

REVIEW ARTICLE



The synapse as a treatment avenue for Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder with devastating symptoms, including memory impairments and cognitive deficits. Hallmarks of AD pathology are amyloid-beta ($A\beta$) deposition forming neuritic plaques and neurofibrillary tangles (NFTs). For many years, AD drug development has mainly focused on directly targeting the $A\beta$ aggregation or the formation of tau tangles, but this disease has no cure so far. Other common characteristics of AD are synaptic abnormalities and dysfunctions such as synaptic damage, synaptic loss, and structural changes in the synapse. Those anomalies happen in the early stages of the disease before behavioural symptoms have occurred. Therefore, better understanding the mechanisms underlying the synaptic dysfunction found in AD and targeting the synapse, especially using early treatment windows, can lead to finding novel and more effective treatments that could improve the lives of AD patients. Researchers have recently started developing different disease-modifying treatments targeting the synapse to rescue and prevent synaptic dysfunction in AD. The main objectives of these new strategies are to halt synaptic loss, strengthen synaptic connections, and improve synaptic density, potentially leading to the rescue or prevention of cognitive impairments. This article aims to address the mechanisms of synaptic degeneration in AD and discuss current strategies that focus on the synapse for AD therapy. Alzheimer's disease (AD) is a neurodegenerative disorder that significantly impairs memory and causes cognitive and behavioural deficits. Scientists worldwide have tried to find a treatment that can reverse or rescue AD symptoms, but there is no cure so far. One prominent characteristic of AD is the brain atrophy caused by significant synaptic loss and overall neuronal damage, which starts at the early stages of the disease before other AD hallmarks such as neuritic plaques and NFTs. The present review addresses the underlying mechanisms behind synaptic loss and dysfunction in AD and discusses potential strategies that target the synapse.

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SYNAPSE STRUCTURE, SYNAPSE LOSS AND SYNAPTIC DYSFUNCTION IN AD

Synapses are critical for brain function since they are required for proper communication between neurons. The synapse is the space formed by interneuronal connections that enables neurons to pass electrical and chemical signals to one another. Neuronal communication is initiated at the presynaptic terminal, where vesicles containing different neurotransmitters are released. The neurotransmitter-synthesizing enzymes, type of neurotransmitters released, and transporters are different between inhibitory and excitatory presynaptic terminals. For instance, the most common excitatory neurotransmitter of the central nervous system (CNS) is glutamate [1], whereas GABA is the predominant inhibitory neurotransmitter of the adult brain. On the other hand, the postsynaptic terminals at dendritic spines use specific neurotransmitter receptors such as N-methyl-D-aspartic acid receptors (NMDA receptors) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA receptors) to receive and transduce the incoming signals [2].

Synapses are dynamic and change their number, structure, and function, leading to strengthening or weakening their

synaptic contacts. This process is called synaptic plasticity and is crucial for cognitive functions such as learning and memory [3]. Synaptic degeneration has been associated with Alzheimer's disease (AD) [4], and it is clearly found in patients with mild cognitive impairment (MCI) [5]. Other studies have also indicated that synaptic density significantly decreases at stages preceding amyloid plaque deposition in several mice models of AD [6, 7]. Synaptic loss and dysfunction can drastically damage signal transduction and neuronal communication, leading to network failures and abnormalities in the CNS. Thus, synaptic degeneration may be one of the first underlying mechanisms behind memory impairment and cognitive deficits, rather than the accumulation of amyloid-beta ($A\beta$) plaques and neurofibrillary tangles (NFTs) [8]. Better understanding the causes of synaptic loss in AD might be of critical importance to finding new therapeutic targets for a novel disease-modifying treatment. Although the cause of synaptic loss in AD has not yet been fully elucidated, $A\beta$, tau, apolipoprotein E (ApoE), and microglia seem to be major players contributing to synaptic dysfunction and neurodegeneration (Figs. 1 and 2).

