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REVIEW

Deregulation of excitatory neurotransmission underlying synapse failure in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common form of dementia in the elderly. Memory loss in AD is increasingly attributed to soluble oligomers of the amyloid-β peptide (AβOs), toxins that accumulate in AD brains and target particular synapses. Glutamate receptors appear to be centrally involved in synaptic targeting by AβOs. Once bound to neurons, AβOs dysregulate the activity and reduce the surface expression of both *N*-methyl-D-aspartate (NMDA) and 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) types of glutamate receptors, impairing signaling pathways involved in synaptic plasticity. In the extracellular milieu, AβOs promote accumulation of the excitatory amino acids, glutamate and D-serine. This leads to overactivation of glutamate receptors, triggering abnormal calcium signals with noxious impacts on neurons. Here, we review key findings linking AβOs to deregulated glutamate neurotransmission and implicating this as a primary mechanism of synapse failure in AD. We also discuss strategies to counteract the impact of AβOs on excitatory neurotransmission. In particular, we review evidence showing that inducing neuronal hyperpolarization via activation of inhibitory $GABA_A$ receptors prevents AβO-induced excitotoxicity, suggesting that this could comprise a possible therapeutic approach in AD.

Keywords: Aβ oligomers, AMPA receptors, D-serine, GABA receptors, NMDA receptors, synapse dysfunction.

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Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia in the elderly, affecting about 35 million people worldwide (Querfurth and LaFerla 2010). This devastating disease deprives patients from their memories and from their capacities of insight, reasoning, abstraction and language (Duyckaerts *et al.* 2009). Neurofibrillary tangles and senile plaques are hallmark pathological lesions found in the brains of affected individuals. While neurofibrillary tangles are intraneuronal aggregates of paired helical filaments of abnormally hyperphosphorylated Tau protein (Goedert *et al.* 1988), senile plaques are extracellular aggregates mainly composed of the amyloid-β peptide (Aβ), surrounded by dystrophic neurites, activated microglia, and reactive astrocytes (Masters *et al.* 1985). Aβ is thought to play a causal role in the pathogenesis of AD (Masters and Selkoe 2012). In particular, soluble Aβ oligomers trigger a cascade of injurious events, which includes the deregulation of excitatory neurotransmission (Ferreira and Klein 2011). Excitatory abnormalities have also been related to Tau pathology in AD as reduction in endogenous tau was found to protect against excitotoxicity and to ameliorate Aβ-induced memory deficits in an AD mouse model. (Roberson *et al.* 2007).

Acute brain insults including hypoglycemia, neurologic trauma, stroke, and epilepsy give rise to an imbalance in excitatory glutamatergic neurotransmission and are known to cause synapse dysfunction and massive cell death in the central nervous system (Esposito *et al.* 2011; Frasca *et al.* 2011). Cognitive impairment and dementia are frequently associated with recovery from such insults, suggesting that common pathological mechanisms may be at play in those

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Abbreviations used: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid-β peptide; AβOs, amyloid-β oligomers; CICR, calcium-induced calcium release; EAAT, excitatory amino acid transporters; LTD, long-term depression; LTP, long-term potentiation; mGluR5, metabotropic glutamate receptors; PrP^C, cellular prion protein; ROS, reactive oxygen species.

neurological conditions and in other forms of dementia, including AD. Indeed, several neurodegenerative disorders have been linked to excitatory neuronal damage, including Parkinson's disease and Huntington diseases, and amyotrophic lateral sclerosis (Mattson 2003; Koutsilieri and Riederer 2007). Here, we review evidence that glutamate receptors are centrally involved in the mechanisms by which amyloid-β oligomers (AβOs) attack synapses and render them dysfunctional. In particular, we discuss the role of *N*-methyl-D-aspartate (NMDA) receptors as common initiators of various forms of neuronal dysfunction/damage instigated by AβOs. Finally, we discuss possible neuroprotective strategies to prevent excitotoxicity and synapse failure in AD.

AβOs

It has been proposed that Aβ causes neuronal dysfunction and cognitive impairments characteristic of both familiar and sporadic forms of AD (Selkoe and Wolfe 2007). Aβ is generated by successive proteolytic processing of the amyloid precursor protein (APP) by β- and γ-secretases (Gralle and Ferreira 2007). Genetic studies of familial forms of AD revealed mutations in different genes, including those coding for APP, presenilin 1 and presenilin 2 (components of the γ-secretase enzymatic complex) (Tanzi and Bertram 2005). The amino acid sequence of Aβ contains part of the transmembrane domain of APP, explaining its high propensity for self-aggregation in aqueous medium. The length of the transmembrane domain of APP present in the Aβ sequence is variable and peptides of different lengths, varying from 38 to 43 residues, are released in the normal brain (Kakuda *et al.* 2012).

