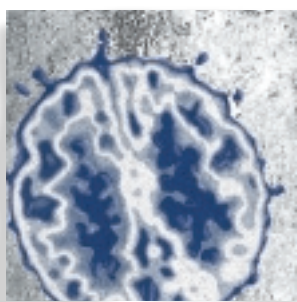# Basic research

Postmortem studies in Parkinson's disease

Andreas Hartmann, MD

No animal model to date perfectly replicates Parkinson's disease (PD) etiopathogenesis, and the anatomical organization of the nigrostriatal system differs considerably between species. Human postmortem material therefore remains the gold standard for both formulating hypotheses for subsequent testing in in vitro and in vivo PD models and verifying hypotheses derived from experimental PD models with regard to their validity in the human disease. This article focuses on recent and relevant fields in which human postmortem work has generated significant impact in our understanding of PD. These fields include Lewy body formation, regional vulnerability of dopaminergic neurons, oxidative/nitrative cellular stress, inflammation, apoptosis, infectious and environmental agents, and nondopaminergic lesions.

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The history of human postmortem studies in Parkinson's disease (PD) begins at the end of the 1950s with two seminal papers: Carlsson's original suggestion that dopamine (DA) may be a transmitter in the central nervous system (CNS) and be involved in the control of motor function, and thus in the parkinsonian syndrome[1]; and the article by Ehringer and Hornykiewicz,[2] which proved the significant reduction in DA concentration in the neostriatum of patients suffering from sporadic PD. In 1973, these initial observations were followed by demonstration of a correlation between DA cell loss in the substantia nigra pars compacta (SNpc, *Figure 1*) and striatal DA concentrations in PD.[3] Interestingly, this study suggested that the classical parkinsonian symptom triad of PD appears when striatal DA concentrations are lowered by approximately 80%; however, this decline corresponds to only 50% of SNpc DA neuron loss, suggesting that a subset of nigral DA neurons may be morphologically intact, but functionally impaired. This concept is supported by the finding that 15% of melanized neurons in the human SNpc no longer express tyrosine hydroxylase (TH; the rate-limiting enzyme in DA synthesis), but remain morphologically intact.[4] This concept of the suffering, ie, metabolically compromised, neuron is important in pathophysiological and therapeutic terms, since it suggests that a subpopulation of nigral DA neurons are amenable to restorative therapies.

After a general outline on the potential and limitations of human postmortem studies in PD, I will explore major questions regarding etiology, pathogenesis, and treatment of PD with reference to human postmortem studies.

## The role of human postmortem studies in PD research

There has been considerable debate over the importance of human postmortem studies in PD research. This controversy is based on the many limitations of postmortem research. Human postmortem studies in PD suffer from

Basic research

Selected abbreviations and acronyms

DA	*dopamine*
DAT	*dopamine transporter*
DLB	*dementia with Lewy bodies*
LB	*Lewy body*
MPTP	*1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine*
PD	*Parkinson's disease*
SNpc	*substantia nigra pars compacta*
VMAT2	*vesicular monoamine transporter 2*

tissue confounds of aging, end-stage disease, and chronic treatments. Moreover, human postmortem studies cannot answer the question of whether the changes observed are a cause or a consequence of neuronal death in PD. On the other hand, no animal model to date perfectly replicates PD etiopathogenesis, and the anatomical organization of the nigrostriatal system differs considerably between humans and lower species. Thus, human postmortem material remains the gold standard for (i) formulating hypotheses for subsequent testing in in vitro and in vivo PD models (cell culture, yeast, *Drosophila*, rodent, and primates models of the disease, based on toxins and/or genetic manipulations); and (ii) verifying hypotheses derived from experimental PD models with regard to their validity in the human disease. This review will emphasize the interaction of findings from postmortem and experimental PD studies.

Control   **Parkinson**
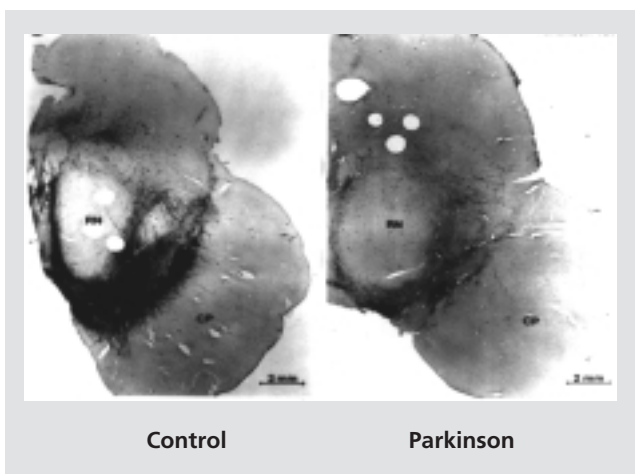
Figure 1. Axial cut of human mesencephalon. The substantia nigra pars compacta (SNpc) can be identified by tyrosine hydroxylase (TH) immunostaining reflecting the presence of dopaminergic (DA) neurons. Cell loss in the parkinsonian SNpc can be appreciated macroscopically by decrease of staining density compared to the control case. RN, red nucleus; CP, cerebral peduncle.

