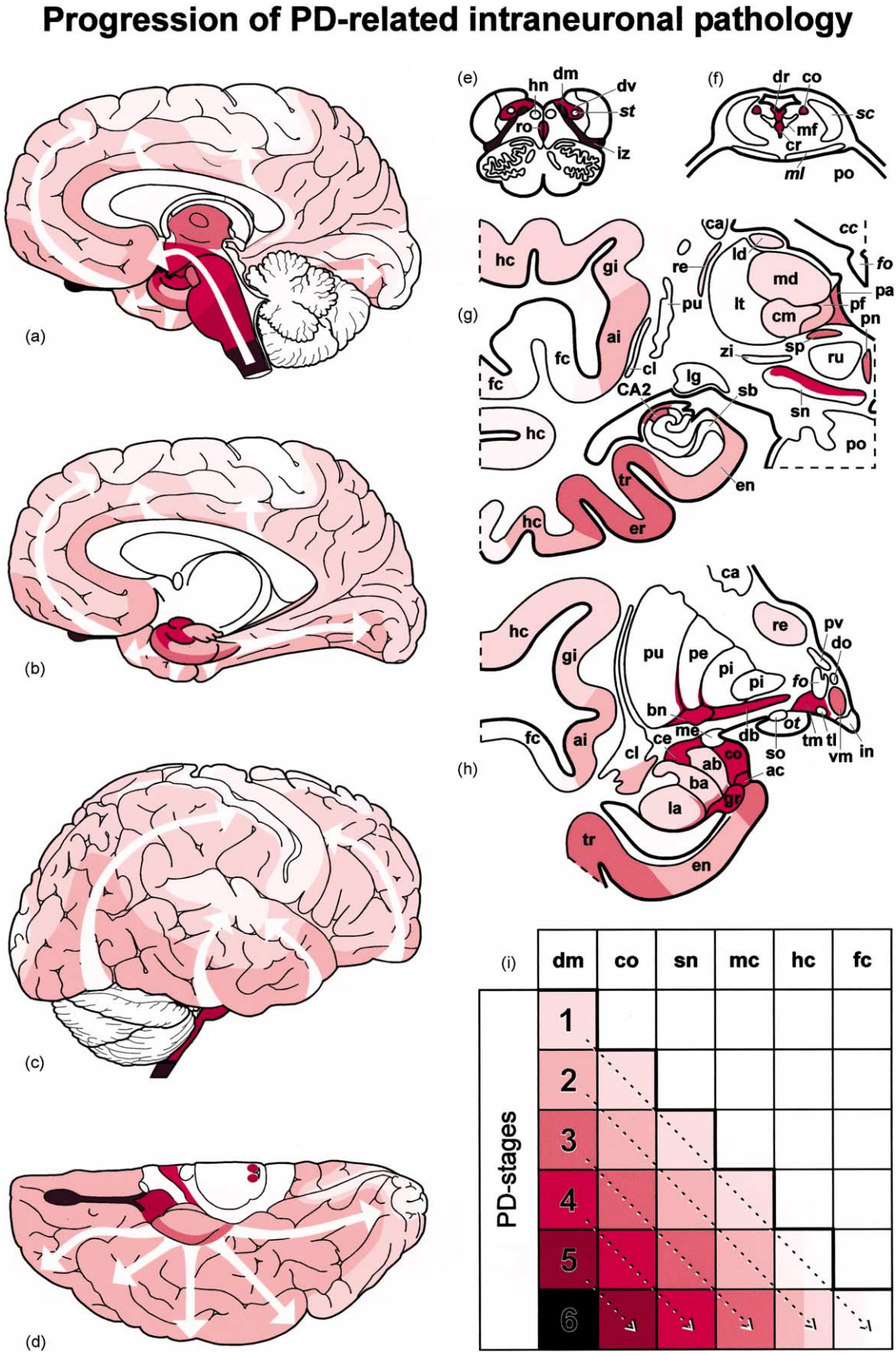


Fig. 3. Development of PD-related cortical lesions. Second sector of the Ammon's horn. (a) The plexus of LNs begins to develop at stage 4 (case 63, arrows point to LNs), (b and c) increases in density (cases 68 and 98), and (d) reaches its maximum at stage 6 (case 106). Bar in (a) also applies to (b–d). Anteromedial temporal mesocortex (e–h). (e) At stage 3, this portion of the cortex is still free of pathology (case 37). (f) The first LNs (arrows) appear in the supragranular layers and LBs occur in the deeper layers at stage 4 (case 68). At stages 5–6, the LB-containing neurons and network of LNs increase even further in density (cases 91 and 101; g and h). High order sensory association areas (here, part of the second temporal gyrus; i and k). In contrast to the anterior temporal mesocortex, the mature neocortex does not develop a thick plexus of LNs in the supragranular layers. The number of projection cells containing LBs increases considerably from (i) stage 5 (case 91) to (k) stage 6 (case 101). The cortical lesions of the final two stages correspond to the neuropathology described as characterizing diffuse LB disease. Bar in (e) also applies for (f–k). α-synuclein immunoreactions, 100 µm thick PEG-sections.

centralis, and dorsalis), whereas, remarkably, the projection neurons virtually devoid of lipofuscin granules and interspersed among the pigment-laden cells of the supratrochlear portion of the dorsal raphe nucleus fail to develop the PD-related pathology. The lipofuscin-rich projection neurons of the compact portion of the pedunculopontine tegmental nucleus are severely affected, and a thick web of long LNs indicates the position of the nucleus. Isolated LB/LNs even appear in portions of the tectum and central gray.

Stage 4 cases show severe involvement of the magnocellular nuclei of the basal forebrain (Fig. 2l), and lesions of similar densities also are encountered in the hypothalamic tuberomamillary nucleus. At the same time, the inclusion bodies develop in the interstitial nucleus of the stria terminalis, the accessory cortical and central nuclei of the


Progression of PD-related intraneuronal pathology
(a)
(b)
(c)
(d)
(e)
dm
hn
dv
st
ro
iz
(f)
dr
co
sc
mf
cr
ml
po
(g)
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CA2
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amygdala, and the ventral claustrum [11]. Similarly, lesions are seen in specific subnuclei of the thalamus [63].