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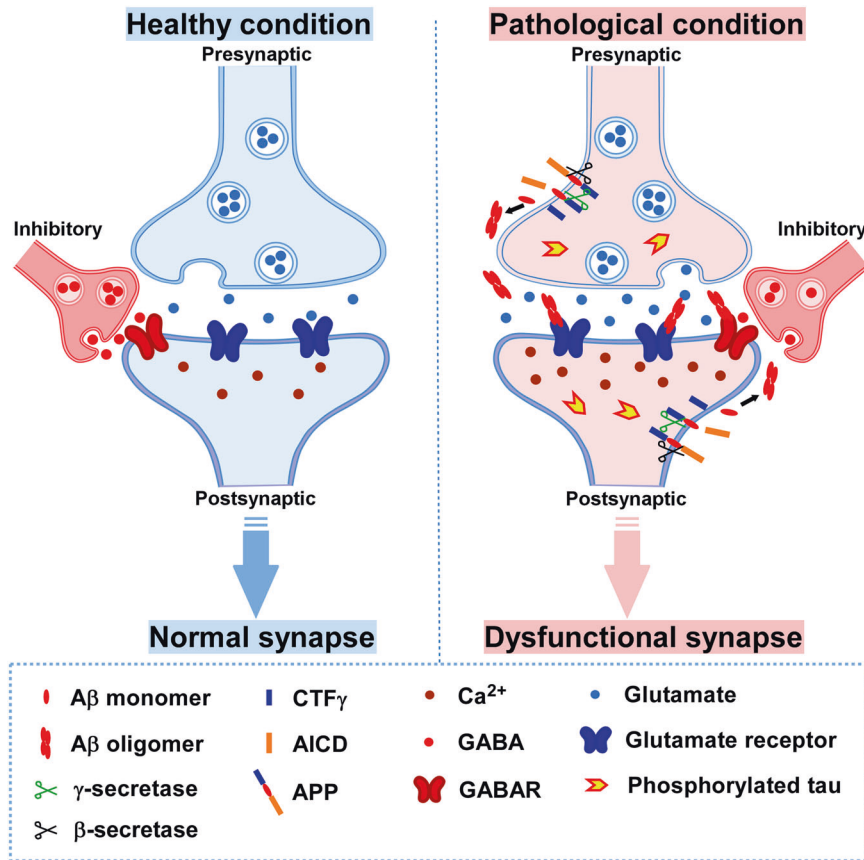


Fig. 1 Oligomeric A β and phosphorylated tau induce neural toxicity causing synaptic loss and dysfunction. In healthy conditions, there is a balance between inhibitory and excitatory connections mainly mediated by GABAergic and glutamatergic neurons. In the pathological condition, abnormal APP processing by β -secretase at the Asp1 site increases A β monomer generation and A β oligomer formation. A β oligomers lead to increased neuronal activity by disrupting glutamatergic/GABAergic balance and resulting in LTP impairments. A β oligomers can also cause tau missorting from the axon to the somatodendritic compartment and increase tau phosphorylation, which causes further neural toxicity and synaptic dysfunction.

A β TOXICITY MEDIATED SYNAPSE LOSS AND SYNAPTIC DYSFUNCTION

APP processing and A β generation

A β is a 38–43 amino acid residue peptide produced through the sequential cleavage of the amyloid-beta precursor protein (APP) by β - and γ -secretases. APP is a type I integral membrane glycoprotein ubiquitously expressed, with isoform APP695 predominantly expressed in the CNS [9–12]. APP can undergo different competitive series of sequential cleavage through either an amyloidogenic or non-amyloid pathogenic pathway. Most of the APP undergoes non-amyloidogenic cleavage processes [13], typically processed by α -secretase at the Leu17 site, producing a large soluble fragment (sAPP α) and a C-terminal fragment (CTF) of 83 amino acids (C83). C83 is further cleaved by γ -secretase, generating a P3a fragment and CTF γ . Beta-site APP cleaving enzyme 1 (BACE1) can also process APP at the Glu11 site, generating C89, which is cleaved by γ -secretase to create a truncated A β_{1-40} as part of the non-amyloidogenic pathways [14, 15]. On the other hand, APP is cleaved first by BACE1 at the Asp1 site for the amyloidogenic pathway to create a secreted form of APP (sAPP β) and C99. C99 is then further cleaved by γ -secretase, producing A β and intracellular CTF γ [16, 17]. Under AD pathological conditions, overproduced A β can aggregate and form soluble oligomers, which can then change their conformation into cross- β -sheet fibrils, creating amyloid plaques. Increasing evidence suggests the critical role of soluble A β oligomers in the pathogenesis of AD [18, 19]. Unlike many thought, large insoluble

A β fibrils are not the main responsible form behind neuronal damage.

A β oligomers connection to synapse loss and dysfunction in AD

The original amyloid cascade hypothesis mainly focused on large insoluble A β fibrils, while very few studies investigated the role of soluble A β oligomers in AD-related neuronal toxicity. However, research has found that A β oligomers can exist independently of fibrils [20]. Different studies have detected a correlation between the concentration of soluble A β oligomers in the brain and synaptic loss and dysfunction [18, 19].

