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REVIEW

Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine – searching for the connections

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β-amyloid (Aβ) is widely accepted to be one of the major pathomechanisms underlying Alzheimer's disease (AD), although there is presently lively debate regarding the relative roles of particular species/forms of this peptide. Most recent evidence indicates that soluble oligomers rather than plaques are the major cause of synaptic dysfunction and ultimately neurodegeneration. Soluble oligomeric Aβ has been shown to interact with several proteins, for example glutamatergic receptors of the NMDA type and proteins responsible for maintaining glutamate homeostasis such as uptake and release. As NMDA receptors are critically involved in neuronal plasticity including learning and memory, we felt that it would be valuable to provide an up to date review of the evidence connecting Aβ to these receptors and related neuronal plasticity. Strong support for the clinical relevance of such interactions is provided by the NMDA receptor antagonist memantine. This substance is the only NMDA receptor antagonist used clinically in the treatment of AD and therefore offers an excellent tool to facilitate translational extrapolations from *in vitro* studies through *in vivo* animal experiments to its ultimate clinical utility.

Abbreviations

AA, arachidonic acid; Aβ, β-amyloid; AChEI, AChEI inhibitor; AD, Alzheimer's disease; ADDLs, Aβ-derived diffusible ligands; AMPK, AMP-activated protein kinase; APP, amyloid precursor protein; CA1, *Cornu ammonis* area 1; CamKII, Ca^{2+}/calmodulin-dependent protein kinases II; CaMKK β, calmodulin-dependent protein kinase-β; CDK5, cell division protein kinase 5; CPP, 3-[(*R*)-2-carboxypiperazin-4-yl]-prop-2-enyl-1-phosphonic acid; CREB, cAMP response element-binding; D-APV, D-AP5, D-amino-phospovaleric acid; DG, dentate gyrus; EAAT2, excitatory amino acid transporter 2; FB, Aβ; fibril; GFAP, glial fibrillary acidic protein; GLAST, glial glutamate transporter; GLT-1, glutamate transporter 1; GSK-3β, glycogen synthase kinase 3β; HNE, 4-hydroxy-2-nonenal; iNOS, inducible NOS; IR, insulin receptor; KPI, Kunitz protease inhibitory domain; KPI-APPs, KPI containing APPs; LTD, long-term depression; LTP, long-term potentiation; mGluR5, metabotropic glutamate receptor 5; MoA, mechanism of action; MWM, Morris water maze; NBM, nucleus basalis of Meynert; NFTs, neurofibrillary tangles; NR2B, NMDA receptor subunit; p-, phosphorylated; PF, protofibril; PFC, prefrontal cortex; PLA, phospholipase A; PMA, phorbol myristate acetate; PP2B, protein phosphatase 2B; PS, population spike; PS1, presenilin 1; PSD-95, post-synaptic anchoring protein 95; PTP, post-tetanic potentiation; ROS, reactive oxygen species; sAPP, soluble amyloid precursor protein; ATPase Na^+/K^+, sodium-potassium adenosine triphosphatase pump; STEP, striatal enriched tyrosine phosphatase; TBOA, threo-β-benzyloxyaspartic acid; TG, transgenic; VgluT, vesicular glutamate transporter

Pathophysiology of Alzheimer's disease

The pathophysiology of Alzheimer's disease (AD) is characterized by chronic, progressive neurodegeneration. The precise aetiology of AD is still not fully clarified but is known to be complex and multifactorial, with a notable overlap between familial and non-familial forms but also with different forms of dementia such as vascular dementia. The neurodegeneration seen in AD involves early synaptotoxicity and loss of neurophil, neurotransmitter disturbances,

accumulation of extracellular β-amyloid (Aβ) deposits (amyloid/senile plaques) and intracellular neurofibrils (neurofibrillary tangles, NFTs), gliosis and only at later stages overt loss of neurons and associated brain atrophy (Yankner, 1996; Heininger, 1999; Bell and Claudio Cuello, 2006; Citron, 2010). At early stages of the disease, the entorhinal cortex and hippocampus are particularly affected, and this is associated with deficits in cognition/memory (Braak *et al.*, 1993). Over the course of AD, up to 80% of neurons in the hippocampus die, and the progressive symptoms of AD manifest themselves as cognitive disturbances, reduced ability to cope with everyday life and worsening of clinical global impression score (Morris, 1986).

Aβ

As described by Alois Alzheimer himself (Alzheimer, 1907), one of the key histopathological hallmarks of the AD brain is the presence of extracellular 'amyloid/senile plaques' around neurons and glia. Such amyloid plaques are insoluble, quasi-crystalline deposits (Lesne *et al.*, 2006), the main component of which is Aβ – a peptide (most commonly 40–42 amino acids in length) that is formed by enzymatic cleavage of the transmembrane amyloid precursor protein (APP) (Hardy and Higgins, 1992; Citron, 2010). Due to its neurotoxic effects and accumulation in AD, Aβ is believed to be a crucial pathogenic factor in disease development, both in familial and non-familial forms. Aβ is produced by the enzymatic cleavage of APP by β-secretase (extracellular cleavage) and γ-secretase (cuts in the middle of the membrane), whereas cleavage by α-secretase precludes formation of Aβ. The 42-amino-acid form, $Aβ_{1–42}$, has a higher tendency to aggregate than $Aβ_{1–40}$ and has been ascribed to be the main pathogenic form of this peptide (Citron, 2010) – but see also (Schlenzig *et al.*, 2009). Aβ is continually released from neurons and glial cells into the extracellular environment where, at very low nM concentrations and possibly in monomeric form it may also play a physiological role (Puzzo *et al.*, 2008).

Soluble Aβ oligomers

More recent evidence indicates that soluble oligomeric forms of Aβ, rather than the insoluble deposits, are primarily responsible for both the neurodegeneration and especially the impairment of synaptic function in AD (Barghorn *et al.*, 2005; Ferreira *et al.*, 2007; 2011; Lacor *et al.*, 2007; Parsons *et al.*, 2007; Demuro *et al.*, 2010; Xia, 2010; Ferreira and Klein, 2011; Wilcox *et al.*, 2011). For example, $Aβ_{1–42}$ and Aβ oligomers were recently reported to be dramatically increased in the soluble fraction of Alzheimer's disease brain extracts, with oligomer levels 20-fold higher in aqueous compared with detergent extracts. Multiple oligomeric forms, including small oligomers, 56 and 200 kDa assemblies were found and proposed by the authors to contribute to synaptic dysfunction (Sokolow *et al.*, 2011). However, contradictory findings have also been reported by others (e.g. van Helmond *et al.*, 2010).

APP transgenic (TG) mice expressing the E693Delta mutation, which is reported to cause AD by enhanced Aβ oligomerization without fibrillization, displayed age-dependent accumulation of intraneuronal Aβ oligomers starting at 8 months but no extracellular amyloid deposits even at 24 months (Tomiyama *et al.*, 2010). These mice indeed already showed deficits in synaptic plasticity, learning, synaptic markers, microglial activation and tau phosphorylation at 8 months, indicating that they might be a useful model of Aβ oligomer-induced pathology in the absence of amyloid plaques (Tomiyama *et al.*, 2010). Soluble Aβ associated with (Q22, Dutch) or (G22, Arctic) mutant APP peptides was approximately 100-fold more potent than wild-type Aβ in inhibiting long-term potentiation (LTP) (Klyubin *et al.*, 2004).

These soluble Aβ oligomers are thought to promote disturbances in glutamatergic neurotransmission and also increase the phosphorylation of tau (De Felice *et al.*, 2007b). For example, chronic treatment with nanomolar concentration of Aβ oligomers was recently reported to induce NMDA receptor-dependent inward calcium ion (Ca^{2+}) currents, mitochondrial Ca^{2+} overload/membrane depolarization, oxidative stress and apoptotic cell death in primary dissociated entorhinal cortex/hippocampal organotypic cultures (Alberdi *et al.*, 2010; Bieschke *et al.*, 2011).

Aβ oligomers are now believed to impair neuronal function and cognition, even before the appearance of overt toxicity (Lesne *et al.*, 2006). However, the exact pathogenic role of deposits versus soluble forms and, in the latter case especially the major oligomeric species of Aβ involved (e.g. dimer, trimer or dodecamer), is still controversial (Barghorn *et al.*, 2005; Selkoe, 2008; Bao *et al.*, 2011). In contrast to soluble oligomeric forms of Aβ, this peptide in its soluble monomeric form has recently even been ascribed a physiological function and can enhance LTP at low pM concentrations (Puzzo *et al.*, 2008), increase synaptic release probability (Abramov *et al.*, 2009) and even protect against excitotoxic insults (Giuffrida *et al.*, 2009).

It is not the intention of the present review to deepen this discussion; rather, we took as our starting point the present widely supported hypothesis that the soluble oligomeric forms of Aβ are primarily responsible for Aβ pathology and, what is perhaps more pertinent to this review, dysfunction of synaptic plasticity, which is expressed in patients as cognitive deficits.

Toxicity

Most early studies used high concentrations of synthetic Aβ (e.g. $Aβ_{1–40}$, $Aβ_{1–42}$ or even the truncated toxic fragment $Aβ_{25–35}$) for studies on toxic effects. For example, $Aβ_{1–42}$ (40 μM) enhanced glutamate neurotoxicity in human cerebral cortical cell cultures, whilst scrambled peptide was without effect (Mattson *et al.*, 1992). $Aβ_{1–42}$ was ineffective when applied alone; the insult required prolonged incubation for 4 days to develop and reflected general compromised ability of the neurons to handle elevated intracellular calcium levels following various glutamate agonists and even a calcium ionophore. All effects were associated with changes in intracellular Ca^{2+} levels (Mattson *et al.*, 1992). Similarly, the selective NMDA receptor antagonist D-amino-phosponovaleric acid (D-APV, D-AP5) completely blocked $Aβ_{1–42}$ (15–30 μM) uptake/internalization and subsequent up-regulation of cathepsin D and activation of microglia in organotypic hippocampal cultures (Bi *et al.*, 2002).

However, more recently, it has been accepted by many working in the field that only much lower concentrations of Aβ are really relevant for chronic toxic effects in AD. For

example, oligomeric $A\beta_{1-42}$ (1 μM) induced reactive oxygen species (ROS) production from cultured cortical neurons through activation of NADPH oxidase. ROS derived from NADPH oxidase led to activation of ERK1/2, phosphorylation of cytosolic phospholipase A(2)α [cPLA(2)α] and arachidonic acid (AA) release (Shelat *et al.*, 2008). The involvement of NMDA receptors in mediating these effects was shown by their reversibility by D-APV (10 μM) and memantine (5 μM) (Shelat *et al.*, 2008).

In mixed neuronal-glial cultures from rat cerebellum, 250 nM $A\beta_{1-42}$ induced about 30% loss of neurons and synapses after 2 to 3 days of treatment, whereas reverse peptide had no effect. There was no signal for overt apoptosis or necrosis, but $A\beta_{1-42}$ rather increased the phagocytic capacity of microglia (Neniskyte *et al.*, 2011).

Pathophysiologically relevant, even lower concentrations (pM) of naturally secreted Aβ oligomers (dimers and trimers, but not monomers) extracted directly from the cerebral cortex of subjects with AD produced a progressive loss of hippocampal synapses in organotypic hippocampal cultures (Shankar *et al.*, 2007). Since this was prevented by the competitive NMDA receptor antagonist 3-[(*R*)-2-carboxypiperazin-4-yl]-prop-2-enyl-1-phosphonic acid (CPP, 20 μM) and associated with decreased Ca^{2+} influx, the involvement of NMDA receptors was postulated (Shankar *et al.*, 2007).

Neurofibrillary tangles

Another characteristic histopathological feature of AD is the deposition of NFTs within neurons (Alzheimer, 1907; Braak *et al.*, 1994). These abnormal protein bundles consist of flame-shaped, helical deposits of hyperphosphorylated tau protein. Under normal physiological conditions, phosphorylation of the tau protein (at five epitopes) helps to maintain cytoskeletal structure. The balance of phosphorylated and unphosphorylated tau regulates the stability of microtubules in the cytoskeleton, which act as an intracellular transport system and maintain the axoplasmic flow (Goedert, 1993; Goedert *et al.*, 2006). In AD, there is probably an imbalance between phosphorylating protein kinases and dephosphorylating protein phosphatases, leading to excessive tau phosphorylation (at up to 21 epitopes), microtubule instability and, consequently, cell death (Noble *et al.*, 2003; Goedert *et al.*, 2006). Glycogen synthase kinase 3β (GSK-3β) and cell division protein kinase 5 (CDK5) seem to play pivotal roles in this hyperphosphorylation (Gong and Iqbal, 2008). The hyperphosphorylated tau protein accumulates inside the cell, dimerizing to paired helical filaments, which aggregate to form the typical NFTs seen in AD. The role of tau will not be addressed further, but readers are referred to recent reviews (Churcher, 2006; Goedert *et al.*, 2006).

The glutamatergic neurotransmitter system

Glutamate is the major fast excitatory neurotransmitter and is involved in almost all CNS functions, especially in cortical and hippocampal regions – 70% of all excitatory synapses in the CNS utilize glutamate as a neurotransmitter (Watkins and Evans, 1981; Danysz *et al.*, 1995; Parsons *et al.*, 1998; 2002). Ionotropic glutamate receptors are ligand-gated ionic channels permeable to the monovalent cations Na^+ and K^+ and, depending on the subtype, also to the divalent cation Ca^{2+}. AMPA receptors show very fast activation/inactivation kinetics, are largely postsynaptic, impermeable to Ca^{2+} and participate in most forms of fast excitatory synaptic neurotransmission (Watkins and Evans, 1981; Shinozaki, 1988; Parsons *et al.*, 2002). In contrast, NMDA receptors are normally only synaptically activated under certain physiological conditions, for example during the induction of synaptic plasticity (Cotman *et al.*, 1988; Collingridge and Singer, 1990).

