Acta Neuropathol (2010) 120:131–143
DOI 10.1007/s00401-010-0711-0

REVIEW

The synaptic pathology of α-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia

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Received: 28 February 2010 / Revised: 31 May 2010 / Accepted: 11 June 2010 / Published online: 20 June 2010

Abstract Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are usually associated with loss of dopaminergic neurons. Loss of substantia nigra neurons and presence of Lewy body inclusions in some of the remaining neurons are the hallmark pathology seen in the final stages of the disease. Attempts to correlate Lewy body pathology to either cell death or severity of clinical symptoms, however, have not been successful. While the pathophysiology of the neurodegenerative process can hardly be explained by Lewy bodies, the clinical symptoms do indicate a degenerative process located at the presynapse resulting in a neurotransmitter deficiency. Recently it was shown that 90% or even more of α-synuclein aggregates in DLB cases were located at the presynapses in the form of very small deposits. In parallel, dendritic spines are retracted, whereas the presynapses are relatively preserved, suggesting a neurotransmitter deprivation. The same α-synuclein pathology can be demonstrated for PD. These findings give rise to the notion that not cell death but rather α-synuclein aggregate-related synaptic dysfunction causes the neurodegeneration. This opens new perspectives for understanding PD and DLB. If presynaptic α-synuclein aggregation, not neuronal loss, is the key issue of the neurodegenerative process, then PD and DLB may eventually be treatable in the future. The disease may progress via trans-synaptical spread, suggesting that stem cell transplants are of limited use. Future therapies may focus on the regeneration of synapses.

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Keywords α-Synuclein · Protein aggregates · Synapse · Neurodegeneration · Dendritic spines

Introduction

Synuclein proteins were identified independently by different groups. In 1988 Maroteaux et al. [93] described a protein associated with cholinergic vesicles in the electric organ of the Pacific electric ray (*Torpedo californica*) and a related 140 amino acid sequence in a rat brain cDNA library. Based on the initial findings of its synaptic and nuclear localization, the protein was denominated by the acronym 'synuclein'. Nakajo et al. and George et al. [39, 108] found brain-specific proteins in bovines and songbirds, and Ueda et al. [139] identified a 35 amino acid, non-A-β peptide in amyloid preparations of Alzheimer's disease patients that belongs to a 140 amino acid protein. Jakes et al. [59] demonstrated the similarities of these proteins by cloning two human homologues named α and β synuclein. Later, additional sequences were identified in rats and humans [1, 65] which are more closely related to the original *Torpedo* synuclein sequence and called γ-synuclein [22]. Gamma-synuclein was identified as being equivalent to the breast cancer-specific gene 1 (BCSG1) that is overexpressed in breast cancer cDNA [65]. For the field of neurodegenerative diseases, not only was the identification of synuclein as a non-A-β component in some Alzheimer's cases a milestone, but also the detection of α-synuclein as the major component of Lewy bodies [6, 128]. The discovery of mutations in the α-synuclein gene [77, 114, 156] and overexpression of α-synuclein [123] as being associated with Parkinson's disease or dementia with Lewy bodies (DLB) strengthens the association between protein misfolding and disease.

Although an intranuclear localization was reported initially, it was not confirmed any earlier than the discovery that nuclear inclusions in multiple system atrophy (MSA) are composed of α-synuclein [36, 127, 138, 140, 142]. Meanwhile, physiological α-synuclein can be identified in neurons [152]. It is upregulated in early-stage neuronal development [157], binds to histones [42] and affects histone acetylation [70]. In cell culture experiments, the C-terminal domain directs recombinant α-synuclein into the nuclear compartment, whereas presynaptic targeting depends on the presence of its N-terminal and core region [126]. The presynaptic localization was reported as early as the detection of the protein and confirmed by many groups [57, 59, 107, 137, 150]. Alpha-synuclein was shown to be related to phospholipids [108] and an interaction with the presynaptic membrane was reported [20, 34, 66]. Alpha-synuclein seems to be important for the size of the presynaptic vesicular pool and vesicle recycling [18, 19, 105] and plays an important role in neurotransmitter release [31, 90], especially for dopamine [2, 33, 91, 122, 151].

Lewy bodies in the pathophysiology of disease

Currently the neuropathological diagnosis of Parkinson's disease and dementia with Lewy bodies is based on the detection and quantification of Lewy bodies [8, 9, 38, 97–99]. These are insoluble protein aggregates forming fibrils and composed mainly but not exclusively of α-synuclein (Fig. 1a, b) [6, 141]. In Parkinson's disease, Lewy bodies are mainly found at predilection sites of neuronal loss, i.e. the substantia nigra and locus coeruleus. This has led to the conclusion that Lewy bodies are somehow related to nerve cell loss. The number of Lewy bodies in patients with mild to moderate loss of neurons in the substantia nigra is higher than in patients with severe neuronal depletion. It was thus interpreted that Lewy body-containing neurons are the dying neurons [141]. On the other hand, Lewy bodies may not always accompany nerve cell degeneration and it is indeed unlikely that every dying nerve cell goes through a stage of Lewy body formation [32]. It was shown that the presence of Lewy bodies does not predispose substantia nigra neurons to undergo apoptotic cell death to a greater degree than the general population of substantia nigra neurons and most neurons that undergo cell death do not contain Lewy bodies [136]. Substantia nigra neurons, whether they contain Lewy bodies or not, are similarly affected, for example, by morphological dendritic abnormalities or biochemical changes, indicating that the neurons in general are involved in the disease process [11, 52, 61, 113].

Consequently, attempts to correlate the density of either cortical or brain stem Lewy bodies with clinical disease symptoms in Parkinson's disease and DLB were not successful. Most studies failed to correlate Lewy body density with disease duration, early onset, different symptoms at onset, presence or absence of cognitive fluctuations, visual hallucinations, delusions, recurrent falls, severity of parkinsonism or cognitive decline [43–45, 94, 144]. However, one study showed a weak correlation between the density of Lewy bodies in the cingulate gyrus and cognitive decline [94]. It could be demonstrated that the presence of symptoms may be related to the involvement of defined regions as measured by the occurrence of Lewy bodies [47–49], supporting the observations that the α-synuclein aggregation pathology spreads through the brain involving anatomical structures sequentially [13]. Interestingly, in a percentage of Parkinson's patients who developed dementia, no Lewy bodies could be detected in cortical areas or in other areas outside the brain stem [37, 87]. These findings indicate that other mechanisms of disease-spread may exist than is reflected by the development of Lewy bodies.

The incidence of Lewy bodies in brains of asymptomatic individuals increases with advanced age. This raises the question of whether Lewy bodies reflect presymptomatic Parkinson's disease, as proposed by Dickson et al. [29], or are a feature of normal aging [63]. In a series of 904 autopsies, Lewy bodies were found in 106 individuals but only 32 had been diagnosed as suffering from a neurodegenerative disease [112]. Gibb [41] found an age-dependent increase in the prevalence of Lewy bodies from 3.8 to 12.8% between the sixth and ninth decade of age, exceeding the prevalence of age-related Parkinson's disease by about 3- to 6-fold, and many other studies show similar findings (for review see [64]).

In conclusion, although Lewy bodies are the neuropathological hallmark of the diagnosis, the pathophysiology of the neurodegenerative process can hardly be explained by them since the number of Lewy bodies is far too low for the severe symptoms. They appear to be neither associated with the cell loss nor do they correlate with the severity of clinical symptoms. Robert D. Terry [131], who did a lot of work explaining neurodegenerative diseases by synaptic failure, resumed: "It seems that not the α-synuclein of the Lewy body (…) is fatal to the neuron. We had better look elsewhere in that regard."