Genes and PD

The etiology of sporadic PD remains unknown. It is generally believed that sporadic PD is the result of complex interactions between genetic susceptibility and environmental factors. In both cases, postmortem studies serve to confirm rather than to generate new hypotheses, with a few notable exceptions.

Genetics is a rapidly growing field in PD research. Three major mutations have been identified to date in affected kindreds: α-synuclein or *Park1*[5]; parkin or *Park2*[6]; and DJ-1 or *Park7*.[7] A fourth mutated gene product, UCHL1 (*Park5*), is associated with gene expression; it may not be able to provoke a parkinsonian syndrome alone,[8] but may be a susceptibility gene.[9] Autopsy specimens from families with these mutations remain rare, but have nevertheless yielded results that have expanded our understanding of sporadic PD, such as the data on α-synuclein.

Three different point mutations in α-synuclein, *A53T*, *A30P*, and *E46K*, have been associated with PD in separate families with dominantly transmitted PD.[5,10,11] These are gain-of-function mutations. There is also evidence that α-synuclein promoter variants contribute to the lifetime risk of sporadic PD.[12-14] In general, alleles that increase α-synuclein expression are associated with an increased risk for PD. Recent work has shown that triplication of the α-synuclein gene is sufficient to cause PD and, in human postmortem brain, is accompanied by doubling of α-synuclein protein expression.[15,16] Similarly, postmortem studies in sporadic PD show that α-synuclein mRNA is upregulated in the SNpc of affected individuals.[17]

The link between α-synuclein and sporadic PD is found in Lewy bodies (LBs), the pathological hallmark of PD, since α-synuclein has been shown to be the primary constituent of LBs.[18-22] LBs are eosinophilic fibrillar cytoplasmic inclusions in DA neurons that can be detected in both the SNpc and the cortex of PD patients *(Figure 2)*. LBs are located in the cell body, axons, and dendrites of neurons, and are composed of neurofilaments 7 to 25 nm in diameter; these neurofilaments are believed to be inappropriately phosphorylated, proteolytically truncated, and ubiquitinated.[23] LBs have been reported to include a wide range of proteins (including ubiquitin, parkin, and tau), heat shock proteins (HSPs), torsin A, neurofilaments, oxidized/nitrated proteins, proteasomal elements, and others.[22,24-31] Interestingly, many proteins that interact with α-synuclein and parkin have also been

identified as components of LBs, for instance, parkin-associated endothelin-like receptor (Pael-R; a transmembrane polypeptide),[32] synphilin-1,[33] and p38 (a structural component of the mammalian aminoacyl-tRNA synthetase complex).[34] LBs ectopically express the cell cycle protein cyclin B; this may be related to cyclin B's interaction with α-synuclein, which predisposes nigral LB-bearing DA neurons to undergo apoptosis.[35] Another protein colocalized with α-synuclein in LBs is tissue transglutaminase (tTGase), which induces cross-linking of α-synuclein in vitro.[36] tTGase inhibition could therefore be a novel therapeutic target in PD, provided that LB formation is indeed a cytotoxic event.

Parkin is widely distributed protein in DA and non-DA neurons in normal human brain and in sporadic PD. It is mostly located in large cytoplasmic vesicles and in the endoplasmic reticulum (ER).[37] The initial postmortem studies from five parkin-positive cases initially failed to find LBs—an observation used to argue that parkin is required for LB formation.[38,39] However, this conclusion was challenged when Farrer et al[40] showed the presence of LBs in one parkin-positive autopsy case. These studies highlight the importance of postmortem study data and their conclusions. They guide our clinical formulations, and thus our experimental and therapeutic approaches.

Finally, studies with DJ-1 are in very early stages. Its distribution has been analyzed in postmortem brain of control and PD subjects in two recent studies. DJ-1 does not colocalize with LBs, but with tau inclusions; it is mainly expressed by astrocytes; and it appears to be sensitive to oxidative stress.[41,42] At present, a functional interpretation of these data is lacking.

The role of LBs in DA cell death

There remains much debate over whether LBs are neuroprotective, constitute an age-related epiphenomenon, or are cytotoxic; postmortem end points may supply some answers. Recently, Conway et al[43,44] suggested that accelerated formation of nonfibrillar α-synuclein oligomers is the critical process in PD pathogenesis, ie, LB formation is neuroprotective by sequestering toxic protein species. Once this issue is resolved, drug therapy can be aimed at promoting the healthy process. Two observations from pathological examination of human brain contribute to this dialogue:

• SNpc DA neurons containing LB appear to be "healthier" than neighboring neurons,[45] whereas the nigral DA

neurons undergoing apoptotic-like cell death do not contain somal LBs. Tompkins and Hill[45] suggested that the majority of SNpc neurons die before or without forming LBs and that SNpc neurons that survive the initial pathological insult suffer damage that leads to LB formation.

• It is not uncommon to observe "incidental" LBs at autopsy of aged asymptomatic individuals. An alternative explanation for this finding is that these individuals have not lived long enough to develop a parkinsonian phenotype. Also, if LBs were protective, one might speculate that controls should have more LBs than PD patients, which is clearly not the case.