In the process of amyloid aggregation, metastable or transient states of aggregation including oligomers, protofibrils, and pre-fibrillar soluble aggregates are formed in addition to the more stable and insoluble amyloid fibrils (Ahmed *et al.* 2010). Although fibrils are present in amyloid plaques and are neurotoxic (Louzada *et al.* 2001, 2004; Paula-Lima *et al.* 2003, 2005, 2009), soluble AβOs appear better related to synapse loss and cognitive impairment than the presence of fibrils (Ferreira and Klein 2011). The term AβOs generally refers to different multimers, ranging from dimers and trimers to dodecamers or higher n-assemblies, but more specific terms have been proposed to distinguish different types of Aβ oligomers. Thus, Aβ derived diffusible ligands include from soluble trimers up to 35–60 kDa oligomers shown to be potent neurotoxins to the central nervous system at non-lethal nanomolar concentrations, and capable of blocking long-term potentiation (LTP) and the reversal of long term depression (LTD) (Lambert *et al.* 1998; Wilcox *et al.* 2011). Naturally secreted cell-derived oligomers, mainly composed of dimers, also block LTP (Walsh *et al.* 2002; Li *et al.* 2011). Yet another type of assembly, termed Aβ*56, a specific dodecamer, was responsible for memory impairments in a transgenic mouse model of AD (Lesné *et al.* 2006). In fact, there is still a huge controversy concerning the exact oligomer species that account for neuropathology in AD (Benilova *et al.* 2012; Larson and Lesné 2012). In this regard, we very recently found that both low-n and high-n Aβ oligomers attack neurons and lead to memory impairment in mice, albeit via different mechanisms (Figueiredo *et al.* 2013). Recent evidence thus suggests that low and high molecular weight oligomers act in an orchestrated manner to cause synapse failure and neuronal dysfunction, leading to memory impairment in AD (Figueiredo *et al.* 2013).

In this review, we mainly focus on results involving Aβ-derived diffusible ligands preparations, but other conformations including naturally secreted cell-derived Aβ dimers (Walsh *et al.* 2002) and dodecameric Aβ*56 (Lesné *et al.* 2006) have also been also considered. Despite their distinct and specific conformations, the general molecular mechanisms of synaptotoxicity of AβOs appear to involve disruption of neuronal calcium homeostasis (De Felice *et al.* 2007; Paula-Lima *et al.* 2011), oxidative damage to neurons (De Felice *et al.* 2007, 2009; Decker *et al.* 2010a), inflammatory processes (Bomfim *et al.* 2012; Ledo *et al.* 2012), mitochondrial fragmentation and dysfunction (Saraiva *et al.* 2010; Paula-Lima *et al.* 2011; SanMartin *et al.* 2012), and disruption of fast axonal transport (Decker *et al.* 2010b) leading to synaptic dysfunction, synapse elimination and, ultimately, cell death (De Felice *et al.* 2007, 2009; Zhao *et al.* 2010; Renner *et al.* 2010; Fig. 1). These deleterious cellular effects are a consequence of the capacity of AβOs to selectively bind excitatory synaptic sites (Lacor *et al.* 2004).

AβOs interact with a multi-protein receptor complex that includes NMDARs as a central component

Considerable evidence indicates that neuronal surface proteins mediate the interaction of AβOs with synaptic membranes, acting as toxin receptors (reviewed in Ferreira and Klein 2011). AβO binding to synapses displays saturable kinetics (Renner *et al.* 2010) and is lost upon controlled treatment of neurons with tripsin (Lambert *et al.* 1998). Glutamate receptors appear to be centrally involved in neuronal binding of AβOs. AβOs interact with complexes containing the GluA2 subunit of AMPA receptors, as suggested by co-immunoprecipitation and photoactivated amino acid cross-linking studies (Zhao *et al.* 2010). Pharmacological inhibition or removal of surface AMPA receptors reduce AβO binding to neurons (Liu *et al.* 2010; Zhao *et al.* 2010; Small 2012). AβO binding and clustering at synapses also appear to require metabotropic glutamate receptors (mGluR5), as demonstrated by single particle tracking of quantum dot-labeled AβOs (Renner *et al.* 2010).