Synaptic pathology in neurodegenerative diseases

Alzheimer's disease and prion diseases

In Alzheimer's disease, the discussion on the impact of the large Aβ aggregates in the form of plaques on the clinical disease course has lasted for decades. Although there is an

association between the frequency and extension of tangles and Aβ-plaques and the occurrence of the disease [55, 104], Terry et al. [132] have shown that the cognitive decline in AD patients only correlates weakly with the quantity of Aβ-plaques or that of neurofibrillary tangles, but it does correlate with synapse loss detected post-mortem. Moreover, the synapse loss precedes the cortical neuron loss. A quantitative morphometric analysis of temporal and frontal cortical biopsies in AD patients revealed a greater loss of synapses than of neurons. On average, 30–38% fewer synapses per surviving neuron were detected in the temporal cortex in patients 3.4 years after onset of the disease and 16% fewer synapses in the frontal cortex 2.3 years after onset [25]. It was assumed that the cognitive impairment in AD is related to a synaptic failure [121, 124, 130]. In prion diseases the synaptic pathology is evident. Kitamoto et al. [68] have shown the parallels in distribution of prion aggregates and synaptophysin. This results in a loss of presynaptic terminals followed by synaptic spine degeneration [10, 62]. The presynaptic prion protein aggregate deposition could be linked to the decrease of neurotransmitter release [12]. Obviously, the most aggressive form of prion deposits is linked to the very faint synaptic aggregates. Whereas patients who suffer from prion diseases that form plaques survive for several years, as it is seen in hereditary Gerstmann–Sträussler–Scheinker syndrome or associated with stop mutations [40, 74], the youngest patients seen with prion diseases die after a much shorter clinical disease course and exhibit synaptic prion protein deposits that frequently can be visualized only by using the very sensitive PET blot technique [101, 120].

Evidence for synaptic pathology in Parkinson's disease and DLB

The clinical symptoms in Parkinson's disease and dementia with Lewy bodies suggest that a failure of synapses is the pathophysiological mechanism of disease. Tremor at rest, rigidity, akinesia/bradykinesia and postural instability are the four cardinal features in Parkinson's disease [60]. In DLB, progressive dementia with deficits in attention and executive functions, fluctuating cognition and recurrent visual hallucinations occur before or concurrently with the parkinsonian syndrome [96]. Akinesia/bradykinesia is assumed to be the result of a disruption of motor cortex activity (for review see [60]); tremor and rigidity are related to nigrostriatal dopaminergic deficits and the dopamine replacement treatment was the major breakthrough for Parkinson's disease patients in the last century [53]. In DLB and PDD, extrastriatal dopaminergic and particularly cholinergic deficits play a central role in mediating dementia (for review see [96]). Various studies of in vivo imaging of synaptic functions of the CNS found compelling evidence for presynaptic neurotransmitter deficiencies in Parkinson's disease, PDD and DLB (overview by [110]). All of these findings indicate that in PD, PDD and DLB the degenerative process is located at the presynapse [88] and results in a neurotransmitter deficiency syndrome.

Synuclein aggregate-related pathology at the synapse

PET blot results

By drawing parallels between α-synuclein aggregation diseases and prion diseases, we assumed that the synaptic pathology in Parkinson's disease and DLB could be linked to α-synuclein aggregates at synapses. To prove this hypothesis we used the PET blot method that we ourselves developed. For the protein aggregate detection by PET blot, paraffin-embedded tissues were cut the same way as for conventional histology but the slides were placed onto a nitrocellulose membrane. By protease digestion, protein aggregates were mobilized from the tissue, bound to the nitrocellulose membrane and epitopes in the aggregates were demasked. An additional guanidine isothiocyanate pretreatment may enhance the immunoreaction. The proteins were detected by an antibody reaction using the formazan reaction for visualization [120]. This method is the most sensitive topographical detection method for protein aggregates [120] and is widely used in prion research for this purpose [116, 119, 120, 146], as well as to characterize lesion patterns in humans and animals [85, 95, 135, 145] and to detect extracerebral involvement [3, 78, 133, 134]. Indeed we were able to visualize a significant amount of tiny α-synuclein aggregates throughout the cortex of DLB patients using the PET blot method (Fig. 2). These aggregates appear to be orders of magnitude smaller than Lewy bodies. The aggregates were most dense in the cingulate cortex. The amount of fine α-synuclein aggregates exceeds that of Lewy bodies or Lewy neurites by more than one order of magnitude. The distribution of these aggregates is identical with that of synaptophysin as can well be demonstrated by immunohistochemistry, suggesting a synaptic localization of the small α-synuclein aggregates. This is in keeping with many prion disease cases and fits well with our initial hypothesis that the synaptic pathology in DLB is linked to α-synuclein aggregates. Synaptic α-synuclein aggregates can be visualized by the PET blot technique also in cortical areas of PDD and brain stem areas of Parkinson's disease patients (Fig. 3).

Why was this significant amount of tiny α-synuclein aggregates not detected earlier? With regular immunohistochemical methods it is not possible to differentiate

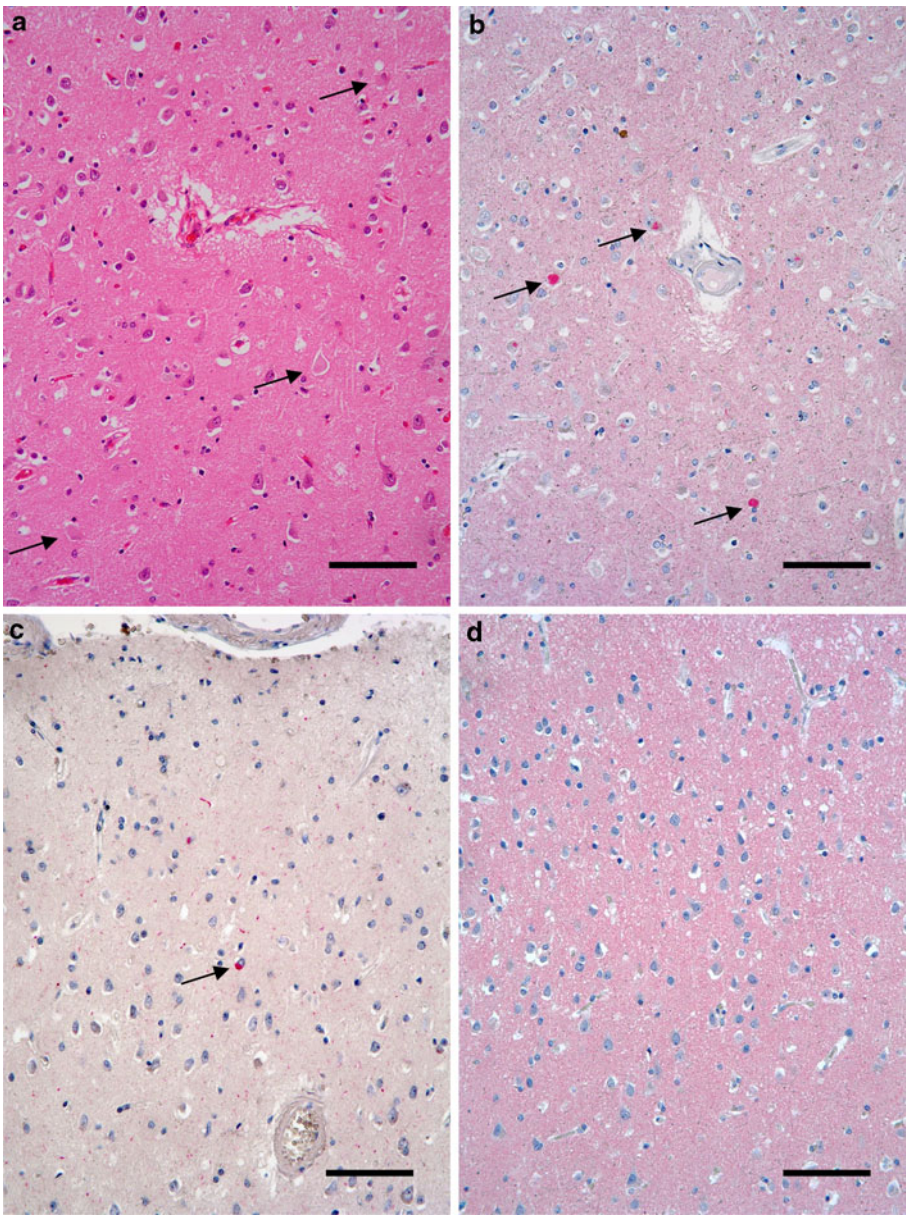

Fig. 1 Detection of α-synuclein deposits in DLB with conventional methods. H&E (**a**) shows 3 Lewy bodies (*arrows*). Immunohistochemically, Lewy bodies are detectable (**b**), whereas in the neuropil α-synuclein deposits are not distinguishable from the physiological α-synuclein staining (**d**) as compared to a control case (mAB 4B12, 1:1,000, abcam). With an antibody against phosphorylated α-synuclein (**c**), more deposits than just Lewy bodies are detectable (polyAB pSer129, 1:500, LifeSpan BioScience). *Bar* 100 μm

between physiological α-synuclein at the synapses and the tiny aggregates (Fig. 1b, d). In contrast to prion diseases, where neuropathologists have in principle the same problem, the amount of physiological α-synuclein is so high that staining occurs in all gray matter areas and the aggregates are so small that they are not easily distinguishable from the physiological staining (Fig. 1d). Because aggregates have been shown to contain phosphorylated α-synuclein [35], antibodies that specifically recognize the Ser129 phosphorylation site are able to recognize aggregates that do not co-react with physiological α-synuclein [117]. This immunohistochemical pattern (see Fig. 1c), however, differs from that seen with the PET blot (Fig. 2d). In our experience, only a limited amount of tiny α-synuclein aggregates is phosphorylated, as shown by PET blot in Fig. 2d (phosphorylated α-synuclein aggregates) in comparison to Fig. 2c (all α-synuclein aggregates), whereas all Lewy bodies can be labelled with both antibodies.