Alternatively, LBs may occur as an epiphenomenon of the primary pathology and have little or no effect on neuronal viability. In contrast to the observations by Tompkins and Hill,[45] Gibb and Lees[46] reported that SNpc neurons with and without somal LBs generally appear to be similarly affected by the disease process. Moreover, cell size and nucleolar size do not differ between LB-positive and LB-negative SNpc neurons.[47] Also, dendritic morphological abnormalities found in parkinsonian SNpc are similar in LB- and non–LB-containing neurons.[48] Finally, neurofilament mRNA levels also show a
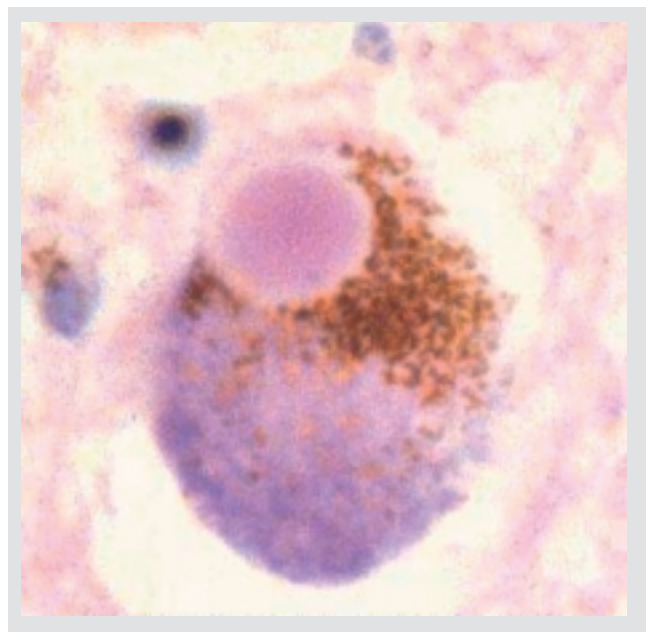

Figure 2. A Lewy body (LB). The LB is shown as a dense eosinophilic inclusion bordered by neuromelanin, the auto-oxidation product of dopamine (DA), which allows identification of DA neurons in the human substantia nigra pars compacta (SNpc).

similar level of reduction for both LB- and non–LB-containing neurons.[49]

We favor the hypothesis that the presence of LBs is an indicator of neuronal distress, although it is impossible to deduce from postmortem work whether LBs are, as such, neurotoxic. The percentage of LB-containing neurons that are positive for caspase-3 and Bax, two proapoptotic proteins, is significantly higher than the percentage of Bax– and caspase-3–positive DA neurons not containing LBs,[50,51] which indicates that LB-containing DA neurons are more predisposed to undergo apoptosis. Furthermore, aggregated proteins bind HSPs in LBs, and thus prevent their potentially protective chaperone action.[52] Entrapment of vital cellular organelles in LBs has been described and may compromise cellular viability.[53] Also, LBs may inhibit axonal transport, probably resulting in a "dying back" phenomenon from the synapse to the cell body.[52] Clinical neuropathological studies in patients with dementia with LBs (DLB) report a correlation between numbers of cortical LBs and the degree of cognitive impairment.[54,55] Finally, we have recently conducted a human postmortem study, where the genetic fingerprints of mesencephalic DA neurons containing LBs versus mesencephalic DA neurons not containing LBs were compared in five PD patients. Total RNA from single neurons of both neuronal subpopulations was obtained by immuno-laser capture microdissection (LCM). Subsequently, RNA arbitrarily primed polymer chain reaction (RAP-PCR) was employed to generate expression profiles from the extracted RNA. Seven expressed sequence tags (ESTs) of interest were selected for further quantitative expression analysis by real-time quantitative reverse transcription PCR (rtq RT-PCR). DA neurons bearing LBs, according to their genetic profile, appeared sicker than their LB-negative counterparts, which were preferentially endowed with prosurvival genes. This suggests that inhibition of LB formation indeed represents a therapeutic strategy in PD (Lu and Hartmann, unpublished results).
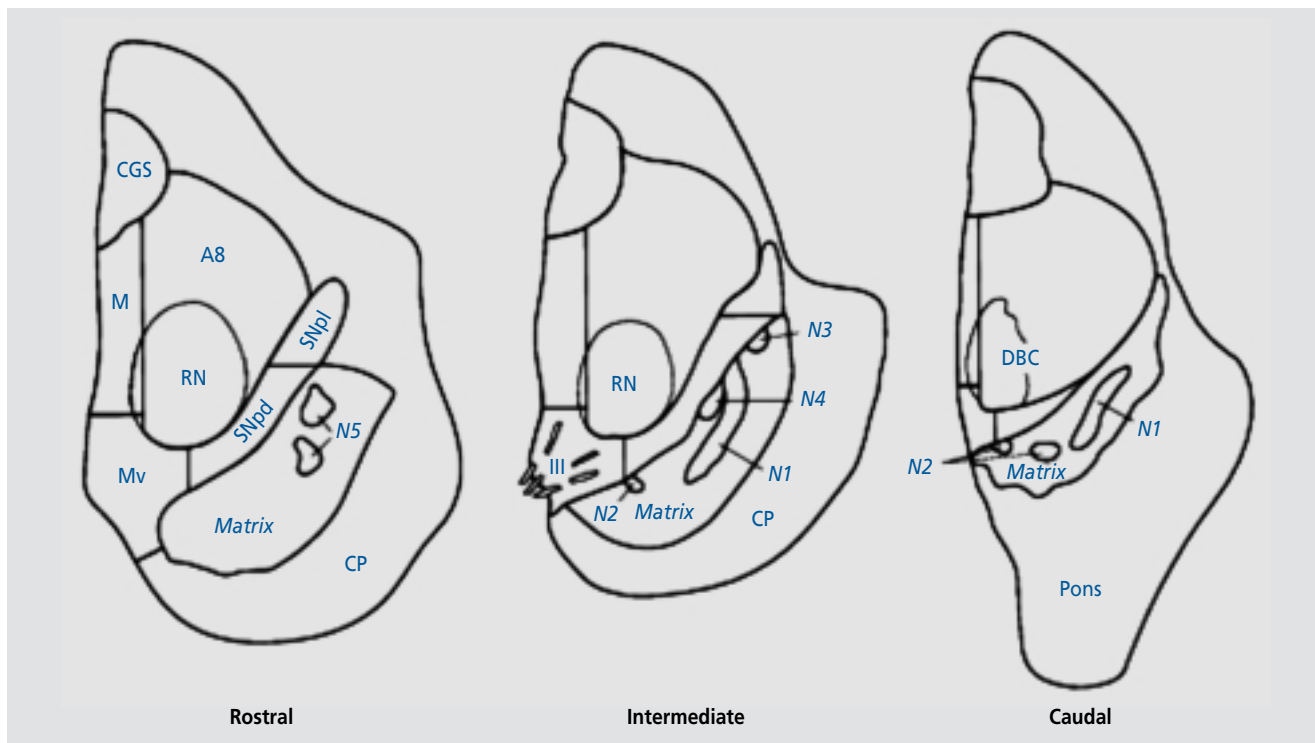
Figure 3. Summary of midbrain subdivisions illustrated at three representative transverse levels. CGS, central gray substance; M, medial group; Mv, medioventral group (M and Mv constitute the ventral tegmental area [VTA]); A8, dopaminergic group A8; SNpd, substantia nigra pars dorsalis; SNpl, substantia nigra pars lateralis; N, nigrosome; RN, red nucleus; DBC, decussation of the brachium conjunctivum; CP, cerebral peduncle; III, exiting third cranial nerve fibers.