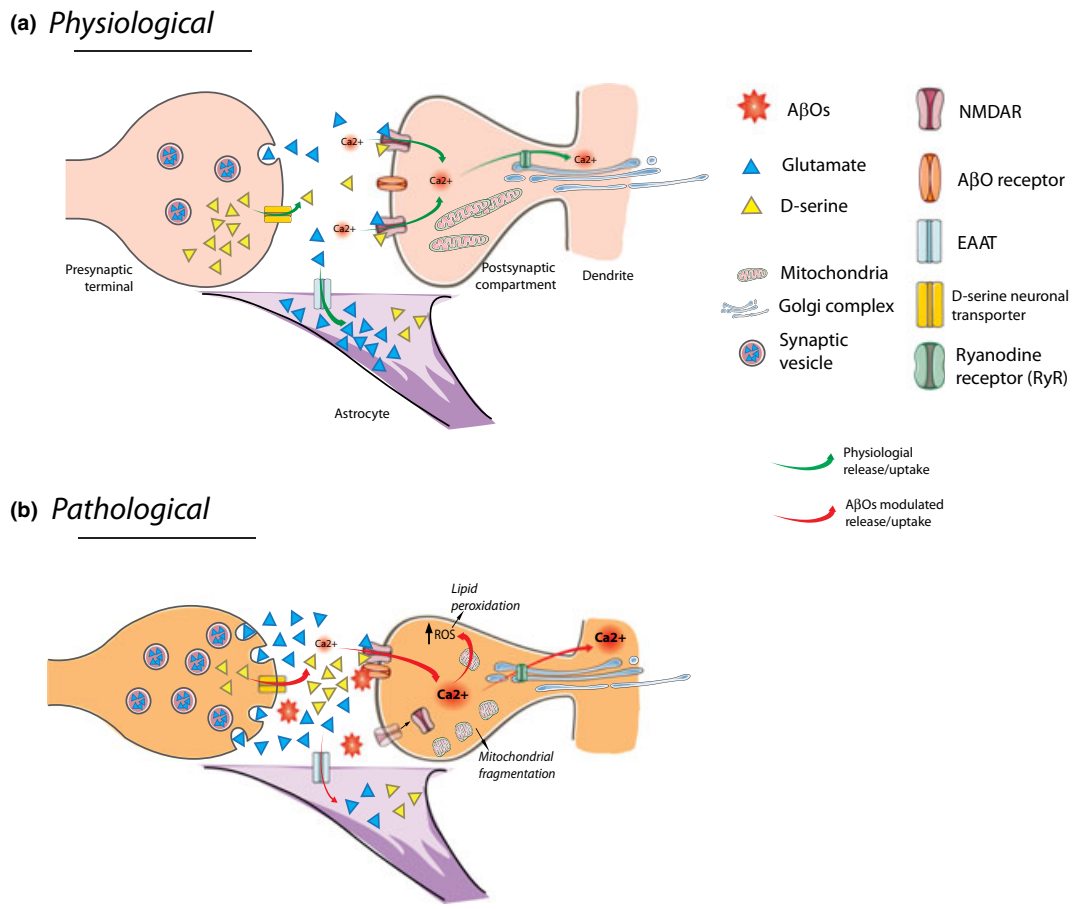

Fig. 1 Physiological events and Aβ oligomers (AβOs)-induced dysfunction in excitatory synapses. (a) *Physiological*: Neurotransmitter vesicles fuse in a Ca^{2+}-dependent manner with the presynaptic membrane, releasing glutamate in the synaptic cleft. Astrocytes play a crucial role in controlling glutamate levels in the synaptic cleft via uptake by excitatory amino acids transporters (EAATs). In the post-synaptic compartment, Ca^{2+} entering through NMDARs causes Ca^{2+}-induced Ca^{2+} release (CICR) from the endoplasmic reticulum by activation of ryanodine receptors, amplifying the NMDAR-initiated signal that will lead to changes in gene expression in the nucleus. D-serine, a co-agonist at NMDARs, can be released from both astrocytes and neurons, but most appears to come from a neuronal pool. (b) *Pathological*: AβOs bind to a putative receptor at the post-synaptic membrane and interact with additional co-receptors (including the NMDAR), not yet fully characterized. AβO binding triggers aberrant activation of NMDARs and abnormal increase in post-synaptic Ca^{2+}. This triggers a variety of toxic pathways in the post-synaptic compartment, including increased generation of reactive oxygen species (ROS) and membrane lipid peroxidation, mitochondrial fragmentation, abnormal CICR and deregulation of Ca^{2+}-mediated phosphorylation pathways involved in regulation of protein synthesis associated with synaptic plasticity, leading to alterations in spine morphology and, ultimately, spine elimination. Neuronal depolarization associated with elevated post-synaptic intracellular Ca^{2+} levels cause an increase in spontaneous pre-synaptic glutamate release (at the axon terminal of the same neuron). Synaptic levels of D-serine also increase, likely because of increased neuronal release stimulated by AβO-induced over-activation of NMDARs.

In addition, NMDA receptors (NMDARs) co-immunoprecipitate with AβOs from detergent-extracted oligomer-treated rat synaptosomal membranes, and an N-terminal (extra-cellular) antibody against the constitutive NR1 subunit of NMDARs markedly reduced oligomer binding to dendrites (De Felice *et al.* 2007). Texidó and coworkers further showed that AβOs directly interact with and activate NMDARs heterologously expressed in *Xenopus laevis* oocytes (Texidó *et al.* 2011). Remarkably, AβO binding is virtually abolished in dendrites of hippocampal neurons in which NMDARs were silenced by a viral vector carrying an amplicon expressing an anti-sense for NR1 (Decker *et al.* 2010a) and NMDAR knock-down abrogated neuronal oxidative stress induced by AβOs, which is mediated by aberrant activation of NMDARs (Decker *et al.* 2010a). In line with a close involvement of NMDARs in interactions with AβOs, pharmacological blockade of NMDARs prevents aberrant calcium signals induced by AβOs (De Felice *et al.* 2007; Paula-Lima *et al.* 2011).

Interestingly, although NMDARs appear specifically required for pathogenic AβO binding to dendrites, NMDARs do not appear to be sufficient for oligomer binding. In fact, not all neurons in a typical hippocampal culture are attacked by AβOs (Lacor *et al.* 2004; Zhao *et al.* 2009), and AβO-attacked and AβO-spared neurons exhibit similar levels of surface NMDARs (Decker *et al.* 2010a). This suggests that AβO binding to synapses may involve NMDAR-promoted assembly of a multi-protein receptor complex that function as a receptor for oligomers (Ferreira and Klein 2011; Fig. 1).