Biochemical analyses

Although the distribution of the small α-synuclein aggregates shows striking similarities to synaptophysin, this does not predicate where at the synapse the aggregates are located. To address this question we used a protocol for subcellular fractionation and synaptosome preparation from autopsy tissues in combination with a protocol for the separation of Lewy bodies on the basis of sucrose gradients

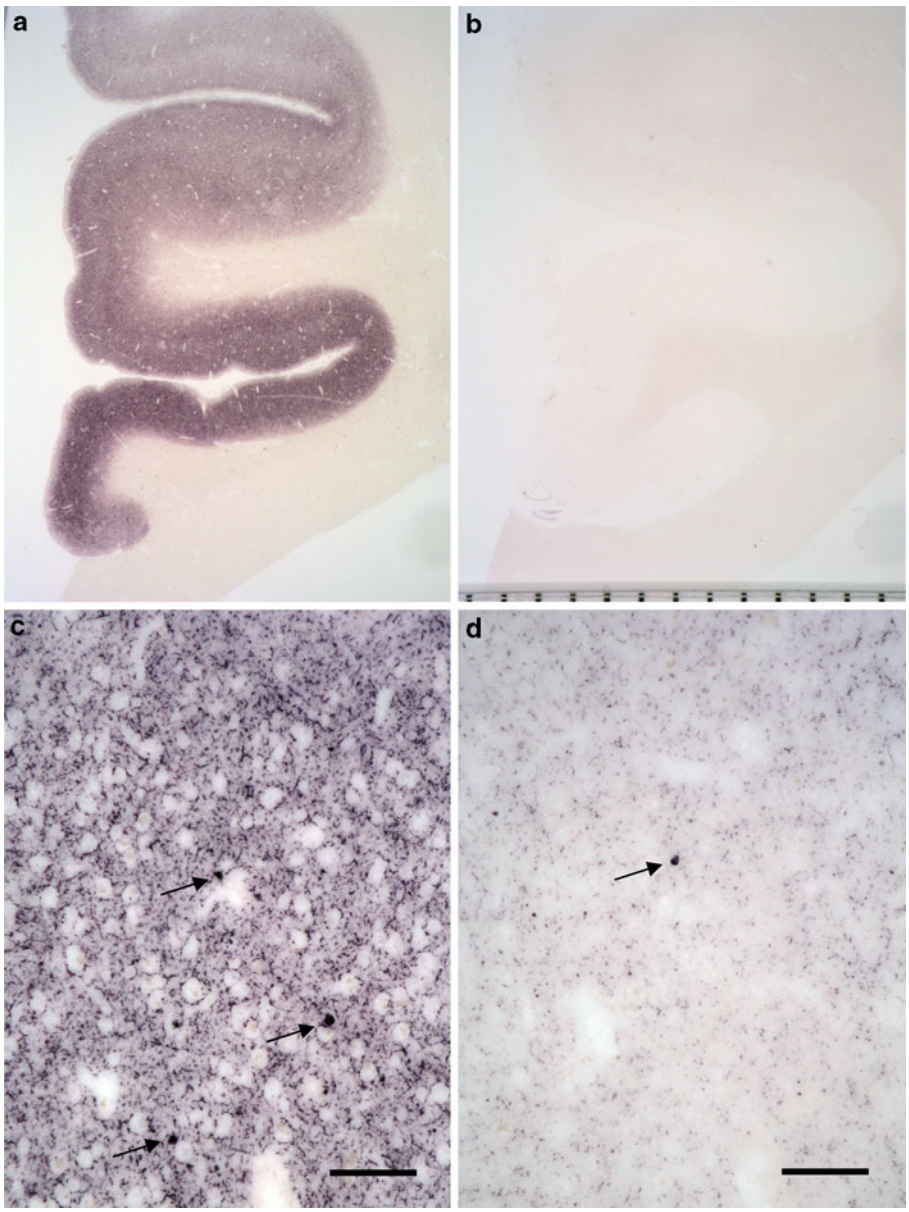

Fig. 2 More than 90% of α-synuclein aggregates are located outside of Lewy bodies at synapses in the frontal cortex of DLB. Frontal cortex and cingulate gyrus of a DLB- and control patient as seen using a dissection microscope (**a**, **b**). The higher magnification of the PET blot (**c**) shows the synaptic distribution of aggregates much smaller than Lewy bodies (LB indicated by *arrows*; mAB 4B12, 1:10,000). Using an antibody against phosphorylated α-synuclein (**d**), only a fraction of proteinase K-resistant aggregates is detectable (polyAB pSer129, 1:5,000). The detectability of phosphorylated α-synuclein is strongly influenced by the fixation period. Here the tissue was fixated short term using buffered formaldehyde. *Bar* 100 μm

[50, 58]. The main problem to solve was how to analyse the gradient fractions to gain information about their α-synuclein aggregate content. In contrast to prions, α-synuclein aggregates, independent of the disease in which they occur, are known to be highly insoluble [103], resulting in a smear at higher molecular weights in Western blot analysis, even after extraction with guanidinium hydrochloride, urea or formic acid [6, 67, 82, 89]. Even with the harshest pretreatment, the majority of aggregates get stuck at the bottom of the loading pocket of the gel for electrophoretic separation. Thus with Western blot a quantification of α-synuclein aggregate content is impossible [75].

We solved the problem by using a protein aggregate filtration (PAF) assay. Based on previously described methods [143, 149], the sucrose gradient fractions were sucked through a 200-nm-pore-size membrane, and the aggregates were retained and separated from soluble α-synuclein [75]. Undesirable binding of soluble proteins to the membrane was blocked with an amphiphilic polymer [51]. With this method it was possible to detect α-synuclein aggregates reliably and most sensitively and to quantify the amount of aggregates, as shown in dilution series [75].

As a result of the subcellular fractionation we found three peaks of α-synuclein aggregates. The smallest represented the Lewy body fraction containing 0.02–11% of the amount of α-synuclein aggregates. The largest fraction, corresponding to 50–92% of α-synuclein aggregates, ran at the 1.0/1.2 M sucrose interface, known for collecting the