A differential vulnerability of mesencephalic DA neurons to degeneration

There is evidence that the loss of DA neurons in PD is heterogeneous. DA neurons in the SNpc are affected, as are those in other mesencephalic structures, eg, the ventral tegmental area (VTA) and the central gray substance (CGS). These DA neuronal populations display a differential vulnerability to cell death in PD. SNpc DA neurons are most affected, with a cell loss averaging 80% to 90% in PD patients, whereas cell loss in the VTA is intermediate at 40% to 50%. Finally, only 2% to 3% of DA neurons degenerate in the CGS in PD.[56] For the PD midbrain, the correlation is simple and direct: the greater the number of pigmented neurons normally present in the DA cell groups, the larger the loss of neurons in the cell groups in the diseased brains. Moreover, within each cell group, nonpigmented neurons are spared relative to the total population of TH-positive neurons and relative to the population of pigmented neurons.[4] There is also a regional selective vulnerability of DA neurons within the SNpc to cell death in PD.[57-59] On the basis of calbindin D28K (CD28K) immunohistochemistry, the SNpc can be divided into a calbindin-rich region (matrix) and five calbindin-poor pockets (nigrosomes 1-5) *(Figure 3)*. The depletion of DA neurons begins in nigrosome 1, and then spreads to other nigrosomes and matrix along the rostral, medial, and dorsal axes. Depletion is maximum (98%) in nigrosome 1, located in the caudal and mediolateral part of the SNpc. Progressively, less cell loss is detectable in more medial and more rostral nigrosomes. A parallel, but lesser, caudorostral gradient of cell loss is observed for DA neurons included in the matrix. Because the nigrosome/matrix analysis refers to compartmental subdivisions within the SNpc, the most obvious conclusion would be that compartmental locality in SNpc itself is a key to differential vulnerability. DA neurons in different compartments may have different expression patterns of genes implicated in PD pathogenesis. The DA neurons relatively spared from the disease process may be endowed with a range of protective mechanisms, which has sparked research aiming to identify these protective or deleterious mechanisms.

Whether CD28K determines neuronal vulnerability itself is controversial, and both positive and negative results have been reported. CD28K-positive neurons have been shown to be relatively resistant to degeneration in PD[59] and in certain animal models of PD.[60-62] There is also a sig-

nificant decrease in CD28K protein and mRNA in the SN, but not in the cerebellum and neocortex of PD patients compared with controls.[63] However, on the basis of the viability assessment of midbrain DA neurons in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion paradigm using CD28K-deficient mice, CD28K-containing neurons are not spared by the pathological process, suggesting that endogenous CD28K is not required for protection of these neurons.[64] Thus, CD28K may be a marker of resistance of DA neurons to the degenerative process in PD, but not the causative agent itself.

