

Glutamate system, amyloid β peptides and tau protein: functional interrelationships and relevance to Alzheimer disease pathology

Timothy J. Revett, PhD; Glen B. Baker, PhD, DSc; Jack Jhamandas, MD, PhD; Satyabrata Kar, PhD

Revett, Jhamandas, Kar — Department of Medicine Centre for Prions and Protein Folding Diseases, University of Alberta, Edmonton, Alta.; Baker, Kar — Department of Psychiatry, Centre for Prions and Protein Folding Diseases, University of Alberta, Edmonton, Alta.

Alzheimer disease is the most prevalent form of dementia globally and is characterized premortem by a gradual memory loss and deterioration of higher cognitive functions and postmortem by neuritic plaques containing amyloid β peptide and neurofibrillary tangles containing phospho-tau protein. Glutamate is the most abundant neurotransmitter in the brain and is essential to memory formation through processes such as long-term potentiation and so might be pivotal to Alzheimer disease progression. This review discusses how the glutamatergic system is impaired in Alzheimer disease and how interactions of amyloid β and glutamate influence synaptic function, tau phosphorylation and neurodegeneration. Interestingly, glutamate not only influences amyloid β production, but also amyloid β can alter the levels of glutamate at the synapse, indicating that small changes in the concentrations of both molecules could influence Alzheimer disease progression. Finally, we describe how the glutamate receptor antagonist, memantine, has been used in the treatment of individuals with Alzheimer disease and discuss its effectiveness.

Introduction

In 2005 there were an estimated 24.3 million individuals with dementia worldwide, with Alzheimer disease accounting for more than 60% of these cases.¹ The etiology of Alzheimer disease is not yet fully understood; however, genetic and environmental factors contribute to disease pathogenesis and progression. Linkage studies indicate that a small proportion of individuals with Alzheimer disease have an autosomal dominant pattern of inheritance and implicate 3 genes in early onset familial forms of Alzheimer disease: those for amyloid β precursor protein (*APP*), presenilin 1 (*PSEN1*) and *PSEN2*.² A small proportion of early-onset Alzheimer disease cases are linked to *APP*, which is situated on chromosome 21. This gene is triplicated in Down syndrome, which invariably leads to the development of Alzheimer disease pathology, indicating that increased expression of *APP* could facilitate Alzheimer disease progression. Whereas mutations in this gene can also lead to the development of early-onset Alzheimer disease by altering/increasing the proteolytic

cleavage of *APP*, most cases are linked to point mutations in the *PSEN* genes, especially *PSEN1*.^{2,3} Although these findings are critical in deducing the biological pathogenesis of Alzheimer disease, it must be remembered that mutations in these 3 genes are responsible for only 30%–50% of early-onset Alzheimer disease cases, which in turn only represents 6%–8% of all Alzheimer disease cases, suggesting that many other factors are involved in disease pathology.^{2,3} There are also genetic risk factors associated with late-onset Alzheimer disease, but in most instances there has been no genetic cause of the disease identified as yet. One such genetic risk factor is possession of 1 or 2 copies of the $\epsilon 4$ allele of the gene for apolipoprotein E, which has been shown to increase the probability of Alzheimer disease developing, whereas the presence of an $\epsilon 2$ allele appears to protect against the disease.² A number of recent studies have shown that variants in some other genes, such as those for clusterin on chromosome 8,^{4,5} phosphatidylinositol-binding clathrin assembly protein on chromosome 11,^{4,5} complement receptor 1 on chromosome 1,⁵ bridging integrator 1 on chromosome 2⁶ and sortilin-related

Correspondence to: S. Kar, Centre for Prions and Protein Folding Diseases, Departments of Medicine (Neurology) and Psychiatry, University of Alberta, Edmonton AB T6G 2M8; skar@ualberta.ca

J Psychiatry Neurosci 2013;38(1):6-23.

Submitted Dec. 8, 2011; Revised Apr. 3, 30, 2012; Accepted May 7, 2012.

DOI: 10.1503/jpn.110190

receptor 1 on chromosome 11,⁷ may be associated with an enhanced risk for Alzheimer disease, but these susceptibility genes need to be validated in future studies.^{8,9} The lack of conclusive genetic linkage in most Alzheimer disease cases not only highlights the possible existence of unidentified genetic risk factors, but also the importance of environmental factors in the development of disease pathology. There is increasing evidence that lower educational and occupational attainment, female sex, low mental ability in early life and reduced mental and physical activity during late life increase the risk for the disease. Moreover, several epidemiological studies have shown that head trauma,¹⁰ stroke and heart disease,¹¹ atherosclerosis, hypercholesterolemia, smoking, obesity and diabetes¹² can also increase the risk for Alzheimer disease.¹³ Whether these are true causal risk factors driving the pathogenic process or whether they add to the clinically silent disease pathology remains to be established. However, among all these factors, age is considered to be by far the greatest risk factor for late-onset Alzheimer disease. Therefore, as the baby boomers enter their mid-60s, the prevalence of Alzheimer disease will increase rapidly, possibly by as many as 4.6 million new cases per year.¹