A strong candidate to integrate such a multi-protein receptor complex for AβOs is the cellular prion protein (PrP^C) (Laurén *et al.* 2009). Although AβOs bind to hippocampal slices from PrP^C knockout, oligomers do not reduce LTP under these conditions (Laurén *et al.* 2009). In accord, transgenic PrP^C-null mice presenting AD-related mutations show Aβ plaques, but do not display deficits in spatial learning and memory (Gimbel *et al.* 2010). Corroborating those findings, antibodies against PrP^C co-precipitate insoluble Aβ from AD brain tissue (Zou *et al.* 2011) and prevent oligomer toxicity in transgenic AD models (Barry *et al.* 2011).

Interestingly, memantine, a NMDAR blocker, abrogates toxic signaling mediated by PrP^C in rat cortical cells (Müller *et al.* 1997). Indeed, the concept of a multi-protein complex that could modulate glutamatergic signaling via PrP^C has been previously proposed and several distinct receptors are regulated by PrP^C, including mGluR1 and 5 (Beraldo *et al.* 2011). The notion that PrP^C and NMDARs may be functionally related in neurons, and that they may be both members of a neuronal receptor complex for AβOs, a regulatory physiological association between PrP^C and NMDARs has also been demonstrated: PrP^C-null mouse neurons exhibit prolonged NMDA-evoked currents as a result of a functional up-regulation of NMDARs (Khosravani *et al.* 2008). In addition, PrP^C was shown to interact with NMDARs inducing a reduction in glycine binding to the co-agonist site, while AβOs induce exactly the opposite effect (You *et al.* 2012). These latter studies reveal a physiological role for PrP^C in regulating excessive NMDAR activity and excitatory neuronal damage, and suggest a molecular mechanism in which AβOs interact with and disrupt PrP^C-dependent regulation of NMDAR activity, leading to synaptic injury.

Once bound to synaptic membranes, a major pathophysiological mechanism by which AβOs instigate synapse failure appears to be the down-regulation of plasticity-related receptors, caused by abnormal trafficking or degradation. AβOs have been shown to promote endocytosis of NMDARs by a mechanism requiring α-7-nicotinic receptors (Snyder *et al.* 2005). More recently, AβOs were found to interact with ephrin B2 (EphB2) receptors, leading to receptor depletion from the plasma membrane and, consequently, impaired modulation of NMDA receptors (Cissé *et al.* 2011). As EphB2 receptors recruit active NMDA receptors to excitatory synapses by tyrosine phosphorylation, AβO-induced depletion of EphB2 receptors would also promote a decrease in surface NMDARs. This, in turn, would trigger LTP impairment and deficits in learning and memory. Consistent with this prediction, reversion of EphB2 receptor depletion rescued cognitive functions in an Alzheimer model (Cissé *et al.* 2011).

In agreement with these findings, neurons from a transgenic mouse model of AD express reduced amounts of surface NMDARs (Priller *et al.* 2009). Consistently, AβO application produced a rapid and persistent depression of NMDA-evoked currents in cortical neurons (Snyder *et al.* 2005). An NMDAR-dependent reduction in the expression of post synaptic density-95, a post-synaptic scaffold protein crucial for anchoring and stabilization of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPARs) and NMDARs at synapses was also observed in cultured cortical neurons exposed to soluble AβOs, a condition in which a decrease in GluA2 AMPAR subunit has also been demonstrated (Roselli *et al.* 2005). Mechanistically, activation of phosphatases in AβO-exposed neurons appears to underlie removal of glutamate receptors from synapses. Kurup *et al.* (2010) have proposed a mechanism in which phosphatase STEP(61) mediates Aβ-induced NMDAR removal from synaptic membranes. Fyn activation was also proposed to occur in response to Aβ-PrP^C interaction, yielding phosphorylation of the NR2B subunit of NMDARs, leading to loss of surface NMDARs (Um *et al.* 2012). Finally, several groups have shown that AβO-instigated activation of calcineurin, mediates dephosphorylation of NMDARs and AMPARs at specific residues critical for insertion at extrasynaptic and synaptic membranes, resulting in receptor internalization in hippocampal neurons (Shankar *et al.* 2007; Kurup *et al.* 2010; Jurgensen *et al.* 2011).

Yet another aspect of NMDAR dysfunction in AD is the fact that the activation of NMDARs and mGluRs promotes the amyloidogenic processing of APP, increasing Aβ levels *in vitro* (Lesné *et al.* 2005; Kim *et al.* 2010). AβOs thus instigate a positive feedback cycle, in which AβO-induced accumulation of glutamate gives rise to glutamate receptor overactivation, leading to the generation of more Aβ. Altogether, the findings described above suggest that AβO-induced depressed synaptic output is a possible mechanism to explain early synaptic dysfunction in AD. Because synaptic depression is linked in development to synapse pruning, it was predicted that AβOs would initiate synapse loss (Wang *et al.* 2002), a prediction that has indeed been confirmed (Lacor *et al.* 2007; De Felice *et al.* 2009). Collectively, results suggest that NMDARs are specifically required for pathogenic AβO binding to dendrites and that binding induces the endocytosis of NMDAR and other plasticity-related membrane receptors, culminating in synapse failure and elimination.