Soluble A β oligomers can bind at the synapse and cause synaptic loss and synaptic changes in shape, size, and composition [21]. A β oligomers have been found to colocalize with PSD-95 in AD transgenic mice, indicating their presence in the postsynaptic terminal and modifying the synaptic structure, composition, and function [22]. A β oligomers exposure can lead to fewer functional spines and abnormally shaped spines similar to those found in mental retardation [21]. Likewise, soluble A β oligomers from the cerebral cortex of AD subjects or released by human APP-transfected cells were found to change the number, composition, and shape of synapses, leading to synaptic plasticity deficits and cognitive function impairments [23, 24].

Several studies using in vitro and in vivo samples have shown that soluble A β oligomers can induce inhibition of long-term potentiation (LTP) [24, 25]. Recordings of the field excitatory

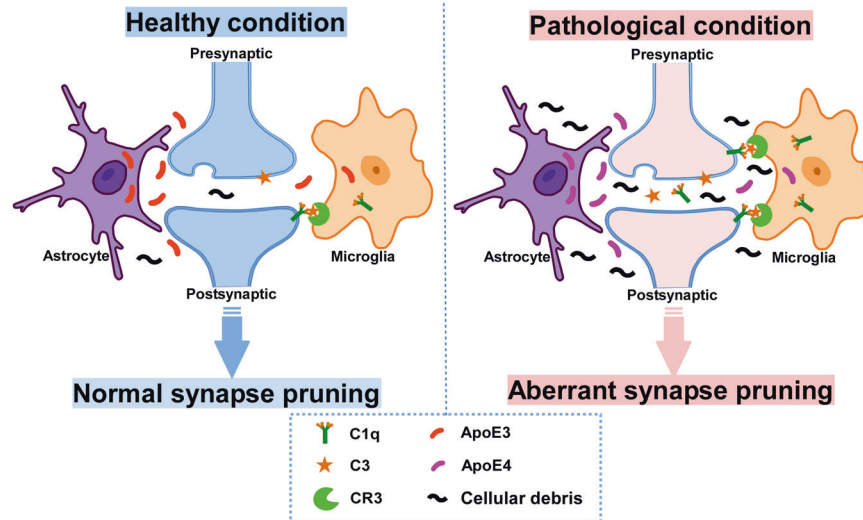


Fig. 2 General representation of the role of microglia, astrocytes and ApoE isoform on abnormal synaptic pruning and synaptic dysfunction. Microglia play a critical role in synaptic refining and elimination. In the pathological condition, there is abnormal microglia activation that leads to synaptic loss. ApoE4 can exacerbate this effect by increasing the C1q accumulation resulting in overactive microglia and increased synaptic loss. ApoE4 can also decrease the phagocytic capacity of astrocytes, leading to abnormal synaptic pruning and increased synaptic debris accumulation, overall causing synaptic dysfunction.

postsynaptic potentials (fEPSP) in wild-type mouse samples treated with A β oligomers indicated that hippocampal LTP impairments were caused by disruption of the glutamatergic/GABAergic balance [26]. Furthermore, synthetic A β aggregates can inhibit NMDA receptor-dependent LTP and diminish AMPA receptor mEPSC (miniature excitatory postsynaptic current) amplitude and frequency [27, 28]. All these studies support the hypothesis that A β oligomers can have a toxic effect on neural connections, resulting in synaptic loss and dysfunction.

Drug development research has also focused on the oligomer theory and placed substantial efforts to create a novel therapy for AD treatment. However, most attempts to date have failed to provide an effective disease-modifying cure for AD.

TAU TOXICITY LEADS TO SYNAPTIC LOSS AND DYSFUNCTION

Tau mediates A β -induced synaptic loss and dysfunction

Tau is a microtubule-associated protein (MAP) that is mostly enriched in the axons of neurons. Aggregation of A β and formation of tau tangles into NFTs are hallmarks of Alzheimer's disease (AD). Synaptic density loss has also been correlated with increased phosphorylated tau (p-Tau), and A β levels [29], indicating that p-Tau also plays a role in synaptic toxicity in AD. For instance, endogenous tau was found to move to the somatodendritic area after treatment with A β oligomers, causing spinal density loss [30].

Decreased levels of endogenous tau can also reduce A β -induced synaptic loss and dysfunction as well as improve cognitive function in AD transgenic mice expressing human APP [31]. These findings suggest that tau can mediate synaptic dysfunction in the presence of A β oligomers and A β aggregates.

Tau directly induces synaptic loss and dysfunction

Tau can have toxic effects on the synapse resulting in density loss and synaptic dysfunction independent from A β [32]. Tau is predominantly found in axons, but misfolded tau oligomers can be located in the pre and postsynaptic terminals in AD [33]. Interestingly, high tau levels in the postsynaptic terminal have been linked with synaptic loss in tau-transgenic mice [34, 35], and were shown to cause synaptic dysfunction [36, 37]. Of relevance, tau-induced synaptic loss was found to occur before neurodegeneration

in transgenic mice models [38]. Transgenic mice with overexpression of human tau have also been associated with fewer synaptic proteins, loss of spinal density and LTP impairment leading to cognitive and memory deficits [39, 40]. Missorting tau from the presynaptic to the postsynaptic compartment can happen during the early onset of AD in mouse models, suggesting that this abnormal tau localization could be partially responsible for the early synaptic dysfunction seen in AD [41]. More research needs to be conducted to understand further the early role of tau on AD-related synaptic dysfunction.