Alzheimer disease is characterized by 3 distinct major neuropathological abnormalities: intracellular neurofibrillary tangles, extracellular plaques and neuronal loss.^{14,15} Neurofibrillary tangles are intracellular aggregates of the hyperphosphorylated microtubule-associated protein, tau. In normal healthy neurons, tau stabilizes microtubules that form the cytoskeleton of the cell by a process involving phosphorylation and dephosphorylation of the protein.¹⁶⁻¹⁸ Phosphorylated tau is unable to bind microtubules and instead polymerizes with other tau molecules, forming straight filaments that subsequently form paired helical filaments. This may cause a failure of neuronal transport that eventually leads to cell death.^{17,18} Tangles are particularly abundant in the entorhinal cortex; hippocampus; amygdala; association cortices of the frontal, temporal and parietal lobes; and certain subcortical nuclei that project into these areas.^{15,19} Interestingly, the number and distribution of cortical tangles correlates positively with cognitive deficits and serves as a good marker of disease progression.^{15,19}

Extracellular plaques are formed by the aggregation of amyloid β , a short peptide of 39–42 amino acids in length. This peptide is produced by the sequential cleavage of APP, which exists in the brain predominantly in 3 different isoforms. The shortest isoform, APP695, is mostly expressed by neurons, whereas the 2 longer isoforms, APP751 and APP770, are expressed predominantly in glial cells, such as astrocytes.²⁰⁻²³ At extracellular sites within the brain parenchyma, amyloid β forms compact deposits, often seeded by aggregation of the longer amyloid β 1–42 peptide, that are associated with dystrophic neurites, activated microglia and reactive astrocytes.^{15,23} Extracellular amyloid β -containing plaques are apparent in most areas of the brain in Alzheimer disease individuals and are associated with neuronal loss in areas such as the entorhinal cortex, hippocampus and association cortices.¹⁹ The number of plaques present in the brain does not correlate with the severity of dementia, but a clinical

correlation between elevated levels of total amyloid β peptide in the brain and cognitive decline has been reported.²⁴ There is also a growing consensus that amyloid β peptide increases tau phosphorylation and initiates multiple pathways that lead to neuronal cell death.²⁵ These pathways include increased oxidative stress,²⁶ altered Ca^{2+} homeostasis^{27,28} and excitotoxicity,^{14,29} which trigger apoptotic dependent and independent cell death mechanisms. The significance of amyloid β peptide in disease pathology has been highlighted by the fact that a number of small compounds that inhibit amyloid β formation are currently undergoing clinical testing as potential therapies for Alzheimer disease.³⁰ Additionally, there is strong experimental data from animal studies to suggest that active amyloid β immunotherapy has disease modifying potential in individuals with Alzheimer disease. Although the first clinical trials with AN1792 (from Elan/Wyeth), a synthetic amyloid β 1–42 peptide along with QS21 as adjuvant, were halted prematurely when subacute meningoencephalitis developed in 6% of the vaccinated individuals, the drug was effective in clearing amyloid β plaques and improving performance on some neuropsychological tests. However, autopsy studies of some individuals revealed no association between vaccination with AN1792 and onset of severe dementia or survival, even among individuals whose brains were virtually devoid of plaques.^{31,32} Other clinical trials with passive immunotherapy confirmed the occasional appearance of microhemorrhage and the occurrence of vasogenic edema in some individuals, particularly those with apolipoprotein E e4 genotypes.^{31,33} Nonetheless, several ongoing clinical trials with different amyloid β fragments and with humanized amyloid β antibodies are likely to indicate not only the importance of amyloid β in Alzheimer disease pathogenesis, but also whether antiamyloid β therapy can modify the disease pathology.

Degenerating neurons and synapses in the brains of patients with Alzheimer disease are usually located within regions that project to or from areas displaying high densities of plaques and tangles. Severely affected regions include the hippocampus, entorhinal cortex, amygdala, neocortex and some subcortical areas, such as basal forebrain cholinergic neurons, serotonergic neurons of the dorsal raphe and noradrenergic neurons of the locus coeruleus.^{15,34} There is evidence that glutamatergic neurons located in the hippocampus and in the frontal, temporal and parietal cortex are severely affected, whereas similar neurons in the motor and sensory cortex are relatively spared.^{19,35} Since the hippocampus and cortex are essential for learning and memory, it is possible that degeneration of glutamatergic neurons could be an early event in the pathogenesis of the disease. A recent study investigating changes in the glutamatergic system has shown a greater correlation between reduced glutamatergic presynaptic bouton density and cognitive deficits than that for tangle or amyloid β burden, as determined by comparing mini mental status examination scores of healthy individuals to those of individuals with mild or severe Alzheimer disease.³⁶ Interestingly, the study also found that there was an increase in glutamatergic synapses in individuals with mild cognitive impairment, suggesting that there may be an initial