Aberrant activation of glutamatergic neurotransmission in AD

An immediate consequence of the binding of AβOs to neurons is calcium influx through the open channel of NMDARs (De Felice *et al.* 2007). This generates a fast and transient increase in intracellular calcium levels, which, in turn, activates ryanodine receptor-mediated calcium-induced calcium release from endoplasmic reticulum stores, generating abnormally long-lasting calcium increases (Paula-Lima *et al.* 2011). Aberrant activation of these channels with the ensuing excessive neuronal influx of sodium and calcium, leads to persistent neuronal depolarization, activation of synaptotoxic calcium-dependent pathways and, ultimately, to cell death (Ferreira and Klein 2011). An early autoradiography study revealed a selective increase in NMDAR-sensitive glutamate binding in the striatum of Parkinson's disease and AD patients (Ułas *et al.* 1994). There is also evidence indicating that glutamatergic neurons are more susceptible to synaptic damage in AD brains (Tannenberg *et al.* 2004), and previous works have demonstrated that, in AD brain, senile plaques localize at hippocampal subfields positive for NMDAR (Geddes *et al.* 1986), and considerable amounts of glutamate receptors are lost in these regions (Aronica *et al.* 1998).

Another important mechanism leading to glutamate receptor overactivation is the accumulation of glutamate at the synaptic cleft, which is highly toxic to both neurons (Camacho and Massieu 2006; Sattler and Rothstein 2006) and oligodendrocytes (Ruiz *et al.* 2010), triggering a cell death cascade known as excitotoxicity. This phenomenon comprises a self-propagating cycle in which glutamate overactivates glutamate receptors causing abnormal calcium signaling and cell death; dead neurons leak additional glutamate to the extracellular milieu, which in turns kills more neurons (Gillessen *et al.* 2002; Mattson 2003).

In healthy brains, efficient transport systems in neurons and astrocytes keep the extracellular glutamate concentration at tightly controlled levels, about 3–4 μM in the extracellular fluid and 10 μM in the cerebrospinal fluid (CSF) in humans (Hamberger and Nyström 1984). In contrast, glutamate levels stored in synaptic vesicles of glutamatergic neurons reach very high concentrations (~ 10 mM; Wu *et al.* 2007). Glutamate transport is an energy-dependent process that allows glutamate uptake to take place very fast after its release from pre-synaptic nerve terminals. The only way to rapidly reduce glutamate concentration is to remove it from the synaptic cleft through the action of transporter proteins. These transporter proteins are present in the plasma membranes of both neuronal and glial cells and five transporter subtypes have been identified. In humans, they were named excitatory amino acid transporters (EAAT) 1–5, and in rodents they are named GLAST, GLT, and EAAC1 (Maragakis and Rothstein 2004). Glutamate transporter dysfunction leads to extracellular accumulation of glutamate and has been correlated with excitotoxicity (Sattler and Rothstein 2006).

Using non-characterized Aβ preparations, early works demonstrated that Aβ infusion into the rat nucleus basalis promotes glutamate accumulation in the extracellular space (Harkany *et al.* 2000). Additional studies showed that fibrillar Aβ inhibits both glial (Harris *et al.* 1996; Parpura-Gill *et al.* 1997) and neuronal glutamate uptake (Parpura-Gill *et al.* 1997), and aberrant expression of the glutamate transporter EAAT1 was reported in the cerebral cortex in AD brains (Scott *et al.* 2002). Moreover, EAAT2-immunoreactive neurons exhibiting tau deposits were observed throughout the cortex, striatum, hypothalamus, and reticular formation in AD brain tissue (Thai 2002). Reduced expression of hippocampal EAAT1 and EAAT2 in AD has also been described, reinforcing the notion of a deficit in glutamate clearance in AD brains (Jacob *et al.* 2007).

In addition to uptake defects, abnormal release of glutamate from vesicle stores has been implicated as a source of excess extracellular glutamate in AD (Fujimoto *et al.* 2004). It was recently shown that AβOs instigate calcium-dependent pre-synaptic vesicular release and extracellular accumulation of glutamate in hippocampal neuronal cultures (Brito-Moreira *et al.* 2011), and this was accompanied by enhanced spontaneous post-synaptic activity. In a more recent study, Russell *et al.* (2012) demonstrated in hippocampal cultures that AβOs interact with synaptophysin in the presynaptic terminal, causing an increase in excitatory post-synaptic potential through vesicular neurotransmitter release. Taken together, these results highlight a potential role of AβOs in deregulated excitatory transmission (Parodi *et al.* 2010; Brito-Moreira *et al.* 2011).

Accumulation of glutamate causes overactivation of NMDARs and results in activation of calcium-dependent intracellular pathways impairing energy metabolism, generating highly reactive free radical species, oxidative stress, and culminating in cell death (Mattson 2007). One of the calcium-dependent enzymes activated in excitotoxicity is nitric oxide synthase, which produces nitric oxide. When combined to superoxide anion, nitric oxide forms peroxynitrite, a highly reactive species that triggers further oxidative and nitrosative stress in part via mitochondrial injury (Bossy-Wetzel *et al.* 2004). In this context, it is noteworthy that AβO-induced generation of reactive oxygen species (ROS) in primary hippocampal neurons is calcium-dependent, appears to be derived from mitochondrial sources and is initiated by activation of NMDARs (De Felice *et al.* 2007).

