Recent advances in the understanding of the role of synaptic proteins in Alzheimer's Disease and other neurodegenerative disorders

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Synaptic damage is an early pathological event common to many neurodegenerative disorders such as Alzheimer's disease (AD) and is the best correlate to the cognitive impairment. Several molecules involved in AD and in other neurodegenerative disorders play an important role in synaptic function and when misfolded aggregate and form amyloid fibrils. Synaptic proteins with an amyloid domain include amyloid β-protein precursor, prion protein, huntingtin, ataxin-1 and α-synuclein. Two of the possible mechanisms by which alterations in synaptic proteins lead to synapse damage are: 1) misfolded or aggregated synaptic molecules have lost their normal function and/or 2) they have gained a toxic capacity. Recent studies support the possibility that while oligomers are toxic, polymers might be inactive. The mechanisms by which oligomers trigger synapse loss could be related to their ability to triggers stress signals once they enter the nucleus and/or accumulate at the endoplasmic reticulum.

1. Introduction

Neurodegenerative disorders are characterized by damage to selective neuronal populations [22], synapse loss, formation of inclusion bodies, extracellular deposition of amyloid proteins and gliosis (for review see [37,39]). There exists significant controversy in understanding which of these pathological events are primary and which ones are secondary (Fig. 1). Studies in the brains of patients with pre-clinical Alzheimer's disease (AD) [3,36], as well as in transgenic animal models [51], support the notion that synaptic damage occurs early in disease progression. This early disruption of synaptic connections in the brain results in neuronal dysfunction that, in turn, leads to the characteristic symptoms of dementia and/or motor impairment observed in several neurodegenerative disorders [37,39, 44,65]. However, additional studies are necessary to confirm this possibility especially in view of reports that have not shown neuronal loss in amyloid β-protein precursor (AβPP) transgenic mice [25]. In addition, other studies have implicated the formation of neurofibrillary tangles (NFTs) as responsible for synapse loss [7]. A possible explanation of why some studies show early synapse loss in AD while others do not, might be related in part to the region of the brain analyzed, the sensitivity of the markers and assays used, and the compensatory changes in the molecular response to neuronal injury [40]. In this regard, in AD, disruption of the perforant pathway circuitry is probably the earliest pathological event, followed by neuronal loss, and plaque and tangle formation [3,5,24]. This indicates that in AD the region of the brain where synapse loss might occur is the outer molecular layer of the dentate gyrus and that synaptic alterations in other brain regions might be a later event. Interestingly, recent studies have shown that many of the neuronal molecules affected in neurodegenerative disorders play an important role in the maintenance and functioning of the synaptic apparatus [18,41], leading to the hypothesis that mutations and other alterations of synaptic proteins might result in particular neurodegenerative diseases (Table 1). In this context, the concept of synapse loss has expanded our understanding of neurodegeneration and has helped to further elucidate the pathogenic mechanisms of this process.

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Table 1
Synaptic proteins involved in neurodegenerative disorders

Molecule	Synaptic localization	Proposed function	Disease
Amyloid β precursor protein	pre- and post-synaptic	LTP, glutamate transport	Alzheimer's disease
Presenilin	post-synaptic	γ-secretase, Notch signal	ing Alzheimer's disease
α-synuclein	pre-synaptic	synaptic signaling regulation (?)	Alzheimer's disease Lewy body disease Multiple system athrophy
Huntingtin	Post-synaptic	?	Huntington's disease
Ataxin-1	neuronal, post-synaptic	LTP	Spinocerebellar ataxia
Prion Protein	pre-synaptic	LTP, neuroplasticity	CJD and other spongiform encephalitis
Frataxin	pre-synaptic	Mitochondrial regulation	Friedreich ataxia