The damage seen in the anterior olfactory nucleus usually is severe at stage 4 (Fig. 1g and h), whereas that seen in cortical areas associated with this nucleus is subject to a relatively marked degree of variability. The conspicuous plexus of LNs in the second sector of the Ammon's horn increases considerably in thickness (Fig. 3b). A recurrent feature of this fourth stage, however, is the development of lesions in the anteromedial temporal mesocortex ($P < 0.01$). The outer cellular layers of this part of the cortex develop a network of filiform LNs while the inner ones show spherical LBs within small to medium-sized pyramidal neurons [69] (Fig. 3f). The nerve cells scattered throughout the white matter below the temporal mesocortex sustain heavy damage (lamina cellularis profunda, [8]). The density of the lesions seen at stage 4 in the anteromedial temporal mesocortex rapidly dwindles when one follows the cortex in the direction of the medially adjoining entorhinal region and laterally adjoining high order sensory association areas of the temporal neocortex (Tables 1 and 2).

3.3. Stages 5 and 6: severe involvement of the brain, including neocortical areas

The degree of damage seen in all of the previously mentioned subcortical and mesocortical structures increases further during stages 5 and 6. The vulnerable portions of the substantia nigra become almost denuded of melanin-laden neurons and, consequently, have an obvious pallor upon macroscopic inspection (Fig. 2h and i). The number of LNs and LBs gradually decreases, whereas that of the extraneuronal neuromelanin aggregations increases. Beyond the reaches of the substantia nigra, a visible loss of melanized neurons within the dorsal IX/X motor nucleus, intermediate reticular zone, reticular formation, and coeruleus–subcoeruleus complex occurs.

At stage 5, the affection of olfactory areas is severe. The network of long LNs present within the second Ammon's horn sector extends into adjoining portions of the first and the third sectors and becomes a conspicuous element of the allocortical pathology (Fig. 3c and, at stage 6, Fig. 3d). The destructive process involving the neocortex takes the temporal mesocortex as its point of departure (Figs. 3g and 4). From there, it makes inroads into the adjoining sensory association areas of the neocortex, agranular and granular insular fields, the anterior cingulate cortex, and prefrontal areas ($P < 0.01$). Large numbers of pyramidal cells containing LBs appear in the infragranular layers of the neocortex, whereas the net of supragranular LNs is much less dense (Fig. 3i and k) than that found in the anterior mesocortex (Fig. 3h). The density of LB-bearing pyramidal cells gradually lessens in the direction of the more distant sensory association areas and prefrontal fields. The first order sensory association and premotor areas as well as the primary sensory and motor areas are free of LNs and LBs at stage 5.