As mentioned, amyloid plaques and NFTs are both pathological hallmarks that have been associated with the progression of AD and ongoing synaptic loss. However, some research suggests that synaptic dysfunction may occur earlier than these common AD symptoms [6, 7]. Therefore, other mechanisms and pathways that trigger synaptic dysfunction in AD need to be investigated, such as genetic factors, inflammation and phagocytosis. Modelling all these interlinked pathological pathways using novel *in vitro* and *in vivo* models that best represent the synaptic degeneration occurring in AD, instead of using the traditional A β toxicity cells or animals, might lead to more promising treatment avenues.

Genetic risk factor ApoE4 exacerbates synapse loss and dysfunction

Apolipoprotein E (ApoE), specifically ApoE4, is the strongest genetic risk factor associated with sporadic AD [42]. In humans, ApoE has three allelic genetic variants leading to three protein isoforms, ApoE2, ApoE3 and ApoE4, with ApoE3 being the most common isoform (77.9%), while ApoE2 (8.4%) and ApoE4 (13.7%) are much less common and thus can be considered variants [43]. ApoE2 can play a protective role and reduce the risk of AD [44, 45]. On the other hand, inheriting even one copy of ApoE4 is associated with a 3-fold increased risk of AD, whereas two copies of ApoE4 is associated with a 15-fold increased risk [43]. It has been reported that ApoE4 can promote synaptic loss and degeneration through abnormal synaptic pruning by astrocytes, microglia, protein changes or through A β , and tau-dependent pathways. However, the role of ApoE in AD is very complex and needs to be further studied.

Proteomic comparative analysis of AD synapses showed that ApoE4 was associated with increased synaptic protein changes in

AD [46]. In AD patient induced pluripotent stem cells (iPSCs)-derived cerebral organoids, ApoE4 can aggravate synaptic loss and neurodegeneration [47]. Moreover, synaptic pruning by astrocytes is mediated and dependent on the ApoE allele, with ApoE2 increasing the phagocytic capacity of astrocytes and ApoE4 decreasing the rate of synaptic pruning by astrocytes, leading to the accumulation of synaptic debris and synaptic dysfunction [48]. Similarly, other studies also showed that ApoE4 could decrease synaptic density in ApoE4 transgenic mice independently of A β presence [49].

On the other hand, by injecting AAV4-human-ApoE4 into the lateral ventricles of traditionally used APP/PS1 mice, ApoE4 enhanced A β oligomer concentration in tissue fluid, increased abnormal neurites and caused synaptic loss around plaques [50]. ApoE4 can also aggravate tau pathology in AD patient-derived cerebral organoids [47] and lead to synaptic degeneration. For instance, in MAPT mice infected with ApoE4, there is significant hippocampal synaptic loss [51]. In a recent study using a new mice model with selective deletion of ApoE4 from astrocytes, removal of ApoE4 reduced tau-induced synaptic degeneration and microglial phagocytosis at the synapse [52]. These new findings suggest that ApoE4 may contribute to synaptic degeneration via both A β /tau-dependent and independent mechanisms. While targeting ApoE, especially ApoE4, may be a promising treatment that rescues synaptic damage, the role of ApoE in synaptic loss in AD needs to be further examined.

Microglia mediates synaptic loss and dysfunction by playing a protective role

Microglia are the main macrophages located in the CNS. They are especially important during brain development since they play a crucial role in synaptic remodelling, pruning and plasticity [53, 54]. However, there is still much to investigate regarding the precise mechanisms behind the communication between synapses and microglia and how microglia conduct synaptic refining and pruning. One potential mechanism is the classical complement cascade [55, 56]. In this mechanism, microglia are characterized as the primary source of complement component 1q (C1q) and complement component 3 (C3) protein [57, 58], which are part of the innate immune response. C1q can activate C3, which then modulates synaptic refining by eliminating dysfunctional synapses. C1q and C3 are developmentally regulated, presenting upregulated levels during early developmental stages and down-regulated expression in the mature CNS [55, 59, 60]. C1q or C3 Knockout (KO) mice show synaptic pruning and refining deficits, causing neural circuit issues [55, 59].