compensatory event occurring in the brains of such individuals that decays with disease progression. This phenomenon has already been observed in the cholinergic system, where there is an increase in choline acetyltransferase activity in individuals with mild cognitive impairment and a subsequent reduction in the enzyme activity as Alzheimer disease worsens.^{37,38} Studies from animal models of Alzheimer disease have shown that upregulation of cholinergic presynaptic boutons occurs before the involvement of glutamatergic terminals, thus raising the possibility that a compromised cholinergic system may affect the functioning/survival of glutamatergic neurons in the brain.³⁹ Indeed, pyramidal neurons of the cortex that use glutamate as their primary transmitter are known to possess both cholinergic and glutamatergic receptors and receive inputs from the basal forebrain cholinergic neurons.³⁵ There is evidence that cholinesterase inhibitors can promote the release of glutamate from pyramidal neurons, possibly by increasing cortical acetylcholine levels and subsequent activation of the cholinergic receptors.⁴⁰ In addition, it has been demonstrated that uncoupling of the postsynaptic muscarinic M1 receptor from G-proteins can correlate with the loss of N-methyl-D-aspartate (NMDA) receptor density and protein kinase C (PKC) activity in the postmortem frontal cortex of individuals with Alzheimer disease.⁴¹ These results provide a neurochemical basis of interactions between cholinergic and glutamatergic systems and their potential implications in triggering pathological abnormalities in Alzheimer disease. However, further investigations using complementary approaches are needed to evaluate other components of their functions during aging and the progression of Alzheimer disease. This review gives an overview of the interactions between the central glutamate system amyloid β peptides and tau protein and their relevance in the development of Alzheimer disease pathology.

Neuropathological changes in the glutamate system in Alzheimer disease

Glutamate is the major excitatory neurotransmitter in the central nervous system and is known to be involved in a variety of functions, including synaptic transmission, neuronal growth and differentiation, synaptic plasticity and learning and memory.^{14,35} The glutamatergic neurons are situated in areas that are known to be affected in Alzheimer disease, and initial damage begins with the pyramidal neurons in layers III and V of the neocortex^{42,43} and glutamate innervated cortical and hippocampal neurons.³⁵

The role of the glutamatergic system is to convert nerve impulses into a chemical stimulus by controlling the concentration of glutamate at the synapse (Fig. 1). In presynaptic neurons, the vesicular glutamate transporters, VGLUT1 and VGLUT2, maintain the level of glutamate stored in vesicles,⁴⁴ and when a neuron is depolarized, glutamate is released into the synaptic cleft where it binds glutamate receptors on pre- and postsynaptic neurons.⁴⁵⁻⁴⁷ There are 2 families of glutamate receptors located at the plasma membrane of neurons, ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors. The iGluR family is further divided into 3 classes of

receptor, which are based on specific agonists and/or permeability to different cations; NMDA receptors (NR1, NR2A–D and NR3A–B) are predominantly Ca^{2+} ion permeable, whereas α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; GluR1–4) and kainate (KA; GluR5–7, KA1–2) receptors are predominantly permeable to Na^+ and K^+ ions.⁴⁵ Currently, there are 8 metabotropic G-protein coupled glutamate receptors, which are classified on the basis of their structure and physiologic function into 3 distinct groups. Group 1 receptors (mGluRs 1 and 5) are coupled to phospholipase C through G_q and G_{11} proteins, whereas group 2 (mGluRs 2 and 3) and group 3 (mGluRs 4 and 6–8) receptors are coupled with adenylyl cyclase through G_i and G_o proteins (Tables 1 and 2).⁴⁵ There is evidence that almost every step in the signalling pathways associated with mGluRs requires or is modulated by Ca^{2+} . For example, the efficacy of mGluR signalling is known to be regulated by physiologically relevant changes in extracellular Ca^{2+} , such that Ca^{2+} depletion in the synaptic cleft can lead to substantial inhibition of postsynaptic mGluR function. There is also increasing experimental data to suggest that Group 1 mGluRs can mobilize intracellular Ca^{2+} not only through inositol 1,4,5-P₃-sensitive Ca^{2+} stores, but also through the ryanodine-sensitive Ca^{2+} stores.^{48,49}

Unlike iGluRs, which are predominantly situated in the postsynaptic membrane to mediate fast excitatory transmission, mGluRs are located in various membrane compartments of both neuronal and glial cells in the brain.⁴⁸ The mGluRs 1, 3, 5 and 7 are widely distributed throughout the brain, whereas mGluRs 2, 4 and 8 are localized in specific brain regions. The only mGluR not found in the brain is mGluR 6, which is expressed in the retina.^{48,50} Whereas most mGluRs in the adult rat are found in neuronal cells, mGluR 3 is localized extensively in glial cells in the brain. There is also evidence that mGluRs 3 and 5 are expressed in reactive astrocytes; that mGluRs 3–5 are localized in cultured astrocytes; and that mGluRs 2, 4, 5 and 8 are apparent in cultured microglia.^{48,50} At the subcellular level, Group 1 mGluRs are mostly localized in somatodendritic domains of neurons, especially in perisynaptic regions, whereas Group 2 mGluRs are evident in the somatodendritic compartment and axonal domains. The Group 3 mGluRs, with the exception of mGluR 6, are present predominantly in the presynaptic active zone of axon terminals.⁴⁸ Functionally, there is convincing evidence that Group 1 mGluRs mediate presynaptic inhibitory effects, that Group 2 mGluRs are activated by the spillover of glutamate from distant synapses and that Group 3 mGluRs may function as autoreceptors. The mechanisms of presynaptic inhibition by Group 2 and Group 3 mGluRs possibly involve suppression of presynaptic voltage-dependent Ca^{2+} channels, activation of K^+ channels and direct inhibition of regulated exocytosis.^{48,51} Following synapse activation, the majority of glutamate is cleared from the synaptic cleft by astrocytes, which express high levels of the glutamate transporters GLAST and GLT-1 in rats.⁵²⁻⁵⁴ The human homologues are the excitatory amino acid transporters 1 and 2 (EAAT1 and 2).^{52,55} In astrocytes glutamate is converted to glutamine by the enzyme glutamine synthetase and transported back to neurons, where glutamine is converted back to glutamate by