The key feature of stage 6 is the involvement of nearly the entire neocortex (Figs. 3i, k and 4). The premotor areas, the primary motor field, the first order sensory association and primary sensory areas usually are subject to relatively mild pathological changes ($P < 0.01$; Tables 1 and 2, Fig. 4).

Fig. 4. Progression of PD-related intraneuronal pathology. The pathological process targets specific subcortical and cortical induction sites (a–i). (a and e) Lesions initially occur in the dorsal IX/X motor nucleus and frequently (a and d) in the anterior olfactory nucleus as well. Thereafter, less susceptible brain structures gradually become involved (see white arrows). The pathology in the anterior olfactory nucleus expands less readily into related areas than that evolving in the brain stem. The brain stem pathology takes an upward course (see white arrows). (a–d, g–h) Cortical involvement follows, commencing with the anteromedial temporal mesocortex (tr and er in g and h). From there, the neocortex succumbs, beginning with high order sensory association and prefrontal areas. First order sensory association/premotor areas and, thereafter, primary sensory and motor fields follow suit. In (a–h), the gradual decrease in shading intensity is intended to represent the topographical expansion of the lesions during the course of the disease. Simplified diagram (i) showing the topographic expansion of the lesions (from left to right: dm to fc) and, simultaneously, the growing severity on the part of the overall pathology (from top to bottom: stages 1–6). With the addition of further predilection sites, the pathology in the previously involved regions increases. List of abbreviations: ab, accessory basal nucleus of the amygdala; ac, accessory cortical nucleus of the amygdala; ai, agranular and dysgranular insular cortex; ba, basal nucleus of the amygdala; bn, basal nucleus of Meynert; ca, caudate nucleus; CA1, first sector of the Ammon's horn; CA2, second sector of the Ammon's horn; *cc*, corpus callosum; ce, central nucleus of the amygdala; cl, claustrum; cm, centromedian nucleus of the thalamus; co, coeruleus–subcoeruleus complex; cr, nucleus raphes centralis; db, interstitial nucleus of the diagonal band; dm, dorsal motor nucleus of the glossopharyngeal and vagal nerves; do, dorsomedial nucleus of the hypothalamus; dr, nucleus raphes dorsalis; dv, dorsal nuclear complex of the glossopharyngeal and vagal nerves containing melanized projection neurons; en, entorhinal region; er, ectorhinal region (mesocortex); *fo*, fornix; fc, first order sensory association areas, premotor areas, as well as primary sensory and motor fields; gi, granular insular cortex; gr, granular nucleus of the amygdala; hc, high order sensory association areas and prefrontal fields; hn, motor nucleus of the hypoglossal nerve; in, infundibular nucleus of the hypothalamus; iz, intermediate reticular zone; la, lateral nucleus of the amygdala; ld, laterodorsal nucleus of the thalamus; lg, lateral geniculate body of the thalamus; lt, lateral nuclei of the thalamus; me, medial nucleus of the amygdala; *ml*, medial lemniscus; *mf*, medial longitudinal fascicle; mc, anteromedial temporal mesocortex; *ot*, optic tract; pa, paraventricular nucleus of the thalamus; pe, pallidum, external segment; pf, parafascicular nucleus of thalamus; pi, pallidum, internal segment; pn, parabrachial pigmented nucleus; po, pontine nuclei; pu, putamen; pv, paraventricular nucleus of the hypothalamus; re, reticular nucleus of the thalamus; ru, red nucleus; ro, nucleus raphes obscurus; sb, subiculum; sc, superior cerebellar peduncle; sn, substantia nigra; so, supraoptic nucleus; sp, subparafascicular nucleus; *st*, solitary tract; tl, lateral tuberal nucleus of the hypothalamus; tm, tuberomamillary nucleus of the hypothalamus; tr, transentorhinal region (mesocortex); vm, ventromedial nucleus of the hypothalamus; zi, zona incerta.