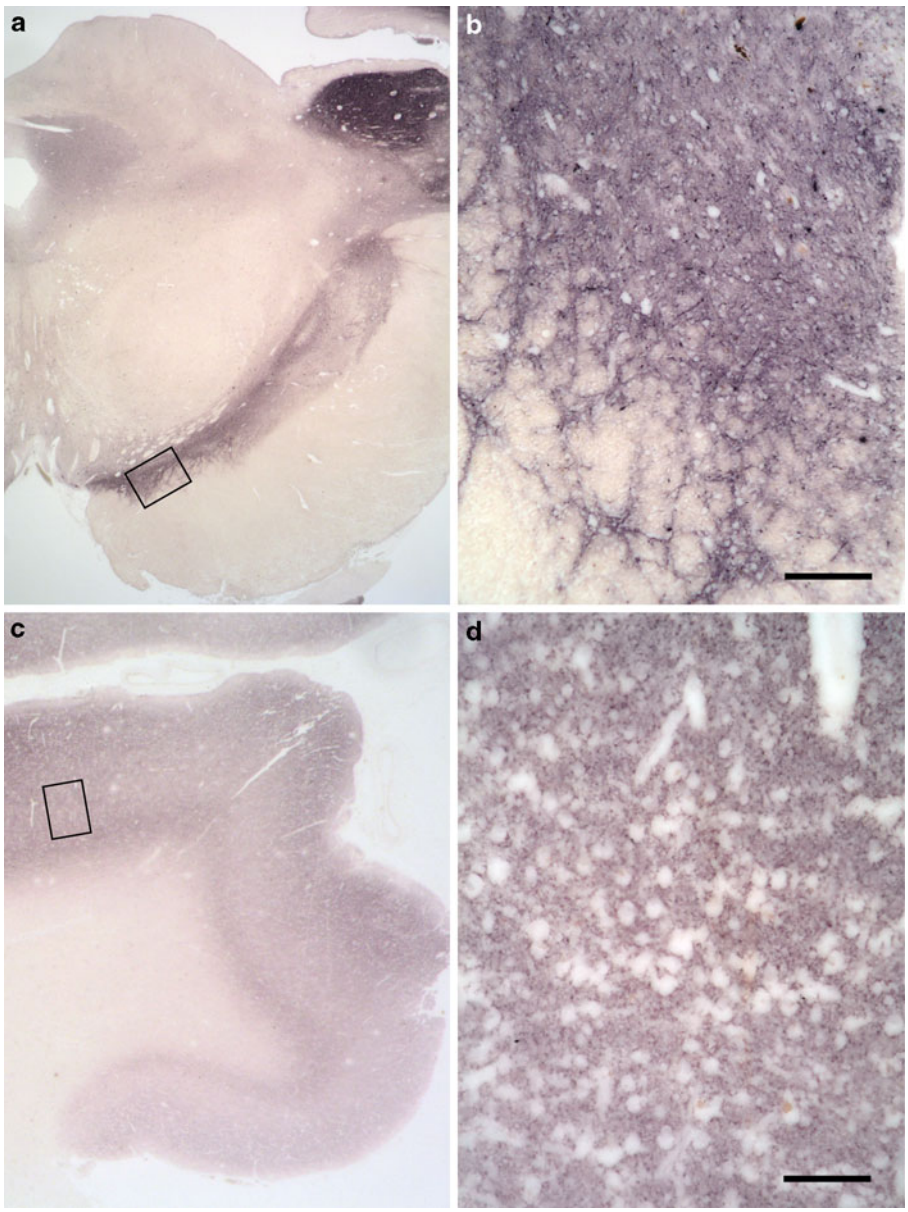

Fig. 3 Synaptic α-synuclein aggregates are the main synuclein pathology in Parkinson's disease as seen in DLB. The substantia nigra shows several proteinase K-resistant α-synuclein aggregates besides Lewy bodies (**a**). In a Parkinson's disease patient with dementia (**b**), the frontal cortex shows a lot of tiny α-synuclein aggregates even though no Lewy bodies are detectable (mAB 4B12, 1:10,000). *Bar* 100 μm

synaptosomes [50] that are detached synapses [147]. Indeed, exclusively in this fraction we found the synaptic vesicle marker synaptophysin and the synaptic membrane marker syntaxin [76] and identified the synaptosomes by electron microscopy. To confirm the presynaptic localization we analysed whether α-synuclein aggregates were trapped in synaptosomes. By hypertonic lysis, synaptosomes were disrupted [54] and α-synuclein aggregates located inside shifted in the sucrose gradient. Indeed, the α-synuclein aggregates shifted so that only one peak besides the Lewy body fraction was observed after hypotonic lysis of the synaptosomes. Analysing the sucrose gradient fractions by Western blot, it was shown that the synaptic vesicle protein syntaxin moved with the α-synuclein aggregates to the higher molecular interface, whereas the synaptic membrane protein synaptophysin was found at lower molecular weight in the sucrose gradient [76]. Moreover, the α-synuclein aggregates that were not migrating with the Lewy body fraction were one to two orders of magnitude smaller in size than the Lewy bodies.

In conclusion, the results from the biochemical analyses confirmed what we have seen with the PET blot method. With a magnitude of 1–2 orders more than Lewy bodies, by far most of the α-synuclein aggregates have the form of much smaller aggregates than Lewy bodies and they are located at the presynapse of neurons. In contrast to oligomeres, these aggregates are detergent insoluble as was shown with the aggregate filtration assay and also proteinase K-resistant as shown with the PET blot.

Do oligomeres or protofibrils explain the neurodegeneration?

The next question is whether small aggregates might be of relevance for the pathophysiology of the neurodegenerative process. In the past, many hypotheses related to small aggregates in neurodegenerative diseases have been generated. Because of the problems of small aggregate detection, it is more common to deal with soluble "oligomeres" that can be analysed by Western blot instead of investigating aggregates that are insoluble. The future will show whether this research approach advances neuroscience or is misleading. Even most of the research hypotheses dealing with small aggregates aim to elucidate the pathways leading to cell death. Because attempts to explain cell death by detectable fibrillar aggregates failed, the toxicity of intermediates was suggested [81]. Using in vitro fibrillogenesis, an oligomerisation of α-synuclein was found in tissue preparations of patients [23, 80]. These protofibrils, enriched in β-sheet structure, form spherical structures that can anneal in a linear fashion forming chains [23] or anneal in circular fashion forming ring structures. The spherical protofibrils have a higher tendency to bind to membranes than monomeric α-synuclein or fibrils. In membranes, the protofibrils can form pore-like structures and cause membrane permeabilization [30]. The mechanism of cytotoxicity can be reproduced using designed proteins not associated with clinical diseases, but only when they were added to cultured cells in their prefibrillar state [16]. A concentration-dependent cytotoxicity was described [7], and an increase in free calcium and reactive oxygen species levels [15], a dysfunction of mitochondria and different pattern of cell death were found [17].

Mitochondrial dysfunction gained increased relevance when researchers found that a syndrome nearly identical to parkinsonism could be induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication [79]. A metabolite of MPTP inhibits the mitochondrial complex I activity that is involved in the electron transport of the respiratory chain. As a result, mitochondrial ATP production is reduced. There may be an increase of reactive oxygen species, free radicals and induction of apoptosis by mitochondrial downstream processes, inducing neuronal cell death. It is assumed that α-synuclein aggregation accelerates mitochondrial dysfunction (for reviews see [46, 109, 148]).

In summary, it is questionable whether the hypotheses related to the toxicity of oligomeres or protofibrils address the key issues leading to neurodegeneration.

The research on oligomeres and protofibrils was aimed mostly at explaining cell death, although cell death does not seem to be the cause of neurodegeneration in DLB and Parkinson's disease, and is perhaps only an incidental consequence.

Are the presynaptic α-synuclein aggregates of pathophysiological relevance?

From neurophysiological studies it is known that the formation of postsynaptic dendritic spines is associated with presynaptic activity. Spine shapes are regulated dynamically by synaptic activity and changes in shape play an important role in synaptic plasticity. Long-term potentiation induces formation of new dendritic spines and deprivation causes a reduction [106] (for review see [153]). We assumed that the huge amount of presynaptic tiny α-synuclein aggregates have a pathological impact on dendritic spines. Analysing pre- and post-synaptic markers in DLB cases, we found a 50% reduction of the presynaptic markers synuclein and syntaxin as compared to controls [76]. This is in line with previous reports showing a reduction of presynaptic structures in DLB and Parkinson's disease [110, 115]. Looking at postsynaptic markers, there is a decrease in the postsynaptic scaffold protein PSD95 but the most considerable changes were seen in an almost complete loss of drebrin [76]. Drebrin is an f-actin-binding postsynaptic protein that is known to be involved in organizing the dendritic pool of actin for the formation of spines [5]. It is reported to modulate spine size and its content correlates with the spine head size [69]. In double transgenic mice serving as an Alzheimer's disease model (APP- and presenilin-1 mutation), a drebrin loss in the hippocampus and the entorhinal cortex precedes the onset of the AD pathology [4].

Because drebrin was beyond the Western blot detection threshold in frontal cortex samples of all DLB cases, we were interested in spine morphology. A Golgy–Cox silver impregnation with modifications according to Davenport was used [24, 118]. By visualizing the dendritic tree of single cells, we observed a nearly complete loss of dendritic spines in frontal cortical neurons of DLB patients, whereas in age-matched controls the dendrites were densely packed by spines (Fig. 4). A reduction of dendritic spines has been reported before in medium spiny neurons of the caudate nucleus in DLB patients [154]. Similar reports of a selective reduction of dendritic spines in Parkinson's disease suggest that the same pathophysiological changes at the synapse underlie Parkinson's disease as was shown for DLB. Selective loss of dendritic spines were reported for neurons of the prefrontal cortex and basal ganglia using the 6-hydroxy dopamine model for Parkinson's disease [56, 125] or in reserpine-treated mice [26] and for striatal regions and the substantia nigra in human Parkinson's disease tissues [28, 100, 113, 129, 155].