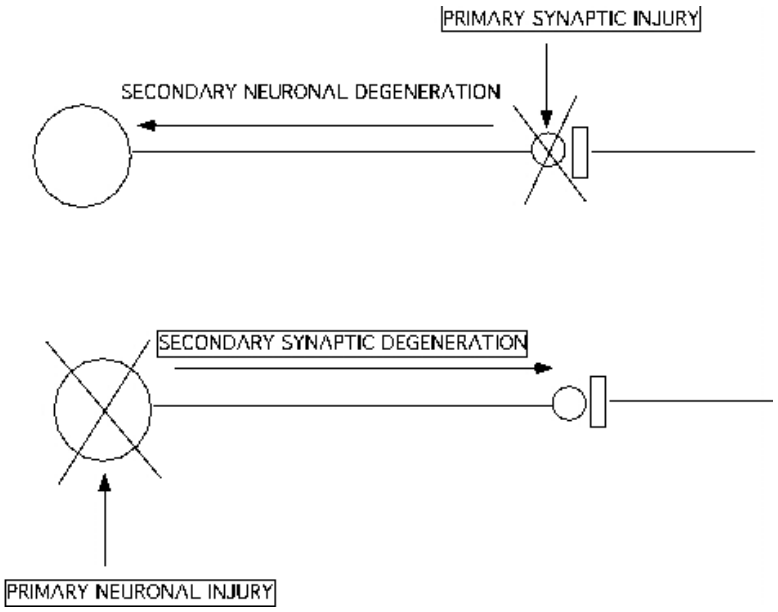

Fig. 1. Synaptic loss can be a primary or secondary event in the process leading to neurodegeneration.

This manuscript reviews some new thoughts as to the potential mechanisms underlying the pathogenesis of synaptic loss in neurodegenerative disorders.

2. Synaptic proteins in neurodegenerative disorders

Several molecules involved in AD and other neurodegenerative disorders play an important role in synaptic function (Table 1). Although, of course, there are many other examples of neuronal proteins involved in neurodegeneration that do not concentrate in the synapses such as superoxide dismutase 1. Another important common feature is that these synaptic proteins contain an amyloidogenic domain, suggesting that mutations and other stress-inducing factors might lead to aggregation and abnormal conformation which, in turn, can compromise synapse function (Fig. 2). Synaptic proteins with an amyloid domain include, among others, AβPP, prion protein (PrP), huntingtin (htt), ataxin-1 and α-synuclein (Table 1).

Remarkably, and in support of this concept, studies have shown that in Lewy body disease (LBD) α-synuclein accumulates in Lewy bodies (LBs) [62, 63,68]. LBD is a heterogeneous group of disorders presenting with parkinsonism and LB formation [47]. In accordance with the CDLB International Workshop [47], this group includes Parkinson's disease (PD), Diffuse LBD, Lewy body variant of AD and combined PD + AD. Furthermore, mutations in the α-synuclein gene are associated with rare familial forms of PD [32, 56]. α-Synuclein, a 140 aa molecule, was originally identified in human brains as the precursor protein of the non amyloid-β (Aβ) component (NAC) of AD