D-serine in excitotoxicity

Activation of NMDARs requires, in addition to glutamate, binding of a co-agonist (Shleper *et al.* 2005; see Fig. 1). The

co-agonist site was originally named glycine site because its first described ligand was glycine. However, recent findings have established that the D-amino acid D-serine is the main co-agonist at NMDARs (Shleper *et al.* 2005). D-serine is as potent as glycine in activating NMDARs, but its concentration is higher in frontal cortex (Matsui *et al.* 1995) and especially high levels of D-serine were detected in mouse hippocampus (Nagata *et al.* 1998). D-serine has been detected in pyramidal neurons of rat cerebral cortex, in microglia, astrocytes, and in retinal amacrine cells from different vertebrate species (Snyder and Kim 2000; Yasuda *et al.* 2001; Stevens *et al.* 2003; Williams *et al.* 2006). Significant amounts of D-serine were also detected in primary neuronal cultures (Kartvelishvily *et al.* 2006). Interestingly, reduced immunoreactivity for D-serine has been detected in cortex and hippocampi from aged animals (Williams *et al.* 2006). In the human brain, the highest levels of D-serine have been found in the hippocampus and corpus callosum (De Miranda *et al.* 2000).

Binding of D-serine enhances NMDAR function (Martina *et al.* 2003), promotes NMDAR recycling to the plasma membrane (Nong *et al.* 2003), and is important for synaptic plasticity and LTP in the hippocampus (Mothet *et al.* 2000; Henneberger *et al.* 2010). Decreased D-serine concentration attenuates NMDAR activation as measured by whole-cell patch-clamp and by determination of NMDAR-mediated calcium signals (Mothet *et al.* 2000). Interestingly, in transgenic mice carrying point mutations at the glycine-binding site, which reduce affinity for the co-agonist, recovery in NMDAR-mediated hippocampal LTP was observed by administration of D-serine (Ballard *et al.* 2002). Mice treated with D-serine presented improvements in tasks involving recognition, learning, and working memory, and these positive effects were blocked by MK-801, an NMDAR blocker (Bado *et al.* 2011). In line with these results, Papouin *et al.* (2012) published an interesting set of results demonstrating that, in rat hippocampal slices, D-serine specifically activates synaptic NMDARs, while glycine binds to extrasynaptic NMDARs. LTP and NMDA-induced neurotoxicity were dependent only on receptors present at synapses, but LTD required both synaptic and extrasynaptic receptors (Papouin *et al.* 2012).

Steinmetz *et al.* (2002) showed that D-serine potentiated glutamate-induced death in a fibroblast cell line expressing NMDAR subunits, the first piece of evidence linking D-serine to excitotoxicity. Interestingly, treatment of rat brain slices with D-serine acid oxidase, which degrades D-serine, prevented NMDA-induced excitotoxicity (Katsuki *et al.* 2004). Neuroprotection by D-serine acid oxidase was abrogated in the presence of excess D-serine. Results from other groups have further indicated decreased NMDAR-mediated excitotoxicity, upon degradation of endogenous D-serine in different experimental models (Shleper *et al.* 2005; Kartvelishvily *et al.* 2006). In line with these findings, the physiological glycine site antagonist kynurenic acid has been investigated as a potentially protective agent against excitotoxicity (Németh *et al.* 2005).

Recent studies indicate that D-serine may be involved in excitotoxic nerve cell damage in AD. Increases in serine racemase expression and D-serine levels were observed in microglial cultures treated with Aβ (Wu *et al.* 2004). We recently showed that neuronal cultures exposed to non-lethal doses of AβOs exhibit increases in extracellular levels of both glutamate and D-serine (Brito-Moreira *et al.* 2011). This increase in D-serine levels could be mediated by neuronal alanine-serine-cysteine transporter-1, recently implicated in D-serine release and modulation of LTP in neuronal cultures (Rosenberg *et al.* 2013). Collectively, these data reinforce the role of NMDAR activation in neuronal excitotoxic damage in AD.

Glutamate receptor antagonists as blockers of Aβ toxicity

Given the relevance of aberrant glutamatergic neurotransmission in AD, several studies performed since the 1990s have investigated the neuroprotective potential of ionotropic and/or metabotropic glutamate receptor antagonists against Aβ toxicity (e.g., Louzada *et al.* 2001).

Chin and coworkers reported that (RS)-alpha-methyl-4-carboxyphenylglycine (MCPG), a non-selective group I/II mGluR antagonist, prevented the reduction induced by Aβ in miniature excitatory post-synaptic currents in rat forebrain slices (Chin *et al.* 2007). Similarly, LTP was rescued when AβO-exposed murine hippocampal slices were pre-treated with the mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (Rammes *et al.* 2011). Interestingly, MCPG and SIB1757 (an mGluR5 antagonist) also prevented LTD facilitation in rat hippocampal slices induced by AβOs extracted from AD brains (Shankar *et al.* 2008). Moreover, AβOs induce clustering of mGluR5 in hippocampal cultures, which increases intracellular calcium levels and triggers synapse deterioration (Renner *et al.* 2010). However, cultures pretreated with SIB1757 or prepared from mGluR5 knockout mice exhibited marked reductions in these neurotoxic effects (Renner *et al.* 2010).