glutaminase⁴⁷ and then returned to vesicles in the presynaptic membrane by VGLUT1 and 2. This cycle and transamination of cytosolic aspartate are essential for maintaining glutamate levels in presynaptic terminals (Fig. 1).⁵²

Deficiencies in many stages of the glutamate cycle have been shown to occur in Alzheimer disease, leading to increased concentrations of glutamate around the neurons of the synapse (Table 3). There have been a number of studies that noted reduced levels of VGLUT1 and 2 in the prefrontal cortex of individuals with Alzheimer disease, with VGLUT1 showing a much greater reduction than VGLUT2.^{56,57} More recent studies using synaptosome-enriched fractions also

showed a trend for reduced levels of VGLUT1 and 2 in the parietal cortex of individuals with Alzheimer disease compared with controls, but they did not reach significant levels.⁵⁸ Whether this could be due to the region of the brain examined or the relatively small number of samples used in the study remains to be established. Nevertheless, this study showed that amyloid β peptide accumulates more in VGLUT1/2-containing terminals than in non-VGLUT terminals, thus suggesting that these peptides may preferentially affect presynaptic glutamatergic terminals in the cortex of individuals with Alzheimer disease.⁵⁸ The gene expression and protein level of EAAT1 and EAAT2, on the other hand,

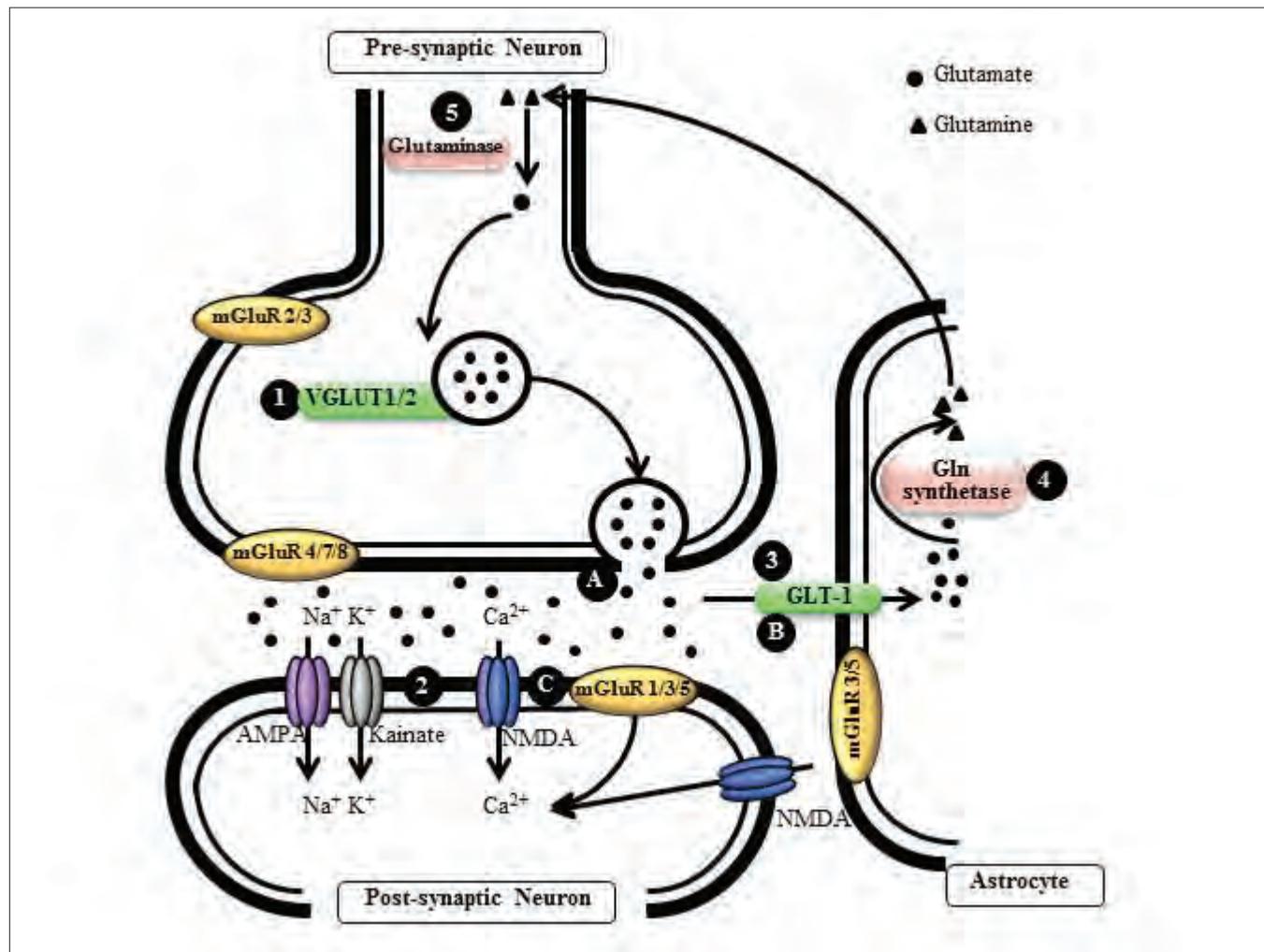


Fig. 1: The glutamate cycle. Glutamate is the most abundant neurotransmitter in the brain, and the levels of the amino acid are maintained in vesicles in the presynaptic neurons by 2 glutamate transporters, (1) VGLUT1 and 2. When the synapse is activated, glutamate is released into the synaptic cleft and activates 2 families of glutamate receptors, (2) ionotropic (iGluRs; N -methyl-D-aspartate [NMDA], α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] and kainate) and metabotropic (mGluRs) receptors. N -methyl-D-aspartate receptors only become fully active after synchronised AMPA receptor activation and depolarization of the postsynaptic membrane. Glutamate receptor activation stimulates further downstream cellular pathways. (3) Glutamate is removed from the synaptic cleft by another group of glutamate transporters, which are mostly expressed in astrocytes, GLT-1 and GLAST in mice or EAAT1 and 2 in humans. Within astrocytes, (4) glutamate is converted to glutamine by glutamine synthetase and (5) returned to the presynaptic terminals where glutaminase converts glutamine back to glutamate. Many aspects of the glutamate cycle are affected in Alzheimer disease, implicating glutamate in disease progression. Amyloid β -related peptides have been shown to (A) increase glutamate release, (B) inhibit clearance of glutamate by astrocytes and (C) affect glutamate receptor activity.