4. Discussion

The α-synuclein-containing inclusion bodies found in the incidental and symptomatic cases are pathological and usually not considered to be normal concomitants of brain aging [12,24]. On account of their immunocytochemical profile, they hardly can be mistaken for the lesions occurring in other neurodegenerative disorders that do not belong to the group of synucleinopathies. The induction sites and cell types involved in multiple system atrophy or Hallervorden–Spatz disease differ from those affected in PD [35,41]. Accordingly, the inclusion bodies under consideration here are regarded as being indicative of incidental and symptomatic cases of sporadic PD.

The findings of the present study corroborate the assumption that the key lesions in PD begin developing—as in other neurodegenerative diseases—a considerable time prior to the appearance of somato-motor dysfunctions. LNs and LBs of incidental cases show the same morphological characteristics and immunocytochemical profiles, and notably develop in the same subsets of nerve cells and at the same predilection sites as those of the symptomatic cases. The lesional pattern seen in the incidental cases is perfectly complemented by that displayed in fully-developed PD cases (Table 2). Only the limited topographical extent and comparably mild degree on the part of the lesions distinguish incidental cases from those with clinically manifest PD.

The vulnerable nerve cell types most likely vary in their proclivities to undergo the pathological changes and, as such, become involved at different times in the course of the disease. The evolving topographical progression of the lesions readily can be used to stage the pathological process (Table 2). Whereas the lesional pattern remains relatively consistent throughout all cases within a given stage, the severity of the pathology varies slightly from one person to another (Table 2). For staging purposes, however, assessment of the degree of involvement is less important than assessment of the anatomical distribution of the lesions.

Among olfactory structures, the first pathological changes appear in the anterior olfactory nucleus and olfactory bulb. Subsequently, they also are seen in closely related areas, such as the olfactory tubercle, piriform cortex, periamygdaloid cortex, and the entorhinal cortex covering part of the ambient gyrus [61]. From there, the lesions do not advance into non-olfactory cortical areas. The material examined here contains only a single instance showing relatively early involvement on the part of olfactory cortical areas (Table 2, case 17). It also should be pointed out in this connection that none of our cases demonstrates the expansion of LNs and LBs from olfactory areas into the neocortex. Rather, all of the cases at our disposal show neocortical involvement only after the appearance of lesions in the anteromedial temporal mesocortex. A prerequisite for neocortical affection appears to be that the subcortical lesions have expanded to such an extent that they include the magnocellular nuclei of the basal forebrain. Therefore, the leading predilection sites of the pathological process are located in the lower brain stem (Tables 1 and 2): the dorsal IX/X motor nucleus and intermediate reticular zone. As additional nuclear grays become involved, the pathology assumes a predominantly upward course, eventually reaching the neocortex (Fig. 4).

4.1. Stages 1 and 2

Recognition of the first and second stages is important for any study seeking to compare data from symptomatic patients with those of age- and gender-matched controls. It is a must that “controls” not include incidental cases. Ideally, such post-mortem studies would compare the situation seen in neuropathologically staged clinical cases with both that of staged incidental cases and that of controls free of any disease-related pathology.

Stages 1 and/or 2 cases provide the opportunity to examine initial PD-related alterations in the absence of the damage seen in more advanced phases of the illness. It should be noted that stage 1 and 2 cases escape recognition if the search for incidental cases is solely based upon evaluating sections through the substantia nigra [23a,59].

It has been reported that non-motor symptoms usually antedate the PD-associated somato-motor dysfunctions by many years [51,66]. According to some clinical studies, PD-patients frequently complain of an impaired sense of smell years prior to the appearance of somato-motor impairments [45,46,57,71]. The present findings point to the early presence of lesions within olfactory structures. Given the existence of advanced techniques for reliable measurement of olfactory performance, it does not appear inappropriate to use impairments in olfaction as one of the markers for early phases of PD [36,64]. The same may apply for signs of autonomic dysfunctions, which also are reported to precede the symptoms of impaired somato-motor functions [42,52,56].