In AD mice models and human patients, microglia quickly surrounds amyloid plaques and are found in high numbers around newly created neuritic plaques [61], with different studies suggesting its involvement in A β clearance [62]. C1q is significantly increased in AD mice models and even in wild-type mice injected with A β oligomers [63, 64]. C1q depletion or exposure to C1q activator blockers in AD mouse models decreases synaptic loss and dysfunction [63, 65]. Similarly, A β oligomer treatment did not cause synaptic dysfunction in C1q-KO mice [63]. Interestingly, accumulation of C1q protein in the hippocampus could be dependent on the ApoE allele present, with a recent study suggesting that in ApoE4 KI mice, there is significantly higher C1q accumulation in hippocampus [48], which could lead to synaptic dysfunction. These results support the crucial role of C1q on synaptic elimination and suggest that overactive microglia and overexpression of C1q might be partially responsible for synaptic dysfunction in AD.

Microglia can also express NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3). Interestingly, NLRP3, the adaptor protein ASC and pro-caspase 1, form the NLRP3 inflammasome, a crucial part of the innate immune response [66]. NLRP3 inflammasome can colocalize with amyloid plaques leading to the reduction of A β clearance and more A β aggregates [67]. In a recent study, the deficiency of functional NLRP3 inflammasome caused significant reduction in tau phosphorylation and aggregation [68]. An inhibitor of NLRP3 inflammasome called dapansutrile or OLT117 improved synaptic plasticity and caused less microglia activation in AD mouse models [69]. These studies suggest the validity of researching neuroinflammation as a potential target for AD treatment that could rescue synaptic density and plasticity.

As explained in this initial section, there is plenty of evidence to show a significant increase in synaptic loss and dysfunction during the early stages of AD. This synaptic dysfunction can be linked to A β oligomers, A β toxicity, tau phosphorylation, ApoE isoform, increased microglia activity and inflammation. Research should continue investigating the causes of synaptic loss in neurodegenerative diseases since it is one of the early markers of these pathologies, particularly in AD. In the next section, current treatments that specifically target the AD-related synaptic changes will be reviewed.

Targeting the synapse for Alzheimer's Disease treatments

Potential AD disease-modifying therapies have also focused on the synapse and preventing or rescuing synaptic density loss. This section will examine these AD treatments, what specific part of the synapse they target, and whether the US Food and Drug Administration (FDA) has approved them to treat AD (Table 1).

Table 1. Summary of Alzheimer's disease (AD) treatment focusing on the synapse.

Target	Drug name	AD Clinical Trials and FDA Approval	References
Kinase activity	AZD0530 or saracatinib	Phase 2 for AD Approved for cancer	[73–77, 81]
	Masitinib	Phase 3 for AD	[79–81]; NCT01872598
	Fasudil	Preclinical only	[83, 84]
	FSD-C10	Preclinical only	[85, 86]
Glutamatergic pathway	Memantine	Approved for AD	[88–94]
	NitroSynapsin	Preclinical only	[95–97]
	Riluzole	Phase 2 for AD Approved for ALS	[98–100]
Cholinergic pathway	Donepezil	Approved for AD	[101–103]
	Galantamine	Approved for AD	[93, 104, 105]
	Rivastigmine	Approved for AD	[93, 106, 107]
Serotonin pathway	SSRIs	Approved for depression	[113–115, 127]
Neurotrophic factors	P021	Preclinical only	[138–141]
	LM11A-31	Phase 2 for AD	[144]; NCT03069014

Targeting kinase activity to improve synaptic density

One of the drug candidates to restore synaptic dysfunction is AZD0530 or saracatinib. AZD0530 inhibits a family of protein kinases known as Src [70]. Several preclinical trials have tested this medication for AD using transgenic AD mouse models taking advantage of the inhibition caused by this compound on Fyn kinase. Of relevance, the formation of A β oligomers can lead to activation of Fyn, which results in tau phosphorylation, and is linked to AD [71, 72]. Oral administration of AZD0530 can block the activation of Fyn kinase in neuronal synapses, potentially leading to synaptic density improvements under pathological conditions. Preclinical trials have shown significant improvement of synaptic density without affecting A β plaques [73], indicating that this compound truly focuses on the synapse rather than targeting A β . Other preclinical studies in AD transgenic mice indicated that AZD030 could rescue synaptic loss and memory impairments [74] and increase synaptic density in the hippocampus [75]. A phase Ib clinical trial found that AZD0530 is safe and relatively well tolerated in AD patients and can pass the blood-brain barrier [76]. However, a recent phase 2a randomized clinical trial using this compound on AD patients found no statistical significance compared to placebo-controlled groups in cerebral metabolic decline and other biomarkers of the pathology [77]. More research should be conducted to understand the mechanism of this compound better and if it has other off-target effects on other members of the Src family besides Fyn kinase inhibition. Furthermore, the effect of different dosages and long-term administration should be further investigated in clinical trials for AD treatment.

