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Synaptic pathology: a shared mechanism in neurological disease

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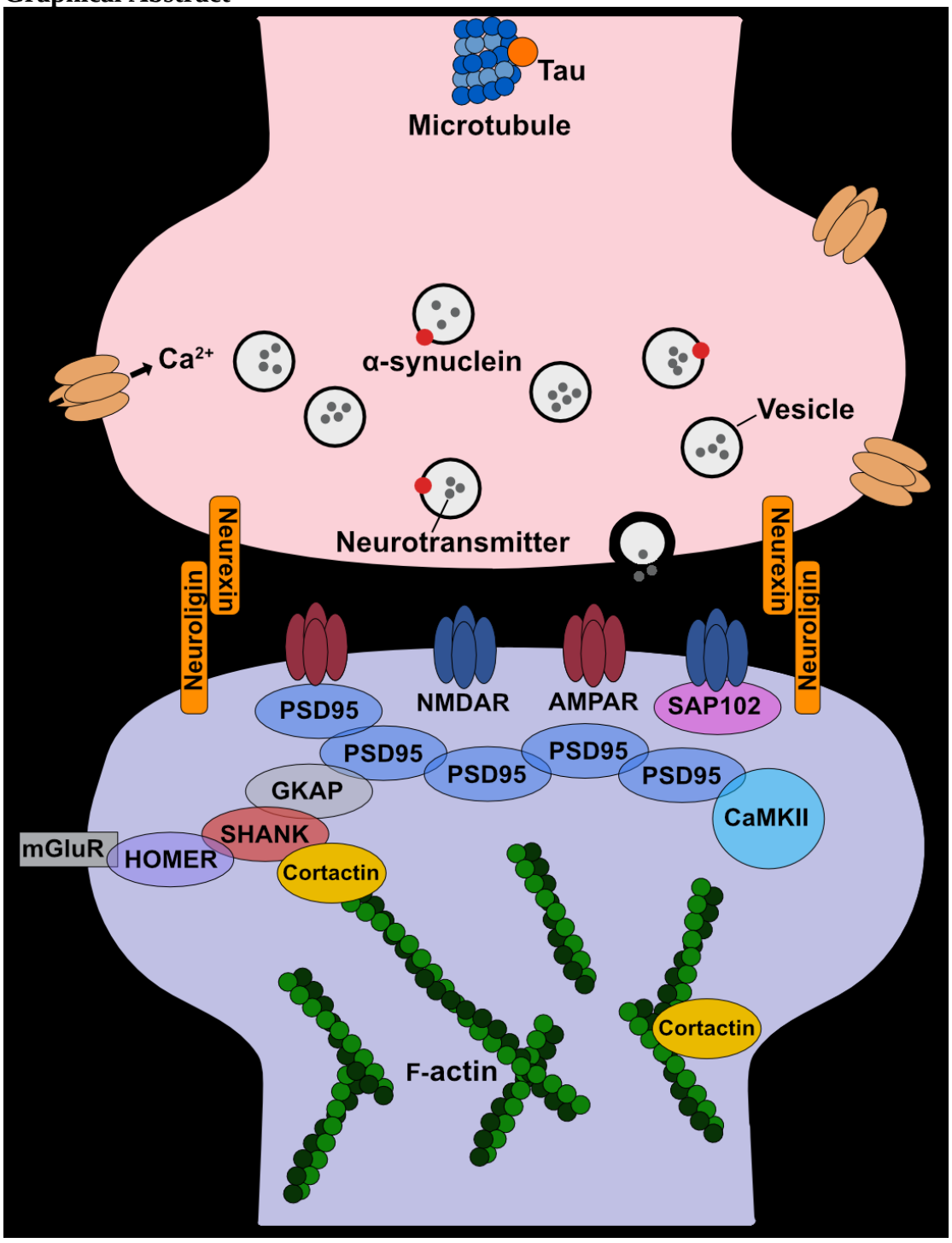
Graphical Abstract



Highlights

- Synaptic changes occur early in most neurodegenerative diseases.
- Synaptic changes likely contribute to cognitive decline in ageing.
- Targeting synaptic pathology is a promising therapeutic strategy.

Abstract
Synaptic proteomes have evolved a rich and complex diversity to allow the exquisite control of neuronal communication and information transfer. It is therefore not surprising that many neurological disorders are associated with alterations in synaptic function. As technology has advanced, our ability to study the anatomical and physiological function of synapses in greater detail has revealed a critical role for both central and peripheral synapses in neurodegenerative disease. Synapse loss has a devastating effect on cellular communication, leading to wide ranging effects such as network disruption within central neural systems and muscle wastage in the periphery. These devastating effects link synaptic pathology to a diverse range of neurological disorders, spanning Alzheimer's disease to multiple sclerosis. This review will highlight some of the current literature on synaptic integrity in animal models of disease and human post-mortem studies. Synaptic changes in normal brain ageing will also be discussed and finally the current and prospective treatments for neurodegenerative disorders will be summarised.

Keywords
Synapse loss, neurodegeneration, pathology, Alzheimer's, ageing

Introduction

The approximately one hundred billion neurons found within the human brain act in beautifully intricate arrangements to generate and control our every thought, memory, emotion and dream. They also control our ability to sense the world, to communicate those sensations to others and decide how to plan our lives. These remarkable abilities are only possible if neurons can efficiently coordinate with other cells in the network, and the transfer of information occurs at specialised compartments called synapses (Figure 1).

Depending on the chemical signal released, synapses can have excitatory or inhibitory effects on the target cell. Excitatory synapses most commonly form on small dendritic protrusions known as spines, where the synapse can be isolated from the main dendritic branch and become highly specialised. Inhibitory synapses tend to form directly onto the dendritic branch or onto the neuronal cell soma, although some exceptions to this general rule do occur. Once formed, these synaptic contacts are not rigid and can strengthen in response to increased activity or become shrunken and

even lost following lack of activity [1]. This plasticity is thought to play a fundamental role in the formation, storage and removal of memory [2]. Furthermore, spine dynamics can often be used as a quantifiable means of analysing circuit activity as spine number and morphology change in response to fluctuations in neuronal activity [1]. See Figure 1D for a summary of this concept.

Given the critical role synapses play in normal neurophysiology, it is not surprising that loss of synaptic integrity may underlie many of the most common neurodegenerative diseases. Synaptic dysfunction or synaptic loss often precedes late-stage features of many neurological conditions such as Alzheimer's disease [3], Motor Neuron disease [4, 5], Huntington's disease [6], Parkinson's disease [7, 8] and multiple sclerosis [9]. While synaptic pathology is a common feature of these disorders, the nature of the synaptic change is not necessarily consistent, which illustrates how critical normal neuronal function is for brain health. Given the plasticity of synapses and the malleability of dendritic spines, it raises the possibility of exploiting these features as potential therapeutic targets. If we can prevent synaptic loss or strengthen existing connections between neurons, we may be able to slow or even reverse disease-driven neurological change.