Several lines of evidence have also suggested blockade of ionotropic glutamate receptors as a possible neuroprotective approach in AD. For example, exposure of retinal cell cultures to Aβ resulted in excitotoxic cell death, which was blocked by an AMPAR and kainate receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione, and by MK-801, an NMDAR blocker (Louzada *et al.* 2001, 2004). Similarly, neuronal cell lines exposed to Aβ showed increased calcium influx, which was normalized by pre-treatment with an AMPAR antagonist (Blanchard *et al.* 2004). Even the neurotoxicity induced by a mixture of Aβ and kainate injected in rat hippocampi could be prevented when kainate receptors were previously blocked by

6-cyano-7-nitroquinoxaline-2,3-dione (Morimoto and Oda 2003). NMDAR blockade by MK-801 also protected neurons exposed to Aβ in rat magnocellular nucleus basalis (Harkany *et al.* 2000) and hippocampus (Nakamura *et al.* 2006). Interestingly, we have found that ROS formation induced by AβOs in hippocampal neurons is prevented when cultures are pre-treated with (2R)-amino-5-phosphonovaleric acid, an NMDAR antagonist, or with memantine, a moderate affinity blocker of NMDARs (De Felice *et al.* 2007).

Memantine is a well-tolerated drug shown to block excitotoxic cell death (Lipton 2006) and approved for treatment of moderate to severe AD by the European Union, the United States Food and Drug Administration (FDA) and regulatory agencies of several countries. Initial studies revealed that memantine showed clinical efficacy (Lipton 2006). Clerici *et al.* showed that patients taking 20 mg memantine daily during the study period had a statistically significant higher probability to experience behavioral improvement than those who discontinued treatment or did not complete memantine titration (Clerici *et al.* 2012). In another pilot study, agitation and aggressive behavior were found to be lower in institutionalized patients with moderate to severe AD following treatment with memantine (Herrmann *et al.* 2011). Patients with moderate to severe AD receiving once-daily memantine (20 mg) treatment significantly improved cognition and functional communication (Schulz *et al.* 2011). Many other recent studies have pointed to positive effects of memantine treatment (Winblad *et al.* 2007; Emre *et al.* 2008). However, a recent multicenter study showed that combination therapy of memantine plus rivastigmine (an acetylcholinesterase inhibitor) did not show any advantage over rivastigmine patch monotherapy on efficacy analyses (Choi *et al.* 2011). In line with the latter study, another multicenter study reported lack of benefit of memantine in mild AD, and meager evidence for its efficacy in moderate AD (Schneider *et al.* 2011). There are two possible explanations for these contradictory results: (i) synaptic dysfunction in AD is, in part, caused by the internalization of NMDARs, and/or (ii) receptors/membrane proteins other than NMDARs are involved in AβO-induced synaptotoxicity (Fig. 1). In conclusion, the efficacy of memantine in the treatment of AD remains a controversial issue, and there are now few hopes that it might constitute a disease-modifying or curative drug.

Neuronal hyperpolarization as a therapeutic approach

The lack of effective treatments underscores an urgent need to develop alternative therapeutic approaches in AD. It has been suggested that, along with aberrant glutamatergic signaling, AD is accompanied by a dysfunction in GABA signaling (Lanctôt *et al.* 2004; Rissman and Mobley 2011). As GABA is the major inhibitory neurotransmitter in the CNS, this could lead to an excitatory/inhibitory imbalance in the AD brain.

Multiple lines of evidence indicate disruption in GABAergic signaling in AD, including results from post-mortem analysis, neuroimaging and studies using GABA markers in the CNS. As pointed out by Lanctôt and coworkers, post-mortem studies comprise the bulk of evidence of GABAergic dysfunction in AD. Measurements of GABA concentrations and benzodiazepine binding in AD brain converge to show reduced frontal, temporal and parietal GABA concentrations (Lanctôt *et al.* 2004), with the temporal lobe as the most affected region.

Three different membrane receptors are activated by GABA in the mammalian brain, namely $GABA_A$, $GABA_B$, and $GABA_C$ receptors (Korpi *et al.* 2002). The $GABA_A$ receptor, of particular interest to the current discussion, is a neurotransmitter-gated ion channel of the Cys-loop family, which permits the influx of chloride when activated. $GABA_A$Rs are crucially involved in synaptic plasticity (Collingridge *et al.* 2004). For example, they help to ensure that NMDARs are activated mainly during high-frequency synaptic transmission and not by low-frequency spontaneous synaptic activity (Collingridge *et al.* 2004).

GABA is physiologically released by neurons in response to glutamate excitotoxicty (Saransaari and Oja 1997; Vignes 2001), suggesting that it may take part in neuronal defense mechanisms to compensate for glutamatergic overactivation. Different classes of GABA receptor agonists have been shown to decrease neuronal vulnerability to excitotoxic damage in several models (Lapchak *et al.* 2000; Schwartz-Bloom *et al.* 2000; Velasco and Tapia 2002; Pisani *et al.* 2006; Zhang *et al.* 2007).