Detection of synaptic α-synuclein aggregates raises a novel concept of neurodegeneration

Our result of virtually complete dendritic spine loss in frontal cortex neurons was surprising because the loss of

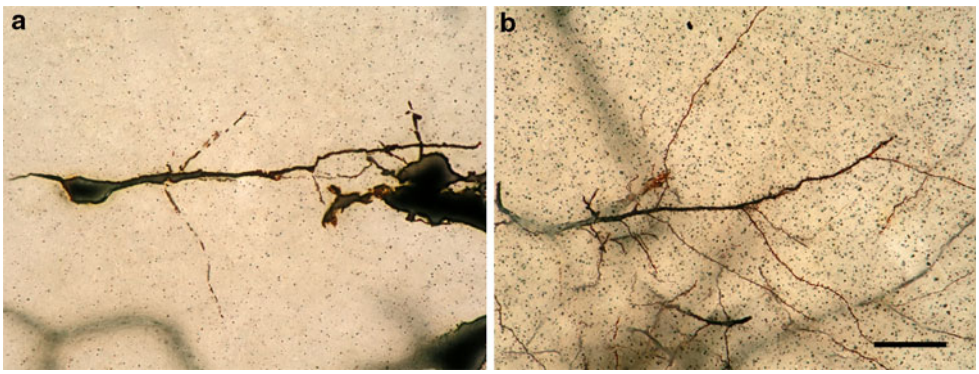

Fig. 4 An almost complete loss of dendritic spines accompanies the presynaptic α-synuclein aggregates. Golgy–Cox–Davenport staining of a neuronal dendrite of a frontal cortex neuron in a DLB patient (**a**) is compared to a control patient of the same age (**b**). *Bar* 50 μm

dendritic spines in diseased patients diverges from the moderate reduction of presynaptic markers. Together with the notion that nerve cell death is not the key in the pathophysiology of α-synuclein aggregating diseases, our findings give rise to a novel concept of neurodegeneration. It seems that a presynaptic accumulation of small α-synuclein aggregates are linked to dendritic spine degeneration. One possibility is that presynaptic α-synuclein aggregation interferes with neurotransmitter release. It has been shown in brain slice preparations of C57/Bl6 mice that the depletion of the neurotransmitter dopamine leads to profound loss of dendritic spines [26]. The imbalance of dendritic spine changes in relation to the relative preservation of presynaptic terminals may be explained by the finding that the bidirectional synaptic plasticity is based on the morphological plasticity of the dendritic spines [106].

The direct link between α-synuclein aggregation and synaptic pathology paves the way towards explaining the clinical symptoms of these neurodegenerative diseases. It serves as a basis for understanding the effect of L-dopa-therapy at the beginning of symptoms and its failure later in the disease. Moreover, the observation that a loss of function of still-existing nerve cells and not nerve cell loss itself is responsible for the clinical symptoms in DLB, Parkinson's disease and PDD makes it conceivable that these diseases may eventually be treatable in future. An animated illustration summing up the pathological findings and building a hypothesis of neurodegeneration based on presynaptic α-synuclein aggregation can soon be found at http://www.prionresearch.de.

How does the disease spread?

Many questions still remain to be answered. One of the most important issues is how the disease progresses. Neuropathological studies implicate a spread of α-synuclein aggregate pathology [13] because in early stages the pathological changes are restricted to certain areas that are constantly involved in later stages. These studies, however, are of limited use here because they assume Lewy bodies, rather than synaptic aggregates, to be an equivalent for the spread of pathology. The pattern of spread shows some parallels to prion diseases, although an infectivity of α-synuclein aggregates has not yet been shown. In prion diseases a trans-synaptic spread is evident [95]. This pathway can easily be explained because the physiological prion protein is anchored at the outer surface of nerve cells and lymphocytes, and prion aggregates can be found extra- as well as intracellularly. In contrast, α-synuclein is a cytoplasmatic protein. The mode of spread is an actual matter of debate. Recent findings that α-synuclein pathology spreads to implanted grafts, focused research efforts on this topic. In three autopsy studies of patients who received transplants of foetal mesencephalic neurons 11–14 years earlier, Lewy body-like inclusions reacting with antibodies against α-synuclein were detected [71, 72, 86]. These findings suggest that α-synuclein aggregation may spread from host to graft. The Lewy body-like pathology in grafted neurons does not necessarily mean their functional impairment. Our findings of synaptic α-synuclein pathology, although not yet shown in grafted neurons, may be one step towards explaining the graft pathology because the integration of grafts by synaptic contacts was demonstrated [73], and a trans-synaptic spread is one possible explanation. Oligomeres of α-synuclein have been shown to be released from cultured cells by exocytosis [83], and can be taken up via endocytosis [84]. Recently a neuron-to-neuron spread of α-synuclein pathology was shown in a cell culture model using adenovirus-mediated α-synuclein overexpression. Additionally, a neuron-to-neuron spread was demonstrated using cortical neural stem cells that were implanted into α-synuclein transgenic mice [27]. Other experiments have shown evidence that the pathological conformation of α-synuclein may act as a seed for forming aggregates in target cells [92]. For β-amyloid [102] and tau [21] seeding effects have been shown to induce the aggregation of the respective protein in transgenic animal models. Interestingly, an induction of α-synuclein aggregates into the enteric nervous system by oral application of

rotenone, which causes non-detectable levels of the toxin in blood or brain, was followed by a spread of α-synuclein pathology to the dorsal motor nucleus of the vagus, the intermediolateral nucleus in the spinal cord and the substantia nigra [111]. With these recent findings, a transsynaptic spread of α-synuclein pathology seems to be a more likely explanation for the propagation of the disease than alternative explanations such as inflammatory processes, oxidative stress or loss of neurotrophic support [14]. The latter aspects may be more of interest for explaining the initiation of the α-synuclein aggregation. They may also make cells vulnerable to amplify aggregates.

Conclusion

The clinical symptoms suggest that a synaptic dysfunction is the cause of the neurodegenerative process in Parkinson's disease and dementia with Lewy bodies. The discovery of a link between these diseases and mutations in the α-synuclein gene or a synuclein dose–response effect, respectively, highlight the importance of protein aggregation-related toxicity as a key issue in neurodegeneration. The recent finding that 90% or even more of α-synuclein aggregates are not localized in Lewy bodies but at the presynapse in the form of much smaller aggregates than Lewy bodies, may contribute towards explaining the synaptic dysfunction. In consequence, dendritic spines are retracted most obviously because of neurotransmitter deprivation. If synaptic α-synuclein aggregation is indeed the key issue in PD and DLB, then it is not cell death but a synaptic dysfunction that causes the neurodegenerative symptoms. Although synaptic α-synuclein aggregation has been neither postulated nor demonstrated before, a synaptic dysfunction has been assumed in explaining neurodegeneration for decades. The link between α-synuclein aggregation and the synaptic pathology opens a completely new avenue towards understanding these diseases as well as treatment options. If the presynaptic α-synuclein aggregation is the key issue, then PD and DLB may eventually be treatable in future, because the neuronal cells and their presynapses are, in principle, still present and the postsynapse is able to regenerate. Seen in the light of these new findings, therapeutic strategies against nerve cell death and stem cell implantation strategies may well be obsolete. As postulated by Terry, it can be assumed that a synaptic dysfunction in other neurodegenerative diseases such as Alzheimer's disease, ALS and Huntington's disease is linked to protein aggregates other than plaques or tangles.

Acknowledgments The skillful technical assistance of Tatjana Pfander and Kerstin Brekerbohm and the support of Chris Crozier in preparing the manuscript are acknowledged. The author is grateful to Christina Behrens and Uwe Hahmann for their helpful comments on the manuscript. The work of the Prion and Dementia Research Unit is funded by the VolkswagenStiftung (ZN 2168).