In this review, we will highlight a selection of neurodegenerative disorders that exhibit synaptic dysfunction as an early feature of the disease, discuss the changes that occur during normal brain ageing and discuss the current and prospective ways in which synaptic function can be targeted for therapeutic exploitation.

Synapse Structure

Synapses are the point of contact between two neurons and can exist as either electrical or, more often, chemical synapses. In both cases the cells don't touch, but communicate by passing ions (electrical synapse) or neurotransmitters (chemical synapse) across a small gap known as the synaptic cleft. Adhesion proteins such as neuroligins and neurexins span this cleft, physically holding the synapse in place [10] (Figure 1C). Intriguingly, these cleft-spanning proteins are critical for synaptic integrity and mutations in the genes for these proteins have been implicated in neurological disorders [10].

The presynaptic bouton contains the complex machinery required for synthesis, storage and release of neurotransmitters [11] (Figure 1C). This is a tightly regulated process, ensuring efficient and accurate transmitter release following action potential propagation. Synaptic vesicles, packed with neurotransmitter, undergo calcium-dependent fusion with the presynaptic membrane and release their contents into the synaptic cleft. Altered protein homeostasis in the presynaptic terminal has been linked to neurological disorders. The abundant presynaptic protein alpha-synuclein forms striking pathological aggregates in a group of neurological disorders known as synucleinopathies [12].

Once released from the presynaptic terminal, neurotransmitters cross the synaptic cleft and interact with receptors in the postsynaptic membrane. Ligand-gated ion channels (ionotropic receptors) open rapidly upon neurotransmitter binding and allow the direct flow of ions into the postsynaptic neuron, altering the local membrane potential. G-protein coupled receptors (GPCRs; metabotropic receptors) induce an array of downstream signalling cascades following neurotransmitter binding, which

are important for local protein homeostasis and dendritic spine morphology (Figure 1C).

The receptors are held in place by a vast protein scaffold known as the postsynaptic density (PSD), which contains almost 1500 proteins [13] and can be seen as an electron-dense structure under the electron microscope (Figure 1B). Disruptions of this critical protein scaffold can have severely detrimental effects on synaptic function, and altered expression of PSD proteins are a common feature of many neurological disorders [13]. Figure 1 shows an example of a typical excitatory synapse with some important proteins highlighted.

In summary, to ensure efficient information transfer between neurons, the synapse must be anatomically intact. Disruptions in synaptic composition can have severe effects on synaptic function, leading to altered network activity and ultimately the clinical manifestation of disease.

Alzheimer's Disease

Alzheimer's disease (AD) is a devastating neurodegenerative disorder exhibiting striking brain atrophy. The brains of AD patients contain two definitive hallmarks of the disease, insoluble beta-amyloid plaques and hyperphosphorylated-tau positive tangles. Plaques are formed extracellularly by the deposition of insoluble amyloid beta peptides, whereas the neurofibrillary tangles are found intracellularly as long tau fibrils characteristically tangled around the nucleus in neuronal cell soma. Neurons and synapses are progressively lost during the disease in tandem with the spread of tau pathology through the brain [14]. However, the early clinical manifestations of memory impairment are likely due to synapse dysfunction and loss rather than to neuron loss or the accumulation of plaques and tangles. A large amount of evidence suggests that the fibrillar plaques and neurofibrillary tangles are not in themselves toxic. These lesions are often found in cognitively normal aged brains [15-17]. The strongest pathological correlate with cognitive change is synapse loss [18], suggesting that the loss of synapses is sufficient to drive AD-related cognitive decline, before the loss of neurons [3, 19]. What causes the synaptic loss is yet to be fully elucidated but it appears that both soluble amyloid and soluble tau have a role to play [20]. In mouse models of amyloid pathology and human post-mortem tissue, synapses are predominantly lost around mature, dense-core amyloid plaques [21, 22]. Interestingly, there is no increased synapse loss in or around diffuse plaques, which don't appear to affect the surrounding neuropil and are made up of small scattered bundles of amyloid fibrils [23]. This may suggest that dense-core amyloid plaques release synaptotoxic molecules in their vicinity. In support of this idea, work in transgenic mouse models revealed a halo of oligomeric amyloid around the edge of plaques and some of this soluble amyloid is found within synapses [21, 22], as independently observed using electron microscopy [24]. The presence of soluble amyloid in the synapse appears to drive shrinkage and ultimately loss of that connection perhaps through microglial-mediated mechanisms regulated by C1q and complement 3 [25]. Before synapses are lost synaptic function is significantly disrupted by the presence of soluble amyloid. Mouse models have shown that the application of synthetic amyloid species results in impaired LTP [26, 27] and enhanced LTD [28], ultimately weakening the synapse. These effects are due to the dysregulation of numerous signalling pathways, but it is evident that the synaptic AMPA [29] and NMDA [30] receptors play a critical role. LTP causes an increase in spine size and requires high calcium levels within the

spine, as a result of rapid influx through NMDA receptors [31]. LTD causes spine shrinkage, as a result of a slower influx through NMDA receptors and ultimately a lower level of calcium in the spine [32]. Oligomeric amyloid can bind NMDA receptors and block NMDA-evoked currents resulting in slower calcium dynamics, which favours the induction of LTD in the spine [33]. Furthermore, the effect of numerous kinases within the spine critical for LTP induction such as JNK [34], p38 MAPK [34] and CaMKII [35, 36] can be altered by oligomeric amyloid. Also, the calcium-dependent phosphatase Calcineurin is activated by amyloid [37], leading to the internalisation of AMPA [37, 38] and NMDA [33] receptors away from the synapse, leading to the loss of synapses and spines. This synapse-specific loss of NMDA receptors is important in a number of ways. Firstly, it reduces excitatory synaptic activity leading to synapse weakening and secondly, it leads to a change in the balance of synaptic and extra-synaptic signalling. Synaptic NMDA receptors are thought to induce pro-survival signalling pathways, whereas NMDA receptors outside the synapse (extrasynaptic) promote toxic, cell-death pathways [39], therefore a loss in synaptic signalling will lead to a greater influence of toxic, extrasynaptic signalling. It has been shown in cultured mouse cortical neurons and in synaptic/non-synaptic fractions of homogenized mouse hippocampal slices that extrasynaptic NMDA receptor levels are not changed following amyloid-β treatment, whereas synaptic levels significantly decrease [33, 40]. Therefore, amyloid-β may induce synapse loss via a combination of synaptic weakening and a shift towards toxic extrasynaptic signalling. Another way in which amyloid-β may significantly alter synaptic function is by disrupting mitochondrial physiology. Mitochondria are critical for maintaining the high-energy supply required for efficient synaptic function and it is thought that mitochondrial dysfunction plays an important role in AD pathogenesis [41]. For example, a recent study has found a direct molecular link between amyloid-β and Abeta-binding alcohol dehydrogenase (ABAD), leading to mitochondrial dysfunction, oxidative stress and cell death [42].