Perturbation of regulated balance between DAT and VMAT2

It has been proposed that the process underlying PD is the selective degeneration of DA nerve terminals in the striatum expressing dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2).[65-68] DAT and VMAT2 are essential for normal DA neurotransmission: DAT terminates the actions of DA by rapidly removing it from the synapse; and VMAT2 loads cytoplasmic DA into synaptic vesicles for storage and subsequent release. Cytosolic DA can quickly form reactive oxygen species, and so DA that has been synthesized or transported into the neuron from the extracellular space is rendered harmless by rapid storage in small synaptic vesicles. Hence, DAT activity increases cytoplasmic DA concentrations, whereas VMAT2 activity decreases them. Detailed neuroanatomical analyses of brain from control and PD cases have shown that the regions with the highest DAT/VMAT2 ratio—the caudate and the putamen—are the most sensitive to damage in PD and MPTP-induced parkinsonism.[67,69,70] A recent study showed that α-synuclein negatively modulates human DAT activity,[71] whereas an earlier study found opposite results.[72] The *A53T* mutation of expression of α-synuclein also reduced levels of VMAT2. Taken together, the defective sequestration of DA mediated by the interplay of DAT, VMAT2, and α-synuclein may be a key event in the DA cell death in sporadic PD.[73]

To extend the one gene/one protein approach to the search for the differences in mesencephalic regional vulnerability to cell death, we compared the genetic fingerprints of mesencephalic DA neurons that are particularly prone to degenerate during PD (DA neurons in nigrosome 1 within the SNpc) and mesencephalic DA neurons that are particularly resistant to the disease course (DA

neurons in the CGS) in five control subjects. We found that SNpc DA neurons do not per se reveal many distinctive deleterious genes; rather, it appeared as if CGS DA neurons were just embodied with more cellular defenses against degeneration, suggesting that the transfer of these factors might endow SNpc DA neurons with the same resistance against neuronal death in PD (Lu and Hartmann, unpublished results).
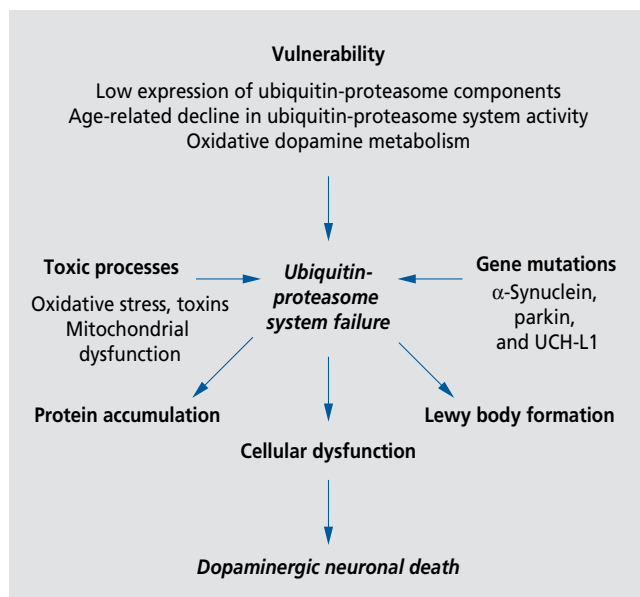
Defects of the ubiquitin-proteasome system in PD

A growing body of evidence suggests that proteolytic stress underlies both familial and sporadic PD. Interest in the role of the proteasome in the pathophysiology of PD has been triggered by observation of parkin, an E3 ubiquitin ligase, which tags (potentially neurotoxic) proteins for degradation by the proteasome. Proteasomes are multicatalytic proteases found in the cytoplasm, ER, perinuclear region, and the nucleus of eukaryotic cell.[74] The accumulation of oxidized proteins in SNpc suggests that protein clearance is inadequate in this brain region.[75] Compared with the brains of age-matched controls, in the brains of subjects with sporadic PD, there is a marked loss of α- but not β-subunits of 20S proteasome core within nigral DA neurons.[76] Levels of PA28 (a multisubunit proteasome activator) are very low in the SNpc of

both control and PD subjects.[77] These findings point to a primary defect in proteasome-mediated proteolysis of nigral DA neurons in sporadic PD. Dysfunction at any point on the proteasome pathway may result in nigral DA neuronal degeneration, accounting for the particular vulnerability of nigral DA neurons to neurodegeneration. Although low proteasomal activity has been linked to LB formation by some,[78] it has been refuted by others.[79] The production of abnormal proteins that resist and inhibit proteolysis (α-synuclein mutations), defects in protein ubiquitination (parkin mutations), reduced de-ubiquitination (*UCH-L1/Park5* mutations), and proteasomal dysfunction (sporadic PD) have all been implicated in the etiopathogenesis of PD *(Figure 4)*.

Oxidative stress

One of the first and most relevant hypotheses for PD pathogenesis relates to increased oxidative/nitrative stress in mesencephalic DA neurons. In PD, DA can auto-oxidize into toxic dopamine-quinone species, superoxide radicals, and hydrogen peroxide. DA auto-oxidizes into neuromelanin, the phenotypic marker of midbrain DA neurons in humans. Accordingly, the neuromelanin content and distribution in the parkinsonian mesencephalon has been linked to the vulnerability of DA neurons to undergo cell death.[80] Oxidative/nitrative stress may result in protein oxidation/nitration[81,82]; decreased neuronal glutathione and glutathione peroxidase content, which prevents inactivation of hydrogen peroxide and enhances formation of toxic hydroxyl radicals[83-85]; basal lipid peroxidation, which results in membrane damage[86]; DNA and RNA oxidation[75,87]; and formation of LBs.[88]