References

1. Akopian AN, Wood JN (1995) Peripheral nervous system-specific genes identified by subtractive cDNA cloning. J Biol Chem 270:21264–21270
2. Al-Wandi A, Ninkina N, Millership S, Williamson SJ, Jones PA, Buchman VL (2010) Absence of alpha-synuclein affects dopamine metabolism and synaptic markers in the striatum of aging mice. Neurobiol Aging 31:796–804
3. Andreoletti O, Simon S, Lacroux C et al (2004) PrPSc accumulation in myocytes from sheep incubating natural scrapie. Nat Med 10:591–593
4. Aoki C, Mahadomrongkul V, Fujisawa S, Habersat R, Shirao T (2007) Chemical and morphological alterations of spines within the hippocampus and entorhinal cortex precede the onset of Alzheimer's disease pathology in double knock-in mice. J Comp Neurol 505:352–362
5. Aoki C, Sekino Y, Hanamura K et al (2005) Drebrin A is a postsynaptic protein that localizes in vivo to the submembranous surface of dendritic sites forming excitatory synapses. J Comp Neurol 483:383–402
6. Baba M, Nakajo S, Tu PH et al (1998) Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 152:879–884
7. Baglioni S, Casamenti F, Bucciantini M et al (2006) Prefibrillar amyloid aggregates could be generic toxins in higher organisms. J Neurosci 26:8160–8167
8. Beach TG, Adler CH, Lue L et al (2009) Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. Acta Neuropathol 117:613–634
9. Beach TG, White CL, Hamilton RL et al (2008) Evaluation of alpha-synuclein immunohistochemical methods used by invited experts. Acta Neuropathol 116:277–288
10. Belichenko PV, Brown D, Jeffrey M, Fraser JR (2000) Dendritic and synaptic alterations of hippocampal pyramidal neurones in scrapie-infected mice. Neuropathol Appl Neurobiol 26:143–149
11. Bergeron C, Petrunka C, Weyer L, Pollanen MS (1996) Altered neurofilament expression does not contribute to Lewy body formation. Am J Pathol 148:267–272
12. Bouzamondo-Bernstein E, Hopkins SD, Spilman P et al (2004) The neurodegeneration sequence in prion diseases: evidence from functional, morphological and ultrastructural studies of the GABAergic system. J Neuropathol Exp Neurol 63:882–899
13. Braak H, Del TK, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211
14. Brundin P, Li JY, Holton JL, Lindvall O, Revesz T (2008) Research in motion: the enigma of Parkinson's disease pathology spread. Nat Rev Neurosci 9:741–745
15. Bucciantini M, Calloni G, Chiti F et al (2004) Prefibrillar amyloid protein aggregates share common features of cytotoxicity. J Biol Chem 279:31374–31382

16. Bucciantini M, Giannoni E, Chiti F et al (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416:507–511
17. Bucciantini M, Rigacci S, Berti A et al (2005) Patterns of cell death triggered in two different cell lines by HypF-N prefibrillar aggregates. FASEB J 19:437–439
18. Cabin DE, Shimazu K, Murphy D et al (2002) Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. J Neurosci 22:8797–8807
19. Chandra S, Fornai F, Kwon HB et al (2004) Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. Proc Natl Acad Sci USA 101:14966–14971
20. Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC (2005) Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 123:383–396
21. Clavaguera F, Bolmont T, Crowther RA et al (2009) Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol 11:909–913
22. Clayton DF, George JM (1998) The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. Trends Neurosci 21:249–254
23. Conway KA, Harper JD, Lansbury PT Jr (2000) Fibrils formed in vitro from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. Biochemistry 39:2552–2563
24. Davenport HA, Combs CM (1954) Golgi's dichromate-silver method. 3. Chromating fluids. Stain Technol 29:165–173
25. Davies CA, Mann DM, Sumpter PQ, Yates PO (1987) A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. J Neurol Sci 78:151–164
26. Day M, Wang Z, Ding J et al (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259
27. Desplats P, Lee HJ, Bae EJ et al (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci USA 106:13010–13015
28. Deutch AY (2006) Striatal plasticity in parkinsonism: dystrophic changes in medium spiny neurons and progression in Parkinson's disease. J Neural Transm Suppl (70):67–70
29. Dickson DW, Fujishiro H, DelleDonne A et al (2008) Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol 115:437–444
30. Ding TT, Lee SJ, Rochet JC, Lansbury PT Jr (2002) Annular alpha-synuclein protofibrils are produced when spherical protofibrils are incubated in solution or bound to brain-derived membranes. Biochemistry 41:10209–10217
31. DiRosa G, Puzzo D, Sant'Angelo A, Trinchese F, Arancio O (2003) Alpha-synuclein: between synaptic function and dysfunction. Histol Histopathol 18:1257–1266
32. Forno LS (1996) Neuropathology of Parkinson's disease. J Neuropathol Exp Neurol 55:259–272
33. Fortin DL, Nemani VM, Voglmaier SM, Anthony MD, Ryan TA, Edwards RH (2005) Neural activity controls the synaptic accumulation of alpha-synuclein. J Neurosci 25:10913–10921
34. Fortin DL, Troyer MD, Nakamura K, Kubo S, Anthony MD, Edwards RH (2004) Lipid rafts mediate the synaptic localization of alpha-synuclein. J Neurosci 24:6715–6723
35. Fujiwara H, Hasegawa M, Dohmae N et al (2002) alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol 4:160–164
36. Gai WP, Power JH, Blumbergs PC, Blessing WW (1998) Multiple-system atrophy: a new alpha-synuclein disease? Lancet 352:547–548
37. Galvin JE, Pollack J, Morris JC (2006) Clinical phenotype of Parkinson disease dementia. Neurology 67:1605–1611
38. Gelb DJ, Oliver E, Gilman S (1999) Diagnostic criteria for Parkinson disease. Arch Neurol 56:33–39
39. George JM, Jin H, Woods WS, Clayton DF (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron 15:361–372
40. Ghetti B, Piccardo P, Spillantini MG et al (1996) Vascular variant of prion protein cerebral amyloidosis with tau-positive neurofibrillary tangles: the phenotype of the stop codon 145 mutation in PRNP. Proc Natl Acad Sci USA 93:744–748
41. Gibb WR, Lees AJ (1988) The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 51:745–752
42. Goers J, Manning-Bog AB, McCormack AL et al (2003) Nuclear localization of alpha-synuclein and its interaction with histones. Biochemistry 42:8465–8471
43. Gomez-Isla T, Growdon WB, McNamara M et al (1999) Clinicopathologic correlates in temporal cortex in dementia with Lewy bodies. Neurology 53:2003–2009
44. Gomez-Tortosa E, Irizarry MC, Gomez-Isla T, Hyman BT (2000) Clinical and neuropathological correlates of dementia with Lewy bodies. Ann NY Acad Sci 920:9–15
45. Gomez-Tortosa E, Newell K, Irizarry MC, Albert M, Growdon JH, Hyman BT (1999) Clinical and quantitative pathologic correlates of dementia with Lewy bodies. Neurology 53:1284–1291
46. Gubellini P, Picconi B, Di FM, Calabresi P (2010) Downstream mechanisms triggered by mitochondrial dysfunction in the basal ganglia: from experimental models to neurodegenerative diseases. Biochim Biophys Acta 1802:151–161
47. Harding AJ, Broe GA, Halliday GM (2002) Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe. Brain 125:391–403
48. Harding AJ, Halliday GM (2001) Cortical Lewy body pathology in the diagnosis of dementia. Acta Neuropathol 102:355–363
49. Harding AJ, Stimson E, Henderson JM, Halliday GM (2002) Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. Brain 125:2431–2445
50. Hardy JA, Dodd PR, Oakley AE, Kidd AM, Perry RH, Edwardson JA (1982) Use of post-mortem human synaptosomes for studies of metabolism and transmitter amino acid release. Neurosci Lett 33:317–322
51. Haycock JW (1993) Polyvinylpyrrolidone as a blocking agent in immunochemical studies. Anal Biochem 208:397–399
52. Hill WD (1996) Altered neurofilament expression does not contribute to Lewy body formation. Am J Pathol 149:728–729
53. Hornykiewicz O (2008) Basic research on dopamine in Parkinson's disease and the discovery of the nigrostriatal dopamine pathway: the view of an eyewitness. Neurodegener Dis 5:114–117
54. Huttner WB, Schiebler W, Greengard P, De CP (1983) Synapsin I (protein I), a nerve terminal-specific phosphoprotein. III. Its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation. J Cell Biol 96:1374–1388
55. Hyman BT, Trojanowski JQ (1997) Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. J Neuropathol Exp Neurol 56:1095–1097
56. Ingham CA, Hood SH, Arbuthnott GW (1989) Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. Brain Res 503:334–338
57. Iwai A, Masliah E, Yoshimoto M et al (1995) The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. Neuron 14:467–475