Intriguingly, a very recent study utilising human tissue and rodent models has shown that amyloid binds to and disrupts an adhesion protein that spans the synaptic cleft and holds the synapse in place. The authors suggest that amyloid-dependent breakdown of NCAM2 leads to synapse disassembly [43].

The role of tau in synapse loss is less well established. Tau is a microtubule binding protein which during the course of AD becomes hyperphosphorylated and accumulates in neuronal somata and dendrites [44]. Despite the historical belief that tau is confined to the axon, recent studies have revealed an important role for tau in the PSD [45]. Furthermore, imaging studies have revealed the abnormal accumulation of tau in spines from both mouse models and human AD post-mortem tissue [46-49]. Given the important role tau plays in microtubule stabilisation and subsequent protein trafficking, it is easy to imagine that pathological tau dislocation would result in failed trafficking of critical proteins required for synaptic function. In support of this, it has been shown that expression of hyperphosphorylated tau disrupts the trafficking of glutamate receptor subunits [50, 51]. Furthermore, mitochondrial transport is significantly disrupted when tau is overexpressed, resulting in disrupted ATP production and calcium buffering, and altered mitochondrial distribution in tau over-expressing neurons in transgenic mice and post-mortem AD brain [49, 52, 53].

Intriguingly, the spread of phosphorylated tau follows a remarkably predictable pattern throughout the brain. Abnormal deposits of tau first appear in the transentorhinal region before spreading into the nearby entorhinal cortex [54]. The pathological spread then appears to follow the flow of synaptic connections from the entorhinal cortex into the hippocampus and then from there, out into other cortical and subcortical regions [54]. This predictable spread of pathology has led some to believe that phosphorylated tau is passed between neurons that are synaptically connected [55-58]. However, the route of transmission and the identity of the propagating toxic tau species are yet to be fully elucidated [59, 60]. Research is now beginning to focus on potential synergistic or hierarchical effects of amyloid and tau in synapse loss [20]. One interesting potential link between amyloid-β and tau pathology is the finding that specific activation of extrasynaptic NMDA receptors enhances tau phosphorylation in cultured mouse hippocampal neurons [61]. This may suggest that amyloid-β not only induces synaptic dysfunction, but drives tau pathology via extrasynaptic NMDA receptor signalling. Treating primary neurons with physiological concentrations of amyloid-β induces synapse loss, which has recently been associated with tau mislocalization to dendrites [62]. Further, genetically removing endogenous mouse tau prevents some of the synaptic deficits associated with overexpressing mutant amyloid [63, 64].

In summary, the vulnerability of synapses in AD is striking and is supported by a vast literature describing presynaptic, postsynaptic and even trans-synaptic sites of damage following the generation of pathological species of amyloid and tau.

α-synucleinopathies: Parkinson's disease and Dementia with Lewy Bodies

Parkinson's disease (PD) and dementia with Lewy bodies (DLB) belong to a group of neurodegenerative disorders called α-synucleinopathies. Patients' brains contain pathological aggregates of the presynaptic α-synuclein protein, which can exist as neuronal cytoplasmic aggregates called Lewy bodies or longer fibril-like structures in the neuronal processes, known as Lewy neurites. Aggregates can also be found in glial cells in other α-synucleinopathies, such as multiple system atrophy. It is currently unknown why this protein leaves the synapse, or why it aggregates.

The loss of dopaminergic neurons in the substantia nigra causing a dramatic reduction of striatal dopamine release leads to the clinical motor problems (rigidity, tremor, bradykinesia, freezing and postural instability) which are characteristic of PD [65]. However, pathology is not restricted to the substantia nigra, as evidenced by a number of other non-motor clinical symptoms such as constipation, hyposmia, depression and sleep disturbance [66]. In PD it is proposed that the presence of Lewy bodies begins before overt clinical symptoms as evidenced by the widely reported incidental Lewy body disease (iLBD). The hypothesis put forward by Heiko Braak states that Lewy bodies spread in a predictable pattern from the brainstem to subcortical structures and finally throughout the cortex in severe late stage cases [67]. Whether Lewy body formation is a result or cause of neuronal degeneration is a matter of debate, although the progression and severity of disease appears to follow their presence [68].

DLB is a common form of dementia, following Alzheimer's disease, vascular dementia, and mixed AD/vascular dementias in prevalence. The classical core features of DLB include fluctuating cognitive impairment with loss of attention and executive function, visual hallucinations and Parkinson's-like motor problems

(rigidity, tremor, bradykinesia, freezing and postural instability) [69]. As in PD, the loss of neurons in the nigrostriatal pathway can account for the motor symptoms observed in DLB, yet the number and location of cortical Lewy bodies in DLB don't necessarily track with the severity of disease [69]. However, high incidence of Lewy bodies in the anterior and inferior temporal lobe, which is important for forming complex visual images, does associate with the presence of visual hallucinations [69].

In both PD and DLB the severe brain degeneration observed post-mortem cannot be explained purely by the presence of Lewy bodies and alternative factors likely play a role. One alternative is that small synaptic α-synuclein inclusions may drive a loss of synapses in the brain of patients. Indeed, loss of excitatory synapses in the striatum has been described in animal models of PD and in human post-mortem brain [7, 70-72]. Using a PET blot technique, a sucrose gradient fractionation technique and electron microscopy, one group have suggested that up to 90% of aggregated α-synuclein exists in small presynaptic inclusions rather than large somatic Lewy bodies, in PD and DLB brains [73, 74]. In DLB cases, this associated with a significant decrease in the levels of presynaptic proteins such as synaptophysin. Furthermore, filled neurons in DLB brain had significantly fewer dendritic spines, corresponding with a decrease in synaptic levels of PSD95. In an α-synuclein overexpressing mouse model of DLB, significant loss of presynaptic terminals are observed in the hippocampus at 8 months, a few months after α-synuclein aggregates begin to appear [75]. However, when the overexpressing transgene was later switched off, the synapse loss was reversed and the α-synuclein pathology cleared.