have also been shown to be reduced in the cortex and hippocampus of individuals with Alzheimer disease,^{59,60,71} suggesting that glutamate clearance from the synaptic cleft is attenuated. In one study, it has been demonstrated that reduced levels of EAAT2, produced mainly by astrocytes, inversely correlates with APP751/770 mRNA levels, thus indicating that abnormal functioning and/or processing of APP may be involved in regulating EAAT2 levels/functions.⁷² Changes in glutamate transporter expression have been identified at an early stage of Alzheimer disease, indicating

that dysfunction in glutamate transport might be an early event in the disease pathology.⁷¹ Concurrent with reduced glutamate transporters there is a reduction in glutamine synthetase in Alzheimer disease,⁶¹ thus preventing the transport of glutamate back to presynaptic neurons. High levels of glutamate in astrocytes lead to the risk of glutamate being returned to the synaptic cleft if mitochondrial membranes are depolarized, a process that is thought to occur in Alzheimer disease because of reduced energy supplies.^{73,74} Taken together, these changes in glutamate transport and conversion to glutamine can lead to increased concentrations of glutamate at the synapse, which can subsequently trigger excitotoxic cell death via NMDA receptor-mediated increase of intracellular Ca²⁺ concentrations.^{75,76} Elevated Ca²⁺ levels lead to neuronal death through a number of known pathways, including increased activation of calpain,⁷⁷ release of reactive oxygen species and depolarization of the mitochondrial membrane, which leads to reduced energy metabolism and cytochrome c release.⁷⁷ This ultimately results in neuronal loss through apoptotic and necrotic pathways.⁷⁶

Alterations in the levels of iGluRs remain inconclusive. Some reports suggest that there is an increase in NMDA-sensitive glutamate binding in the striatum of individuals with Alzheimer disease,⁷⁸ whereas other studies indicate that there

Table 1: Classification of the ionotropic glutamate receptors

Receptor agonist	Subunit	Composition	Ion permeability
NMDA	NR1A–H	The functional protein contains a dimer of NR1 or NR2, with or without an NR3 subunit	Ca ²⁺ , Na ⁺
	NR2A–D		
	NR3A–B		
AMPA	GLUR1–4	Heterotetrameric protein containing a dimer of GluR2 and a dimer of either GluR1, 3 or 4	Na ⁺ , K ⁺
Kainate	GLUR5–7 KA 1–2	Heterotetrameric protein containing dimers of GluR5–7 with or without KA 1–2	Na ⁺ , K ⁺

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA = N-methyl-D-aspartate.