58. Iwatsubo T, Yamaguchi H, Fujimuro M et al (1996) Purification and characterization of Lewy bodies from the brains of patients with diffuse Lewy body disease. Am J Pathol 148:1517–1529
59. Jakes R, Spillantini MG, Goedert M (1994) Identification of two distinct synucleins from human brain. FEBS Lett 345:27–32
60. Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79:368–376
61. Javoy-Agid F, Hirsch EC, Dumas S, Duyckaerts C, Mallet J, Agid Y (1990) Decreased tyrosine hydroxylase messenger RNA in the surviving dopamine neurons of the substantia nigra in Parkinson's disease: an in situ hybridization study. Neuroscience 38:245–253
62. Jeffrey M, Halliday WG, Bell J et al (2000) Synapse loss associated with abnormal PrP precedes neuronal degeneration in the scrapie-infected murine hippocampus. Neuropathol Appl Neurobiol 26:41–54
63. Jellinger KA (2004) Lewy body-related alpha-synucleinopathy in the aged human brain. J Neural Transm 111:1219–1235
64. Jellinger KA (2008) A critical reappraisal of current staging of Lewy-related pathology in human brain. Acta Neuropathol 116:1–16
65. Ji H, Liu YE, Jia T et al (1997) Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing. Cancer Res 57:759–764
66. Jo E, Darabie AA, Han K, Tandon A, Fraser PE, McLaurin J (2004) alpha-Synuclein–synaptosomal membrane interactions: implications for fibrillogenesis. Eur J Biochem 271:3180–3189
67. Kahle PJ, Neumann M, Ozmen L et al (2001) Selective insolubility of alpha-synuclein in human Lewy body diseases is recapitulated in a transgenic mouse model. Am J Pathol 159:2215–2225
68. Kitamoto T, Shin RW, Doh-ura K et al (1992) Abnormal isoform of prion proteins accumulates in the synaptic structures of the central nervous system in patients with Creutzfeldt–Jakob disease. Am J Pathol 140:1285–1294
69. Kobayashi C, Aoki C, Kojima N, Yamazaki H, Shirao T (2007) Drebrin a content correlates with spine head size in the adult mouse cerebral cortex. J Comp Neurol 503:618–626
70. Kontopoulos E, Parvin JD, Feany MB (2006) Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. Hum Mol Genet 15:3012–3023
71. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med 14:504–506
72. Kordower JH, Chu Y, Hauser RA, Olanow CW, Freeman TB (2008) Transplanted dopaminergic neurons develop PD pathologic changes: a second case report. Mov Disord 23:2303–2306
73. Kordower JH, Freeman TB, Snow BJ et al (1995) Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. N Engl J Med 332:1118–1124
74. Kovacs GG, Puopolo M, Ladogana A et al (2005) Genetic prion disease: the EUROCJD experience. Hum Genet 118:166–174
75. Kramer ML, Behrens C, Schulz-Schaeffer WJ (2008) Selective detection, quantification, and subcellular location of alpha-synuclein aggregates with a protein aggregate filtration assay. Biotechniques 44:403–411
76. Kramer ML, Schulz-Schaeffer WJ (2007) Presynaptic alpha-synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies. J Neurosci 27:1405–1410
77. Kruger R, Kuhn W, Muller T et al (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18:106–108
78. Lacroux C, Simon S, Benestad SL et al (2008) Prions in milk from ewes incubating natural scrapie. PLoS Pathog 4:e1000238
79. Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219:979–980
80. Langston JW, Sastry S, Chan P, Forno LS, Bolin LM, Di Monte DA (1998) Novel alpha-synuclein-immunoreactive proteins in brain samples from the Contursi kindred, Parkinson's, and Alzheimer's disease. Exp Neurol 154:684–690
81. Lansbury PT Jr (1999) Evolution of amyloid: what normal protein folding may tell us about fibrillogenesis and disease. Proc Natl Acad Sci USA 96:3342–3344
82. Lee HJ, Lee SJ (2002) Characterization of cytoplasmic alpha-synuclein aggregates. Fibril formation is tightly linked to the inclusion-forming process in cells. J Biol Chem 277:48976–48983
83. Lee HJ, Patel S, Lee SJ (2005) Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. J Neurosci 25:6016–6024
84. Lee HJ, Suk JE, Bae EJ, Lee JH, Paik SR, Lee SJ (2008) Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. Int J Biochem Cell Biol 40:1835–1849
85. Lezmi S, Bencsik A, Baron T (2006) PET-blot analysis contributes to BSE strain recognition in C57Bl/6 mice. J Histochem Cytochem 54:1087–1094
86. Li JY, Englund E, Holton JL et al (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med 14:501–503
87. Libow LS, Frisina PG, Haroutunian V, Perl DP, Purohit DP (2009) Parkinson's disease dementia: a diminished role for the Lewy body. Parkinsonism Relat Disord 15:572–575
88. Linazasoro G (2007) Classical Parkinson disease versus Parkinson complex—reflections against staging and in favour of heterogeneity. Eur J Neurol 14:721–728
89. Lippa CF, Fujiwara H, Mann DM et al (1998) Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer's disease patients with mutations in presenilin and amyloid precursor protein genes. Am J Pathol 153:1365–1370
90. Liu S, Ninan I, Antonova I et al (2004) alpha-Synuclein produces a long-lasting increase in neurotransmitter release. EMBO J 23:4506–4516
91. Lotharius J, Brundin P (2002) Impaired dopamine storage resulting from alpha-synuclein mutations may contribute to the pathogenesis of Parkinson's disease. Hum Mol Genet 11:2395–2407
92. Luk KC, Song C, O'Brien P et al (2009) Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc Natl Acad Sci USA 106:20051–20056
93. Maroteaux L, Campanelli JT, Scheller RH (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. J Neurosci 8:2804–2815
94. Mattila PM, Rinne JO, Helenius H, Dickson DW, Roytta M (2000) Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. Acta Neuropathol 100:285–290
95. McBride PA, Schulz-Schaeffer WJ, Donaldson M et al (2001) Early spread of scrapie from the gastrointestinal tract to the central nervous system involves autonomic fibers of the splanchnic and vagus nerves. J Virol 75:9320–9327
96. McKeith I (2007) Dementia with Lewy bodies. In: Koller WC, Melamed E (eds) Parkinson's disease and related disorders, Part II. Handb Clin Neurol 84:531–548
97. McKeith IG (2006) Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the Consortium on DLB International Workshop. J Alzheimers Dis 9:417–423