Vesicular monoamine transporter 2 (VMAT2) and the dopamine transporter (DAT) are important for the vesicular storage of dopamine in the presynaptic terminal and studies have shown a significant reduction in levels and activity of these in human PD [76, 77]. Importantly, these proteins appear to inversely correlate with the level of α-synuclein in the substantia nigra suggesting that increased α-synuclein deposition leads to decreased levels of VMAT2 and DAT [77]. In support of a presynaptic dysfunction in Parkinson's, mouse models of the disease exhibit a redistribution of numerous critical presynaptic proteins to sites of aggregated α-synuclein, resulting in reduced dopamine release in the striatum [78]. Human brain imaging has reinforced the case for synaptic failure in PD pathology and has revealed presynaptic disruption of numerous neurotransmitter systems [79].

Despite the growing literature describing presynaptic dysfunction in PD, it is clear that other synaptic compartments can be affected. For example, exogenously applied oligomers of α-synuclein to rat hippocampal slices can disrupt LTP via postsynaptic, NMDA receptor-dependent mechanisms [80], and in cultured dopaminergic neurons, application of α-synuclein to the culture media leads to internalisation of NMDA receptors [81]. In cultured hippocampal neurons, α-synuclein also internalises NMDA receptors and affects NMDA-induced Ca^{2+} changes, leading to decreased NMDA-dependent currents [82]. Given the importance of these receptors in synaptic signalling and spine morphogenesis, it is no surprise to find in human post-mortem tissue and numerous models of PD that spine densities are altered [83, 84]. Furthermore, postsynaptic calcium disruption has been shown in striatopallidal medium spiny neurons, leading to rapid loss of glutamatergic axospinous synapses and disconnection of the motor system [7].

It is also clear that mitochondrial dysfunction plays a prominent role in PD and DLB pathogenesis and may explain some of the synaptic deficits. Some of the known genes associated with familial PD play important roles in normal mitochondrial function, such as PINK-1 and Parkin [85]. Also, in numerous model systems, over-expression of mutated human α-synuclein can lead to mitochondrial degeneration [86, 87]. Furthermore, one of the most common models of PD is the MPTP-induced breakdown of dopaminergic neurons and this neuronal death occurs due to inhibition of mitochondrial complex I, resulting in massive reactive oxygen species accumulation and mitochondrial damage [88, 89]. In DLB post-mortem tissue it has been shown that there is a significant loss of mitochondria from neuronal processes, with aggregation of mitochondria around cytoplasmic Lewy bodies [90]. Furthermore, once the mitochondria are engulfed by the expanding Lewy body their membranes rupture and the mitochondria are destroyed [90]. In both PD and DLB, mitochondrial dysfunction will hamper energy supply to synapses and this may be a driving force in synaptic disconnection.

The overall picture emerging from the current literature is that synaptic pathology is an early feature of PD and DLB and that α-synuclein aggregation and deposition can affect the synapse both pre- and post-synaptically. This assault from both sides of the synapse leads to significant neurophysiological disruption and subsequent anatomical change, resulting in spine alterations which affect overall neuronal function and circuit activity, leading to neuronal death and clinical manifestation.

Motor Neuron Disease

Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease and is characterised by the loss of upper and lower motor neurons. This leads to progressive muscle weakness and atrophy, and the denervation of respiratory muscles is often the cause of death, commonly occurring within 5yrs of diagnosis [91]. Catastrophic motor neuron loss represents the final step in disease progression, however mounting evidence suggests that synaptic disconnection at the neuromuscular junction occurs prior to cell death [4, 5, 92]. This has led to the "dying back" hypothesis of disease progression, which states that following toxic insult at the neuromuscular synapse, the axon disconnects from the target muscle, leading to axonal degeneration and finally neuronal death. Both cell autonomous and non-autonomous factors have been suggested as the initiating insult [91]. Betz cells are giant pyramidal cells residing in layer five of the motor cortex and project directly onto the lower motor neurons in the spinal cord. Research has shown that synaptic terminals on the soma of normal-looking Betz cells in ALS patients appear dysmorphic and exhibit a degenerative appearance [93]. Loss of synaptic input may contribute to the dysfunction and loss of these critical cells in muscle control. Recent studies using ALS mouse models have revealed that cortical synapse loss may be an early presymptomatic feature of ALS. Presymptomatic spine loss was observed in the motor cortex of the $hSOD1^{G93A}$ transgenic model [94] and an early shrinkage/loss of synapses was observed in the sensorimotor cortex of the FUS-R521C mouse [95]. Furthermore, disconnection of the upper and lower motor neuron circuit appears to be supported by a loss of synapses onto lower motor neurons in the ventral horn of the spinal cord [96-98].

Although the source of the synaptotoxic insult has yet to be fully elucidated, interestingly, as described above for Alzheimer's and Parkinson's disease, protein

aggregates also feature in the neuropathology of motor neuron disease. Mutations in the Cu/Zn superoxide dismutase (SOD1) gene are found in approximately 20% of familial forms of ALS which corresponds to 1-2% of all ALS cases and much of the pre-clinical work has been performed in mouse models containing mutations in SOD1 [91]. SOD1 protein is located on the outer mitochondrial membrane and plays an important role in mitochondrial physiology [99]. Aggregated forms of mutant SOD1 are found in sporadic and familial ALS, as well as in SOD1 transgenic models, in the form of cytoplasmic inclusions [100-102]. Whether these inclusions are also found in synapses has yet to be fully elucidated. Intriguingly, cultured motor neurons show a propensity for accumulating SOD1 aggregates when compared to cultured dorsal root ganglion or hippocampal neurons transfected with similar levels of mutant SOD1 [103]. However, things are further complicated by the fact that these aggregates often include one or more other proteins known to play a role in ALS such as TAR DNA-binding Protein 43 (TDP-43), ubiquitin, Fused in Sarcoma (FUS) and Sequestesome-1 (p62/SQSTM1) [104]. Interestingly, the two RNA-binding proteins TDP-43 and FUS have been shown in cultured mouse neurons to traffic into dendritic spines and their synaptic levels significantly increase following neuronal activity [105, 106]. Therefore, given the high incidence of cytosolic protein aggregates it's possible that sequestration of these important synaptic proteins away from their target site contributes to synaptic failure and loss. In support of this, it was recently shown in primary mouse neurons and human-derived motor neurons that ALS-associated mutations in TDP-43 led to reduced motility of TDP-43+ve mRNA granules along axons and a decrease in synapse-associated mRNA [107]. New protein synthesis is critical for long-lasting changes in synaptic remodelling and local synaptic mRNA trafficking and processing plays an important role. Therefore, disrupting either the temporal or spatial processing of synaptic mRNAs can have severely deleterious effects on neuronal function.