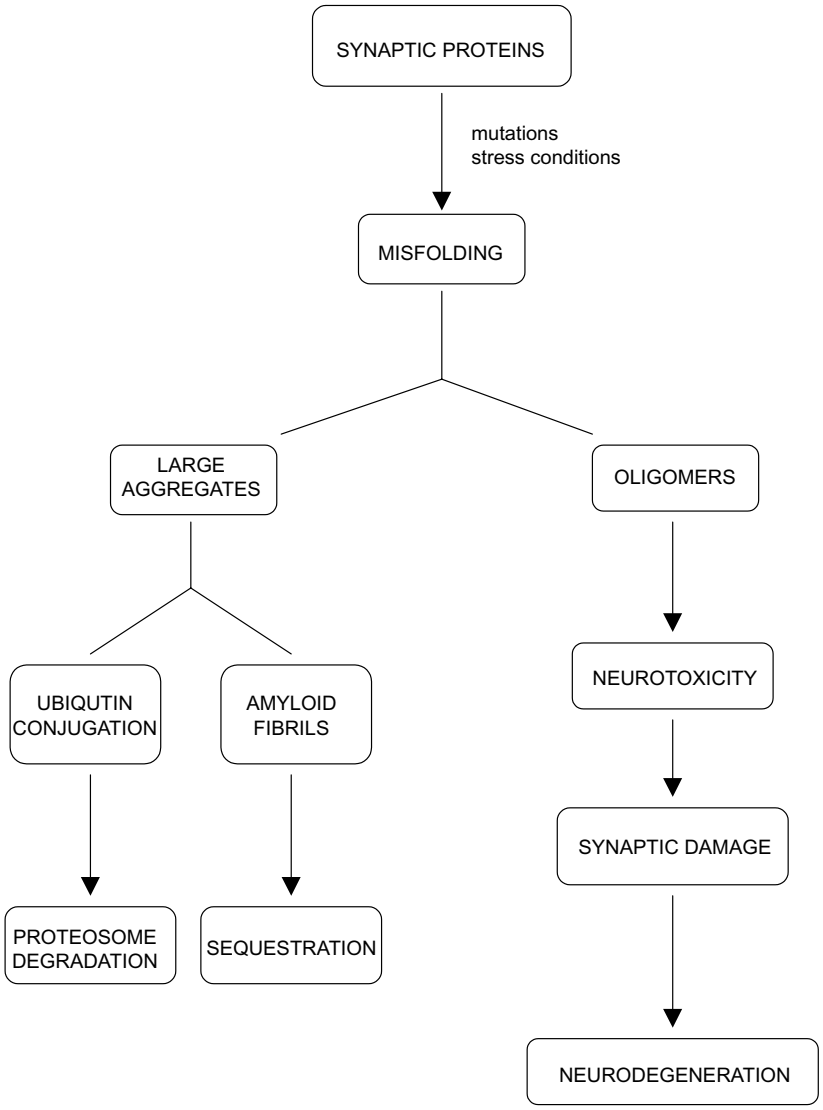

Fig. 2. Misfolded synaptic proteins can form polymers and/or oligomers.

plaques [35,63]. NAC is a highly hydrophobic 35 aa domain within the α-synuclein molecule that is involved in amyloid formation [27]. α-Synuclein is related to other molecules, including human β-synuclein/bovine phosphoneuroprotein 14 [29,52], which are highly homologous with (α-synuclein, but do not possess an amyloidogenic domain [26]. This molecule has been independently identified in a variety of biological systems in torpedo [34], rat [35] and human [29] and as "synelfin" in the song bird [16]. Additional studies have shown that α-synuclein is a presynaptic nerve terminal protein [16,26,29], leading to a hypothesis that it may play a critical role in synaptic events, such as neural plasticity during development, learning [16] and degeneration of nerve terminals under pathological conditions in AD, PD and other disorders [6].

The α-synuclein molecule is capable of self-aggregation to form amyloid-like fibrils [19]. Conditions promoting this aggregation include mutations [53] and oxidative stress [20,21]. Taken together, this suggests that both mutations and stress conditions act in a similar manner by promoting protein misfolding and aggregation which, in turn, could result in the formation of either inactive fibrils (polymers) or toxic oligomers (Fig. 2). This is supported by recent studies in transgenic mice [43] and *Drososphila* [13], showing that expression of the wildtype or mutant α-synuclein protein leads to dopaminergic synapse loss, inclusion

body formation and motor deficits. These observations are further supported by recent studies in α-synuclein-transfected neuronal cell systems where accumulation of this molecule results in dopaminergic cell death [75], mitochondrial dysfunction and oxidative stress [23].