A potential signaling pathway between oxidative stress and subsequent cell death has been explored by Hunot et al.[89] They showed that nuclear translocation of the nuclear factor–κB (NF-κB), which is triggered by oxidative stress and precedes the engagement of an apoptotic program, is increased 70-fold in nigral DA neurons from PD subjects compared with control subjects. Oxidative stress has also been implicated in altered iron, ferritin, and trace metal contents of nigral DA neurons and may increase the susceptibility of these neurons to cell death.[90-92] Prior to causing cell death, increased iron in the brain has been suggested to trigger LB formation and initiation of inflammatory responses.[93] Interestingly, the detection of redox-active iron in situ showed a strong labeling of LBs in the SNpc of PD patients, whereas cortical LBs remained

Vulnerability

Low expression of ubiquitin-proteasome components
Age-related decline in ubiquitin-proteasome system activity
Oxidative dopamine metabolism

Toxic processes

Oxidative stress, toxins
Mitochondrial dysfunction

Ubiquitin-proteasome system failure

Gene mutations

α-Synuclein, parkin, and UCH-L1

Protein accumulation

Lewy body formation

Cellular dysfunction

Dopaminergic neuronal death

Figure 4. Possible relationship and interplay between proteolytic stress and other prominent observations in Parkinson's disease.

unstained; this indicates a fundamental difference between cortical and brain-stem LBs.[94] Similarly, Giasson et al[95] reported that nitrated α-synuclein is present in the major filamentous building blocks of LBs, underlining the importance of oxidative/nitrative stress in PD.

Inflammation

The degeneration of DA neurons is associated with a strong glial reaction, which is generally considered to be a nonspecific consequence of neuronal degeneration. However, there is increasing evidence that inflammation is an active phenomenon in PD, continuously triggering DA cell death in this neurodegenerative disorder. The glial reaction in the SN of PD patients is a well-known neuropathological characteristic of the disease. In their seminal study, McGeer and McGeer [96] reported a large number of reactive human leukocyte antigen-DR (HLA-DR)–positive microglial cells in the SN of PD patients. Such a glial reaction has also been described in the affected brain regions in other neurological disorders, such as Alzheimer's disease and brain infarct,[97] as well as in animal models of PD.[98] These data suggest that glial activation is not specific to PD and that it most likely represents a common phenomenon in neurodegenerative disorders. However, recent evidence supports the notion that a subpopulation of activated glial cells may be deleterious in PD, particularly for highly dysfunctional neurons that are metabolically compromised. Strong support for this hypothesis came from a study of young drug addicts who developed a parkinsonian syndrome after MPTP intoxication.[99] In a recent study, the same authors reported a postmortem neuropathological study of three subjects with MPTP-induced parkinsonism.[100] Interestingly, gliosis and clustering of microglial cells around DA neurons were detected, despite survival times ranging from 3 to 16 years. These findings not only indicate an ongoing nerve cell loss after a time-limited insult, but also suggest that activated microglial cells may perpetuate neuronal degeneration. One may thus speculate that after a primary insult of environmental and/or genetic origin, the glial reaction may perpetuate the degeneration of DA neurons.

The mechanism by which microglial cells can amplify injury to nigral DA neurons is not yet known. However, the factors involved in this deleterious effect are very likely cytokines, including tumor necrosis factor α (TNF-α), interleukin 1β (Il-1β), and interferon γ (IFN-γ). Accordingly, several studies have reported a marked increase of cytokine levels in the brain and cerebrospinal fluid (CSF) of PD patients.[101] In addition, a higher density of glial cells expressing TNF-α, Il-1β, and IFN-γ was observed in the SN of PD patients compared with age-matched control subjects.[102,103] Some of these cells were close to blood vessels and degenerating DA neurons, suggesting their involvement in the pathophysiology of PD. Two mechanisms, which are not mutually exclusive, may explain the deleterious role of cytokines in the parkinsonian SNpc:

• Proinflammatory cytokines induce the production of nitric oxide in glial cells.[104]
• TNF-α receptors directly activate DA neurons of the human SN.[102]

The question of whether inflammation plays a prominent role in PD pathogenesis cannot be resolved by postmortem studies alone, and experimental PD models have much contributed to strengthening this hypothesis, making inflammation a prime candidate for neuroprotective studies in PD patients.[98] Importantly, recent primate studies have replicated chronic glial activation in the SNpc following a time-limited MPTP insult[105-107] and may thus represent a valuable model to study the long-term consequences of this process.