Synaptic plasticity

The hippocampus, with its high density of glutamate receptors and in particular NMDA receptors, is known to be extremely important for some forms of learning and memory. Glutamatergic synapses can show pronounced plasticity in terms of the number and strength of individual synapses and are also characterized by their ability to express LTP – a long-lasting strengthening of synaptic transmission (Cotman *et al.*, 1988; Collingridge and Singer, 1990). This remodelling at the cellular and molecular level is widely accepted to be an underlying synaptic mechanism for learning and memory (Collingridge and Singer, 1990; Butterfield and Pocernich, 2003). Signal cascades triggered by the activation of postsynaptic NMDA receptors are fundamentally important for LTP induction and, thereby, for neuronal plasticity.

The NMDA receptor has three cardinal features that permit its 'co-incidence' detector function in Hebbian synaptic plasticity: high permeability to Ca^{2+} ions, voltage-dependent block by magnesium ions (Mg^{2+}) and relatively slow ligand gated kinetics. The resting membrane potential of a healthy neuron is normally around –70 mV, and the Ca^{2+} channel of the NMDA receptor is blocked by Mg^{2+} ions. As a consequence, normal resting conditions are associated with a low background level of postsynaptic intracellular Ca^{2+}. Even during normal fast excitatory glutamatergic neurotransmission, postsynaptic intracellular Ca^{2+} levels remain low due to the above discussed biophysical properties of NMDA receptors. Only during, for example the induction of LTP does the stronger/more prolonged pulsatile glutamate release and a more pronounced influx of Na^+ ions into the postsynaptic neuron via AMPA receptors decrease membrane potential for long enough to remove the block of the NMDA receptor channel by Mg^{2+} at which stage, Ca^{2+} ions can freely enter the cell via the NMDA receptor channel and trigger a cascade of second messenger processes that are involved in the fixation of increased synaptic strength.

At this juncture, one should emphasize the crucial physiological role of endogenous Mg^{2+} ions in this process which function as a switch to keep NMDA receptors blocked under resting or normal fast synaptic transmission conditions but allow Ca^{2+} ion influx when the pattern of activation has features characteristic for those required for learning processes, that is temporal and spatial convergence (cooperativity). This transient influx of Ca^{2+} is clearly distinguished

against the low levels of background Ca^{2+} noise and, through downstream second-messenger processes, leads to detection of the neuronal plasticity/'learning' signal.

Glutamate excitotoxicity

Consistent with the involvement of the glutamatergic system in learning and memory, disturbances in glutamate neurotransmission have been linked with the pathophysiological processes underlying AD (Hardy and Cowburn, 1987; Greenamyre and Young, 1989; Palmer and Gershon, 1990; Cacabelos *et al.*, 1999; Francis, 2003; Wenk *et al.*, 2006). Chronic, mild activation of NMDA receptors ultimately leads to neurodegeneration – an effect termed chronic 'excitotoxicity' (Greenamyre and Young, 1989; Mattson *et al.*, 1989; Braak *et al.*, 1994; Dodd *et al.*, 1994; Holscher, 1998; Butterfield and Pocernich, 2003). Notably, in this regard, the Mg^{2+} blockade of the NMDA receptor channel can be lifted by even moderate depolarization of the cell plasma membrane as well as by other factors discussed below. This triggers the pathological influx of Ca^{2+} ions into postsynaptic neurons. The prolonged Ca^{2+} overload leads first to loss of synaptic function, followed by synaptotoxicity and ultimately cell death, which correlates with the loss of memory function and learning ability in AD patients (Parsons *et al.*, 1998; Danysz and Parsons, 2003; Miguel-Hidalgo *et al.*, 2003; Wenk *et al.*, 2006).

Factors that can influence the sensitivity of the glutamatergic system

Various pathologies such as the deposition of Aβ in plaques, soluble Aβ oligomers, hyperphosphorylated tau protein in NFTs, oxidative stress, mitochondrial dysfunction, energy deficits, chronically elevated concentrations of glutamate and neuronal inflammation have been associated with increased sensitivity and/or activity of the glutamatergic system, resulting in neuronal dysfunction and cell death in AD (Gray and Patel, 1995; Mattson *et al.*, 1999; Wenk, 2006; Wenk *et al.*, 2006; De Felice *et al.*, 2007a; Parihar and Brewer, 2007; Gasparini and Dityatev, 2008; Parameshwaran *et al.*, 2008).

Resting levels of glutamatergic agonists

The numerous factors that can influence the levels of endogenous glutamate receptor agonists and their downstream effects are presented in a schematic way in Figure 1. Some of these factors are outside of the scope of the present review, but the reader is recommended to refer to one of the following overviews for more information (Greenamyre and Young, 1989; Maragakis and Rothstein, 2001; Butterfield and Pocernich, 2003; Francis, 2003; Wenk *et al.*, 2006; Jacob *et al.*, 2007; Parsons *et al.*, 2007; Bojarski *et al.*, 2008).

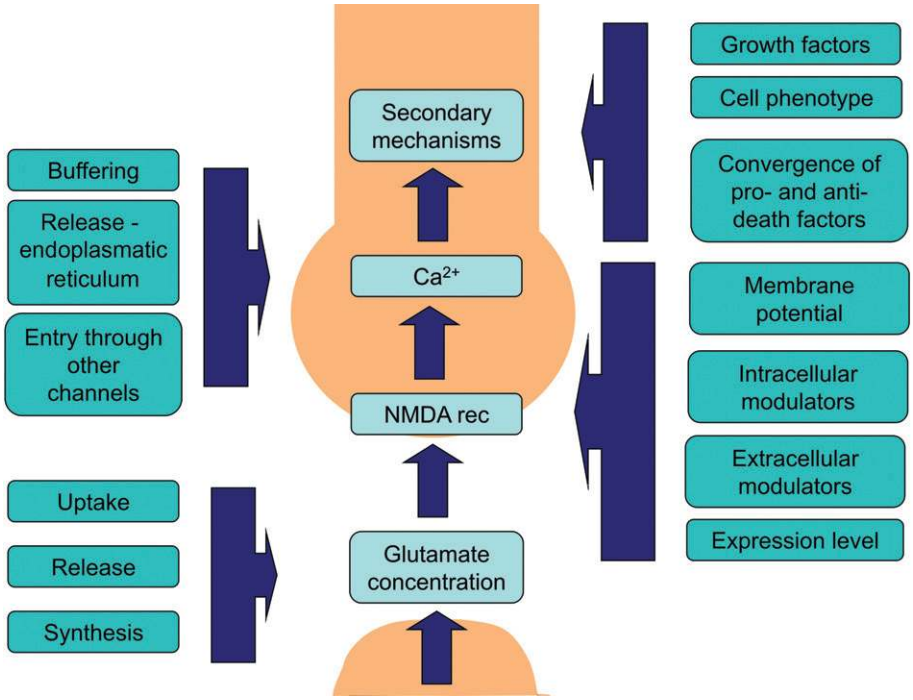

Figure 1

Schematic illustrating the factors directly involved in normal physiological NMDA receptor-mediated synaptic transmission/plasticity and associated processes/factors that can modulate such NMDA receptor activation/transmission both under physiological, but more importantly also under disturbed pathological conditions. For simplification, the roles of other receptors (e.g. AMPA) and feedback inhibition in synaptic plasticity have been omitted from this cartoon (see also Figure 2A). The points where such secondary factors interact with this signalling cascade are indicated by the vertical blue boxes with associated arrows pointing to the light blue boxes.

Resting glutamate concentrations under physiological conditions are normally in the low micromolar range. Only during synaptic transmission do these levels transiently reach mM concentrations for a few milliseconds (Clements *et al.*, 1992). These low background levels of extracellular glutamate are normally regulated following transient physiological synaptic glutamate release by tightly controlled, very efficient re-uptake processes and intracellular metabolism to glutamine by glutamine synthetase in, for example, glial cells that serves to recycle glutamate released at these synapses. Glutamine passively diffuses to the presynaptic button where it is recycled into glutamate by glutaminase (Fagg *et al.*, 1986; Danbolt, 2001).

Glutamate uptake/recycling mechanisms can be severely impaired in AD due to deficits in glutamate transporter expression. For example, glutamate transporter capacity (B_{max}, K_d) and protein expression is reduced in the frontal/temporal cortex of AD patients, and there is also a selective loss of the vesicular glutamate transporter (VGluT) (Masliah *et al.*, 1996; 2000; Li *et al.*, 1997; Kirvell *et al.*, 2006). It has also recently been reported that the excitatory amino acid transporter 2 (EAAT2), which is concentrated in perisynaptic astrocytes, also undergoes disease- and pathology-specific changes, with relatively greater expression of splice variants with reduced function in AD (Scott *et al.*, 2010).

Glutamate is not the only endogenous agonist for NMDA receptors, and the levels of other endogenous agonists have been reported to be tonically elevated in AD. One clear example is homocysteic acid. This, probable non-neurotransmitter, endogenous amino acid is an agonist at both NMDA and metabotropic glutamate receptor 5 (mGluR5) receptors, and its levels have been reported to be chronically elevated in AD due to deficits in folic acid metabolism (Bleich *et al.*, 2003).

NMDA receptor sensitivity

One prominent feature of NMDA receptors is their ability to be directly modulated by numerous endogenous factors. Probably, the most physiologically relevant are the co-agonists glycine/D-serine, the continuous (i.e. non-synaptically released) presence of which is an absolute prerequisite for NMDA receptor activation by glutamate (Kleckner and Dingledine, 1988; Danysz and Parsons, 1998). D-serine is the predominant endogenous co-agonist of the NMDA receptor in the forebrain and may be involved in controlling the extent of NMDA receptor-mediated neurotoxic insults observed in CNS disorders, including AD (Danysz and Parsons, 1998). Serine racemase knockout mice showed an approximately 90% decrease in forebrain D-serine content and reduced neurotoxicity induced by both NMDA and $A\beta_{1-42}$ injections into the forebrain *in vivo* (Inoue *et al.*, 2008). Conditioned medium from $A\beta_{1-42}$ (15 μM) treated microglia also contained elevated levels of D-serine. This conditioned media was toxic to cultured hippocampal neurons, an effect that could be blocked by the NMDA receptor glycine site antagonist 5,7-dicholorokynurenic acid and by enzymatic degradation of D-amino acids by D-amino acid oxidase (Wu *et al.*, 2004). Serine racemase mRNA levels were reported to be elevated in both these microglia cultures as well as in AD hippocampus (Wu *et al.*, 2004). In contrast, others have reported that $A\beta_{25-35}$ (3 nmol i.c.v.) – induced learning deficits in spontaneous alternation and step-down passive avoidance were reversed by the NMDA glycine site partial agonist D-cycloserine (1–30 $mg \cdot kg^{-1}$ i.p.) and the glycine prodrug milacemide (3–100 $mg \cdot kg^{-1}$ i.p.) (Maurice *et al.*, 1996).

However, other factors have a somewhat more subtle but still very important influence on NMDA receptor function. Some examples would be endogenous polyamines like spermine (Williams, 1997), which have multiple effects on NMDA receptors, the most important of which is their ability to positively modulate NMDA receptors containing the NR2B subunit. Other important factors are free radicals, redox potential, inflammation, pH, etc., which clearly change during pathological processes such as those occurring in AD (Wenk *et al.*, 2006).

The sensitivity of NMDA receptors to detect physiological/pathological signals does not just depend on the presence of agonists/modulators. One obviously important factor is their expression level. In general, the overall expression level of NMDA receptors in AD is reduced rather than increased, but this probably reflects compensatory reactions of the biological system in an attempt to compensate the pathological changes (i.e. receptor down-regulation as an adaptive change), as well as blatant loss of neuronal cells and synapses expressing these receptors as a long term consequence of chronic excitotoxicity (Geddes *et al.*, 1986; Procter *et al.*, 1989; Ninomiya *et al.*, 1991; Hynd *et al.*, 2001).

The synaptic/non-synaptic distribution of NMDA receptors is also of paramount importance in determining which receptors are available for physiological activation and which might rather be available for excitotoxic processes. In this regard, also the subunit composition is of importance as, for example, NR2B subunit containing receptors have been deemed by some to be extra synaptic 'death' receptors (Hardingham *et al.*, 2002; Bordji *et al.*, 2011). The postsynaptic localization and thereby the ability of NMDA receptors to be activated by physiologically, synaptically released glutamate also depends on their association with postsynaptic anchoring proteins such as PSD-95. The phosphorylation status of NMDA receptors can also influence their sensitivity to both activation by agonists (Zheng *et al.*, 1997) and modulation by the endogenous channel blocker Mg^{2+} (Chen and Huang, 1992) (Figure 1).

Perhaps one of the most important factors, however is the resting membrane potential. The NMDA receptor is almost unique in its combined ligand and voltage-gating properties (Nowak *et al.*, 1984) (i.e. physiological sensitivity to synchronise transient changes in both neurotransmitter concentration and membrane potential). Precisely, these properties render NMDA receptors their ability to act as coincidence detectors, essential for their role in synaptic plasticity (Cotman *et al.*, 1988; Collingridge and Singer, 1990). The caveat is that this voltage dependency can be a burden in chronic disease states such as those occurring in AD. Factors that disturb the normal resting membrane potential of neurons can have severe impact on the normal function of NMDA receptors as these can lead to a tonic relief of their voltage-dependent modulation by Mg^{2+}.

Specific issues to be addressed in this review

The ultimate goal of this review is to try to address the following questions:

1) How does Aβ affect homeostasis of the glutamatergic system?
2) How does Aβ impact directly on NMDA receptor function?
3) What is the resulting effect of Aβ on synaptic function/plasticity, partially independent from overt toxicity?
4) What is the evidence that NMDA receptor antagonists like memantine may reverse/prevent these negative effects of Aβ?

How does Aβ affect homeostasis of the glutamatergic system (Table 1)?

Aβ influences glutamate concentrations in the synaptic cleft

Most studies on the effects of Aβ on glutamate transport mechanisms have been performed with toxic sub fragments of Aβ. Studies in hippocampal slices indicate that glutamate uptake is impaired in the aged hippocampus, and that $A\beta_{25-35}$ augments the release and/or inhibits the uptake of glutamate and aspartate, especially in aged animals (Arias *et al.*, 1995; ParpuraGill *et al.*, 1997). $A\beta_{25-35}$ (0.25–15 μM) applied for 30 min to cultured neurons and astrocytes increased glutamate levels in media by decreasing glutamate uptake (Harris *et al.*, 1996; Fernandez-Tome *et al.*, 2004). These effects were associated with overt toxicity, but surviving neurons showed enhanced uptake of glutamate, possibly as a reactive protective mechanism (Fernandez-Tome *et al.*, 2004).