Further evidence of synaptic disruption in ALS is the growing revelation of excitotoxicity in the disease. The strongest argument for a role of excitotoxicity in ALS is that the only drug capable of slowing disease progression in patients is riluzole, a suppressor of excitatory synaptic activity [108]. Furthermore, in human synaptoneurosomes preparations it has been found that glutamate re-uptake is significantly decreased compared to samples from control or non-ALS patients [109] and that CSF levels of glutamate are significantly higher in ALS patients [110, 111]. Loss of glutamate transporters in ALS is found in both post-mortem tissue and rodent models [112, 113]. Motor neurons appear to be intrinsically vulnerable to excitotoxicity due to their high expression of Ca^{2+}-permeable AMPA receptors and low expression of Ca^{2+} buffering proteins [114]. Therefore, subtle changes in their Ca^{2+} buffering capacity could render the postsynapse vulnerable to Ca^{2+}-dependent excitotoxicity. In support of this idea it has been shown that mutated SOD1 can accumulate within vacuolated mitochondria [115], significantly disrupt mitochondrial function [116] and diminish the Ca^{2+}-buffering capacity of these organelles [117]. Furthermore, corticostriatal plasticity is significantly altered in a mutated SOD1 mouse model. In acute slices, tetanic stimulation (100 Hz, 1-s, 6-s interval) induced LTD in control slices but induced LTP in SOD1 mutants [118]. This synaptic alteration would shift the excitatory/inhibitory balance and transfer a physiological network into a hyperexcitable one. Altered physiology is not just limited to the brain however, as SOD1 transgenic mice also show alterations in spinal motor neuron activity. Electrically stimulated motor neurons from transgenic mice fired shorter

action potentials in a higher frequency, due to decreased repolarization time [119]. Also, spinal motor neuron hyperexcitability appears to be a presymptomatic feature in SOD1 mouse models [120].

In summary, although it has been well established that neuromuscular synaptic loss is an early presymptomatic feature of ALS progression, the nature and source of the initiating toxic insult has yet to be convincingly described. Furthermore, the multi-faceted pathology suggests that ALS is a multifactorial disorder, likely affecting synapses in numerous diverse ways, ultimately rendering them extremely vulnerable in the early stages of disease pathogenesis.

Huntington's Disease

Huntington's disease (HD) is caused by a trinucleotide (CAG) repeat expansion in the huntingtin gene, resulting in a polyglutamine expansion in the huntingtin protein. Patients exhibit classical movement disorders such as chorea and bradykinesia, cognitive deficits which progress into dementia and psychiatric symptoms such as depression [121]. These clinical features can be attributed to significant neuronal cell death in the striatum in the early stages (up to 95% loss of striatal medium spiny neurons), followed by more global brain atrophy [122]. Preceding neuronal death, alterations in spines and synapses are evident in both human post-mortem tissue and animal models of the disease [123]. In human post-mortem striatum, dendrites of medium-sized spiny neurons appear tortuous with recurved endings and exhibit a loss of spines [124, 125]. Similar findings are described in layer five of the prefrontal cortex [126]. Rodent models expressing mutant huntingtin transgenes (R6/1 HD mice) have revealed changes in dendritic spine density and anatomy, in HD-relevant brain regions [127]. Earlier attempts to generate rodent models of the disease involved intrastriatal injections of quinolinic acid, an endogenous NMDA receptor agonist, which produced neurodegenerative lesions that appeared neurochemically similar to those in human post-mortem tissue [128]. This suggested a prominent role for the excitatory glutamatergic system in HD pathogenesis. Furthermore, it has been shown in a mouse model of HD that an early increase in extrasynaptic NMDA receptor signalling may contribute to disease pathogenesis [129]. The authors revealed a significant increase in extrasynaptic NMDA-dependent currents, which coincided with increased extrasynaptic NMDA receptors. Environmental enrichment induces synapse formation and delays symptom onset and ameliorates symptoms in mouse models of HD, potentially by rescuing axonal transport of BDNF to the striatum and hippocampus [130-132]. Furthermore, it has been shown that wild-type huntingtin protein exists in postsynaptic membranes, binds PSD-95 [133, 134] and mutant protein can interfere with correct trafficking of postsynaptic receptors [135, 136]. Interestingly, specific loss of PSD-95 and GluR1-containing glutamatergic receptors occurs before the onset of spine loss [137], which suggests that spine loss is a result of synaptic dysfunction rather than a cause. As expected from the anatomical changes described, electrophysiological alterations are apparent in numerous model systems, with both resting and activity-dependent changes in neuronal physiology [138-140].

Mitochondrial dysfunction is also evident in HD and appears to be a very early stage of pathogenesis, occurring presymptomatically [141, 142]. Striatal mitochondria containing mutant huntingtin have a reduced calcium uptake capacity than wild type cells [143]. Furthermore, mutant huntingtin binds to the mitochondrial outer membrane and directly lowers the threshold required for Ca^{2+}-induced mitochondrial

permeability [144]. Therefore, studies suggest that the dysfunction of mitochondrial Ca^{2+} handling may render HD neurons unable to cope with excessive neuronal activity, leading to early synaptic loss, followed by cell death.

From the current literature, one can imagine a scenario in which mutated huntingtin initiates a cascade of disruption, starting with perturbed protein trafficking, synaptic dysfunction and spine loss, leading to network disconnection and ultimately neuronal death.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic, autoimmune disorder exhibiting inflammatory lesions in the CNS and subsequent axonal demyelination and neurodegeneration. The pathological hallmarks of the disease are sclerotic plaques, which represent the end-point of a destructive process involving inflammation, demyelination, gliosis and axonal/neuronal death [145]. Clinically, neurodegeneration leads to progressive physical disability as neuronal networks and muscle control are lost [145]. Also, in addition to the sensory and motor deficits, up to 65% of MS patients present with cognitive deterioration [146]. The clinical course is complex, as the disease tends to wax and wane under the control of the inflammatory episodes with patients improving during remission. However, recovery from each relapse is usually incomplete and 65% of patients will advance into a secondary progressive form of neurodegeneration [145].

Despite the well-described pathology, the exact order of events that lead to the formation of sclerotic plaques is hotly debatable. Recently, focus has centred on the role of inflammation-driven synapse alteration in MS pathogenesis. Using a non-invasive imaging technique called Transcranial Magnetic Stimulation (TMS) to measure cortical activity in MS patients, it has been shown that intracortical facilitation is evident in MS patients [147] and that cortical hyperexcitability correlates with increased levels of pro-inflammatory cytokines in the CSF [148]. Furthermore, elevated levels of glutamate have been found in the CSF [149] and brain [150] of MS patients, pointing towards glutamate-induced excitotoxicity in the CNS. In support of this hypothesis, pharmacological blockade of glutamate receptors in rodent models of MS (experimental autoimmune encephalitis (EAE)), perturb disease progression and severity, reduce neurological deficits and decrease damage to axons and myelinating cells (oligodendrocytes), despite having no effect on CNS inflammation [151, 152]. Furthermore, in acute brain slices from EAE models it has been shown that LTP is enhanced and LTD is reduced, leading to an overall hyperexcitable environment, similar to the human cortex [153-155].