It is important to note that the somato-motor dysfunctions experienced by PD-patients—rather than solely reflecting a loss of dopaminergic neurons within the nigrostriatal system—may be traceable to extranigral impairment of the motor system [10,13]. In fully-developed cases of PD, it is difficult to distinguish clinically the significance of the pathology at separate, but multiple, sites. In the course of PD, three major components of the somato-motor and emotional motor systems suffer deterioration, one after the other: (1) the gain setting nuclei of the lower brain stem (gigantocellular reticular nucleus, caudal raphe nuclei, coeruleus–subcoeruleus complex), (2) the substantia nigra, and (3) specific nuclei of the thalamus and many prefrontal association fields, which are important for normal motor functions. The first alterations target the gain setting nuclei at stage 2 [13]. The destruction of the substantia nigra follows at stages 3 and 4 and is exacerbated by deterioration of specific thalamic nuclei as well as neocortical areas at stages 5 and 6. Thus, the first two stages could open up the possibility of studying features of incipient motor dysfunction related to deterioration within the gain setting nuclei in

individuals still in possession of a normal nigrostriatal system as well as an intact thalamus and neocortex. Diagnostic tools designed to detect impaired functions of the gain setting system might be suitable markers for early phases of PD [13,21,55,68]. In view of the findings presented earlier for stages 1 and 2, a diagnostic panel that would combine assessment of olfactory impairments, autonomic regulatory dysfunctions, and abnormalities of the gain setting system might yield the best results.

4.2. Stages 3 and 4

The pars compacta of the substantia nigra is heterogeneously composed, which means that its neuronal constituents display regionally variable traits [5,19,43]. Subtle regional differences may, in turn, be reflected in differential susceptibilities of nigral melano-neurons to develop LNs and LBs. The results of the present study underscore the particularly early involvement of the posterolateral subnucleus, a finding that corresponds to the severe damage of this subnucleus in clinically overt cases [20,38,39,44].

Sectioning of the substantia nigra often is regarded as sufficient for neuropathological evaluation of questionable PD cases. In this context, it should be emphasized that the substantia nigra is not the first structure in the brain to develop PD-related lesions [23a]. The absence of α-synuclein immunoreactive inclusion bodies in sections through the substantia nigra, therefore, does not mean the presence of PD-related lesions elsewhere in the brain can be ruled out.

Involvement of the anteromedial temporal mesocortex is a hallmark of stage 4. The human mesocortex, a zone of transitional areas between the allocortex and neocortex, is particularly well-developed in the anteromedial portions of the temporal lobe. The latter include the periallocortical transentorhinal region and the proneocortical ectorhinal area [8].

4.3. Stages 5 and 6

The involvement of key neocortical areas permits distinction of the final two stages. At stage 5, the pathology reaches from the temporal mesocortex into the adjoining high-order sensory association areas of the mature neocortex. At stage 6, the cortical pathology extends further into the first order sensory associations areas, premotor fields and, occasionally, even primary sensory and motor fields. In the temporal lobe, stage 6 pathology encroaches upon the cortex covering the first temporal convolution and often reaches the primary auditory area in portions of the transverse gyrus of Heschl.

The damage incurred by important limbic structures (amygdala, hippocampal formation, anteromedial temporal mesocortex) as well as by extensive neocortical territories during stages 5–6 might well be considered to pave the way for declining intellectual faculties [14] and, in fact, impaired cognition is a frequent phenomenon in the end phase of PD [28].

4.4. Concluding remarks

The criteria currently in use for neuropathological diagnosis of sporadic PD only permit distinction of clinically manifest cases, on the one hand, and a broad spectrum of ill-defined cases with less severe brain involvement on the other (incidental cases). As such, a neuropathological staging procedure is called for that allows sufficient differentiation among initial, intermediate, and final stages of PD-related lesions. In addition, such a procedure would permit accurate identification of controls truly devoid of PD-related lesions. Inasmuch as the brains of the elderly frequently are affected by more than one neurodegenerative disease, a large proportion of PD patients exhibits co-occurring AD-related pathology and vice versa. Accurate post-mortem evaluation of such individuals, therefore, requires the use of post-mortem staging systems for both AD and PD.

Acknowledgments

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References

[1] Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VMY, et al. Aggregation of α-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 1998;152:879–84.

[2] Bergeron C, Pollanen MS. Pathogenesis of the Lewy body. In: Perry RH, McKeith IG, Perry EK, editors. Dementia with Lewy bodies. Cambridge, NY: Cambridge University Press, 1996. p. 302–7.