A possible mechanism for this effect is the fact that glutamate transporters are inhibited by oxidative damage from ROS and lipid peroxidation products such as 4-hydroxy-2-nonenal (HNE). $A\beta_{1-42}$ has been reported to increase HNE conjugation to the glutamate transporter resulting in inhibition of glutamate uptake (Lauderback *et al.*, 2001).

When natural length Aβ has been investigated, until recently, extremely high concentrations of up to 100 μM were normally used to demonstrate inhibition of glutamate uptake (Lauderback *et al.*, 1999). However, synaptic glutamate uptake was recently reported to be strongly decreased by much lower levels of soluble Aβ from several sources (synthetic, cell culture, human brain extracts) (Li *et al.*, 2009). This in turn resulted in enhancement of NR2B-containing NMDA receptor currents and extra synaptic responses, an effect mimicked by the glutamate reuptake inhibitor dl-threo-β-benzyloxyaspartic acid (Li *et al.*, 2011).

The important role of inflammatory process through the cascade Aβ > microglia > TNF-α > NMDA should also be considered (Wenk *et al.*, 2006). For example, the $A\beta_{25-35}$ subfragment has even been reported to causes reverse glutamate transport by microglia (Noda *et al.*, 1999). Although only $A\beta_{1-40}$ but not the $A\beta_{25-35}$ subfragment caused a moderate enhancement of glutamate and oxygen free radical production by cultured macrophages (Klegeris and McGeer, 1997), both potentiated the stimulatory effect of phorbol myristate acetate (PMA) when used as priming agents (Klegeris and McGeer, 1997).

The physiological Aβ precursor protein APP has been reported to have 'positive' effects on glutamate transport (Mattson *et al.*, 1993; Masliah *et al.*, 1996; 1998). In contrast, microglia activated by soluble APP (sAPP) have been shown to release excitotoxic levels of glutamate, probably as a consequence of auto protective antioxidant glutathione production within the microglia, ultimately causing synaptic degeneration and neuronal death via NMDA receptor activation (Barger and Basile, 2001).

The opposite effect of Aβ on glutamate levels has also been reported, for example, $A\beta_{1-42}$ (20 μM) incubated for 12–48 h increased glutamate uptake activity in primary cultures of rat cortical astrocytes and neurons (Ikegaya *et al.*, 2002). This effect was associated with an increase in cell-surface expression of the glial glutamate transporter (GLAST). Only modest effects were seen with the less toxic species $A\beta_{1-40}$. Similarly, treatment of astrocytic cultures with $A\beta_{1-42}$ or $A\beta_{25-35}$ (20 μM) for 24–48 h increased expression levels of the glutamate transporter GLAST and uptake of glutamate from the culture media (Abe and Misawa, 2003). However, it is possible that this increased uptake/expression was a secondary protective mechanism of cells surviving toxicity (Fernandez-Tome *et al.*, 2004).

Excitotoxins released into media from brain mononuclear phagocytes (macrophages and microglia) following activation by co-culture with neuronal cells expressing wild-type APP or familial AD-linked APP mutants were neurotoxic, induced ROS and were able to evoke inward currents in *Xenopus* oocytes heterologously expressing NMDA receptors (Ikezu *et al.*, 2003). Similarly, conditioned media from αAPPs/p3- and βAPPs/Aβ-stimulated cultured human monocyte-derived macrophages directly induced inward currents through NR1a/NR2B receptors expressed in *Xenopus* oocytes that were blocked by the NMDA receptor antagonist D-APV (50 μM) but not by the AMPA receptor antagonist CNQX (20 μM) (Xiong *et al.*, 2004).

Taken together, the different effects of toxic Aβ and physiological APP on glutamate transport mechanisms seems likely to contribute to excitotoxicity and the neuronal degeneration observed in AD, whereby Aβ may increase availability/residence of glutamate in the synaptic cleft through inhibition, and even reversal, of uptake mechanisms (Gegelashvili and Schousboe, 1997). This evidence linking Aβ to disturbed homeostasis of glutamate levels has been listed in Table 1. However, one caveat that should be noted is that most studies used very high concentrations of Aβ and/or toxic subfragments of this peptide.

Intracellular signalling cascades

Apart from synaptic consequences of Aβ, certain intracellular mechanisms may also be changed by this peptide leading either to enhancement or inhibition of downstream effects of glutamate receptor activation as illustrated in Figure 1. For example, $A\beta_{1-42}$ oligomers (Aβ derived diffusible ligands ADDLs) have also been shown to induce overexpression of the Arc gene (associated with memory function), leading to a loss of NMDA receptors and altered cell morphology (Klein *et al.*, 2007)

Table 1

Effect of Aβ on glutamate homeostasis (studies showing effects leading to decrease in synaptic glutamate are in bold text)

Experimental system	Aβ type/dose	Effect of Aβ	Reference
Hippocampal slices	$A\beta_{25-35}$ (Macromolecular Analysis Lab) dissolved in water, 10 μM	Enhanced depolarization-stimulated glutamate release- stronger in aged animals – by Aβ pre-incubated for 1 h. in slices. Added acute, did not have any effect.	Arias *et al.* (1995)
Xenopus oocytes expressing heterologous proteins	$A\beta_{1-42}$ (40 μM, Sigma) and $A\beta_{25-35}$ (590 μM, Sigma) in 100 mM CH_3COOH stock stored at –20°C	$A\beta_{1-42}$ (1 μM) inhibited the ATPase Na^+/K^+ pump and glutamate transporter EAAC1	Gu *et al.* (2004)
Glial cultures	$A\beta_{25-35}$ (Bachem) 100 μM dissolved immediately before experiment	Inhibited glial glutamate uptake	Harris *et al.* (1996) Harris *et al.* (1995)
Rat primary glial cultures	$A\beta_{25-35}$ (Univ. of Iowa), 7 days incubation	Astrocytes exposed for 7 days to Aß showed reduced glutamate uptake	ParpuraGill *et al.* (1997)
Cultured macrophages	$A\beta_{1-40}$, $A\beta_{25-35}$, $A\beta_{40-1}$ (Bachem) dissolved in water and stored at –20°C,	$A\beta_{1-40}$ (from 10 μM) but not reverse $A\beta_{40-1}$ or the $A\beta_{25-35}$ sub fragment enhanced glutamate and oxygen free radical (after 15 min) production by cultured macrophages	Klegeris and McGeer (1997)
Human cortical cultures	$A\beta_{1-38\ or\ 25-35}$ (Bachem), stored in water or DMSO at –20°C, 1 and more days incubation in culture	Aβ at 40 μM enhanced the toxicity of NMDA and starting at 20 μM of glutamate.	Mattson *et al.* (1992)
Microglia cultures	$A\beta_{25-35}$ (Peptide Inst, Osaka) (stored at –80°C until use) 5 μM final concentration	Reversed glutamate transporter activity	Noda *et al.* (1999)
Cultured neurons and astrocytes	$A\beta_{25-35}$ (Neosystem) in water, allowed to aggregate (confirmed by microscope)	$A\beta_{25-35}$ (0.25–15 μM) applied for 30 min to cultured neurons and astrocytes increased glutamate levels in media but astrocytes were more sensitive. These effects were also associated with overt toxicity, but surviving neurons showed enhanced uptake of glutamate.	Fernandez-Tome *et al.* (2004)
Rat cortical synaptosomes	$A\beta_{1-42}$ pre incubation for 24 h in 37°C in PBS	$A\beta_{1-42}$ also increases HNE conjugation to the glutamate transporter resulting in uptake inhibition	Lauderback *et al.* (2001)
Primary microglia cultures	sAPP released from cells	Aβ stimulated microglia release glutamate	Barger and Basile (2001)
Primary hippocampal rat astrocytes	$A\beta_{25-35}$	At 100 μM inhibition by 50% was observed at 30 min. Prevented by scavengers.	Lauderback *et al.* (1999)
Primary rat telencephalonic astrocytes *in vitro*	$A\beta_{25-35}$ custom synthesis	$A\beta_{25-35}$ (100 μM) for 24 h caused depolarization and inhibition of glutamate uptake.	Harkany *et al.* (2000)
Rat magnocellular nucleus basalis (MBN) *in vivo*	$A\beta_{1-42}$ or $A\beta_{25-35}$ custom synthesis (200 μM)- 1 μL into NBM	Aβ infusion via microdialysis caused increased extracellular concentrations of excitatory amino acid neurotransmitters within 20–30 min	Harkany *et al.* (2000)
Primary cortical rat astrocytes and neurons	**$A\beta_{1-40}$ and $A\beta_{1-42}$ (Tokyo Metropolitan Inst. Of Gerontology, Tokyo), prepared in basic conditions to prevent aggregation**	**$A\beta_{1-42}$ (20 μM) incubated for 12–48 h increased glutamate uptake activity in primary cultures of rat cortical astrocytes and neurons (Ikegaya *et al.*, 2002). This effect was associated with an increase in cell-surface expression of the glial glutamate transporter GLAST. Only modest effects were seen with the less toxic species $A\beta_{1-40}$**	**Ikegaya *et al.* (2002)**
Rat cortical astrocytes	**$A\beta_{25-35}$, $A\beta_{1-42}$, $A\beta_{1-40}$ (Sigma)**	**$A\beta_{1-42}$ or $A\beta_{25-35}$ (20 μM) for 24–48 h increased expression levels of the glutamate transporter GLAST and uptake of glutamate from the culture media**	**Abe and Misawa (2003)**

In primary neuronal cell culture and hippocampal slices, Aβ oligomers impaired LTP and spontaneous network activity and induced retraction of synaptic contacts long before major cytotoxic effects were visible (Ronicke *et al.*, 2010). In this same study, the second messenger Jacob was shown to couple extra synaptic NMDA receptor activity to CREB protein dephosphorylation and accumulated in the nucleus after Aβ oligomer administration. The NR2B-containing NMDA receptor antagonists ifenprodil and Ro 25–6981 both blocked all of these effects (Ronicke *et al.*, 2010).

Other factors influencing NMDA receptor second messenger effects include alterations of intracellular Ca^{2+} concentration, buffering, release from intracellular stores and sequestration as well as changes in expression/function of Ca^{2+} target proteins like Ca^{2+}/calmodulin-dependent protein kinases II (CamKII) (Mattson *et al.*, 1993; Koizumi *et al.*, 1998; Brzyska and Elbaum, 2003; Zhao *et al.*, 2004; Cheung *et al.*, 2008). Intracellular mechanisms are not intended as a primary focus of the present review and were previously discussed in an excellent review (Ferreira *et al.*, 2010).

Effects of Aβ on NMDA receptor function

There are many indications that Aβ may directly affect NMDA receptor function (Figure 2 and Table 2). For example, (+)MK-801 or removal of extracellular Ca^{2+} reduced $A\beta_{1-40}$-induced Ca^{2+} transients, NO production and neurotoxicity in cultured neuroblastoma (MES 23.5) cells (Le *et al.*, 1995). (+)MK-801 partially prevented the decrease in cell viability and the energy impairment in HEK293 cells transiently expressing NR1/NR2A or NR1/NR2B subunits exposed to $A\beta_{1-42}$ (Domingues *et al.*, 2007). $A\beta_{1-40}$ treatment of cultured cerebellar granule cells induced a time- and concentration-dependent activation of NF-κB, which was inhibited by (+)MK-801 (10 μM) (Kawamoto *et al.*, 2007). These authors suggested that Aβ activates NF-κB by an NMDA-Src-Ras-like protein through MAPK and PI3K pathways (Kawamoto *et al.*, 2007).

Neuronal activation in primary neocortical cultures was selectively dependent on the assembly state of Aβ. Protofibril (PF)-induced activity was specifically attenuated by the NMDA receptor antagonist D-APV. In contrast, the non-NMDA ionotropic glutamate receptor antagonist, NBQX, preferentially reduced Aβ fibril (FB)-induced activity. Removal of Mg^{2+} from the medium, increased both PF- or FB-induced activation, but D-APV was more effective in attenuating PF-induced excitatory activity (Ye *et al.*, 2004).

Further evidence of Aβ/NMDA receptor interactions is the fact that natural Aβ dodecameric oligomers co-immunoprecipitate with NR1 and NR2A (Venkitaramani *et al.*, 2007). Moreover, $A\beta_{1-42}$ oligomers (ADDLs) bind to glutamatergic neurons expressing NR1 and NR2B but not GABA-ergic neurons (Lacor *et al.*, 2007). $A\beta_{25-35}$ (10 μM) inhibited both [^{3}H]glutamate and [^{3}H]glycine binding (by 20% and 70%, respectively) and stimulated functional [^{3}H]MK-801 binding (Cowburn *et al.*, 1997). These authors concluded that $A\beta_{25-35}$ shows moderate affinity for the agonist recognition sites of the NMDA receptor, but not for other excitatory amino acid receptor types or for L-type voltage-dependent calcium channels, and that this fragment enhances NMDA receptor function (Cowburn *et al.*, 1997). However, others have failed to detect binding of $A\beta_{1-42}$ to any known recognition sites on glutamate receptors (Von Euler *et al.*, 2008).

Most recent evidence indicates that such effects of $A\beta_{1-42}$ on NMDA receptors may be secondary to $A\beta_{1-42}$ binding to postsynaptic anchoring proteins like PSD-95 (De Felice *et al.*, 2007a; Lacor *et al.*, 2007). Exposure of cultured cortical neurons to soluble oligomers of $A\beta_{1-40}$ (0.1–10 μM) reduced levels of the synaptic PSD-95 and AMPA receptors in a concentration- and time-dependent manner (Roselli *et al.*, 2005). This effect was prevented by the NMDA receptor channel blocker (+)MK-801 and the NR2B site antagonist ifenprodil but was not increased by combining Aβ with NMDA indicating possible direct activation of NMDA receptors by Aβ (Roselli *et al.*, 2005). Soluble oligomeric $A\beta_{1-42}$ also down-regulated the levels of PSD-95 and synaptophysin, and that this effect was also blocked by (+)MK-801 and ifenprodil (Liu *et al.*, 2010). The authors proposed that Aβ leads to a loss of these associated synaptic proteins subsequent to binding to PSD-95 and indirect suppression of NR2A function but activation of NR2B function that, in turn, induces caspase-8 and caspase-3 activity (Liu *et al.*, 2010). Indeed, selective enhancement of NR2A activity and/or reduction of NR2B activity has been suggested to be a useful therapeutic approach in AD (Liu *et al.*, 2010).