Interestingly, other molecules also linked to AD pathogenesis have recently been shown to occupy a predominant synaptic location. For example, AβPP has a preferential localization at central and peripheral synaptic sites [2,42,58], suggesting a possible role in neuroplasticity [50]. Furthermore, studies have shown that secreted AβPP (sAβPP) fulfills synaptotrophic [50,57] and neuroprotective functions within the central nervous system (CNS) in response to excitotoxicity [46,49] and ischemia [4,61]. In transgenic mice, abnormal expression of mutant forms of AβPP results not only in amyloid deposition, but also in widespread synaptic damage [15,45,54]. This synaptic pathology occurs early and is associated with levels of soluble Aβ1-42 rather than with plaque formation [51]. Similar results have also been reported in AD [33], suggesting that free amyloid oligomers rather than large aggregates might be involved in the process of synaptic damage in AD (Fig. 2). Another AD-associated molecule – apolipoprotein E, which is primarily produced by glial cells, has been shown to accumulate at the central and peripheral synaptic complexes [1] and to play an important role in synaptic membrane formation after injury [55]. Moreover the presenilins, recently liked to familial AD [10], have been shown to accumulate at the post-synaptic site [48] and to be indispensable for CNS development [70].

Further evidence supporting the concept that abnormal accumulation of synaptic proteins could alter synaptic function has been derived from studies in Creutzfeldt-Jakob disease (CJD), where PrPCJD accumulates in synapses [31] and amyloid plaques. Moreover, in CJD and other prion protein diseases patterns of synaptophysin and SNAP25 (another synaptic-associated molecule) immunostaining are abnormal, indicating a primary synaptic alteration in these conditions [9]. Previous studies have shown that in CJD, depending on the genetic alteration, PrP could accumulate either in a plaque-like fashion or in the synapses [31]. Point mutation in codon 102 or 117/129 results in a plaque-type PrP accumulation [30,31], while a point mutation in codon 200 or no mutations in the PrP gene results in synaptic-type accumulation [30,31]. Studies in PrP-null mice have suggested that this molecule is necessary for normal synaptic function [11].

Finally, other neurodegenerative diseases where gene products have now been shown to be closely associated with synaptic complexes include Huntington's disease (HD) and myotonic dystrophy (DM). Huntingtin was reported to be a membrane-bound protein with a distribution very similar to that of synaptic vesicle protein synaptophysin [71]. Studies in human brain detected htt in perikarya of some neurons, neuropil, varicosities and as punctate staining likely to be nerve endings [66]. In transgenic mice, expression of mutant forms of htt leads to formation of intranuclear inclusions with amyloid-like characteristics. The serine/threonine kinase (DMK), which is the gene product of the DM gene, was found to localize post-synaptically at the neuromuscular junction of skeletal muscle and at intercalated discs of cardiac tissue [69]. DMK was also found at synaptic sites in the cerebellum, hippocampus, midbrain and medulla [69]. Taken together, these findings suggest that abnormal accumulation and functioning of synaptic proteins might play an important role in the pathogenesis of various neurodegenerative disorders and points to the possibility that other disease variants might be linked to not yet described mutations in genes encoding for selected synaptic proteins.

In summary, similarly to α-synuclein, the other synaptic proteins involved in neurodegenerative disorders (such as AβPP, PrP, and htt) all contain a significant amyloidogenic potential. Since mutations and/or other stress factors such as oxidation might lead to protein misfolding and aggregation, the next question is how accumulation of these proteins leads to synaptic damage and neurodegeneration.

3. How does accumulation of synaptic proteins lead to synaptic damage and neurodegeneration?

The mechanisms triggering cell death and synaptic damage in neurodegenerative disorders might be related to a gain of a toxic property and/or loss of function of specific synaptic proteins [59] (Fig. 3). For example, it has been proposed that loss of function might be important in the pathogenesis of DM and Friedreich ataxia. Frataxin mutations result in decreased protein synthesis and mitochondrial dysfunction with oxidative stress [8]. In the case of DM, there is now circumstantial evidence that long (CTG)n repeats may affect the expression of any of at least three genes, myotonic dystrophy protein kinase (DMPK), DMR-N9 (gene 59), and a DM-associated homeodomain protein (DMAHP). Furthermore, new findings suggest that DM is not simply a loss-of-function disorder, but that en-