In human post-mortem hippocampi, dramatic demyelination is observed [156, 157]. The loss of myelin associates with synaptic dysfunction, as although neuron number remains stable, synaptic density is significantly decreased [156, 157]. Interestingly. A role for the compliment system (C1q and C3) has been implicated in hippocampal synapse loss [157], which is a system thought to play an important role in supernumerary synapse elimination during development, but may be erroneously activated during disease [158]. Furthermore, in demyelinated hippocampi the levels of astrocytic glutamate uptake transporters EEAT1 and EEAT2 were significantly decreased [156], likely driving increased synaptic glutamate levels and subsequent synaptic breakdown. This is an important finding as approximately 50% of MS

patients exhibit impaired long-term memory, a process that requires functional hippocampi [159]. Synapse loss has also very recently been documented in the cortex. In demyelinated areas of the post-mortem human insular and frontotemporal lobes, there were fewer intracortical axons and a dramatic decrease in spine numbers [160]. Interestingly, even in MS patients with normal appearing grey matter (without demyelination), spine density was significantly lower than control and not different to the densities in demyelinated patients [160]. Thus in the cortex, it appears synapse loss does not necessarily associate with demyelination.

Excessive excitatory signalling could result from uncontrolled glutamate release, decreased glutamate clearance or increased postsynaptic expression of receptors. In fact, it appears all three may play a role. Glutamate transporters and metabolizing enzymes are lost from oligodendrocytes in and around active MS lesions and infiltrating immune cells express high levels of glutamate-synthesising enzymes, all contributing to the increased levels of synaptic glutamate and localised axon damage [161, 162]. Also, increased expression of glutamate receptors in glial cells and ectopic expression in axons is observed around active MS lesions [163, 164].

Another condition in which network excitation can become excessive, is if inhibitory control is lost. Almost one quarter of neurons in the cortex are inhibitory [165] and they play an important role in regulating rhythmic firing across cortical networks. Numerous studies have shown that the inhibitory GABAergic system is disrupted in MS patients. It has been known for more than 30yrs that GABA levels in the CSF of MS patients are lower that controls [166] and around 10yrs ago it was shown by microarray and confirmed by RT-PCR and western blotting that many components of the GABAergic system were significantly down regulated in MS patients [167]. The authors reported a decrease in GABA receptor subunits, receptor associated proteins and presynaptic proteins involved in GABA synthesis. Also, they discovered that the cortical area covered in parvalbumin (Ca^{2+}-binding protein highly expressed in a subpopulation of inhibitory cells) -positive cells and their processes was almost 30% lower in MS patients. These findings suggest that GABA release is lower in MS patients and the machinery required to send and receive inhibitory signals is significantly hindered in the MS brain.

Another pathological feature described by Dutta et al. [167] in post-mortem MS motor cortex, was the breakdown of mitochondrial function. Interestingly, mitochondrial number and protein composition were the same in MS and control motor cortex preparations, however mitochondrial respiratory chain function was reduced by approximately 50% in MS samples [167]. Mitochondrial DNA (mtDNA) damage can induce significant mitochondrial dysfunction and in human MS cortical grey matter, there are extensive mtDNA deletions, leading to respiratory dysfunction [168]. In the EAE animal model of MS, mitochondrial breakdown and dysfunction appeared as early as three days after EAE sensitisation, long before leukocyte infiltration into the CNS [169]. Due to the mounting evidence in animal models and human tissue, mitochondrial dysfunction is becoming more appreciated as an important factor in MS pathogenesis [170], which may play a significant role in neuronal physiology, leading to synaptic breakdown.

In summary, mounting evidence supports the supposition that changes in the neuronal milieu during inflammatory relapse leads to early synaptic dysfunction and a shift

towards increased excitatory transmission, resulting in hyperexcitation and excitotoxic neurodegeneration. Furthermore, increased energy demands required to propagate signal transduction along demyelinated axons, coupled with decreased energy production due to dysfunctional mitochondria, leads to a virtual hypoxic state, further enforcing neurodegenerative processes.

Normal cognitive ageing

Despite the wealth of literature describing pathological changes in the diseased brain, we are yet to fully understand the changes that occur during normal ageing of the brain and it's important to remember that age is a major risk factor for most neurodegenerative diseases. Normal cognitive ageing is likely influenced by a number of underlying factors and the term refers to age-related changes in cognition in the absence of any known neurologic disease [171]. Interestingly, this trait of age-related cognitive change is not restricted to humans and can be found in other aged species such as rodents and non-human primates [172]. Declarative and working memory are mediated by the hippocampus and dorsolateral prefrontal cortex respectively and are the most vulnerable cognitive processes in ageing [172]. Furthermore, it is known that regional coordination, required for higher order tasks, begins to breakdown during ageing [173] and is thought to be a result of alterations in the connections between these brain regions, driven by a deterioration of white matter physiology [174]. Post-mortem, a number of structural changes are evident such as neuronal loss, white matter deterioration, gliosis, neurovascular changes and increased deposition of pigments and proteins inside cells [175]. Furthermore, glutamatergic signalling and glutamate homeostasis are disrupted in normal brain aging and this has knock-on deleterious effects on other neurotransmitter systems [176]. For example, the breakdown of important neurotransmitter systems such as the dopaminergic and serotonergic systems appears to be an age-dependent process [177, 178]. However, despite all these diverse changes it has been frequently shown that synaptic health is essential in maintaining cognitive performance in older age and it is synaptic density, not neuronal loss, that associates most strongly with age-related cognitive decline [172].

Using genome-scale microarrays it has been shown that genes involved in the regulation of synaptic function are significantly down regulated in aged human brain [179, 180]. Also, genetic variability within genes coding for postsynaptic proteins preferentially associates with the inherent variability in general intelligence [181]. Evidence supporting an age-dependent change in synapses is not merely genetic. In non-human primates there is a significant age-related decrease in volume of the dorsolateral prefrontal cortex, which is not caused by neuronal loss but associates with a dramatic loss of glutamatergic, axospinous synapses [182]. Furthermore, this synaptic loss (specifically in cortical layer 3) correlates with the degree of cognitive decline in the aged animals. This is similar to human ageing studies that have shown an association between high presynaptic protein levels and lower odds of dementia diagnosis in later life [183]. Also, human post-mortem studies have revealed a decrease in synaptic density in an array of cortical regions, including the prefrontal cortex, without changes in neuron number [184-187]. Thus it appears that synaptic loss is a feature of normal brain ageing across a variety of distinct species, but what drives or initiates this process? Interestingly, some genes involved in vital processes such as mitochondrial function, immune regulation and inherent stress responses are changed in an age-dependent manner and these changes are evolutionarily conserved

throughout the animal kingdom from humans to nematode worms [188]. Thus it appears that the brain ages in a similar way across species and that common factors likely drive synaptic loss. For example, genes encoding mitochondrial proteins are decreased across species in aged individuals [180] and studies have shown significant mitochondrial dysfunction in many animal models of ageing [189]. Human mitochondrial DNA deletions increase with age [190], and interestingly deletions were common in the cortex but largely absent from the cerebellum. This may partly explain the more prominent cognitive decline associated with ageing. In rodents, mitochondrial enzyme activity correlates with neurological performance and median life span and ageing associates with increased mitochondrial dysfunction and fragility [189]. Furthermore, in non-human primates, mitochondrial number and morphology in the presynapse correlates with performance in working memory tasks, which declines with age [191].