Similarly, masitinib is another inhibitor of protein kinases. Interestingly, masitinib has also been implicated in blocking Fyn kinases and studied in neurodegenerative diseases [78]. This drug can block the survival and function of mast cells, which are crucial for inflammatory responses linked to synaptic loss in different pathologies. In a recent study using a transgenic AD mice model, masitinib was found to improve spatial learning and cognition by potentially protecting the synapse [79]. A phase 2 placebo-controlled study of this drug was conducted by administering it in conjunction to other compounds on AD patients [80]. As an add-on therapy, masitinib was shown to decrease cognitive decline and was well tolerated [80]. A very recent phase 3 trial for this drug in AD patients is undergoing analysis (ClinicalTrials.gov Identifier: NCT01872598). Remarkably, a study using human induced pluripotent stem cells (hiPSCs) and human neural stem cells found that Fyn kinase inhibitors, including AZD0530 and masitinib, can decrease Fyn kinase activity and A β 42 release [81]. However, these effects were only found for a subset of neurons derived from AD patients with high APP Tyr phosphorylation [81]. These results suggest that certain AD patients with high APP phosphorylation might benefit more from Fyn kinase inhibitor treatment than other patients, indicating the potential importance of using early biomarkers to identify APP phosphorylation levels before treatment administration.

The wntless pathway (Wnt) also relies on kinase activity and is crucial in synaptic formation and maintenance. Inhibitors of specific targets within this signalling pathway have been studied as a potential treatment for reversing synaptic loss observed in AD. Fasudil inhibits ROCK, which is part of the Wnt pathway. Fasudil and its active metabolite hydroxyfasudil can cross the blood-brain barrier and enter the CNS [82]. Fasudil, along with bone marrow stromal cells, was found to rescue synaptic loss and enhance learning and memory in AD transgenic mouse model [83]. Furthermore, in an AD-induced rat model, fasudil was found to improve behavioural symptoms of AD [84]. However, this compound has not been approved for clinical AD trials, potentially due to possible severe side effects and toxicity. Interestingly, preclinical studies using a derivative of fasudil called FSD-C10 suggest more promising results and

fewer safety concerns than fasudil [85] while also benefiting memory and synaptic density [86].

Targeting the Glutamatergic pathway to protect synapses

Memantine is an NMDA receptor antagonist that inhibits Ca²⁺ influx happening extrasynaptically and targets the receptor under pathological conditions. One reported advantage of this drug is that it is a low-affinity, non-competitive antagonist of NMDA receptors [87], avoiding the adverse side effects of prolonged receptor blockage on learning and memory. Memantine was synthesized by Eli Lilly laboratories and was used intravenously in AD patients in 1986 [88]. This project covered only a small sample size of 20 patients with two different doses and showed no benefit on neuropsychiatric functioning, but it detected damaging side effects. In the late 1990s, memantine was used again in a larger, placebo-controlled clinical study of moderate to severe AD patients [89]. This project found positive effects of the use of memantine for behavioural performance and acceptable drug tolerance. Another clinical study in the early 2000s confirmed these findings [90], leading to the approval of this drug by the European Agency for the Evaluation of Medical Products (EMA) and later by the FDA. Since then, it has been associated with decreased neuronal toxicity and linked to improved AD symptoms. In more recent mouse model studies, memantine was shown to rescue symptoms of AD pathology, corroborating previous findings [91, 92]. Although memantine has been approved for AD treatment, other studies have shown limited efficacy of this drug even when administered in conjunction with cholinesterase inhibitors [93]. There is still controversy on how effective memantine is in AD, with researchers suggesting it might depend on the disease stage and symptoms of AD patients [94]. A more selective blocker of abnormal NMDA receptor activity is NitroSynapsin, formerly known as nitromemantine, which protects synapses and has been shown to work in vivo and in vitro [95]. A recent study in rodent models and hiPSC-derived neurons found that using NitroSynapsin can protect against synaptic loss caused by α Syn oligomer damage, which has been previously associated with neurodegenerative diseases such as Parkinson's and AD [96]. Furthermore, NitroSynapsin was able to rescue hyperexcitability of neurons caused by abnormal electrical events in an AD hiPSC neuronal organoid model, showing more promising results than memantine [97].