tirely new pathological pathways at the DNA, RNA, or protein level may play a role in its manifestation [17]. This also appears to be the case in other diseases with trinucleotide repeats such as HD and spinocerebellar ataxia (SCA). In these disorders it was originally postulated that the accumulation of aggregates, particularly in the nucleus, was probably involved in triggering a series of events leading to neurodegeneration. However, a series of experiments with mutant htt and ataxin-1 proteins [12] have shown that neither ubiquitinization nor the presence of nuclear aggregates of the misfolded proteins are necessary for toxicity [14]. Subsequent experiments showed that nuclear localization is necessary for toxicity, but it needs to be emphasized that diffuse nuclear localization of oligomers appears to be the toxic trigger. In contrast, large polymeric intranuclear inclusions might be considered either inactive or a defense mechanism to isolate smaller toxic proteins (Fig. 2).

In fact, in view of the studies discussed, the model of cellular pathogenesis in trinucleotide expansion diseases becomes both more complex and simpler, since neither ubiquitination nor formation of nuclear aggregates appears to contribute to toxicity. Although the mechanisms underlying the case where a toxic portion of the protein may cause disease remain unknown, it has been previously proposed that the expansion may cause the mutant protein to adopt a new conformation, leading to altered interactions with other proteins. Several of these proteins including chaperones and the ubiquitin-dependent proteolytic machinery may not be important to toxicity. On the other hand, interacting proteins such as transcription factors found within the nucleus are prime candidates as the cause of toxicity, which eventually leads to cell death [14].

As to α-synuclein, although by far this protein has a wide range of actions, it is primarily localized to the synapses [26], while small amounts are probably shuttled to the nucleus. This has been difficult to observe in mammalian brain but, in fact, the name "synuclein" derives from finding both synaptic and nuclear localization of the protein in Torpedo [34]. In the brains of transgenic mice, high expression levels of α-synuclein result in intranuclear localization of diffuse and aggregated forms of α-synuclein [43]. As to PrP, attempts to investigate the role of nuclear localization in the pathogenesis of neurodegeneration have shown that the putative nuclear localization signal in PrP is not efficient at targeting the protein to the nucleus [28]. Thus, while the mechanisms by which the protein triggers neurodegeneration remain unclear, alterations in the plasma membrane are suspected to be involved [64].

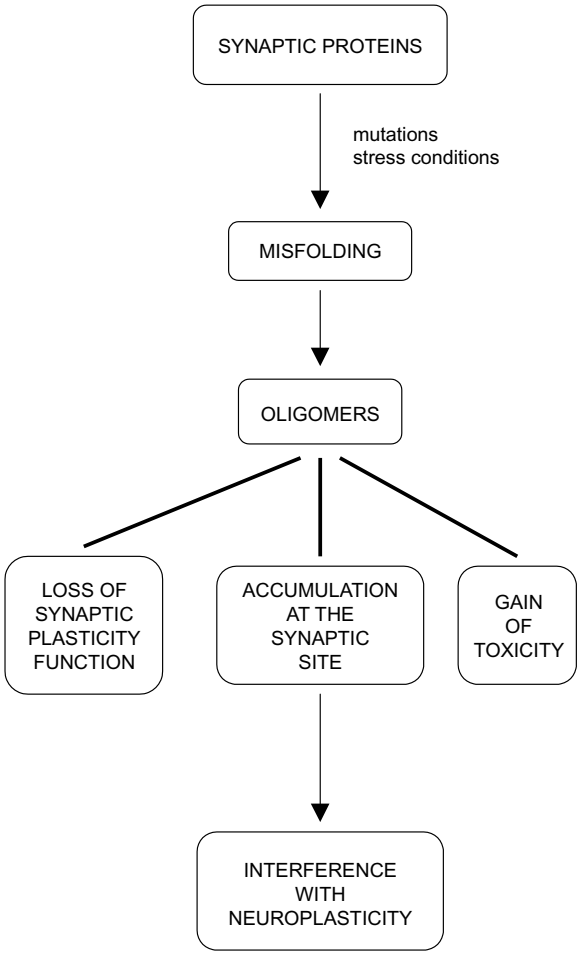

Fig. 3. Abnormal accumulation of synaptic proteins might lead to neurodegeneration via loss of function or gain of toxicity.