Table 2: Classification of the metabotropic glutamate receptors

Group	G protein	Second messenger	Subtype localization	
1	G _q /G ₁₁	Phospholipase C Ca ²⁺	mGluR 1 mGluR 5	Postsynaptic neurons Postsynaptic neurons, reactive astrocytes and cultured microglia
2	G _i /G _o	Adenylyl cyclase	mGluR 2 mGluR 3	Pre- and postsynaptic neurons and cultured microglia Pre- and postsynaptic neurons, reactive astrocytes and cultured astrocytes
3	G _i /G _o	Adenylyl cyclase	mGluR 4 mGluR 5 mGluR 6 mGluR 7 mGluR 8	Presynaptic neurons, cultured astrocytes and microglia Presynaptic neurons, reactive astrocytes and cultured microglia Retina only Presynaptic neurons Presynaptic neurons and cultured microglia

mGluR = metabotropic glutamate receptor.

Table 3: Alterations in the glutamate system in brains of people with Alzheimer disease

Component of the glutamate system	Alteration in the brain
VGLUT1	Reduced protein levels before cell loss and onset of pathology ^{56–58}
VGLUT2	Reduced protein levels before cell loss and onset of pathology ^{56–58}
GLAST/EAAT1	Reduced protein at early clinical stages ^{59,60}
GLT-1/EAAT2	Reduced protein levels at early clinical stages ^{59,60}
Glutamine synthetase	Reduced protein levels ⁶¹
NMDA receptor	Increased protein levels in mild cognitive impairment ³⁶
NR1	Reduced protein levels ⁶²
NR2A–B	Reduced protein levels ⁶³
NR2C–D	Unaffected ⁶³
NR3	Not assessed
AMPA receptor	Variable results indicate that there is initial increase at very early stages before a reduction in GluR1 and increased GluR2/3 at later stages ^{64–66}
GLUR1–3	Not assessed
GLUR4	Reduced receptor binding ⁶⁷
Kainate receptor	Reduced expression ⁶⁸
GLUR5–7	Reduced expression ⁶⁸
Metabotropic receptor	Reduced protein levels ^{67,69}
mGluR 1	Increased protein levels ⁷⁰
mGluR 2	Not assessed
mGluRs 3–7	Not assessed

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; mGluR = metabotropic glutamate receptor; NMDA = N-methyl-D-aspartate.

are no changes in the level of NMDA receptors in the affected areas of the brain in individuals with Alzheimer disease.⁷⁹ However, most reports suggest that there is a decrease in protein and mRNA levels of these receptors, at least in later stages of the disease.^{62,63,80,81} Discrepancies in these results could be explained by differences in the severity of the disease, as there is evidence of increased synaptic activity in individuals with mild cognitive impairment.^{62,81} Autoradiography studies have shown reduced NMDA receptor binding in the CA1 and CA3 regions of the hippocampus, supporting the idea that there are fewer NMDA receptors in these areas of the brain. Western blot analysis and immunohistochemical evidence have also shown changes in the levels of AMPA receptors. These results suggest that there is an initial increase in GluR1 and GluR2/3 levels in membrane preparations taken from the neocortex of individuals with mild cognitive impairment, followed by a reduction in receptor levels in individuals in the early stages of Alzheimer disease, but the levels of GluR2/3 increase in severe cases. A transcriptome analysis of synaptoneuroosomes prepared from Alzheimer disease and control brains also showed an increase in the levels of mRNA encoding GluR2 in the Alzheimer disease samples compared with the controls.⁶⁴ Detailed studies of brains affected by Alzheimer disease have shown that the reductions in AMPA receptors are well correlated to areas of the brain that are affected, but there is substantial variation in AMPA receptor levels among individuals with Alzheimer disease.^{65,66} These researchers showed GluR1 and GluR2/3 loss was restricted to the subiculum and CA1 layer of the hippocampus, which indicates that changes in AMPA receptor levels are likely to be very region-specific. The KA receptors have received less attention than the other classes of iGluRs, and it remains unclear whether there are alterations in the number of these receptors in Alzheimer disease. Autoradiography studies suggest little change in KA binding in areas of the brain that remain unaffected in Alzheimer disease, but there is reduced KA binding in the hippocampus of individuals with Alzheimer disease compared with controls.⁶⁷ Immunohistochemical staining using an antibody directed against GluR5/6/7 also showed some reduction in the levels of the KA receptors in the CA1 region of the hippocampus from individuals with Alzheimer disease compared with controls, but this was most likely due to neuronal loss in this area rather than alterations in the levels of the receptor.⁶⁸

As for mGluRs, radioligand binding assays showed a general reduction in the levels of mGluRs in the cortex and hippocampus of individuals with Alzheimer disease.⁶⁷ Western blot analysis confirmed a reduction in mGluR 1 levels in the CA1 region and subiculum of the hippocampus, but other regions of the hippocampus, such as the CA3 region, that are less susceptible to damage in Alzheimer disease were unaffected.^{67,69} Conversely, increased levels of mGluR 2 are found in the hippocampus of individuals with Alzheimer disease, and this correlates well with elevated phosphorylated tau levels.^{67,70} This increased mGluR 2 expression may result in increased intracellular Ca^{2+} , which may in turn activate tau kinases. It is therefore probable that alterations in mGluR expression are involved in Alzheimer disease pathogenesis.