[3] Braak E, Braak H. Silver staining method for demonstrating Lewy bodies in Parkinson's disease and argyrophilic oligodendrocytes in multiple system atrophy. J Neurosci Methods 1999;87:111–5.

[4] Braak H. Architectonics as seen by lipofuscin stains. In: Jones EG, Peters A, editors. Cerebral cortex. Cellular components of the cerebral cortex, vol. 1. London: Plenum Press, 1984. p. 59–104.

[5] Braak H, Braak E. Nuclear configuration and neuronal types of the nucleus niger in the brain of the human adult. Hum Neurobiol 1986;5:71–82.

[6] Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 1991;82:239–59.

[7] Braak H, Braak E. Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. Brain Pathol 1991;1:213–6.

[8] Braak H, Braak E. The human entorhinal cortex: normal morphology and lamina-specific pathology in various diseases. Neurosci Res 1992;15:6–31.

[9] Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiol Aging 1997;18:351–7.

[10] Braak H, Braak E. Pathoanatomy of Parkinson's disease. J Neurol 2000;247(Suppl 2):3–10.

[11] Braak H, Braak E, Yilmazer D, de Vos RAI, Jansen Steur ENH, Bohl J, et al. Amygdala pathology in Parkinson's disease. Acta Neuropathol 1994;88:493–500.
[12] Braak H, Braak E, Yilmazer D, Schultz C, Bohl J. Age-related changes of the human cerebral cortex. In: Cruz-Sanchez FF, Ravid R, Cuzner ML, editors. Neuropathological diagnostic criteria for brain banking. Biomedical health research, vol. 10. Amsterdam: IOS Press 1995;19:14–9.
[13] Braak H, Rüb U, Sandmann-Keil D, Gai WP, de Vos RAI, Jansen Steur ENH, et al. Parkinson's disease: affection of brain stem nuclei controlling premotor and motor neurons of the somatomotor system. Acta Neuropathol 2000;99:489–95.
[14] Braak H, Del Tredici K, Bohl J, Bratzke H, Braak E. Pathological changes in the parahippocampal region in select non-Alzheimer dementias. Ann N Y Acad Sci 2000;911:221–39.
[15] Braak H, Del Tredici K, Gai WP, Braak E. Alpha-synuclein is not a requisite component of synaptic boutons in the adult human central nervous system. J Chem Neuroanat 2000;20:245–52.
[16] Calne DB, Snow BJ, Lee C. Criteria for diagnosing Parkinson's disease. Ann Neurol 1992;32:125–7.
[17] Cochran WG. Some methods for strengthening the common c2-tests. Biometrics 1954;10:417–51.
[18] Dale GE, Probst A, Luthert P, Anderton BH, Leigh PN. Relationship between Lewy bodies and pale bodies in Parkinson's disease. Acta Neuropathol 1993;83:525–9.
[19] Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. I. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D28K immunohistochemistry. Brain 1999;122:1421–36.
[20] Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopaminergic neurons in Parkinson's disease. Brain 1999;122:1437–48.
[21] Delwaide PJ, Pepin JL, Maertens de Noordhout A. The audiospinal reaction in parkinsonian patients reflects functional changes in reticular nuclei. Ann Neurol 1993;33:63–9.
[22] de Vos RAI, Jansen Steur ENH, Stam FC, Ravid R, Swaab D. Lewy body disease: clinico-pathological correlations in 18 consecutive cases of Parkinson's disease with and without dementia. Clin Neurol Neurosurg 1995;97:13–22.
[23] de Vos RAI, Jansen Steur ENH, Yilmazer D, Braak H, Braak E. Pathological and clinical features of Parkinson's disease with and without dementia. In: Perry RH, McKeith IG, Perry EK, editors. Dementia with Lewy bodies. Cambridge, NY: Cambridge University Press, 1996. p. 255–67;
[23a] Del Tredici K, Rüb U, de Vos RAI, Bohl JRE, Braak H. Where does Parkinson disease pathology begin in the brain? J Neuropathol Exp Neurol 2002;61:413–26.
[24] Dickson DW. Aging in the central nervous system. In: Markesbery WR, editor. Neuropathology of dementing disorders. London: Arnold, 1998. p. 56–88.
[25] Dickson DW. Tau and synuclein and their role in neuropathology. Brain Pathol 1999;9:657–61.
[26] Dickson DW, Ruan D, Crystal H, Mark MH, Davies P, Kress Y, et al. Hippocampal degeneration differentiates diffuse Lewy body disease (DLBD) from Alzheimer's disease: light and electron microscopic immunocytochemistry of CA2-3 neurites specific to DLBD. Neurology 1991;41:1402–9.