As the brain ages, postmitotic neurons that have worked diligently for decades, begin to tire. Underlying degeneration of DNA repair mechanisms and mitochondrial function begin to take their toll on neuronal physiology and the critical points of contact and communication between cells (synapses) start to breakdown. As synapses are lost during ageing, there is an inevitable change in neuronal electrophysiology, and for a long time it has been known that aged animals showing a brain region specific decrease in LTP [192-195]. Also, as Ca^{2+} homeostasis alters, likely due to dysfunction in Ca^{2+}-buffering organelles, synaptic plasticity favours LTD induction rather than LTP [196]. Synapse loss and physiological alterations occur as a prelude to neuronal loss and synapse loss correlates with early cognitive change. However given the inherent malleability of spines and synapses, this could provide a therapeutic opportunity for slowing the progression of not only ageing but also some neurodegenerative diseases, such as those described above.

Current and potential therapies in neurodegenerative disease

During neurodegenerative disease, the processes regulating synaptic signalling and adaptation are hindered and as a result, physiological plasticity is lost. However, by pharmacologically altering synaptic function to regain the delicate balance of synaptic physiology, we could potentially prevent synaptic loss and even induce synaptic growth. This is critically important given that synapse loss often occurs in the early prodromal phases before overt and irreversible neuron death has occurred. This would considerably widen the therapeutic window for such disorders, providing aid to millions of patients around the world.

Most of the treatments currently licenced for neurodegenerative diseases act by boosting diminishing synaptic function or blocking excessive synaptic activity in overactive circuits. For example, acetylcholinesterase inhibitors are used to prevent the breakdown of the neurotransmitter acetylcholine in the brain and thus boost cholinergic signalling. These treatments are based on the early Cholinergic Hypothesis of Alzheimer's pathogenesis from the observations in the 1970s that cholinergic neurons are lost early in the disease process [197]. While these treatments ameliorate symptoms to some extent in some stages of the disease, they do not slow progression because the loss of cholinergic neurons is not the primary cause of the disease. One example of a cholinesterase inhibitor is Donepezil which is used in AD to enhance the signalling capacity of the degenerating cholinergic cells of the basal forebrain, having a small positive effect on cognition and daily living in patients with

mild-to-moderate AD [198]. Rivastigmine (cholinesterase inhibitor) is the favoured treatment for DLB and has produced significant improvement in patients' hallucinations, cognition and behavioural changes in DLB patients over a 96-week treatment period [199], but again this symptomatic relief does not alter disease progression. In PD, the treatments are more effective because loss of a single neurotransmitter, dopamine, does appear to drive the disease process. Levodopa is currently the most effective treatment for the motor symptoms of PD and is used in combination with carbidopa, which inhibits the peripheral breakdown of levodopa allowing more drug to enter the brain [200]. Levodopa counteracts the loss of dopamine producing neurons in the substantia nigra by replacing dopamine in the brain. Another common therapeutic approach is to block excessive excitatory signalling. Memantine is a non-competitive NMDA receptor antagonist that appears to have specificity for open, extrasynaptic channels thus preventing glutamatergic excitotoxicity but leaving normal synaptic function unhindered, however the exact mechanism of action is still debated [201, 202]. Memantine can enhance cognition in patients with moderate-to-severe dementia [203]. Riluzole is used as a neuroprotective drug in ALS. It has many effects on neuronal physiology and certainly inhibits neurotransmitter release and glutamate receptors, leading to the hypothesis that it's effects in ALS are to dampen excitotoxicity [204]. Interestingly, Riluzole is currently in Phase II trials as a combination therapy with two other drugs for treating MS [205-207]. Tetrabenazine has an unknown mode of action, but is believed to deplete levels of monoamines in the presynapse, by inhibiting vesicular monoamine transporter 2 (VMAT2) and is effective at controlling chorea in HD [208].

The number of licensed drugs for these disorders may seem encouraging, however none of these can be cured and most of the drugs have very limited effect if any on slowing disease progression. In fact, despite Riluzole being the only available medication with any proven effect in ALS, it can only prolong life by around two to three months [209], thus new therapeutics and novel approaches are desperately needed. One approach being pursued in a number of diseases is gene therapy. Gene therapy trials have already been run for PD, however despite positive safety results the trials have yet to yield clinical efficacy [210]. Encouraging success in the treatment of the motor neuron disease, spinal muscular atrophy (SMA) has inspired hope in the field of ALS, however given the genetic heterogeneity of the disease, it will likely not prove a viable therapy available for all patients [211]. Gene therapy approaches are also being considered for HD [212], however the fine balance of huntingtin levels will be crucial as conditional removal of the gene in adult mice led to neurodegeneration [213]. While gene therapy represents an exciting potential approach for some neurological disorders, vector properties, cellular targeting and precise control over transgene expression remain considerable hurdles to be cleared before widespread use [214]. Another approach that has reached clinical trial stage for AD, PD, DLB and HD is the specific targeting of the pathological proteins (amyloid-β, tau, α-synuclein and huntingtin) associated with the disease and aiding clearance from the brain. These include increasing protein clearance by enhancing proteasomal function, dampening post-translational modifications associated with pathological forms of protein and preventing protein aggregation [121, 215]. However, this approach should be treated with extreme caution and lessons must be learned from AD in which trials aimed at clearing toxic amyloid from the brain have so far all

failed to reach their primary clinical endpoints [216], highlighting the difficulty of translational medicine in the field of neurodegenerative diseases.