Finally, the case of AβPP in AD although similar in some respects, differs since the proteolytic toxic product of AβPP namely Aβ1-42 is secreted, while in trinucleotide repeat diseases, PrP disease and synucleopathies the proteins are not secreted and are not localized to the endoplasmic reticulum (ER) (Fig. 4). Recent studies in AD also support the notion that large amyloid polymers are probably not the primary source of toxicity, but rather a mechanism for isolating more toxic small molecules (Fig. 2). In contrast, accumulation of intracellular oligomers of Aβ1-42 appears to be highly toxic [60]. Although these mechanisms are not yet clear, accumulation of misfolded proteins in the ER might lead to stress signaling that eventually can result in synapse loss and neurodegeneration via pathways involving transcription factors, apoptosis and caspase degradation [74]. As to this latter possibility, some authors have now proposed the term "synaptosis"

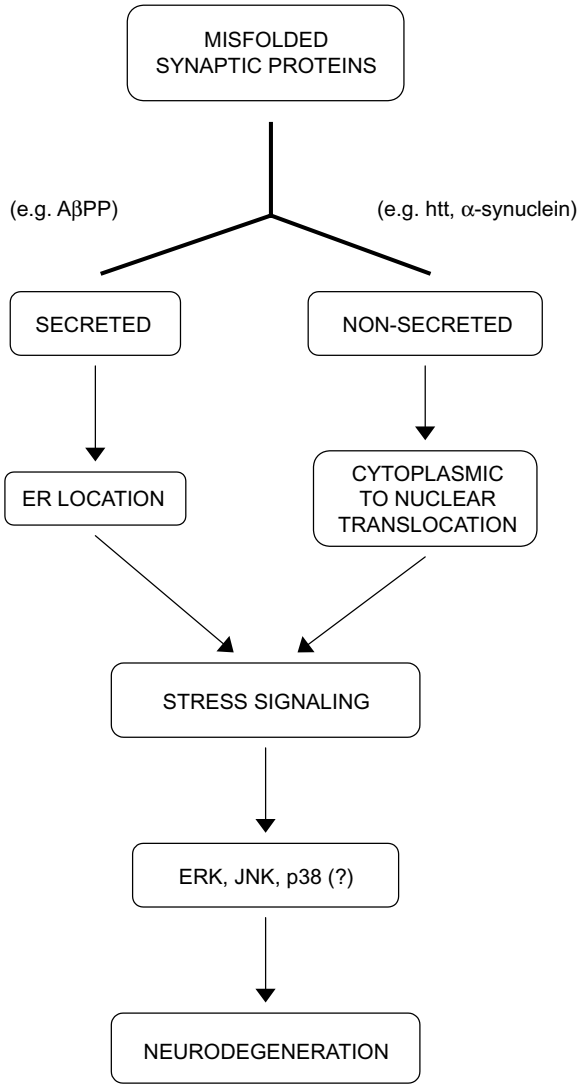

Fig. 4. Stress signaling via nuclear or endoplasmic reticulum localization might lead to neurodegeneration.

to refer to the localized activation of caspases at the synapse, resulting in nerve terminal "pruning" without generalized cell death [73].

In summary, two potential pathways should be considered: one for synaptic proteins that ought to be secreted and another for synaptic proteins that are not secreted (Fig. 4). As to the first pathway, abnormal folding of secreted proteins such as Aβ1-42 might lead to stress response at the ER which, in turn, could trigger signaling events leading to synapse loss and neuronal death [72]. In the second case, misfolding of non secreted synaptic proteins such as htt, ataxin-1 and α-synuclein could result in their translocation to the nucleus where interactions with transcription factors and/or other chaperones might trigger signaling events leading to synapse loss and neuronal death.

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