[27] Dickson DW, Schmidt ML, Lee VMY, Zhao ML, Yen SH, Trojanowski JQ. Immunoreactivity profile of hippocampal CA2/3 neurites in diffuse Lewy body disease. Acta Neuropathol 1994;87: 269–76.
[28] Dubois B, Pillon B. Cognitive deficits in Parkinson's disease. J Neurol 1997;244:2–8.
[29] Duda JE, Lee VMY, Trojanowski JQ. Neuropathology of synuclein aggregates: new insights into mechanism of neurodegenerative diseases. J Neurosci Res 2000;61:121–7.
[30] Fahn S. Parkinson's disease and other basal ganglion disorders. In: Asbury AK, McKhann GM, McDonald WI, editors. Diseases of the nervous system: clinical neurobiology, vol. 1. Philadelphia: Saunders, 1992. p. 1144–58.
[31] Fearnley J, Lees AJ. Pathology of Parkinson's disease. In: Calne DB, editor. Neurodegenerative diseases. Philadelphia: Saunders, 1991. p. 545–54.
[32] Forno LS. Concentric hyalin intraneuronal inclusions of Lewy type in the brain of elderly persons (50 incidental cases): relationship to parkinsonism. J Am Geriatr Soc 1969;17:557–75.
[33] Forno LS. Neuropathology of Parkinson's disease. J Neuropathol Exp Neurol 1996;55:259–72.
[34] Gai WP, Power JH, Blumbergs PC, Culvenor JG, Jensen PH. Alpha-synuclein immunoisolation of glial inclusions from multiple system atrophy brain tissue reveals multiprotein components. J Neurochem 1999;73:2093–100.
[35] Galvin JE, Lee VMY, Trojanowski JQ. Synucleinopathies. Clinical and pathological implications. Arch Neurol 2001;58:186–90.
[36] Geisler MW, Murphy C. Event-related brain potentials to attended and ignored olfactory and trigeminal stimuli. Int J Psychophysiol 2000;37:309–15.
[37] Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson's disease. Arch Neurol 1999;56:33–9.
[38] Gibb WRG. Neuropathology of the substantia nigra. Eur Neurol 1991;31(Suppl 1):48–59.
[39] Gibb WRG, Lees AJ. Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatry 1991;54:388–96.
[40] Gibb WRG, Scott T, Lees AJ. Neuronal inclusions of Parkinson's disease. Mov Disord 1991;6:2–11.
[41] Goedert M. The significance of tau and α-synuclein inclusions in neurodegenerative diseases. Curr Opin Genet Dev 2001;11:343–51.
[42] Goetz CG, Luthe W, Tanner CM. Autonomic dysfunction in Parkinson's disease. Neurology 1986;36:73–5.
[43] Hassler R. Zur Normalanatomie der Substantia nigra. Versuch einer architektonischen Gliederung. J Psychol Neurol 1937;48:1–55.
[44] Hassler R. Zur Pathologie der paralysis agitans und des postencephalitischen Parkinsonismus. J Psychol Neurol 1938;48:387–476.
[45] Hawkes CH, Shephard BC, Daniel SE. Olfactory dysfunction in Parkinson's disease. J Neurol Neurosurg Psychiatry 1997;62:436–5446.
[46] Hawkes CH, Shephard BC, Daniel SE. Is Parkinson's disease a primary olfactory disorder? Q J Med 1999;92:473–80.
[47] Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. Neurology 1967;17:427–42.
[48] Hyman BT. New neuropathological criteria for Alzheimer's disease. Arch Neurol 1998;55:1174–6.
[49] Irizarry MC, Growdon W, Gómez-Isla T, Newell K, George JM, Clayton DF, et al. Nigral and cortical Lewy bodies and dystrophic nigral neurites in Parkinson's disease and cortical Lewy body disease contain α-synuclein immunoreactivity. J Neuropathol Exp Neurol 1998;57:334–7.
[50] Jellinger K. Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol Chem Neuropathol 1991;14:153–97.
[51] Koller WC. When does Parkinson's disease begin? Neurology 1992;42(Suppl 4):27–31.
[52] Korczyn AD. Autonomic nervous system disturbances in Parkinson's disease. Adv Neurol 1990;53:463–8.
[53] Lewy FH. Paralysis agitans. I. Pathologische Anatomie. In: Lewandowski M, editor. Handbuch der Neurologie, Band III. Berlin: Springer, 1912. p. 920–33.
[54] Lowe J. Lewy bodies. In: Calne DB, editor. Neurodegenerative diseases. Philadelphia: Saunders, 1994. p. 51–69.
[55] Loza A, Pepin JL, Rapisarda G, Moglia A, Delwaide PJ. Functional changes of brainstem reflexes in Parkinson's disease. Conditioning of blink reflex R2 component by paired and index finger stimulation. J Neural Transm 1997;104:679–87.
[56] Meco G, Pratesi L, Bonifati V. Cardiovascular reflexes and autonomic dysfunction in Parkinson's disease. J Neurol 1991;238:195–9.