It is clear that new medications are required for neurodegenerative disease, potentially to prevent or reverse synapse loss. One potentially interesting novel approach is the targeting of neuronal extracellular matrix components. The formation of perineuronal nets (PNNs) is thought to be a critical stage of neurodevelopment and results in the formation of neuroprotective barriers around cells, and helps stabilise mature synaptic contacts [217]. Interestingly, in AD it appears that PNNs are lost in plaque cores and cells that retain these nets are devoid of tau pathology, despite being surrounded by severely affected cells [218]. Furthermore, cultured neurons with an intact PNN were protected against treatment with exogenous Aβ1-42, whereas cells without a PNN, degenerated [219]. Many of the extracellular matrix molecules are found at synapses, although their exact role in synaptic physiology and whether they are synaptoprotective has yet to be elucidated [217]. However, this interesting therapeutic avenue is not without it's paradoxes. Recent data suggests that digesting PNNs with chondroitinase actually reverses memory deficits in mouse tauopathies by specifically aiding synaptic plasticity, without altering pathological load [220]. Therefore more research is required into the role(s) of the extracellular matrix in disease pathogenesis, however it is interesting to consider the possibility of altering the PNN defences around synapses to inhibit or even reverse synapse loss in neurodegenerative disease.

Conclusion

While the neurodegenerative diseases mentioned above appear distinct in their causative factors and end-point pathologies, examining their early-stage pathogenesis reveals a coalescent point at the synapse (see Table 1). Our understanding of synaptic structure has expanded immeasurably since the beautiful observations and drawings of dendritic spines by Ramon Cajal in the 19th century. Modern technology now allows us to probe neuronal and network function with a flash of light [221] and to visualise numerous proteins within a 3-dimension nanometre scale, at individually identified synapses [222]. While these are amazing advances for academic research, most are only applicable to transgenic model systems and our ability to perform such experiments in living patients is a long way off. If such techniques were available to assess synaptic function in living patients, neurodegenerative diseases could be diagnosed at prodromal stages.

Currently, most neurodegenerative diseases have their own distinct therapeutic programmes for tackling the disease, with varying levels of successes. Most drugs treat the symptoms of the disease, but a better approach may be to combine efforts in an attempt to identify common factors and focus on preventing or delaying disease pathogenesis. As described above, some medications are useful for more than one disease, which suggests common features must play a part in disease pathogenesis. With greater collaboration between researchers working on different diseases, both in and outside the clinic, our hopes of finding novel synaptoprotective therapies can be achieved.

Table 1: Summary of common disease-related synaptic pathologies

Disease	Synapse loss	Synaptic accumulation	Disrupted synaptic	Disrupted mitochondria	Alterations in synaptic

		of disease-related protein	**plasticity**		**machinery**
AD	Early synapse loss even before amyloid plaque formation and loss correlates with cognitive decline (18, 20, 21, 23, 41)	Amyloid-β and pTau (21, 24, 44-47)	Disrupted LTP and enhanced LTD (26-30, 33, 48, 49)	Amyloid-β induces mitochondrial dysfunction and pTau disrupts mitochondrial trafficking (41, 42, 47, 50, 51)	Disrupted trafficking of synaptic receptors (33, 37, 38, 40, 48, 49)
PD	Early loss of synapses in the striatum (7, 70-72)	α-synuclein (73-75)	Disrupted LTP (80, 81)	PD genetic risk factors are associated with mitochondrial dysfunction (85-89)	Presynaptic disruption of numerous neurotransmitter systems (76-79)
DLB	Loss of hippocampal and cortical synapses (74, 75)	α-synuclein (74, 75)	Disrupted LTP (80, 81)	Mitochondria accumulate and breakdown around Lewy bodies (86, 87, 90)	α-synuclein-induced internalisation of synaptic receptors (80-82)
MND	Early synapse loss in the motor and sensorimotor cortices and onto lower motor neurons of the spinal cord (92-96)	Unknown	Increased excitability and enhanced LTP (118-120)	SOD1 is a mitochondrial protein, when mutated it accumulates in mitochondria and disrupts their function (91, 99, 115-117)	Decreased glutamate clearance in motor cortex and spinal cord (109-113)
HD	Presymptomatic loss of synapses and spines (123-127)	Huntingtin (133-137)	Disrupted LTP (138-140)	Mitochondrial calcium handling defects occur early in disease pathogenesis (141-144)	Disrupted trafficking of synaptic receptors (135, 136)
MS	MS cortex and demyelinated hippocampi contain less synapses (156, 157, 160)	Unknown	Disrupted LTD and enhanced LTP (147, 148, 153-155)	Mitochondrial dysfunction occur early in mouse models of MS and defects are commonly found in patients (167-170)	Altered glutamate release and uptake (147-152, 156, 161-164)
Ageing	Age-dependent synapse loss correlates with cognitive decline (172, 179-187)	Unknown	Disrupted LTP and enhanced LTD (192-195)	Mitochondrial dysfunction increases with age (180, 189-191)	Age-related decline in numerous neurotransmitter systems (176-178)

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Figure Legends

Figure 1: Synaptic structure

A. Neurons within a network frequently communicate by passing excitatory messages from one cell (pink) to another (blue) at small compartments known as synapses. Excitatory synapses often occur between a presynaptic axon terminal and a postsynaptic dendritic spine (lower panel) and this is known as an axospinous synapse. **B.** Electron micrograph from the mouse nucleus accumbens showing an axospinous synapse. The presynaptic terminal (pink) contains the machinery required to release small packets of neurotransmitter inside synaptic vesicles (s.v.) which when released, cross the synaptic cleft and act on the next cell (blue). Synapses require a lot of energy and two small mitochondria (m) can be seen inside the presynaptic terminal. The postsynaptic cell contains a clearly identifiable spine, protruding from the dendritic shaft. The spine head receives the synaptic message from the presynaptic cell at an electron dense accumulation of proteins known as the PSD (within the red arrowheads). Note the dendritic mitochondria (m) in close proximity to the spine. Scale bar = 100nm. **C.** Diagram highlighting a select few of the presynaptic and postsynaptic components of the synapse. Presynaptically, the microtubule-binding protein Tau can be found bound to microtubules and Ca^{2+}-permeable ion channels are located on the terminal's plasma membrane. Synaptic vesicles are presynaptic and contain α-synuclein in the membrane and neurotransmitters within their lumen. Neurexins protrude into the synaptic cleft, looking for their postsynaptic neuroligin partners and help hold the synapse in place. Postsynaptically, ionotropic glutamatergic receptors such as NMDA and AMPA receptors are found directly opposing the presynapse. These are held in place by scaffolding proteins such as PSD95 and SAP102, which form an integral part of the PSD. Other important scaffolding molecules such as Homer, GKAP and Shank combine to hold metabotropic glutamate receptors such as mGluR5 in place, in perisynaptic regions. Spine architecture is maintained by important structural proteins such as F-actin, which are found in the spine neck and base of the spine head. It is believed that interactions between Shank and cortactin allow synaptic changes to influence spine dynamics, via alterations in F-actin.

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