Riluzole is a glutamate modulator that has been FDA approved to treat amyotrophic lateral sclerosis. Preclinical trials using rodent models of AD and ageing have found that riluzole can prevent age-related cognitive decline [98], and protect against A β -induced learning and memory deficits [99]. Even though its mechanisms to improve AD symptoms are not entirely understood, it is suggested that riluzole has neuroprotective effects, decreases glutamatergic toxicity and induces clustering of dendritic spines, which increases synaptic plasticity under pathological conditions. Phase 2 clinical trials for AD using riluzole have been recently conducted and showed less decline in cerebral glucose metabolism than placebo groups, which is correlated with slower cognitive decline [100]. Other clinical trials are ongoing for this drug and its prodrug troriluzole (ClinicalTrials.gov Identifier: NCT03605667).

Targeting the cholinergic pathway to increase synaptic strength

Anti-cholinesterases have been very well studied to treat AD. Within this drug category, galantamine, rivastigmine, and donepezil are FDA-approved for AD treatment. The role of anti-cholinesterases in the treatment of AD is to increase the availability of acetylcholine at the synapse by inhibiting the hydrolysis of acetylcholine, leading to stronger cholinergic synapses. Several preclinical and clinical studies have indicated the efficacy of these drugs as AD treatment. Of relevance, clinical studies have found that donepezil can delay the progression of AD

and improve cognitive function, especially in early AD stages [101–103]. Similarly, large AD clinical studies that indicate the treatment efficacy of galantamine [104, 105] and rivastigmine [106, 107] have also been reported.

Furthermore, anti-cholinesterases have been administered together with NMDA receptor antagonists, such as memantine, to treat neurodegenerative diseases. For example, namzaric is a combination of memantine and donepezil, and it is FDA approved for AD treatment. Several clinical studies in AD patients have found that using memantine together with anti-cholinesterases benefits cognition, memory, and behaviour [108–110]. However, a recent meta-analysis shows that namzaric and other anti-cholinesterases, such as galantamine and rivastigmine, have limited efficacy as an AD therapy [93]. Similarly, another meta-analysis in 2013 indicated that cognitive enhancers such as memantine and anti-cholinesterases did not improve cognition [111]. One reason for this limitation might be that these compounds cannot rescue severe synaptic loss caused by AD, showing once more the importance of early drug administration in AD. A particular characteristic of the cholinergic system is the circadian rhythm in its activity, meaning that it is more active during waking hours [112]. Enhanced acetylcholine levels during sleeping hours can then lead to side effects such as insomnia and other sleep-related disorders. Therefore, analyzing the timing of administration and duration (half-life) of anti-cholinesterases based on their circadian rhythm is necessary to improve current AD treatment.

Targeting the serotonin pathway to strengthen the synapse

Several studies have associated enhancements in cognitive function with the administration of serotonin reuptake inhibitors (SSRI) in dementia patients with depression during AD and MCI progression or in addition to other drugs [113–115]. SSRIs such as fluoxetine and sertraline work by inhibiting serotonin's reuptake, increasing serotonin's availability in the synapse and strengthening communication between neurons. However, there is controversy on the effects of SSRI as an effective treatment for cognitive impairment and dementia associated with AD. Different studies have indicated the benefits of SSRI for cognition in dementia patients [114], while many others have found no significant effects or detrimental effects [116–119].

One SSRI that is commonly studied is fluoxetine. This drug was shown to prevent cognitive decline in AD animal models [120–122], increase the synaptic protein expression as well as spine density in the hippocampus of rodents [123, 124] and protect synapses while preventing neuronal apoptosis in primary cultures and AD animal models. In a more recent study, fluoxetine improved cognitive behaviours in AD transgenic mice while rescuing synaptic density in the early stages of the pathology [122]. These studies show that this compound's positive effects on cognition might be related to rescuing synaptic loss in the early stages of AD. Fluoxetine has also been used to treat depression and anxiety in clinical studies, with reports of improved cognition and reduced depression in AD patients [125]. In contrast, another study and a meta-analysis paper indicated no significant effect in depression scores for depressive AD patients after fluoxetine treatment compared to placebo [126, 127]. Furthermore, as previously mentioned, several other studies find no effect or even detrimental effects of antidepressants such as fluoxetine on dementia and cognition. Overall, SSRI antidepressants have been used as adjuncts with other treatments or alone to treat depression in AD patients. There is still controversy regarding their efficacy in enhancing cognition in dementia patients. Different dosages of the compounds, the timing of the SSRI administration, and the duration of administration of the drugs could be the cause behind these controversial results.

Neurotrophic factors to rescue synaptic loss

Early stages of AD in patients have shown compensatory mechanisms that potentially increase neurogenesis and synaptic plasticity [128–130]. These attempts to reverse the progressive loss of neurons fail in the long term, and the disease progresses. Researchers consider that one potential reason for this failure is insufficient neurotrophic factors in the CNS, specifically at the synapse, to sustain prolonged compensatory mechanisms that improve synaptic plasticity and neurogenesis [130]. For instance, several studies have found abnormal levels of different neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), VGF and nerve growth factor (NGF), in AD patients [131–134].