Similarly, although NMDA receptor knock-down using an amplicon vector abolished $A\beta_{1-42}$ oligomer (ADDLs) binding to dendrites, and associated neuronal oxidative stress, both oligomer-attacked and non-attacked control neurons exhibited similar levels of NMDA receptor surface expression (Decker *et al.*, 2010a). Moreover, insulin treatment down-regulated $A\beta_{1-42}$ oligomer-binding sites in the absence of a parallel reduction in surface levels of NMDA receptors. (Decker *et al.*, 2010a)

Recently, single particle tracking of quantum dot-labelled $A\beta_{1-42}$ membrane-attached oligomers (ADDLs) revealed that, whilst initially moving freely, their diffusion was hindered upon accumulation at synapses (Renner *et al.*, 2010). Concomitantly, individual metabotropic glutamate receptors (mGluR5) also showed reduced lateral diffusion, aberrant clustering at synapses and caused an elevation of intracellular calcium and synaptic loss which was prevented by an mGluR5 antagonist (Renner *et al.*, 2010). In this regard, it should be noted that NMDA and mGluR5 receptors are closely associated with each other in postsynaptic complexes (Tu *et al.*, 1999).

Others have reported that $A\beta_{25-35}$ induced increases in intracellular Ca^{2+} in cultured hippocampal neurones, and that this effect was enhanced by Mg^{2+} removal and blocked by NMDA receptor antagonists (Brorson *et al.*, 1995). However, this was proposed to be due to enhanced glutamatergic synaptic network activity rather than direct activation of NMDA receptors (Brorson *et al.*, 1995). $A\beta_{1-42}$ has also been reported to inhibit the sodium-potassium adenosine triphosphatase (ATPase Na^+/K^+) pump and could thereby cause membrane depolarization and relief of Mg^{2+} blockade of NMDA receptors (Gu *et al.*, 2004). However, application of $A\beta_{1-40}$ by extracellular perfusion (200 nM) or intracellularly via the recording

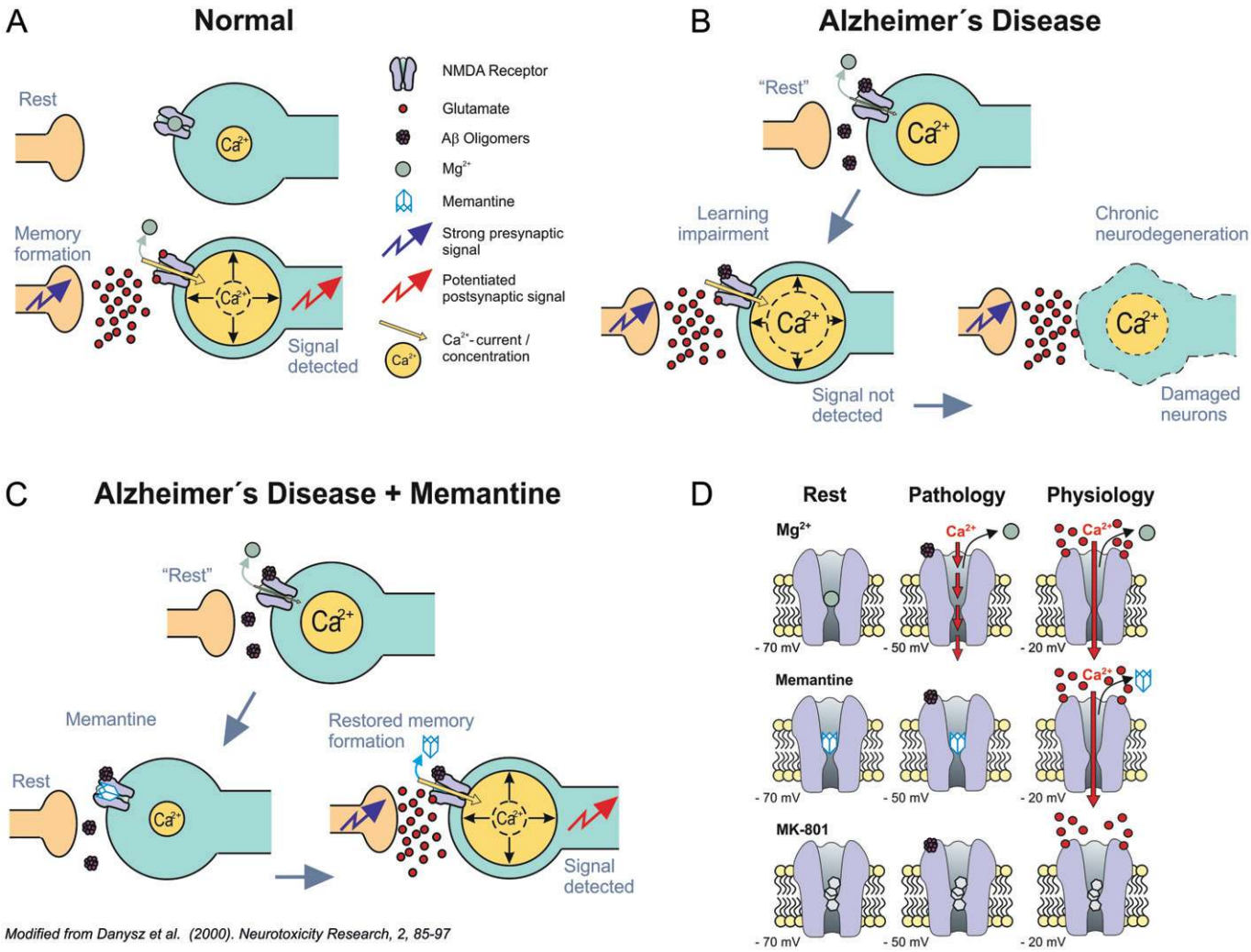

Figure 2

(A) Under normal physiological conditions, synaptic plasticity/learning depends on the detection of a relevant (sufficiently strong) synaptic signal over background noise (here referring to transient high vs. prolonged moderate intracellular Ca^{2+} levels), resulting in a sufficient signal-to-noise ratio. Intracellular Ca^{2+} concentrations at any one time point are represented by different sizes of the yellow Ca^{2+} containing circles. Jagged blue arrow indicates arrival of a presynaptic signal. Jagged red arrow indicates detection of the postsynaptic signal. Mg^{2+} and glutamate are illustrated by large green and small red circles respectively. For simplification, the roles of other receptors (e.g. AMPA) and feedback inhibition have been omitted from this cartoon (please refer to figure 3 in Parsons *et al.*, 2007 for a more detailed depiction of the processes believed to underlie such synaptic plasticity). (B) The signal-to-noise ratio hypothesis assumes that in Alzheimer's disease (AD), due to a tonic over activation of NMDA receptors by, for example soluble β-amyloid oligomers, Mg^{2+} is no longer effective enough to play its 'filtering' function. In turn, synaptic noise rises, impairing detection of the relevant synaptic signal required for learning/plasticity. The light blue straight arrows indicate the proposed course of events (i.e. first symptomatic disturbance of synaptic plasticity) followed by synaptotoxicity and ultimately neuronal death. Soluble β-amyloid oligomers represented as mauve aggregates of small circles – here binding directly to NMDA receptors for simplification, but probably interacting more directly with anchoring protein complexes and thereby affecting the function of their associated proteins such as NMDA receptors. Other symbols have the same meaning as in panel A. (C) Schematic illustrating memantine's proposed MoA in AD based on the signal-to-noise hypothesis. Memantine is able to serve as a more effective filter than Mg^{2+}, blocking pathological 'noise' at glutamatergic synapses and thereby allowing detection of the relevant synaptic signal. Synaptic plasticity is restored and synaptotoxicity/ultimate neuronal death is prevented by the same MoA. Memantine illustrated as a simple light blue adamantane cage. For other aspects, see legends to panels A and B. Modified from Danysz et al. (2000). (D) Schematic illustrating the hypothesis explaining how the fast unblocking kinetics of memantine allow this voltage-dependent compound to differentiate between the physiological and pathological activation of NMDA receptors. Under resting therapeutic conditions [i.e. in their continuing presence at –70 mV (left), Mg^{2+} (top), memantine (middle) and MK-801 (bottom)], all occupy the NMDA receptor channel. Both Mg^{2+} and memantine are able to leave the NMDA receptor channel upon strong synaptic depolarization (–20 mV, right) due to their pronounced voltage dependency and rapid unblocking kinetics, whereas the slow, potent blocker MK-801 remains trapped. However, memantine – in contrasts to Mg^{2+} – does not leave the channel so easily upon moderate prolonged depolarization during chronic excitotoxic insults caused by soluble β-amyloid oligomers tonically activating NMDA receptors (–50 mV, centre). Transient strong and prolonged moderate Ca^{2+} influx illustrated by the full and dashed red arrows respectively. Modified after Kornhuber and Weller (1997) and Parsons et al. (1999).

Table 2

Effect of Aβ on NMDA receptor function (studies showing a decrease of NMDA function after Aβ are indicated by bold text)

Experimental system	Aβ type/dose	Effect of Aβ observed	Reference
Patch clamp – rat hippocampal slices, dentate gyrus	$A\beta_{1-40}$ (Bachem, 100–200 nM)	Applied by perfusion or intracellular via the recording pipette enhanced NMDA receptor-mediated synaptic currents	Wu *et al.* (1995a)
Single unit recordings, iontophoretic application to CA1 *in vivo*	$A\beta_{1-42}$ mixture of different species (fibrils and protofibrils)	Responses to NMDA were potentiated to 260% but to AMPA decreased	Szegedi *et al.* (2005)
Akt phosphorylation – mice slices or primary cortical/hippocampal cultures	Aß length not specified (California Peptide Research), claimed to be in forms of dimers and trimers	Aß stimulated phosphorylation of Akt and this effect was attenuated by the NR2B antagonist ifenprodil	Abbott *et al.* (2007)
Inward currents, Ca^{2+} entry, apoptosis – rat cortical primary cultures and organotypic slices from entorhinal-hippocampal region	$A\beta_{1-41}$ (ABX), stated to be soluble Aß at the time of application	Aß stimulated inward currents (whole cell patch clamp) and Ca^{2+} influx (fluorescence imaging) and these effects were attenuated by AP5, Memantine, (+)MK-801. AMPA antagonist as also active indicating contribution of AMPA receptors.	Alberdi *et al.* (2010)
Axonal trafficking of dense-core vesicles and mitochondria – primary hippocampal cultures	Soluble Aß oligomers (5 μM, incubated for 4 or 18 h)	Aß effect was prevented by AP5, Memantine and (+)MK-801	Decker *et al.* (2010b)
Aß toxicity (LDH release) in HEK293 cells	$A\beta_{1-40}$ (Bachem, 1 μM) aged for 7 days at 37°C to produce ß-sheet fibrils, incubation for 24–48 h.	Aß-induced increase in toxicity was present in NR1/NR2A but not NR1/NR2B expressing cells,	Domingues *et al.* (2007)
Neurite growth – primary rat cortical neurons	$A\beta_{1-42}$ (Athens), freshly prepared at 5 μM, incubation for 3 days.	Aß-induced decrease in neurite growth was attenuated by memantine	Hu *et al.* (2007)
Inward currents – *Xenopus* oocytes expressing NMDA receptor subunits	$A\beta_{1-42}$ (ABX, 100 μM), incubated at 4°C for 24 h to allow aggregation.	Aß-induced inward currents were blocked by memantine, AP5 and (+)MK-801. Ca^{2+} cytosolic increase was attenuated by AP5 but only partially inhibited by ifenprodil (NR2B ant.)	Texido *et al.* (2011)
Spine density – rat organotypic cultures	$A\beta_{1-42}$ containing several oligomeric species, secreted by neurons	Reduction in spine density produced by Aß was attenuated by AP5	Wei *et al.* (2009)
fEPSPs and neuronal damage – organotypic rat hippocampal cultures	$A\beta_{1-42}$ globular form pre-incubated for 12 h at room temperature.	Memantine (1 μM) attenuated fEPSP deficits produced by Aß (82 nM, globulomer). Similar effects were observed against neuronal damage.	Nimmrich *et al.* (2010)
Apoptosis, cGMP – MES 23.5 cell line	$A\beta_{1-42}$ (Bachem), 10 μM	(+)MK-801 (100 μM) prevented cGMP increase and toxicity produced by Aß	Le *et al.* (1995)
Expression of PSD-95 and synaptophysin – primary rat hippocampal cultures	$A\beta_{25-35}$ (American Peptides) 10 μM used without pre-incubation, low N oligomers expected	Down-regulation of PSD-95 and synaptophysin induced by Aß was attenuated by (+)MK-801 (but note that low doses of NMDA were also preventative)	Liu *et al.* (2010)
Surface expression of insulin receptors – cultures of hippocampal neurones	$A\beta_{1-42}$ (American Peptides) 100 μM, pre-incubated overnight to allow formation of ADDLs followed by centrifugation and supernatant was used as soluble Aß oligomers	Memantine (20 μM) and AP5 (50 μM) prevented Aß-induced insulin receptor loss.	Zhao *et al.* (2008)
NR1a/NR2B receptors expressed in X. oocytes	Conditioned media from aAPPs/p3- and bAPPs/Aβ-stimulated cultured human monocyte-derived macrophages	This media produced induced inward currents that were blocked by the NMDA receptor antagonist D-APV (50 μM) but not AMPA antagonist	Xiong *et al.* (2004)