Apoptosis

There has been much interest in whether DA neurons in PD die by apoptosis, necrosis, or some other form of cell death. This is because apoptosis is amenable to pharmacological inhibition and may thus be a therapeutic target in PD. The initial human postmortem studies showed conflicting results,[51] either because of methodological problems, especially the unreliability of 3' DNA end labeling as a marker of apoptotic nuclear degradation,[108] or because the apoptotic changes observed might in fact represent a perimortem effect rather than the primary disease process.[109]

Thus, the next step was to look at the cellular transduction pathways mediating apoptosis in PD brains. In this respect, the signaling pathways downstream of the inflammatory release of proinflammatory cytokines, eg, coupled to the TNF type 1 receptor (TNFR1), are of particular interest, given previous work on inflammation triggering cell death in PD. TNF-α induces trimerization of TNFR1 on binding, which leads to the autoproteolytical activation of caspase-8 via the adaptor molecule TNFR1-associating protein with a death domain and

Basic research

FAS-associated protein with a death domain (FADD). Caspase-8 may in turn either cleave effector caspases, such as caspase-3, directly or amplify the death signal through the mitochondrial release of cytochrome-c into the cytosol.[110] Indeed, in a human postmortem study, we showed a significant decrease in the percentage of FADD immunoreactive DA neurons in the SNpc of PD patients compared with control subjects.[111] Furthermore, this decrease correlated with the known selective vulnerability of nigral DA neurons in PD, suggesting that this pathway contributes to the susceptibility of DA neurons to TNF-mediated apoptosis in PD. One step downstream in this proapoptotic signaling cascade, the proportion of melanized neurons displaying caspase-8 activation in PD was also higher in PD than in control subjects.[112] Similar results were obtained for caspase-3, where we found (i) a positive correlation between the degree of neuronal loss in DA cell groups affected in the mesencephalon of PD patients and the percentage of caspase-3–positive neurons in these cell groups in control subjects; (ii) a significant decrease in caspase-3–positive pigmented neurons in the SNpc of PD patients compared with control subjects; and (iii) a significantly higher percentage of active caspase-3–positive neurons among DA neurons in PD compared with control subjects.[50] Taken together, these studies suggest that the melanized DA neurons expressing the TNFR1 transduction pathway are particularly prone to degeneration in PD if this pathway is activated during the course of the disease.

As regards mitochondrial proteins controlling apoptosis in PD, we have shown a similar distribution of nigral DA neurons immunoreactive for Bax, a proapoptotic mitochondrial protein, in PD compared with control subjects.[113] However, by assessing staining intensity, Tatton[114] reported increased immunoreactivity for Bax and caspase-3 in nigral DA neurons of PD compared with control subjects. We also studied the mRNA expression of Bcl-x_L, a major anti-apoptotic mitochondrial protein in the SNpc of PD patients and controls. We found a significant upregulation of Bcl-x_L mRNA expression in nigral DA neurons from PD patients, as assessed by in situ hybridization, which was accompanied by a redistribution of the protein to the mitochondrial outer membrane, as assessed by electron microscopy.[115] This process suggests a compensatory upregulation of Bcl-x_L in the nigral DA neurons surviving the pathological process in PD. Finally, an experimental link between sublethal activation of apoptotic pathways and LB formation has been suggested by Hashimoto et al,[116] who showed that release of cytochrome-c from mitochondria into the cytosol may also function as a stimulator for α-synuclein aggregation.

Environmental toxins

The seminal study by Langston et al[99] on MPTP as the causative agent for a PD-like syndrome has triggered numerous studies on the role of environmental toxins in the pathophysiology of PD. Since MPTP inhibits complex I of the mitochondrial respiratory chain, a defect in this protein has been investigated in cases of sporadic PD. In 1990, Schapira et al[117] showed that complex I activity is indeed decreased in the SNpc of patients suffering from sporadic PD. Environmental toxins, particularly herbicides and pesticides that inhibit complex I activity, such as rotenone, paraquat, and maneb, have since been studied as potential causative or at least risk factors in PD models and in epidemiological studies.[118] However, only limited human postmortem data have been gathered so far. Fleming et al[119] screened postmortem brain samples from PD patients and control cases for 16 organochloride pesticides. They found a positive association of PD and pesticide concentrations for only one pesticide, dieldrin, a lipid-soluble mitochondrial poison. These results were replicated by another group in separate studies with regard to increased dieldrin concentration in PD brain.[120-122] However, the mode of action of this pesticide strongly supports current concepts of oxidative stress and mitochondrial energy impairment as an important factor in PD pathogenesis. Interestingly, the pesticides dieldrin, paraquat, and rotenone, which are all complex I inhibitors, have been shown to induce an acceleration of α-synuclein fibril formation in vitro, and thus likely Lewy body formation.[123]

Infection

The idea of a putative role of infectious agents in the etiology of PD can be traced back to 1918, when postencephalitic parkinsonism due to influenza A infection was widespread in Europe. Many decades later, observations of sporadic PD suggest that LBs harbor viral and bacterial signatures.[124] A very recent study has convincingly shown that *Nocardia asteroides* 16S rRNA is present in LBs from PD patients and points to a role in bacterial infection in protein aggregation.[125] These findings, however, need to be confirmed in larger samples. The same

authors also showed that one out of two cynomolgus monkeys infected with *N. asteroides* developed intracellular inclusion bodies (immunoreactive for α-synuclein and ubiquitin); the infected monkey also expressed rRNA for *N. asteroides*. Other viral and bacterial pathogens need to be studied in human postmortem brain tissue of PD patients using more recent virological and bacterial detection methods.[126]

Non-DA cell loss in PD

The concept of a specific neurotransmitter deficiency associated with a specific neurological syndrome potentially amenable to replacement therapy, as exemplified by the initial studies of Carlsson,[1] Ehringer and Hornykiewicz,[2] and Bernheimer,[3] has somehow obscured the fact that PD is not a disease restricted to the nigrostriatal system and involving solely DA as neurotransmitter. In fact, postmortem studies have shown that much of the peripheral and central nervous systems (stellate ganglia, cardiac, and enteric plexus, nucleus basalis of Meynert, amygdala, limbic nuclei of the thalamus, parahippocampal and cingulate gyri, insula, and isocortex) and transmitter systems (serotonin, noradrenaline, and acetylcholine) are affected in PD, albeit at varying degrees.[56] This explains the comorbidity of PD with depression,[127] dementia,[128] autonomic dysfunction,[129] and sleep disorders.[130] The common link between degeneration in these structures and/or transmitter systems may be the presence of LBs, which stresses their importance in PD pathogenesis.