[57] Mesholam RL, Moberg PJ, Mahr RN, Doty RL. Olfaction in neurodegenerative disease. A meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. Arch Neurol 1998;55:84–90.
[58] Pearce RK, Hawkes CH, Daniel SE. The anterior olfactory nucleus in Parkinson's disease. Mov Disord 1995;10:283–7.
[59] Perry RH, Irving D, Tomlinson BE. Lewy body prevalence in the aging brain: relationship to neuropsychiatric disorders, Alzheimer-type pathology and catecholaminergic nuclei. J Neurol Sci 1990;100: 223–33.
[60] Pollanen MS, Dickson DW, Bergeron C. Pathology and biology of the Lewy body. J Neuropathol Exp Neurol 1993;52:183–91.
[61] Price JL. Olfactory system. In: Paxinos G, editor. The human nervous system. San Diego: Academic Press, 1990. p. 979–1000.
[62] Rajput AH. Clinical features and natural history of Parkinson's disease (special consideration of aging). In: Calne DB, editor. Neurodegenerative diseases. Philadelphia: Saunders, 1994. p. 555–71.
[63] Rüb U, Del Tredici K, Schultz C, Ghebremedhin E, de Vos RAI, Jansen Steur ENH, et al. Parkinson's disease: the thalamic components of the limbic loop are severely impaired by α-synuclein immunopositive inclusion body pathology. Neurobiol Aging 2002;23:245–54.
[64] Sakuma K, Nakashima K, Takahashi K. Olfactory evoked potentials in Parkinson's disease, Alzheimer's disease and anosmic patients. Psychiatr Clin Neurosci 1996;50:35–40.
[65] Sandmann-Keil D, Braak H, Okochi M, Haass C, Braak E. Alpha-synuclein immunoreactive Lewy bodies and Lewy neurites in Parkinson's disease are detectable by an advanced silver-staining technique. Acta Neuropathol 1999;98:461–4.
[66] Sawle GV. The detection of preclinical Parkinson's disease: what is the role of positron emission tomography? Mov Disord 1993;8:271–7.
[67] Trojanowski JQ, Lee VMY. Aggregation of neurofilament and α-synuclein proteins in Lewy bodies—implications for the pathogenesis of Parkinson-disease and Lewy body dementia. Arch Neurol 1998;55:151–2.
[68] Valls-Solé J. Neurophysiological characterization of parkinsonian syndromes. Neurophysiol Clin 2000;30:352–67.
[69] Wakabayashi K, Hansen LA, Masliah E. Cortical Lewy body-containing neurons are pyramidal cells. Laser confocal imaging of double-immunolabeled sections with anti-ubiquitin and SMI32. Acta Neuropathol 1995;89:404–8.
[70] Wakabayashi K, Takahashi H, Obata K, Ikuta F. Immunocytochemical localization of synaptic vesicle-specific protein in Lewy body-containing neurons in Parkinson's disease. Neurosci Lett 1992;138:237–40.
[71] Wolters EC, Francot C, Bergmans P, Winogrodzka A, Booij J, Berendse HW, et al. Preclinical (premotor) Parkinson's disease. J Neurol 2000;247(Suppl 2):103–9.