Table 2

Continued

Experimental system	Aβ type/dose	Effect of Aβ observed	Reference
Rat primary cortical cultures	$A\beta_{1-40}$ (American Peptides), 0.1–10 μM (claimed to be monomeric to tetrameric)	Caused an NMDA receptor dependent decrease in PSD-95 after 60 but not 15 min. starting at 0.1 μM. Prevented by MK-801 and ifenprodil. NMDA + Aβ was not stronger than Aβ alone.	Roselli *et al.* (2005)
Receptor binding in rat cortical membranes	$A\beta_{25-35}$ (UCB Bioproducts) dissolved immediately before the experiment	$A\beta_{25-35}$ (10 μM) inhibited both [^{3}H]glutamate and [^{3}H]glycine binding (by 20 and 70% respectively) and stimulated functional [^{3}H]MK-801 binding	Cowburn *et al.* (1997)
	Natural	Aβ dodecameric oligomers co-immunoprecipitate with NR1 and NR2A	Venkitaramani *et al.* (2007)
	$A\beta_{1-42}$, freshly prepared in 0.1 N NaOH, pH readjusted to 7.4, and then diluted in serum-free culture medium.	Internalization of Aβ is blocked by NMDA receptor antagonists indicating	Bi *et al.* (2002)
		Activation of NMDA receptors by Aβ was proposed to be secondary to microglial activation and production of TNFα NMDA	Wenk *et al.* (2006)
Differentiated cultures of hippocampal neurons	Soluble Aβ-derived diffusible ligands (ADDLs)	Aβ bound to glutamatergic but not GABA-ergic neurons and to postsynaptic density complexes containing NMDA receptors. After chronic exposure produced abnormal spine morphology and a decrease in their density. Subsequent consequences such as loss of the spine cytoskeletal protein drebrin were prevented by memantine at a concentration of 5 μM	Lacor *et al.* (2007)
Hippocampal neuronal cultures	$A\beta_{1-42}$ (California Peptide) oligomers aged overnight at 4°C, and centrifuged to remove insoluble aggregates	Aβ (starting at 300–500 nM) produced oxidative stress and calcium influx that was prevented by memantine (5 or 10 μM) or anti NMDA receptor antibodies.	De Felice *et al.* (2007a)
		Aβ co-immunoprecipitated together with NMDA receptor	
Hippocampal neuronal cultures and neuroblastoma cells	$A\beta_{1-42}$ (California Peptide) oligomers aged overnight at 4°C, and centrifuged to remove insoluble aggregates (ADDLs)	ADDLs stimulated tau phosphorylation at epitopes characteristically hyperphosphorylated in AD	De Felice *et al.* (2007b)
Hippocampal neuronal cultures	$A\beta_{25-35}$ (Bachem and Sigma), 18 h of incubation	Aβ increased intracellular Ca^{2+} (starting at 11 μM) which was enhanced by Mg^{2+} removal and blocked by NMDA receptor antagonists (claimed to be due to enhanced glutamatergic synaptic network activity but not due to direct effects on NMDA receptors)	Brorson *et al.* (1995)
Wistar rats, field excitatory potentials in CA1 Hippocampal slices recording in DG	$A\beta_{1-40}$ (Bachem) freshly prepared	$A\beta_{1-40}$ caused a long lasting depression of hippocampal EPSPs *in vivo* 24 h. after i.c.v. injection (1 μL of 3.5 mM) (Cullen *et al.*, 1996). This effect was prevented by the NMDA receptor antagonist CPP. The same effect was seen in the DG of hippocampal slices	Cullen *et al.* (1996)

Table 2

Contined

Experimental system	Aβ type/dose	Effect of Aβ observed	Reference
Cultured cerebellar granule cells	$A\beta_{1-40}$ (Bachem, pre-incubated in PBS for 5 days at 37°C to allow aggregation, to cells it was applied at 1 or 2 μM for 6, 12, 24 h)	Aβ produced activation of NFkB (1 μM at 12 h) that was inhibited by MK-801 (10 μM)	Kawamoto *et al.* (2007)
Assessment of oxidative stress (2,3-DHB generation) after application through microdialysis probe in the striatum	$A\beta_{1-40}$ (Bachem) pre-incubation for 4 days at 37°C	Aβ infusion produced oxidative stress that was attenuated by MK-801	Parks *et al.* (2001)
Patch clam whole cell recording from primary rat cortical cultures	$A\beta_{1-42}$ after 35 min of incubation	Aβ produced depression of NMDA mediated current related to NMDA receptor endocytosis that was α7 dependent	Snyder *et al.* (2005)
Cortical neuronal cultures from mice	$A\beta_{1-42}$ (American Peptide) pre-incubation in water for 7 days at 37°C	Conditioned medium from microglia incubated with $A\beta_{1-42}$ produced neuronal death by oxidative mechanisms. This was prevented by memantine (1 μM) and D-APV (10 μM)	Floden *et al.* (2005)
Single unit recording *in vivo* in CA1 iontophoretic application	$A\beta_{1-42}$, $A\beta_{25-35}$ (own synthesis) sonicated to lessen aggregation	Both Aβ forms enhanced NMDA responses	Molnar *et al.* (2004)
Primary rat cerebral cultures	$A\beta_{1-42}$ incubated at 4°C for 24 h in 2%DMSO and Ham F-12 medium	1 μM (30 min) $A\beta_{1-42}$-induced AA release that was inhibited by D-APV (10 μM) and memantine (5 μM)	Shelat *et al.* (2008)
Primary neocortical cultures neurons and glia (patch clamp)	Aβ (Biopolymer facility at Brigham and Women's Hospital) assemblies in a prefibrillar form	1 μM Aβ produced neuronal activation that was attenuated in case of prefibrillar form by the NMDA receptor antagonist D-APV but effect produced by fibrillar form was not significantly attenuated (but was by AMPA antagonist)	Ye *et al.* (2004)
Organotypic hippocampal slices	$A\beta_{1-42}$, freshly prepared in 0.1 N NaOH, pH readjusted to 7.4, and then diluted in serum-free culture medium.	The selective NMDA receptor antagonist D-APV completely blocked $A\beta_{1-42}$ (15–30 μM) internalization, up-regulation of cathepsin D, and activation of microglia	Bi *et al.* (2002)
Cultured cortical neurons	**Supplementary methods not available**	**$A\beta_{1-42}$ (1 μM for 1 h) produced a rapid and persistent depression of NMDA receptor mediated currents and synaptic receptor endocytosis**	**Snyder *et al.* (2005)**
Inward currents – *Xenopus* oocytes expressing NMDA receptors	**$A\beta_{1-42}$, (Bachem) globulomers (dodecamers), 6 h pre-incubation at 37°C,**	**Aß had no effect on NMDA-induced glutamate currents**	**Mezler *et al.* (2011)**
Toxicity (LDH release) – septal rat primary neurons in culture	**$A\beta_{1-42}$ (Peptide Institute, Osaka) dissolved directly before use**	**Memantine (up to 10 μM) and (+)MK-801 (10 μM) did not prevent toxicity produced by Aß measured as LDH release**	**Kimura *et al.* (2005)**
Organotypic hippocampal slices	**Aβ, naturally secreted dimmers and trimers**	**Oligomers decrease Ca^{2+} influx and produced loss of hippocampal synapses that was prevented by 20 μM NMDA antagonists CPP, which at lower concentrations produced synapse loss on its own**	**Shankar *et al.* (2007)**

pipette (100 nM) resulted in a gradual enhancement of NMDA receptor-mediated synaptic currents in granule cells in the rat dentate gyrus (DG) *in vitro* with no effect on AMPA receptor-mediated transmission, resting membrane potential or input resistance (Wu *et al.*, 1995a).

There are also multiple effects of Aβ seen *in vivo* that can be attributed to over activation of NMDA receptors since they are blocked by antagonists of this receptor type (Table 2). *In vivo* responses of single hippocampal neurons to local microiontophoretic NMDA were potentiated by local application of $A\beta_{1-42}$, whereas those of AMPA/kainate receptors were decreased (Molnar *et al.*, 2004; Szegedi *et al.*, 2005). Oxidative stress seen *in vivo* after Aβ application is also blocked by NMDA receptor antagonists (Parks *et al.*, 2001).

Others have reported that $A\beta_{1-42}$ (1 μM for 1 h) produced a rapid and persistent depression of NMDA receptor-mediated currents and synaptic receptor endocytosis secondary to activation of protein phosphatase 2B (PP2B) and striatal enriched tyrosine phosphatase (STEP) (Snyder *et al.*, 2005). Preincubation of primary neuronal cultures with synthetic $A\beta_{1-42}$ oligomers decreased NR2B-immunoreactive synaptic spines and surface expression of NR2B containing NMDA receptors *in vitro* (Dewachter *et al.*, 2009). Prolonged exposure of primary cortical neurons to $A\beta_{1-42}$ oligomers exhibited toxic effects (mitochondrial dysfunction and production of ROS) associated with an attenuation of NMDA receptor-mediated Ca^{2+} influx and inhibition of NMDA-induced AA release (He *et al.*, 2011). However, such changes could reflect reactive endocytosis of NMDA receptors subsequent to their tonic pathological activation (Bi *et al.*, 2002; Snyder *et al.*, 2005; Hsieh *et al.*, 2006).

Neurons from a genetic mouse model of AD also rather showed reduced expression of surface NMDA receptors and dephosphorylation of the NMDA receptor subunit NR2B at Tyr^{1472}, which correlated with receptor endocytosis (Snyder *et al.*, 2005). Decreased concentrations of the NMDA receptor subunit NR2B and PSD-95, impaired NMDA-dependent LTP and decreased NMDA and AMPA receptor currents in hippocampal *Cornu ammonis* area 1 (CA1) region have also been reported in APP[V717I] transgenic mice (Dewachter *et al.*, 2009). Similar, probably reactive effects of glutamatergic and cholinergic neurotransmitter systems have also been reported at the level of PKB/Akt phosphorylation (Abbott *et al.*, 2007).

In conclusion, the majority of the evidence listed above (see also Table 2) indicates increased tonic NMDA receptor stimulation in the presence of soluble forms of Aβ. However, one caveat is that most earlier studies used high concentrations of Aβ, which makes extrapolation of these data to the true disease conditions *in vivo* challenging.

Effects of glutamatergic transmission on Aβ processing/levels

Already two decades ago dysregulation of neuronal calcium homeostasis has been implicated to enhance the production of Aβ in AD (Mattson *et al.*, 1993). Recent results indicate that Ca^{2+} stimulates the formation of oligomers of Aβ (Itkin *et al.*, 2011). It therefore seems plausible that prolonged Ca^{2+} influx via pathological activation of NMDA receptors could also promote the intracellular generation of toxic Aβ oligomers – a kind of positive feedback viscious circle. Also, internalization of $A\beta_{1-42}$ itself has been reported to be blocked by NMDA receptor antagonists (Bi *et al.*, 2002).

Sub-toxic activation of NMDA receptors increases the proportion of Kunitz protease inhibitor domain containing APP that, in turn, favours β-secretase over α-secretase processing resulting in enhanced Aβ production. Prolonged activation of extrasynaptic NMDA receptors, but not synaptic NMDA receptors, was recently reported to increase the neuronal production of Aβ (Bordji *et al.*, 2010). This effect was preceded by a shift from APP695 to Kunitz protease inhibitory domain (KPI) containing APPs (KPI-APPs), isoforms exhibiting greater amyloidogenic potential. Somewhat in line with this notion is the concept that calcium influx through synaptic NMDA receptors actually promotes non-amyloidogenic α-secretase-mediated APP processing (Hoey *et al.*, 2009). In rat organotypic slices, acute overproduction and synaptic release of either axonal or dendritic Aβ reduced spine density and plasticity at nearby dendrites (Wei *et al.*, 2009). However, in this case, only the synaptotoxic effects, but not Aβ production, was sensitive to NMDA receptor blockade by D-APV (Wei *et al.*, 2009).

Others have proposed that general synaptic activity-dependent modulation of endogenous Aβ production/secretion may normally rather participate in a negative feedback loop to depresses excitatory synaptic transmission to keep neuronal hyperactivity in check and that disruption of this feedback system could contribute to disease progression in AD (Kamenetz *et al.*, 2003). More recently, Cirrito *et al.* (2005; 2008) showed, using brain microdialysis *in vivo*, that synaptic activity increases endocytosis of APP and also subsequently increases Aβ levels in the brain extracellular fluid through increased Aβ exocytosis. However, the relationship between synaptic activity and increase in pathogenic Aβ is still controversial. The Gouras group showed that synaptic activity promotes APP intracellular transport to the synapses, decreases intracellular Aβ due to neprilysin activity and protects against Aβ related synaptic changes (Tampellini and Gouras, 2010).

Functional consequences of Aβ (LTP, learning) and the role of NMDA receptors

Effect of Aβ on LTP indicative of a role for NMDA receptors

There are numerous reports that incubation of rodent hippocampal slices with small diffusible $A\beta_{1-42}$ oligomers strongly inhibits the induction LTP in the CA1 and dentate gyrus, but not NMDA receptor-independent LTP – see schematic in Figure 2 (Table 3). These effects occur via interactions with NMDA receptors as they are blocked by many different NMDA receptor antagonists and manifest themselves well before any signs of overt excitotoxicity (Lambert *et al.*, 1998; Wang *et al.*, 2002; Chen *et al.*, 2002b; Rowan *et al.*, 2003; Wang *et al.*, 2004a; Walsh *et al.*, 2005; Puzzo and Arancio, 2006; Townsend *et al.*, 2006; Martinez-Coria *et al.*, 2010) (Table 3).