Glutamatergic receptors and APP processing

The APP is a type 1 integral membrane protein with a long N-terminal extracellular region, a single membrane-spanning domain and a short cytoplasmic C-terminal. The mature APP is known to be proteolytically processed via 2 alternative pathways (Fig. 2). Most APP, following transport to the cell surface, is known to be cleaved by an α -secretase, which is thought to be a member of the ADAM (a distintegrin and metalloprotease) family.^{20,22,82} Currently the best candidates for the α -secretase are ADAM10 and ADAM17.⁸² This pathway precludes the formation of amyloid β as the α -cleavage site is within the peptide sequence and produces an 89 amino acid C terminal fragment (α -CTF) and soluble APP α . The second, the amyloidogenic pathway, is initiated by β -site APP cleaving enzyme and produces a C-terminal fragment containing 99 amino acids (β -CTF) and soluble APP β . The α -CTF and β -CTF are then cleaved by γ -secretase, a tetrameric complex containing PS1 or PS2 and anterior pharynx defective 1, presenilin enhancer 2 and nicastrin, to form P3 or amyloid β peptide, respectively, and the APP intracellular domain.^{20,22,83} The physiologic role of APP remains elusive, but it has been suggested to be involved in a variety of functions, including cell adhesion, regulation of cell/cell or cell/matrix interactions and mediation of neurite outgrowth.^{83,84} Low nanomolar and picomolar concentrations of amyloid β 1–40 are also thought to increase cell viability and may also play a role in synaptic control.⁸⁵ However, prolonged exposure and higher micromolar concentrations of the peptide are known to induce cell death.^{23,84} Relative activities of the 2 alternative APP processing pathways can be influenced by a variety of factors, including the stimulation of receptors for neurotransmitters/neuromodulators, such as acetylcholine, serotonin, glutamate, estrogen, neuropeptides and growth factors.^{20,22,86} The role of glutamate in APP processing has received a lot of attention as the activation of both iGluRs and mGluRs has been shown to differentially regulate the amyloidogenic and non-amyloidogenic pathways.²²

Short treatments with NMDA receptor and mGluR 1 agonists have been shown to increase soluble APP α release and intracellular accumulation of the α -CTF fragment and to reduce amyloid β formation. This is apparent in cultured neurons and in astrocytes, which express mostly Kunitz protease inhibitor domain-containing APP.^{87,88} It is likely that NMDA receptor and mGluR 1 activation increases nonamyloidogenic APP processing by increased trafficking and activation of α -secretase at the cell membrane.^{87–89} There is evidence that the NMDA receptor, by interacting with synapse associated protein 97, can increase ADAM10 trafficking to synaptic membranes,⁸⁹ whereas mGluR 1 activation can increase α -secretase activity.⁸⁸ The increased α -secretase activity appears to be mediated by downstream activation of PKC because phorbol esters, well-characterized PKC activators, and mGluR agonists have both been shown to increase nonamyloidogenic APP processing⁹⁰ and because a PKC inhibitor, GF 109203X, can abolish mGluR 1 agonist-induced soluble APP α release, whereas mGluR 1 antagonists are unable to prevent phorbol ester-induced APP processing.⁸⁸ As most APP is found at the

cell membrane, NMDA receptor-induced trafficking and mGluR 1 activation of ADAM10 at the membrane could facilitate soluble APP α release and reduce amyloidogenic APP processing.^{87,88}

Conversely, prolonged activation of NMDA receptors, a situation analogous to Alzheimer disease pathology, increases amyloidogenic APP processing. When neurons are activated by high glutamate concentrations for longer time periods (i.e., more than 24 h) there is a shift from APP695 expression to APP751 and APP770.^{91,92} This shift is mediated by activation of extrasynaptic NMDA receptors through Ca²⁺/calmodulin-dependent protein kinase (CaMK) IV.^{91,92} The longer APP isoforms have been shown to be more amyloidogenic, and so this shift in expression increases amyloid β levels.⁹¹ Excessive glutamate release from the presynaptic terminals has also been suggested as a mechanism for increased amyloid β production via NMDA receptor-mediated Ca²⁺ influx.⁹² Increased Ca²⁺ levels trigger fusion of synaptic vesicles to the plasma membrane, which enhances the rate of endocytosis to recycle these vesicles. Increased endocytosis subsequently removes APP from the plasma membrane, the site of

nonamyloidogenic α -secretase cleavage, into the endosomes for amyloidogenic processing via β -secretase.⁹³ More recent data have also shown that mGluRs are involved with increased amyloidogenic processing of APP; specifically mGluR 5 increases amyloid β 1–40 production, whereas mGluR 2 increases amyloid β 1–42 release.^{94,95}