The studies of Braak's group are of special interest here. In large series of individuals suffering from PD or in the preliminary stages, the distribution of LBs appears to follow a specific temporal (subdivided into stages 1 to 6) and anatomical distribution: 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 then pursues an ascending course. Cortical involvement ensues, beginning with the anteromedial temporal mesocortex. Next, the neocortex is affected, commencing with higher order sensory association and prefrontal areas. First-order sensory association/premotor areas and primary sensory/motor fields are affected last.[131,132]

Braak et al[133] have speculated that PD might originate outside the CNS, caused by an as yet unidentified pathogen capable of passing the mucosal barrier of the gastrointestinal tract and, via postganglionic enteric neurons, entering the CNS along unmyelinated preganglionic fibers generated from the visceromotor projection cells of the vagus nerve. By way of retrograde axonal and transneuronal transport, such a causative pathogen (a toxin and/or infectious agent?) could reach selectively vulnerable subcortical nuclei and, unimpeded, gain access to the cerebral cortex. At present, the experimental arguments supporting this intriguing hypothesis are sparse, especially because the relationship between neuronal degeneration and LB formation is still unclear.[134] However, considering current evidence, it is plausible that the presence of LBs indicates a disease process and reflects neuronal suffering.

Conclusion and perspectives

Human postmortem studies remain the mainstay of our understanding of PD. Despite considerable advances in modeling PD, none of the experimental models available today reflects all the major characteristics of the disease in humans. Human postmortem studies and experimental PD paradigms should be closely associated to study questions related to etiology and/or pathogenesis. Future major research topics will include the role of protein aggregation, LB formation, and proteasomal dysfunction in pathogenesis, and their relationship to DA metabolism, accounting for the selectivity of lesions in PD. The role of environmental toxins and infectious agents in the etiology of PD and in relation to susceptibility genes should also be an area of vigorous research. The microglial reaction and chronic inflammation will also be major therapeutic targets to slow PD progression. Interestingly, an inverse correlation between the intake of nonsteroidal anti-inflammatory drugs (NSAIDs) and the risk for PD has recently been claimed by an extensive epidemiological study.[135] In this regard, it would undoubtedly be of great value to study the brains of individuals with a long-standing history of NSAID intake to seek the presence (or absence) of PD-like pathology. With respect to these questions, we should emphasize the need to collect donor brains in specialized brains banks to supply the field of human postmortem PD research.[136] Specifically, brain bank characterization of PD brain samples and other neurodegenerative diseases in the postgenomic era must include the genotype and phenotype of the affected individuals as well as thorough clinical data. ❏

Basic research

Estudios postmortem en la enfermedad de Parkinson

A la fecha, ningún modelo animal ha podido reproducir perfectamente la etiopatogenia de la enfermedad de Parkinson (EP). Además, la organización anatómica del sistema nigroestriatal difiere considerablemente entre las especies. Es por esto que los estudios postmortem en humanos continúan siendo el gold standard tanto para la formulación de hipótesis que serán probadas posteriormente en modelos de EP in vivo e in vitro, como también para la verificación de hipótesis que deriven de modelos experimentales de EP respecto a su validez para la enfermedad en el hombre. Este artículo se centra en campos recientes y relevantes en los que el trabajo postmortem en humanos ha generado un impacto significativo para nuestra comprensión de la EP. Estos campos incluyen la formación de cuerpos de Lewy, la vulnerabilidad regional de las neuronas dopaminérgicas, el estrés celular de la oxidación y de la nitratación, la inflamación, la apoptosis, los agentes infecciosos y ambientales, y las lesiones no dopaminérgicas.

Études post mortem dans la maladie de Parkinson

Aucun modèle animal à ce jour ne reproduit fidèlement l'étiopathogenèse de la maladie de Parkinson (MP). Aussi, l'organisation anatomique du système nigrostrié varie considérablement d'une espèce à l'autre. C'est pourquoi les études post mortem chez l'homme restent la meilleure approche soit pour formuler des hypothèses qui seront ensuite testées dans des modèles in vitro et in vivo de la MP, soit pour vérifier des hypothèses dérivées de modèles expérimentaux de la MP, quant à leur validité en pathologie humaine. Dans cet article, l'accent sera mis sur des domaines de recherche récents et pertinents, tels que la formation de corps de Lewy, la vulnérabilité régionale des neurones dopaminergiques, le stress cellulaire lié à l'oxydation et la nitration, l'inflammation, l'apoptose, les agents infectieux et environnementaux ainsi que les lésions non dopaminergiques, où les études post mortem chez l'homme ont contribué de manière significative à notre compréhension de la MP.

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