Table 3

Effect of Aβ on LTP. Majority of studies show disruption of LTP, few studies show an enhancement of LTP by Aβ (indicated by bold text)

Experimental system	Aβ type/dose	Effect of Aβ observed	Reference
LTP in hippocampal slices, CA1	$A\beta_{1-42}$ (Bachem), incubation for 6 h and then for 18 h after dilution at 37°C to produce globular forms	Completely blocked LTP at 42 nM given 80–120 min before tetanus	Barghorn *et al.* (2005)
LTP *in vivo*, CA1	$A\beta_{25-35}$ (Bachem) 10 and 100 nM i.c.v.	At 5 min, 100 but not 10 nM impaired LTP. At 1 h, both were active. The effects of Aβ on LTP were probably mediated via a postsynaptic mechanism because they did not affect paired pulse facilitation	Freir *et al.* (2001)
LTP *in vivo*, CA1	$A\beta_{1-42}$ (Bachem)- stock solution in 0.1% $NH^{4+}OH^{-}$, centrifuged, supernatant stored at –80°C.	$A\beta_{1-42}$ (80 pmol i.c.v.) impaired LTP *in vivo* and this effect was reversed by NR2B and TNFα antagonists	Hu *et al.* (2009)
LTP *in vitro*, CA1, from transgenic APP (V717I) mice	$A\beta_{1-42}$ (Bachem, 100 μM), incubated for 24 h at 4°C for oligomers and 24 h at 37°C in DMSO)	APP Tg mice had impaired LTP in CA1 region (NMDA dependent)	Dewachter *et al.* (2009)
LTP *in vivo*, CA1	$A\beta_{1-42}$ (Bachem)- stock solution in 0.1% $NH^{4+}OH^{-}$, centrifuged, supernatant stored at –80°C.	$A\beta_{1-42}$ (80 pmol i.c.v.) impaired LTP *in vivo* and this effect was reversed by low doses of memantine	Klyubin *et al.* (2011).
LTP rat hippocampal slices CA1	$A\beta_{1-42}$ (AnaSpec), pre-incubated at room temp. for 1–2-days	1 μM Aβ (3 h) moderately inhibited LTP. Co-treatment with a sub-threshold concentration of glutamate (30 μM) strongly impaired LTP.	Nakagami *et al.* (2002); Nakagami and Oda (2002)
LTP rat hippocampal slices CA1	$A\beta_{1-42}$ and $A\beta_{25-35}$ (1 μM) (Peptide Institute, Osaka)	LTP was markedly reduced by i.c.v. $A\beta_{25-35}$ (10 nmol) and completely blocked by $A\beta_{25-35}$ (100 nmol). Aβ did not affect NMDA EPSP. Not NMDA involvement but downstream mechanisms.	Nomura *et al.* (2005)
LTP rat hippocampal slices, CA1	$A\beta_{1-42}$, $A\beta_{1-40}$ (Keck Peptide Synthesis Lab.) or their active fragment $A\beta_{25-35}$ (Bachem) applied for 20 min before HFS	$A\beta_{25-35}$ (10 μM) inhibited both [^{3}H]glutamate and [^{3}H]glycine binding (by 20 and 70% respectively) and stimulated functional [^{3}H]MK-801 binding	Chen *et al.* (2000)
LTP hippocampal slices DG	$A\beta_{1-42}$ (Bachem) applied 20 min before HFFS, no pre-aggregation	Aβ at 200 but not 20 nM inhibited LTP, involving calcineurin mechanisms. Bath application of $A\beta_{1-42}$ (1 μM, 10 min) reduced NMDA receptor-mediated EPSCs.	Chen *et al.* (2002b)
LTP rat hippocampal slices	Diffusible $A\beta_{1-42}$ derived diffusible ligands (ADDLs)	Prevented LTP induction.	Puzzo and Arancio (2006)
LTP rat hippocampal slices CA1	$A\beta_{1-40}$ (200 nM) (US Peptides)	Caused rapid inhibition of LTP and a modest short term inhibition (approx. 25%) of NMDA EPSP but no long-term effect on normal synaptic transmission or LTD.	Raymond *et al.* (2003)
LTP rat hippocampal slices CA1	Aβ dimmers isolated from human brain	Suppressed LTP and enhanced LTD	Shankar *et al.* (2008)
LTP *in vivo*	Aβ naturally secreted from microsomes from hamster ovary	The negative effects of i.c.v. Aβ oligomers on hippocampal LTP *in vivo* were prevented by monoclonal Aβ antibodies. Monomers were inactive.	Walsh *et al.* (2002)
LTP rat hippocampal slices CA1	$A\beta_{1-42}$ (Bachem) globulomers prepared according to Barghorn	$A\beta_{1-42}$ (42 nM) completely blocked LTP and this was reversed by memantine 1 μM	Martinez-Coria *et al.* (2010)

Table 3

Continued

Experimental system	Aβ type/dose	Effect of Aβ observed	Reference
LTP rat hippocampal slices CA1	$Aβ_{1–42}$ (Bachem)- soluble Aβ-derived oligomers (ADDLs)	$Aβ_{1–42}$ (1–50 nM), concentration-dependently blocked LTP and this was reversed by memantine 1 μM as well as by NR2B and mGluR5 receptor negative allosteric modulators	Rammes *et al.* (2011)
LTP rat hippocampal slices CA1	$Aβ_{1–42}$ (MoBiTec) HFIP aliquots dissolved (100 μM) in DMSO, diluted to 20 μM in F12/DMEM and incubated at 4°C for 24 h	$Aβ_{1–42}$ (500 nM) strongly inhibited LTP and this was reversed by the NR2B receptor negative allosteric modulators ifenprodil and Ro25-6981	Ronicke *et al.* (2010)
LTP rat hippocampal slices CA1	$Aβ_{1–42}$ (American Peptide), incubated at 4°C for 24–30 h.	Aβ inhibited LTP but not LTD	Wang *et al.* (2002)
LTP hippocampal slices DG	Aβ naturally secreted (CHO cells expressing human APP751) and synthetic $Aβ_{1–42}$ (Bachem)	Inhibited the induction of LTP (natural at 1 μM and synthetic at 100–200 nM). mGluR5 antagonists blocked this effect of Aβ	Wang *et al.* (2004b)
LTP rat hippocampal slices CA1	$Aβ_{25–35}$	$Aβ_{25–35}$ impaired both PTP and LTP	Costello and Herron (2004)
LTP hippocampal slices DG	$Aβ_{25–35}$ (Bachem) perfused for 40 min (no pre-incubation)	Aβ (500 nM) inhibited induction of NMDA dependent LTP (involving superoxide), but not induction of NMDA-independent LTP or long-term depression (LTD)	Wang *et al.* (2004a)
LTP hippocampal slices DG	Aβ (Bachem, type not specified) perfused for 40 min (no pre-incubation)	Inhibited LTP induction which involved the TNFα and metabotropic glutamate receptors (mGluR5)	Wang *et al.* (2005)
LTP rat hippocampal slices CA1	0.1 mM of the short Aβ fragment $Aβ_{31–35}$ and $Aβ_{25–35}$	Suppressed the induction of LTP of PS in a similar manner to the longer fragment $Aβ_{25–35}$. Had no effect on NMDA receptor mediated multiple PS in Mg^{2+}-free medium, suggesting that these Aβ fragments suppressed the induction of LTP through an NMDA receptor-independent pathway	Ye and Qiao (1999)
LTP in mouse hippocampal slices, CA1	Aβ, soluble oligomers derived from CHO cells expressing human APP_{v717F}, 20 min before HFS	Aβ inhibited LTP	Townsend *et al.* (2006)
LTP hippocampal slices D	$Aβ_{1–42}$ (Bachem) no pre-aggregation applied 20 min before HFS	Aβ (final concentration not known) inhibited LTP	Zhao *et al.* (2004)
LTP hippocampal slices DG	**$Aβ_{1–40}$ (Bachem) stored as water solution at –20°C**	**At 200 nM, Aβ enhanced LTP but not basal responses 25 min after application**	**Wu *et al.* (1995a,b)**
LTP *in vivo*, CA1	**$Aβ_{1–42}$ (Bachem)**	**Facilitated the induction of LTD and depotentiation of LTP in the an NMDA receptor-dependent manner**	**Kim *et al.* (2001)**

For example, soluble Aβ oligomers from different sources (cultured cells, AD cortex or synthetic peptide) consistently inhibit LTP in murine and rat hippocampal slices, and this inhibition can be prevented by the NR2B negative allosteric modulators ifenprodil and Ro 25–6981 (Hu *et al.*, 2009; Ronicke *et al.*, 2010; Li *et al.*, 2011; Rammes *et al.*, 2011). Additionally, $Aβ_{1–42}$ (42 nM) incubated under somewhat artificial conditions – that is, in the presence of SDS to produce stable globular 'dodecameric' forms – bound specifically to dendritic processes/spines of neurons but not glia in hippocampal cell cultures and completely blocked LTP in the CA1 region in hippocampal slices (Barghorn *et al.*, 2005; Albrecht *et al.*, 2008; Martinez-Coria *et al.*, 2010). Similar results were seen when $Aβ_{1–42}$ was prepared under conditions presumably

more closely resembling the pathophysiological situation (e.g. lacking SDS, ADDLs) (Lacor *et al.*, 2007). $A\beta_{1-42}$ (1–50 nM) concentration-dependently blocked LTP, with strong effects already seen at the lowest concentration of Aβ tested (1 nM) (Rammes *et al.*, 2011). Under both conditions, the LTP deficits induced by $A\beta_{1-42}$ were completely reversed by 1 μM memantine, as well as by NR2B negative allosteric modulators and partial knockout of NR2B subunits (Rammes *et al.*, 2011).

Such effects of $A\beta_{1-42}$ are normally not reflected in effects on baseline AMPA receptor-mediated EPSPs, but one study showed a reduction in the amplitude of isolated NMDA receptor-mediated synaptic currents in dentate granule cells *in vitro* via a postsynaptic mechanism (Chen *et al.*, 2002b).

There are indications that the inhibitory effects of $A\beta_{1-42}$ on LTP involves reactive oxygen and nitrogen species (Wang *et al.*, 2004a), and the pro-inflammatory cytokine TNF-α; as such, suppression is not seen in TNF-α-deficient mice (Wang *et al.*, 2005). Further evidence that TNF-α mediates this deleterious action was recently provided by the ability of TNF-α antagonists to prevent $A\beta_{1-42}$ inhibition of LTP *in vivo* and the abrogation of a similar disruptive effect of TNFα using the NR2B selective NMDA receptor antagonist Ro 25–6981 (Hu *et al.*, 2009). Similarly, stimulation of the kinases JNK, CDK5 and p38 MAPK; TNF-α; metabotropic glutamate receptors (mGluR5) and CREB protein have been proposed to be involved in Aβ-induced deficits in LTP (Wang *et al.*, 2004b; 2005; Li *et al.*, 2011).

$A\beta_{1-42}$ also inhibited LTP and associated phosphorylation processes in DG of rat hippocampal slices (Zhao *et al.*, 2004). The authors suggested that activity-dependent CaMKII autophosphorylation and AMPA receptor phosphorylation are essential for LTP in this region and that disruption of such mechanisms could directly contribute to Aβ-induced deficits in hippocampal synaptic plasticity and memory. Similarly, $A\beta_{1-42}$ (200 nM) inhibited LTP of EPSPs and population spikes (PS) in the same region *in vitro* (Chen *et al.*, 2000). Interestingly, in this same study, even the often reported less toxic $A\beta_{1-40}$ blocked LTP of EPSPs at the same relatively low concentration, but was less effective against PS (Chen *et al.*, 2000). In contrast, some authors have reported that $A\beta_{1-40}$ actually enhanced LTP but not basal responses in the DG of hippocampal slices (Wu *et al.*, 1995a,b).

Negative effects of $A\beta_{1-42}$ on LTP were also reported for Schaffer-collateral projecting to CA1 *in vivo* (e.g. Kim *et al.*, 2001; Hu *et al.*, 2009). Such effects of i.c.v. $A\beta_{1-42}$ oligomers on hippocampal LTP *in vivo* were completely prevented by co-administration of monoclonal Aβ antibodies (Kim *et al.*, 2001; Walsh *et al.*, 2002).

In some studies, pretreatment of rat hippocampal slices with $A\beta_{1-42}$ alone only moderately inhibited LTP, but co-treatment with a sub-threshold concentration of glutamate agonists impaired LTP more strongly, implying an interplay between Aβ and the glutamatergic system (Nakagami *et al.*, 2002).

Smaller fragments of the Aβ peptide usually have similar effects. $A\beta_{25-35}$ was also found to impair both post-tetanic potentiation (PTP) and LTP in the hippocampal CA1 *in vitro* and, in agreement with (Wang *et al.*, 2004a,b), these effects were proposed to involve activation of the JNK signalling pathway (Costello and Herron, 2004). Similarly, 0.1 μM of the short Aβ fragment $A\beta_{31-35}$ suppressed the induction of LTP of PSs in CA1 of rat hippocampal slices in a similar manner to the longer fragment $A\beta_{25-35}$, whereas neither treatment changed the amplitude of the baseline PS. These fragments had no effect on NMDA receptor-mediated multiple PSs when recorded in Mg^{2+}-free medium, which was taken to imply that these Aβ fragments suppress the induction of LTP through a NMDA receptor-independent pathway (Ye and Qiao, 1999). A similar conclusion was drawn by others (Nomura *et al.*, 2005).

In vivo LTP was also markedly reduced by i.c.v. $A\beta_{25-35}$ at 10 nmol and completely blocked at 100 nmol (Freir *et al.*, 2001). The effects of this Aβ fragment on LTP were probably mediated via a postsynaptic mechanism because they did not affect paired pulse facilitation (Freir *et al.*, 2001).

$A\beta_{1-42}$ has also been reported to facilitate the induction of LTD and depotentiation of LTP in the CA1 area of the rat hippocampus *in vivo* in an NMDA receptor-dependent manner (Kim *et al.*, 2001). Similarly, soluble Aβ oligomers from several sources (synthetic, cell culture, human brain extracts) facilitated NMDA- and mGluR-dependent LTD in the CA1 region of hippocampal slices (Li *et al.*, 2009). This Aβ-facilitated LTD was mimicked by the glutamate reuptake inhibitor threo-β-benzyloxyaspartic acid (TBOA) and prevented by an extracellular glutamate scavenger system (Li *et al.*, 2009).

In another study, $A\beta_{1-40}$ caused a long-lasting (2–5 days) depression of CA1 hippocampal EPSPs *in vivo* after i.c.v. injection (1 μL of 3.5 nM) that was prevented by the NMDA receptor antagonist CPP 7 $g \cdot kg^{-1}$ i.p. twice, but there was no change in the ability to induce LTP (Cullen *et al.*, 1996). The same effect was seen in the DG of hippocampal slices (Cullen *et al.*, 1996). In contrast, others have reported that application of $A\beta_{1-40}$ by extracellular perfusion (200 nM) or intracellularly via the recording pipette (100 nM) to the same region *in vitro* resulted in a gradual enhancement of the NMDA receptor-mediated synaptic currents which did not reverse upon washout (Wu *et al.*, 1995a,b).

