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

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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-synaptic spread, suggesting that stem cell transplants are of limited use. Future therapies may focus on the regeneration of synapses.

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-Aβ 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-Aβ 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 \( \alpha \)-synuclein \([36, 127, 138, 140, 142]\). Meanwhile, physiological \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-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 A\( \beta \) 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
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 α-synuclein staining (d) as compared to a control case (mAB 4B12, 1:1,000, abcam). With an antibody against phosphorylated α-synuclein (e), more deposits than just Lewy bodies are detectable (polyAB pSer129, 1:500, LifeSpan BioScience). Bar 100 μm

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.
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...
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 \( \alpha \)-synuclein aggregates were trapped in synaptosomes. By hypertonic lysis, synaptosomes were disrupted [54] and \( \alpha \)-synuclein aggregates located inside shifted in the sucrose gradient. Indeed, the \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-synuclein aggregates have the form of much smaller aggregates than Lewy bodies and they are located at the presynapse of neurons. In contrast to oligomers, these aggregates are detergent insoluble as was shown with the aggregate filtration assay and also proteinase K-resistant as shown with the PET blot.

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

![Fig. 4](http://www.prionresearch.de)
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 trans-synaptic 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 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.

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