Treatments using neurotrophic factors have limitations due to the delivery method. Neurotrophic factors do not cross the blood-brain barrier and therefore need different delivery methods to be administered successfully for AD treatment. A phase 1 clinical trial using NGF gene therapy and delivery has been conducted in AD patients showing that it slowed cognitive decline without severe adverse side effects [135]. Similarly, clinical studies using intracerebral injections of adeno-associated viral vectors expressing NGF have shown no adverse side effects in AD patients [136, 137]. However, more studies need to investigate the efficacy of these treatments and their long-term effects. Several studies have focused on orally administering neurotrophic tetra-peptide compound P021 to transgenic AD mouse models during early AD stages. P021 was shown to increase neurogenesis and synaptic plasticity while enhancing cognitive function and rescuing synaptic loss in AD rodent models [138–141]. More studies using neurotrophic factors as AD treatments need to be conducted to investigate dosage and critical administration periods. Furthermore, different delivery methods for neurotrophic factors are currently being examined, such as encapsulated delivery [142, 143] and NGF mimetic peptides or small molecules such as LM11A-31 [144] (ClinicalTrials.gov Identifier: NCT03069014), which can help to overcome the side effects linked to the route of administration in humans.

Summary

In this section, we have mentioned five different targets for AD treatment that focus on improving synaptic function and health. Overall, targeting kinase activity could be a potentially viable avenue for AD treatment. Specifically, Fyn kinase and ROCK inhibitors have the advantage of reducing synaptic dysfunction in different AD models. However, their limitations associated with off-target effects need to be considered more carefully since severe side effects have been associated with these inhibitors. Additionally, further studies to more conclusively determine if subsets of AD patients are more likely to benefit from kinase inhibitors are crucial and could explain the results of using Fyn inhibitors with different degrees of success in humans.

As an alternative approach, targeting the glutamatergic pathway has been studied as a potential target for AD treatment for many years. Memantine, the only glutamatergic drug FDA-approved for AD treatment, has been found to improve synaptic function. However, there is controversy associated with the efficacy of these drugs in AD, with several studies reporting minimal positive effects. Presumably, the reasons behind this controversy could be associated with drug administration at a late AD stage when it is difficult for glutamatergic-mediated drugs to rescue synaptic damage. This shows once more the importance of intervening early and identifying pathogenic windows in AD, wherein drug administration will be safe and therapeutically effective. The other class of FDA-approved AD drugs is anti-cholinesterases. They have been shown to benefit synaptic function and can be administered together with memantine. However, similarly to treatments targeting the glutamatergic pathway, anti-cholinesterases have been shown to have

controversial results, including limited efficacy and adverse side effects.

Regarding the serotonin pathway, SSRIs might offer more benefits to AD patients with depression. These drugs alone or administered in adjunction to other AD-approved treatments have had controversial results regarding their efficacy in improving cognition in dementia. Interestingly, SSRI administration appears to have mixed results, with some studies indicating its benefits to synaptic function in AD, while others report no improvements in cognition and even detrimental effects. Alternatively, treatments using neurotrophic factors might help rescue ongoing synaptic loss in AD, but their main limitation lies in difficulties surrounding their route of administration. Further studies using novel methods such as encapsulated delivery of neurotrophic factors might prove to be very beneficial in AD treatment.

CONCLUSIONS

The study of synaptic changes in AD might be one crucial path to identifying viable targets for AD treatment. Determining these targets that can reverse or prevent synaptic density loss can be an effective avenue for new disease-modifying therapies for AD patients. In this review, several compounds that help prevent or rescue synaptic degeneration were examined regarding their targets within the synapse and their efficacy. Future studies should focus on the pharmacokinetics of the compounds and the critical windows for the treatment administration, while investigating efficient dosage depending on the AD stage. One reason behind the reported failure of AD treatments might be that the medications are administered too late since the first cognitive symptoms usually show after drastic brain changes and severe synaptic loss have already occurred [5]. Therefore, more studies should focus on detecting AD at earlier stages by developing new diagnostic tools such as effective biomarkers targeting the early synaptic changes. Furthermore, almost all studies mentioned in this review did not consider the sex-difference effects of the treatments. Given that AD has been shown in several studies to be more prevalent in females, more studies should examine if the compounds cause different effects depending on the sex of the patients or animal models. Finally, the development of new diagnostic tools to detect synaptic loss at early events of AD pathology could be crucial for the efficacy of timed-treatment administration, allowing the identification of early AD windows and helping prevent significant synaptic damage.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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