Pathophysiologically relevant concentrations of naturally secreted dimeric Aβ extracted directly from the cerebral cortex of subjects with AD potently (pM) inhibited LTP, enhanced LTD and reduced dendritic spine density in normal rat hippocampal slices (Shankar *et al.*, 2008). NMDA receptors were required for the spine loss. Insoluble amyloid plaque cores from AD cortex did not impair LTP unless they were first solubilized to release Aβ dimers, suggesting that plaque cores are largely inactive but sequester Aβ dimers that are synaptotoxic. This same extracted Aβ also disrupted memory in normal rats (Shankar *et al.*, 2008). Similary, i.c.v. injection of Aβ-containing aqueous extracts of AD brain robustly inhibited LTP without significantly affecting baseline excitatory synaptic transmission in the rat hippocampus *in vivo* (Barry *et al.*, 2011).

Effects of Aβ on NMDA receptor-dependent learning

In contrast to *in vitro* experiments, it is much more difficult in behavioural studies to provide evidence supporting a specific effect of Aβ on NMDA receptor-dependent learning. One

type of evidence is based on studies showing that impairment of learning/memory produced by Aβ is attenuated by NMDA receptor antagonists. However, the authors are aware of the fact that it is not really strong evidence since correction of the deficit may be achieved by a different mechanism than that causing the deficit. One example could be the fact that some effects of $A\beta_{1-40}$ are also attenuated by AChE inhibitors (AChEIs) (Yamada *et al.*, 2005). Another type of supporting evidence, even less direct, comes from data obtained in transgenic animals. Here, we have to assume that the learning/memory deficit comes solely from overproduction of Aβ. Being aware of these limitations we provide below selected evidence. We should mention that a review on the effects of exogenous Aβ on behavioural parameters, in general, has been recently published by our group (Chambon *et al.*, 2011).

The only evidence not based on the use of NMDA receptor antagonists, but an agonist, was generated by Sipos *et al.* (2007). They showed that $A\beta_{1-42}$ injected bilaterally into the entorhinal cortex of rats did not affect spatial working memory (alternation task, 10–17 days later) but produced deficits in recognition memory in an object recognition task and the Morris water maze (MWM), where a hidden platform has to be found. This pattern of behavioural deficits mirrored the effects of NMDA administration supporting that similar mechanisms could play a role.

The NMDA receptor channel blocker (+)MK-801 ($2.5\ mg{\cdot}kg^{-1}$) applied 2 h before $A\beta_{1-42}$ injected into the nucleus basalis of Meynert (NBM) prevented passive avoidance learning deficits assessed 2 weeks later (Harkany *et al.*, 1999). It should, however, be stressed that the dose of (+)MK-801 used was very high, and saturation of NMDA receptors would be expected with (+)MK-801 below $0.5\ mg{\cdot}kg^{-1}$. The evidence supporting the role of NMDA receptors using memantine against deficits caused by i.c.v. injection of Aβ in transgenic animals has been discussed in the sections 'Effects of memantine on the toxic actions of Aβ *in vivo*' and 'Effects of memantine in transgenic models of AD' respectively. Nevertheless, in summary, in the majority of studies memantine has been shown to correct many of the deficits, presumably resulting from Aβ administration or overproduction, giving support for the role of NMDA receptors in learning deficits in transgenic AD animals.

Is the net effect hypo- or hyperactivity of NMDA receptor system?

Although it is still not clear whether NMDA receptors are directly or indirectly activated/modulated by Aβ, enhanced NMDA receptor sensitivity might, intuitively, rather be expected to enhance LTP. However, we propose similar mechanisms as discussed above for the impairment of synaptic plasticity/learning during conditions of chronic, non-phasic activation of NMDA receptors. This has been demonstrated, for example, for LTP following reduction in Mg^{2+} concentration (Coan *et al.*, 1989), application of non-toxic concentrations of NMDA (see above) (Parsons *et al.*, 2007) as well as in glutamate transporter 1 (GLT-1) knockout mice (Katagiri *et al.*, 2001).

The direct and indirect effects of Aβ on NMDA receptor function are likely to keep the ion channel tonically open in AD. This chronic pathological activation would be expected to cause a constant mild influx of Ca^{2+}, even under resting conditions, greatly increasing the level of background noise at the postsynaptic terminal. As a result, incoming physiological signals may not be distinguished against this raised background noise and, consequently, synaptic plasticity, LTP and learning/memory could be impaired. Ultimately, the excessive influx of Ca^{2+} ions could cause death of the postsynaptic neuron via associated effects, such as the formation of free radicals, changes in nuclear chromatin and DNA breakage (Danysz *et al.*, 2000; De Felice *et al.*, 2007a; Parsons *et al.*, 2007) – see Figure 1.

Prolonged exposure to $A\beta_{1-42}$ probably also induces a reactive endocytosis of both NMDA and AMPA receptors subsequent to their tonic pathological activation (Bi *et al.*, 2002; Snyder *et al.*, 2005; Hsieh *et al.*, 2006). Even following such reactive receptor down-regulation, the remaining receptors are likely to be sensitized and continue mediating negative effects of Aβ as concentrations of glutamate may also increase due to inhibition of glial uptake (Harkany *et al.*, 2000).

These aspects provide a rational for therapeutic intervention focusing on inhibition of NMDA receptors.

Caveats to most data presented in Tables 1–3

It should be stressed that the vast majority of studies on Aβ suffer from technical obstacles. The most problematic is related to the fact that therapeutically relevant extracellular Aβ concentrations are actually only within the high pM to low nM range. Unfortunately, such concentrations do not usually produce negative effects on neuronal function in the short time frame used for experiments, in particular when the system is not additionally challenged with other insults (e.g. NMDA agonists, oxidative stress, etc.). In turn, much higher concentrations of Aβ are typically used (see Tables 1–3), usually in the 10–100 μM range, to achieve faster effects on neurons. The caveat is that such high concentrations are probably not so relevant for the *in vivo* situation in AD as different aggregation pathways/target protein interactions are likely to be operative. The same caveats apply to the frequently used 'toxic' sub-fragments of Aβ such as the sequence from amino acids 25 to 35.

Memantine

Strong support for the clinical relevance of such interactions between Aβ, glutamate and NMDA receptors in AD is provided by the NMDA receptor antagonist memantine. This substance is the only NMDA receptor antagonist used clinically in the treatment of AD and therefore offers an excellent tool to facilitate translational extrapolations from *in vitro* studies through *in vivo* animal experiments to its ultimate clinical utility.

Mechanism of action of the NMDA receptor antagonist memantine

Memantine is an uncompetitive NMDA receptor antagonist with strong voltage dependency and rapid unblocking kinet-

ics (Kornhuber *et al.*, 1989; Chen *et al.*, 1992; Parsons *et al.*, 1993). These properties have been proposed to allow memantine to prevent the tonic pathological influx of Ca^{2+} and oxidative stress in postsynaptic neurons, whilst preserving the transmission of strong transient physiological signals, which can then be detected against reduced levels of background noise (Parsons *et al.*, 1993; 2007) – see Figure 2. Therefore, as for the Mg^{2+} block in the healthy brain, memantine block of the NMDA receptor channel is transiently relieved during temporally and/or spatially convergent/co-operative activation of glutamatergic synapses – for example, during learning and memory processes.

Memantine has recently been shown to selectively target extrasynaptic 'death' receptors, which are mainly composed of NR2B subunits and coupled to different signalling pathways than the physiologically more relevant subsynaptic receptors (Okamoto *et al.*, 2009; Xia *et al.*, 2010) and may be of particular relevance for pathologically processes in AD (Bordji *et al.*, 2010; Rammes *et al.*, 2011). This moderate selectivity probably has little to do with true pronounced subtype selectivity of memantine at NR2B receptors (see e.g. Bresink *et al.*, 1996), as discussed in these studies, but rather relates to the more moderate but prolonged membrane depolarization at these receptor loci and the voltage dependency of memantine.

Effects of memantine on the toxic actions of Aβ in vivo

In vivo, infusion of memantine prevented the development of errors in the delayed non-matching to sample lever pressing task produced by i.c.v. infusion of $A\beta_{1-40}$ in rats (Yamada *et al.*, 2005). I.c.v. infusion of $A\beta_{25-35}$ in rodents decreased the level of immunoreactive somatostatin and substance P in the hippocampus prior to neuronal loss or caspase activation, which was correlated with the loss of spine density and activation of inducible NOS (iNOS) (Arif and Kato, 2009; Arif *et al.*, 2009). Memantine, attenuated these $A\beta_{25-35}$-induced changes of neuropeptides, their metabolizing enzymes, glial marker proteins and activation of iNOS.

In the hippocampus, s.c. semi-chronic infusions of memantine (15 $mg \cdot kg \cdot day^{-1}$) prevented neuronal cell loss and apoptosis induced by the direct injection of $A\beta_{1-40}$ into this structure (Miguel-Hidalgo *et al.*, 2002). Memantine treatment decreased lesions, glial fibrillary acidic protein (GFAP) staining, ED1-labelled Aβ deposits and the number of pyknotic/fragmented cell nuclei in the hippocampus, indicating that reduction of apoptotic cell death involved in this effect. This study was later extended to demonstrate clear attenuation of apoptosis and active avoidance learning deficits (Miguel-Hidalgo *et al.*, 2006).

Memantine (10, 20 $mg \cdot kg \cdot day^{-1}$ s.c. infusion for 6 weeks) starting 24 h before $A\beta_{1-40}$ injection into the rat hippocampus (followed 2 days later by ibotenic acid) significantly prevented learning deficits in the MWM, which emerged 5 weeks after $A\beta_{1-40}$ injection (Nakamura *et al.*, 2006). A lower dose of memantine (5 $mg \cdot kg \cdot day^{-1}$) and relatively high doses of (+)MK-801 (0.312, 0.624 $mg \cdot kg \cdot day^{-1}$) were without beneficial effect. Neuronal damage in the hippocampus, assessed via elevation in levels of the peripheral-type benzodiazepine-binding site (a gliosis marker for neuronal damage) and Cresyl violet staining, was significantly attenuated by memantine (10, 20 $mg \cdot kg \cdot day^{-1}$) and (+)MK-801 (0.624 $mg \cdot kg \cdot day^{-1}$) (Nakamura *et al.*, 2006). In naïve rats, (+)MK-801 produced a significant learning impairment in the MWM task at a dose of 0.624 $mg \cdot kg \cdot day^{-1}$, whilst memantine (20 $mg \cdot kg \cdot day^{-1}$ s.c. infusion) did not (Nakamura *et al.*, 2006). These results suggest that whilst both memantine and (+)MK-801 exert protective effects on progressive neuronal damage caused by Aβ, only memantine prevents memory impairment in hippocampal-lesioned rats.

Local injection of Aβ into the NBM caused a conformation-dependent enhancement of cortical NOS activity, which was blocked by the NR2B selective antagonist ifenprodil (OMahony *et al.*, 1998). Memantine also rescued the neocortical cholinergic fibres originating from NBM cholinergic neurons, attenuated microglial activation around the intracerebral lesion sides, and improved attention and memory in object recognition and passive avoidance tasks after i.c.v. $A\beta_{1-42}$ injection (Nyakas *et al.*, 2011). In this study, $A\beta_{1-42}$ was claimed to consist mainly of monomers and tetramers. Memantine is also protective against various other toxic insults to this cholinergic structure including NMDA, mitochondrial toxins and LPS (Wenk *et al.*, 1995; 1996; 1997).

Systemic treatment with memantine (1 $mg \cdot kg^{-1}$) prevented the $A\beta_{1-42}$ induced perseveration errors in a food reinforced lever pressing task (Klyubin *et al.*, 2011). Interestingly, in this study $A\beta_{1-42}$ was injected i.c.v. 2 h before testing whilst memantine was injected 45 min before testing, meaning that the effect observed was reversal, rather than prevention, of $A\beta_{1-42}$-induced deficits.

Effects of memantine on the toxic actions of Aβ in vitro

Memantine has been shown to attenuate the deleterious action of Aβ *in vitro* in numerous studies. Early data indicated that the toxic effects of $A\beta_{1-40}$ in cultured cortical neurons were blocked for 48 h by brief exposure to memantine (Tremblay *et al.*, 2000). Similarly, $A\beta_{1-42}$-induced reduction of neurite outgrowth in neuronal cultures was attenuated by memantine (Hu *et al.*, 2007). The vicious circle of Aβ activating NMDA receptors, which then cause a further increase in Aβ production, was blocked by low, 1 μM concentrations memantine (Floden *et al.*, 2005). Memantine also concentration-dependently inhibited extrasynaptic NMDAR-induced KPI-APPs expression as well as neuronal production and release of Aβ (Bordji *et al.*, 2010)

It has also been claimed that the ability of memantine to protect rat cortical cultured neurons against Aβ-induced toxicity is a secondary consequence of attenuating tau phosphorylation (Song *et al.*, 2008). Thus, in primary mouse cortical neurons, calmodulin-dependent protein kinase β (CaMKK β) activation of AMPK in response to $A\beta_{1-42}$ leads to increased phosphorylation of tau at S262/S356 and S396 (Thornton *et al.*, 2011). This effect was blocked by memantine providing a possible mechanism of action (MoA) for its positive effects on the pathophysiological phosphorylation of tau observed in patients (Thornton *et al.*, 2011).

In contrast, neither memantine nor (+)MK-801 showed any neuroprotective effect against overt toxicity in cultured septal cholinergic neurones following 1 week treatment with a high concentration of $A\beta_{1-42}$ (5 μM), whereas donepezil

concentration-dependently reduced LDH efflux (Kimura *et al.*, 2005).