98. McKeith IG, Dickson DW, Lowe J et al (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 65:1863–1872
99. McKeith IG, Galasko D, Kosaka K et al (1996) Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 47:1113–1124
100. McNeill TH, Brown SA, Rafols JA, Shoulson I (1988) Atrophy of medium spiny I striatal dendrites in advanced Parkinson's disease. Brain Res 455:148–152
101. Meissner B, Westner IM, Kallenberg K et al (2005) Sporadic Creutzfeldt–Jakob disease: clinical and diagnostic characteristics of the rare VV1 type. Neurology 65:1544–1550
102. Meyer-Luehmann M, Coomaraswamy J, Bolmont T et al (2006) Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science 313:1781–1784
103. Miake H, Mizusawa H, Iwatsubo T, Hasegawa M (2002) Biochemical characterization of the core structure of alpha-synuclein filaments. J Biol Chem 277:19213–19219
104. Mirra SS, Heyman A, McKeel D et al (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41:479–486
105. Murphy DD, Rueter SM, Trojanowski JQ, Lee VM (2000) Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. J Neurosci 20:3214–3220
106. Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T (2004) Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron 44:759–767
107. Nakajo S, Shioda S, Nakai Y, Nakaya K (1994) Localization of phosphoneuroprotein 14 (PNP 14) and its mRNA expression in rat brain determined by immunocytochemistry and in situ hybridization. Brain Res Mol Brain Res 27:81–86
108. Nakajo S, Tsukada K, Omata K, Nakamura Y, Nakaya K (1993) A new brain-specific 14-kDa protein is a phosphoprotein. Its complete amino acid sequence and evidence for phosphorylation. Eur J Biochem 217:1057–1063
109. Naoi M, Maruyama W, Yi H, Inaba K, Akao Y, Shamoto-Nagai M (2009) Mitochondria in neurodegenerative disorders: regulation of the redox state and death signaling leading to neuronal death and survival. J Neural Transm 116:1371–1381
110. Nikolaus S, Antke C, Muller HW (2009) In vivo imaging of synaptic function in the central nervous system: I. Movement disorders and dementia. Behav Brain Res 204:1–31
111. Pan-Montojo F, Anichtchik O, Dening Y et al (2010) Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. PLoS One 5:e8762
112. Parkkinen L, Kauppinen T, Pirttila T, Autere JM, Alafuzoff I (2005) Alpha-synuclein pathology does not predict extrapyramidal symptoms or dementia. Ann Neurol 57:82–91
113. Patt S, Gertz HJ, Gerhard L, Cervos-Navarro J (1991) Pathological changes in dendrites of substantia nigra neurons in Parkinson's disease: a Golgi study. Histol Histopathol 6:373–380
114. Polymeropoulos MH, Lavedan C, Leroy E et al (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047
115. Revuelta GJ, Rosso A, Lippa CF (2008) Neuritic pathology as a correlate of synaptic loss in dementia with Lewy bodies. Am J Alzheimers Dis Other Demen 23:97–102
116. Ritchie DL, Head MW, Ironside JW (2004) Advances in the detection of prion protein in peripheral tissues of variant Creutzfeldt–Jakob disease patients using paraffin-embedded tissue blotting. Neuropathol Appl Neurobiol 30:360–368
117. Saito Y, Kawashima A, Ruberu NN et al (2003) Accumulation of phosphorylated alpha-synuclein in aging human brain. J Neuropathol Exp Neurol 62:644–654
118. Scheibel ME, Scheibel AB (1978) The methods of Golgi. In: Robertson RT (ed) Neuroanatomical research techniques. Academic Press, New York, pp 89–114
119. Schulz-Schaeffer WJ, Fatzer R, Vandevelde M, Kretzschmar HA (2000) Detection of PrP(Sc) in subclinical BSE with the paraffin-embedded tissue (PET) blot. Arch Virol Suppl 173–180
120. Schulz-Schaeffer WJ, Tschoke S, Kranefuss N et al (2000) The paraffin-embedded tissue blot detects PrP(Sc) early in the incubation time in prion diseases. Am J Pathol 156:51–56
121. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. Science 298:789–791
122. Sidhu A, Wersinger C, Vernier P (2004) Does alpha-synuclein modulate dopaminergic synaptic content and tone at the synapse? FASEB J 18:637–647
123. Simon-Sanchez J, Schulte C, Bras JM et al (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 41:1308–1312
124. Small DH, Mok SS, Bornstein JC (2001) Alzheimer's disease and Abeta toxicity: from top to bottom. Nat Rev Neurosci 2:595–598
125. Solis O, Limon DI, Flores-Hernandez J, Flores G (2007) Alterations in dendritic morphology of the prefrontal cortical and striatum neurons in the unilateral 6-OHDA-rat model of Parkinson's disease. Synapse 61:450–458
126. Specht CG, Tigaret CM, Rast GF, Thalhammer A, Rudhard Y, Schoepfer R (2005) Subcellular localisation of recombinant alpha- and gamma-synuclein. Mol Cell Neurosci 28:326–334
127. Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M (1998) Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. Neurosci Lett 251:205–208
128. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388:839–840
129. Stephens B, Mueller AJ, Shering AF et al (2005) Evidence of a breakdown of corticostriatal connections in Parkinson's disease. Neuroscience 132:741–754
130. Terry RD (2000) Cell death or synaptic loss in Alzheimer disease. J Neuropathol Exp Neurol 59:1118–1119
131. Terry RD (2000) Do neuronal inclusions kill the cell? J Neural Transm Suppl 59:91–93
132. Terry RD, Masliah E, Salmon DP et al (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572–580
133. Thomzig A, Schulz-Schaeffer W, Kratzel C, Mai J, Beekes M (2004) Preclinical deposition of pathological prion protein PrPSc in muscles of hamsters orally exposed to scrapie. J Clin Invest 113:1465–1472
134. Thomzig A, Schulz-Schaeffer W, Wrede A et al (2007) Accumulation of pathological prion protein PrPSc in the skin of animals with experimental and natural scrapie. PLoS Pathog 3:e66
135. Thomzig A, Spassov S, Friedrich M, Naumann D, Beekes M (2004) Discriminating scrapie and bovine spongiform encephalopathy isolates by infrared spectroscopy of pathological prion protein. J Biol Chem 279:33847–33854
136. Tompkins MM, Hill WD (1997) Contribution of somal Lewy bodies to neuronal death. Brain Res 775:24–29
137. Totterdell S, Meredith GE (2005) Localization of alpha-synuclein to identified fibers and synapses in the normal mouse brain. Neuroscience 135:907–913
138. Tu PH, Galvin JE, Baba M et al (1998) Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system

atrophy brains contain insoluble alpha-synuclein. Ann Neurol 44:415–422
139. Ueda K, Fukushima H, Masliah E et al (1993) Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci USA 90:11282–11286
140. Wakabayashi K, Hayashi S, Kakita A et al (1998) Accumulation of alpha-synuclein/NACP is a cytopathological feature common to Lewy body disease and multiple system atrophy. Acta Neuropathol 96:445–452
141. Wakabayashi K, Tanji K, Mori F, Takahashi H (2007) The Lewy body in Parkinson's disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. Neuropathology 27:494–506
142. Wakabayashi K, Yoshimoto M, Tsuji S, Takahashi H (1998) Alpha-synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. Neurosci Lett 249:180–182
143. Wanker EE, Scherzinger E, Heiser V, Sittler A, Eickhoff H, Lehrach H (1999) Membrane filter assay for detection of amyloid-like polyglutamine-containing protein aggregates. Methods Enzymol 309:375–386
144. Weisman D, Cho M, Taylor C, Adame A, Thal LJ, Hansen LA (2007) In dementia with Lewy bodies, Braak stage determines phenotype, not Lewy body distribution. Neurology 69:356–359
145. Wemheuer WM, Benestad SL, Wrede A et al (2009) Similarities between forms of sheep scrapie and Creutzfeldt–Jakob disease are encoded by distinct prion types. Am J Pathol 175:2566–2573
146. Wemheuer WM, Benestad SL, Wrede A et al (2009) Detection of classical and atypical/Nor98 scrapie by the paraffin-embedded tissue blot method. Vet Rec 164:677–681
147. Whittaker VP (1993) Thirty years of synaptosome research. J Neurocytol 22:735–742
148. Winklhofer KF, Haass C (2010) Mitochondrial dysfunction in Parkinson's disease. Biochim Biophys Acta 1802:29–44
149. Winklhofer KF, Hartl FU, Tatzelt J (2001) A sensitive filter retention assay for the detection of PrP(Sc) and the screening of anti-prion compounds. FEBS Lett 503:41–45
150. Withers GS, George JM, Banker GA, Clayton DF (1997) Delayed localization of synelfin (synuclein, NACP) to presynaptic terminals in cultured rat hippocampal neurons. Brain Res Dev Brain Res 99:87–94
151. Yavich L, Tanila H, Vepsalainen S, Jakala P (2004) Role of alpha-synuclein in presynaptic dopamine recruitment. J Neurosci 24:11165–11170
152. Yu S, Li X, Liu G et al (2007) Extensive nuclear localization of alpha-synuclein in normal rat brain neurons revealed by a novel monoclonal antibody. Neuroscience 145:539–555
153. Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24:1071–1089
154. Zaja-Milatovic S, Keene CD, Montine KS, Leverenz JB, Tsuang D, Montine TJ (2006) Selective dendritic degeneration of medium spiny neurons in dementia with Lewy bodies. Neurology 66:1591–1593
155. Zaja-Milatovic S, Milatovic D, Schantz AM et al (2005) Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. Neurology 64:545–547
156. Zarranz JJ, Alegre J, Gomez-Esteban JC et al (2004) The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 55:164–173
157. Zhong SC, Luo X, Chen XS et al (2010) Expression and subcellular location of alpha-synuclein during mouse-embryonic development. Cell Mol Neurobiol 30:469–482