Network excitability is decreased by activation of $GABA_A$ receptors by muscimol (Glykys and Mody 2006). That study is consistent with an important role of tonic inhibition in the control of hippocampal network excitability, and highlights selective enhancers of tonic inhibition as promising therapeutic approaches for diseases involving network hyperexcitability. Furthermore, a large increase in $GABA_A$ receptor levels has been shown to occur following an excitotoxic septal lesion, resulting in decreased glutamate neurotransmission and suggesting that *in vivo* coordinated interactions between inhibitory systems control glutamate-mediated hippocampal excitability (Rodríguez *et al.* 2005). In the AD context, we have shown that $GABA_A$ receptor agonists protect neurons in culture against the neurotoxicity of Aβ and of various glutamate receptor agonists (Paula-Lima *et al.* 2003, 2005; Louzada *et al.* 2004). It is, thus, tempting to propose that stimulation of GABAergic signaling could represent an effective alternative therapeutic approach in AD.

Stimulating GABAergic transmission has indeed been attempted to treat AD (Lanctôt *et al.* 2004; Möhler 2011). Most of the interventions using GABAergic agents aimed to manage behavioral and psychological dysfunctions

associated with dementia, including aggression and agitation. In this regard, benzodiazepines have demonstrated efficacy, but they are limited by tolerance after long-term use and other side effects such as sedation, dizziness, and ataxia (Wallace 2001; Lanctôt *et al.* 2004). Antiepileptic drugs such as vigabatrin, topiramate, and THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) offer novel possibilities to interrupt or retard AD progression in light of their neuroprotective actions against excitotoxicity (Kristensen *et al.* 2003; Eleuteri *et al.* 2009). Interestingly, a recent study demonstrated that Aβ–induced aberrant network activity contributes to synaptic and cognitive deficits, and showed that treatment of AD mouse models with levetiracetam, an antiepileptic drug, reduced abnormal spike activity detected by electroencephalography, reversed hippocampal remodeling, behavioral abnormalities, synaptic dysfunction, and deficits in learning and memory (Sanchez *et al.* 2012).

Consistent with the idea that abnormal network activity is crucial to synaptic and cognitive deficits in AD, endogenous molecules such as melatonin and taurine were found to attenuate or block Aβ-induced excitotoxicity *in vitro* by mechanisms related to $GABA_A$ receptor activation (Louzada *et al.* 2004; Paula-Lima *et al.* 2005). Indeed, Brito-Moreira *et al.* (2011) have recently demonstrated that pre-treatment of neurons with taurine, as well as with the NMDAR blocker MK-801, prevents the release of glutamate induced by non-lethal concentrations of AβOs in primary hippocampal neurons. Collectively, results suggest that the beneficial effects of $GABA_A$ receptor activation may occur at a stage previous to neuronal death.

In particular, taurine (2-aminoethanesulfonic acid) exhibits an interesting profile to prevent AβOs neurotoxicity. Taurine is a naturally occurring β-amino acid that plays important roles in neuronal development and survival in the mammalian CNS (Huxtable 1992). It may induce an increase in Cl^- influx through $GABA_A$ receptors (Saransaari & Oja, 2007) and, thus, counteract neuronal depolarization associated with Aβ-induced excitotoxicity. Interestingly, impairments in taurinergic tonus have been implicated in several pathological conditions, including loss of taurine from epileptic foci in humans (Perry and Hansen 1981) and a higher susceptibility of taurine-deficient rats to seizures (Pasantes-Morales *et al.* 1987). Low levels of taurine have been reported in the brains of AD patients (e.g., Csernansky *et al.* 1996). It is noteworthy that the effectiveness of GABA and taurine as endogenous neuroprotective agents may decline with age. The aging hippocampus seems to lack the capacity to respond to ischemic insults by releasing taurine (Saransaari and Oja 1997). Therefore, it can be hypothesized that age-dependent declines in GABA and taurine signaling may account for a progressive loss of CNS inhibitory tonus and, consequently, to increased excitability and neuronal vulnerability to the toxicity of AβOs in AD.

Conclusion

Several lines of evidence indicate that Aβ, both as amyloid fibrils and as soluble oligomers, increases glutamate and D-serine concentrations at the synaptic cleft, thus aberrantly activating calcium influx through NMDARs and causing a subsequent decrease in surface expression of NMDARs and AMPARs. This results in a vicious cycle in which Aβ induces an increase in extracellular glutamate concentration, by inhibiting glutamate transporters and by stimulating vesicular glutamate release; in turn, increased synaptic glutamate levels cause aberrant activation of NMDARs, which increases glutamate release and Aβ production. From a therapeutic point of view, a strong body of evidence suggests that increasing neural inhibition by selective activation of $GABA_A$Rs could prevent neuronal depolarization, NMDAR activation and abnormal calcium signaling induced by Aβ, without compromising activity-dependent glutamatergic signaling. In particular, results suggest that taurine, an endogenous $GABA_A$ receptor agonist with a favorable pharmacological profile, should be investigated for efficacy in clinical treatment of AD.

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