More recent attention has been placed on the toxic role of low nM concentrations of oligomeric Aβ species *in vitro*. Chronic exposure of hippocampal cultures to soluble $A\beta_{1-42}$ oligomers (ADDLs) produced abnormal spine morphology and a decrease in their density and the formation of ROS (Lacor *et al.*, 2007). Associated consequences of this synaptic deterioration including loss of the spine cytoskeletal protein drebrin were completely prevented by memantine 5 μM (Lacor *et al.*, 2007; Lambert *et al.*, 2007). The same group showed that memantine also completely protected against Aβ-induced ROS (De Felice *et al.*, 2007a).

NMDA and oligomeric $A\beta_{1-42}$ induce ROS production from cortical neurons through activation of NADPH oxidase. ROS derived from NADPH oxidase leads to activation of ERK1/2, phosphorylation of cPLA(2)α and AA release. $A\beta_{1-42}$-induced AA release was inhibited by both memantine and D-APV, providing strong support that these toxic effects of Aβ are mediated via NMDA receptors (Shelat *et al.*, 2008). Memantine, as well as two further NMDA receptor antagonists (D-APV, (+)MK-801), prevented the disruption of axonal trafficking of dense-core vesicles and mitochondria by 500 nM $A\beta_{1-42}$ oligomers (ADDLs) (Decker *et al.*, 2010b). Signal transduction by neuronal insulin receptors (IR) is also strikingly sensitive to disruption by soluble $A\beta_{1-42}$ oligomers (ADDLs) in mature cultures of hippocampal neurons, an effect that was completely blocked by relatively high concentrations of memantine (20 μM, lower concentrations unfortunately were not tested) (Zhao *et al.*, 2008).

Recently, Aβ oligomers were reported to induce inward non-desensitizing currents in *Xenopus* oocytes expressing the NMDA receptor subunits NR1/NR2A and NR1/NR2B that were blocked in the presence of memantine, D-APV and (+)MK-801 (Texido *et al.*, 2011). Interestingly, the responses to Aβ oligomers was greater for NR1/NR2A heteromers than for NR1/NR2B heteromers. Similar increases in the cytosolic concentration of Ca^{2+} induced by Aβ oligomers in cortical neurons were only slightly attenuated by the NR2B preferring NMDA receptor antagonist ifenprodil, indicating that Aβ oligomers directly activate NMDA receptors, particularly those with the NR2A subunit (Texido *et al.*, 2011). In contrast, others found no effect of oligomeric Aβ on glutamate-induced currents in *Xenopus* oocytes expressing NMDA receptors under conditions where clear effects of voltage-gated P/Q-type calcium channels were seen (Mezler *et al.*, 2011).

Vesicular zinc released during neurotransmission has been reported to be critical for targetting synaptically released $A\beta_{1-42}$ oligomers to extrasynaptic NR2B containing NMDA receptors, an effect blocked by memantine (10 μM) as well as by other NMDA receptor antagonists (Deshpande *et al.*, 2009).

To examine the specific effects of memantine on Aβ-induced deficits in LTP, extracellular field EPSPs (fEPSPs) were obtained from the dendritic region of the CA1 region of hippocampal slices from adult mice (Albrecht *et al.*, 2008; Martinez-Coria *et al.*, 2010). Under control conditions, fEPSPs were found to be potentiated 60 min after the stimulus was applied (100 Hz, 1 s). Administration of memantine alone (1 μM) did not affect this synaptic plasticity. After washing in $A\beta_{1-42}$ globulomers (42 nM)(Barghorn *et al.*, 2005), the same stimulus produced only short-term potentiation (returning to baseline after 60 min). However, when memantine was applied together with the Aβ globulomers, LTP was completely developed. Therefore, at a clinically relevant concentration, memantine was able to reverse the complete block of LTP by Aβ oligomeric globulomers.

Bell-shaped dose response relationship of memantine

Similar results were recently published for hippocampal LTP both in the DG *in vitro* (memantine 1 μM) and in the CA1 *in vivo*, although the therapeutic window *in vivo* in this study was quite narrow as effects were lost at higher doses of memantine (acute at 10 but not at 20 $mg \cdot kg^{-1}$ i.p. was effective) (Klyubin *et al.*, 2011). Systemic administration of a sub threshold dose of memantine (1 $mg \cdot kg^{-1}$ i.p.) in an operant learning task also prevented the $A\beta_{1-42}$-mediated increase in perseveration errors in this same study (Klyubin *et al.*, 2011). Low concentrations of memantine (1 μM) prevented $A\beta_{1-42}$ oligomer (82 nM)-induced deficits in neurotransmission in organotypic hippocampal slice cultures, and this effect was also lost at higher concentrations (3 and 10 μM) (Nimmrich *et al.*, 2010).

Such bell-shaped dose–response relationships are often seen with memantine and seem to be inherent to its MoA; that is, positive effects are seen following moderate negative modulation of NMDA receptor function but lost if the NMDA receptor is blocked more strongly by higher concentrations of memantine, which then also block synaptic activation by higher mM concentrations of glutamate (Zajaczkowski *et al.*, 1997; Frankiewicz and Parsons, 1999; Zoladz *et al.*, 2006). Our previous study showed that at the doses producing positive effect there is c.a. 30% NMDA receptor occupancy *in vivo* (More *et al.*, 2008).

Effects of memantine on levels of Aβ

Treatment of human neuroblastoma (SK-N-SH) cells with memantine (50 nM–50 mM) increased the levels of sAPPα in the conditioned media without affecting the levels of total intracellular APP (Chen *et al.*, 2002a). This increase in sAPPα secretion by memantine, without a concomitant increase in the cellular APP levels, was taken to suggest that memantine may enhance the α-secretase (non-amyloidogenic) pathway. Memantine treatment increased cell viability and metabolic activity. In contrast, others have reported that memantine (10 μM) decreased the levels of the secreted form of sAPP, sAPPα and $A\beta_{1-40}$ in similar cells (Ray *et al.*, 2010). Similarly, memantine (1–4 μM) decreased levels of secreted APP, $A\beta_{1-40}$ and $A\beta_{1-42}$ and also lowered $A\beta_{1-42}$ secretion in neuroblastoma cells and primary cultures of cortical neurons (Alley *et al.*, 2009). It is unclear how memantine could affect levels of Aβ and related peptides (see also next section), but this effect could be unrelated to NMDA receptor antagonism and rather associated with the known lysotrophic properties of this amphiphlic molecule (Honegger *et al.*, 1993).

Effects of memantine in transgenic models of AD

In APP/presenilin 1(PS1) double transgenic (TG) mice, memantine (30 $mg \cdot kg^{-1}$ p.o. daily for 2–3 weeks) significantly

improved memory acquisition in the MWM but did not affect swimming speed, locomotor activity or aggressive behaviour (Minkeviciene *et al.*, 2004). These data indicate that memantine improves hippocampus-based spatial learning without inducing non-specific effects on locomotion or exploratory activity. In the same TG mice, oral dosing of memantine (20 mg kg^{-1} day^{-1} for 8 days) significantly reduced the elevated cortical levels of soluble $A\beta_{1-42}$ (Alley *et al.*, 2009).

Memantine has also shown beneficial effects in a study in 4–month-old TG APP23 mice, which develop amyloid plaques, accompanied by astrogliosis and microgliosis, at the age of just 6 months, thus presumably reflecting the prodromal stage of AD (Sturchler-Pierrat *et al.*, 1997; Sturchler-Pierrat and Staufenbiel, 2000). At the age of 6 weeks (i.e. before plaque formation), the mice received an s.c. infusion of memantine at various doses for 2 months or saline. After a wash-out phase of 3 weeks, their learning ability and cognitive function was investigated in the MWM. Treatment with memantine significantly improved both learning (distance covered during training) and memory (accuracy with which the platform was found)(Van Dam and De deyn, 2006). This effect of memantine treatment was observed 3 weeks after treatment termination, thus indicating disease modification (Van Dam and De deyn, 2006).

Long-term administration of memantine (10 and 20 mg kg^{-1} day^{-1} for 6 months) to Tg2576 mice was associated with a significant decrease in Aβ plaque deposition, increases in synaptic density and lowered appearance of degenerating axons (Dong *et al.*, 2008). Administration of a lower dose of memantine (5 mg·kg^{-1}) was also associated with a significant decrease in Aβ plaque deposition and a significant increase in synaptic density, but no significant effect on degenerating axons was observed (Dong *et al.*, 2008). However, memantine did not significantly improve behavioural deficits in these mice in a fear-conditioning paradigm at any dose. Others have reported that memantine treatment reduced the total cortical levels of membrane-bound APP(45–55%) in both Tg2576 mice and non-transgenic mice *in vivo* (Unger *et al.*, 2006).

Tg2576 mice (8-month-old) treated with memantine (30 mg kg^{-1} day^{-1}) with or without folic acid (8 mg kg^{-1} day^{-1}) for 4 months showed learning improvements in the MWM and less neuronal damage accompanied by an up-regulation of CNS genes involved in neurogenesis, neural differentiation, memory and neurotransmission (Chen *et al.*, 2010).

The effects of sub-chronic memantine administration on spatial and non-spatial learning as well as exploratory activity and nest-building in APP/PS1 mutant mice have also been assessed (Filali *et al.*, 2011). Memantine (10 mg·kg^{-1}, i.p.) improved reversal of left–right discrimination but had no effect in the MWM, passive avoidance learning or non-learned behaviours such as elevated plus-maze exploration and nest building. APP/PS1 TG mice treated with memantine for a period of 4 months starting at 3 months of age performed as well as wild-type control mice in a novel object recognition task (Scholtzova *et al.*, 2008). Memantine-treated TG mice had a reduced plaque burden detected with μMRI, which correlated with the improvement in cognitive performance (Scholtzova *et al.*, 2008).

Most recently, LaFerla's group (Martinez-Coria *et al.*, 2010) examined the therapeutic and neuroprotective effects of memantine in triple TG AD mice (APP, PS1 and tau mutations). In the therapeutic arm of the study, adult mice (aged 9 months), with established plaque and NFT pathology, were treated with memantine (30 mg kg^{-1} day^{-1} via drinking water) or placebo for 3 months (model of established AD). In these animals, memantine significantly improved spatial memory (MWM) and fear conditioning, but not novel object recognition. In the preventive arm of the study, young mice (aged 2 months), with no detectable pathology or behavioural deficits, were treated with memantine (30 mg kg^{-1} day^{-1} in drinking water) or 'placebo' for 10 months (model of developing AD). In these animals, memantine significantly improved fear conditioning and retention of spatial memory, but not acquisition of spatial memory or novel object recognition. Interestingly, the same treatment with memantine again resulted in a decrease in plaque load (Luhrs *et al.*, 2006; Martinez-Coria *et al.*, 2010). These results indicate that memantine is able to slow cognitive decline in younger transgenic mice, as well as to reverse established cognitive deficits in older transgenic mice.

Secondary pathological processes blocked by memantine

As introduced above, various processes overlap and influence one another in AD pathogenesis (e.g. Aβ accumulation, excitotoxicity at NMDA receptors, formation of tau neurofibrils, disturbance of mitochondrial function and neuroinflammation) (Peng and Greenamyre, 1998; Duchen, 2000; De Felice *et al.*, 2007a). Neuroprotective effects have been described for memantine in numerous preclinical models of various chronic neurodegenerative diseases asides from AD (Danysz *et al.*, 2000; Rosi *et al.*, 2006). Secondary mitochondrial function disturbances may contribute to the neuronal damage observed in AD. As with the NMDA-induced lesions, long-term treatment with memantine has been shown to protect against the damaging effects of mitochondrial toxins and hypoxia (Schulz *et al.*, 1996; Wenk *et al.*, 1996; Karanian *et al.*, 2006; Volbracht *et al.*, 2006). Inflammatory processes are also thought to contribute to the neurodegenerative changes during AD pathogenesis (Katsuura *et al.*, 1989; Rothwell and Strijbos, 1995; Hanisch *et al.*, 1997; Emerit *et al.*, 2004) and may be linked with the enhanced activation of NMDA receptors [reviewed by (Wenk *et al.*, 2006; Parsons *et al.*, 2007)]. In an animal model of neuroinflammation, memantine (at the therapeutically relevant dose in rats of 20 mg kg^{-1} day^{-1} s.c.) also protected cholinergic neurons from inflammatory processes (Willard *et al.*, 2000). I.c.v. streptozotocin treated rats showed memory deficits and significantly decreased p-GSK3β levels in both the hippocampus and PFC. The memory impairment was reversed by memantine (5 mg·kg^{-1}), but no changes in p-GSK3β levels were seen (Ponce-Lopez *et al.*, 2011).

Neuroprotection in the clinical situation

Whilst numerous preclinical studies have shown the strong neuroprotective potential of memantine, such has been very difficult to translate to the clinical situation, largely due to the difficulty in designing/performing such trials that, in the case of AD, would have to be very large, start early in the prodromal stage of the disease, last several years and include

a wash-out phase. Nonetheless, there are hints from retrospective analyses that memantine does indeed have disease modifying potential in AD patients (Wilkinson and Andersen, 2007; Atri *et al.*, 2008)

Conclusions

The reviewed evidence suggests that the glutamatergic system in general, and NMDA receptors in particular, may play a significant role in the execution of synaptic dysfunction and neuronal death triggered by Aβ in AD. This implies that NMDA receptor antagonists with special features may prevent/attenuate these pathological processes. In fact, memantine, which is an uncompetitive NMDA receptor antagonist with fast, voltage-dependent blocking properties, is able to selectively block pathological tonic NMDA receptor activation in the presence of soluble Aβ oligomers, e.g. (Parsons *et al.*, 2007; Albrecht *et al.*, 2008) whilst preserving their physiological transient synaptic activation. Memantine is hypothesized to provide both symptomatic improvements in, for example, cognition and long-term neuroprotective effects by this same MoA. In fact, several clinical trials have proven such beneficial effects of memantine on symptoms of AD (Reisberg *et al.*, 2003; Tariot *et al.*, 2004; Peskind *et al.*, 2006), and meta-analysis of several trials suggests potential to reduce clinical worsening (Wilkinson and Andersen, 2007; Weiner *et al.*, 2011).

Conflict of interest

Both authors work for a pharmaceutical company.

The drug/molecular target nomenclature used in this review conforms to BJP's Guide to Receptors and Channels (Alexander *et al.*, 2011).

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