Glutamate-mediated synaptic plasticity and amyloid β peptide

Glutamate is essential in establishing new neural networks that form memories and assist learning through a process called long-term potentiation. This process is generated by high-frequency stimulation of the presynaptic plasma membrane, resulting in increased release of glutamate and activation of its receptors at the postsynaptic membrane. The AMPA and mGluRs are activated by the initial glutamate release, whereas NMDA receptors only become fully active after continuous synchronized glutamate stimulation following activation of the AMPA and mGluRs. The NMDA receptor activation allows Ca²⁺ to enter the postsynaptic cell,

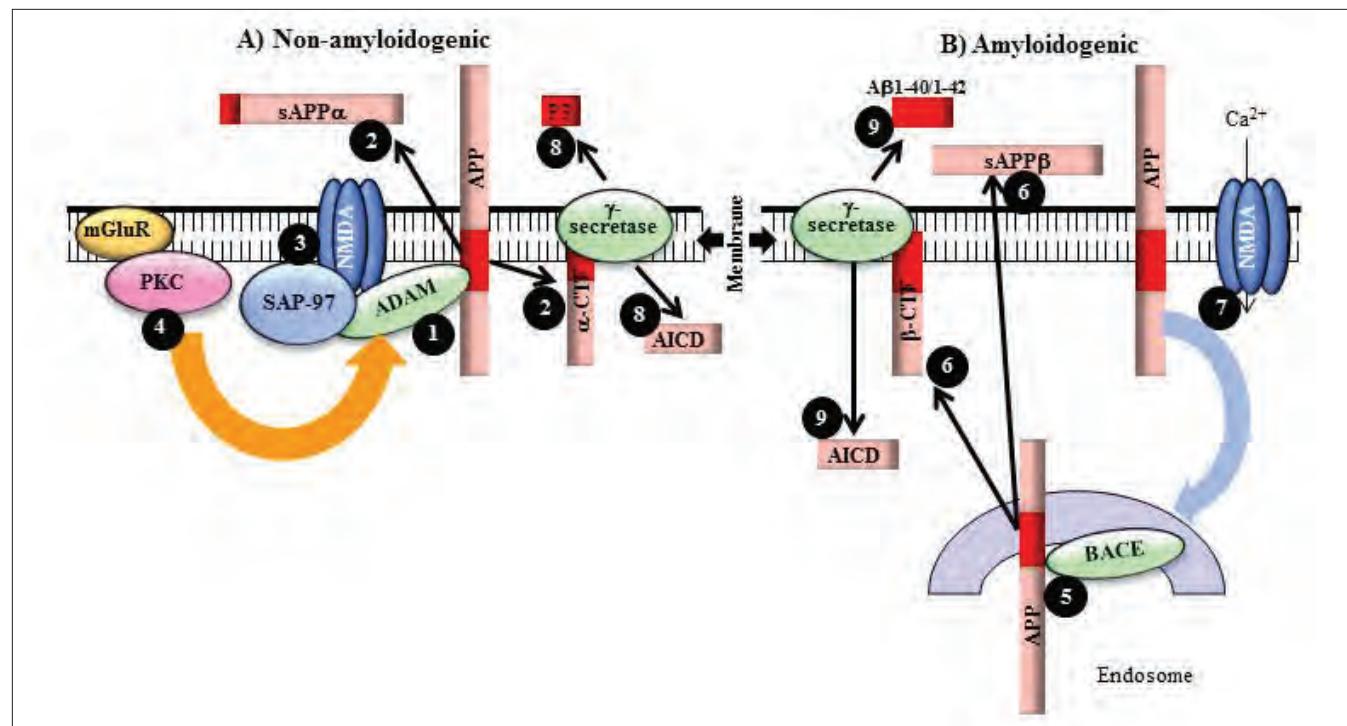


Fig. 2: Effects of glutamate receptor activation on APP processing. The APP is cleaved via (A) nonamyloidogenic and (B) amyloidogenic pathways. (A) The nonamyloidogenic pathway precludes amyloid β production due to (1) α -secretase cleavage, by ADAM10 or ADAM17, of APP within the amyloid β domain, which (2) results in production of soluble APP α and α -CTF. (3) Short-term N-methyl-D-aspartate (NMDA) receptor activation increases the trafficking of ADAM10 to the cell membrane via interactions with SAP-97 and (4) metabotropic glutamate receptor activation of ADAM17 via PKC, thus increasing nonamyloidogenic APP processing. (B) The main β -secretase, BACE1, (5) initiates the production of amyloid β peptide, which occurs partly within the late-endosomes, by (6) cleaving APP into soluble APP β and β -CTF. Prolonged NMDA receptor activation results in high Ca²⁺ levels and activation of CaMK-IV, which in turn enhances transcription of more amyloidogenic APP751 and APP770 isoforms. (7) The high Ca²⁺ levels also increase vesicle binding to the plasma membrane and subsequent transport of APP via endocytosis to late-endosomes, where it can be processed by BACE1. Thus, increased NMDA receptor activation enhances amyloidogenic processing of APP. The α - and β -CTFs generated from nonamyloidogenic and amyloidogenic pathways are further processed by the γ -secretase complex, leading to the formation of (8) AICD fragment, and the P3 or (9) amyloid β peptides, respectively. Activation of mGluRs 2 and 5 is also thought to increase generation of amyloid β peptide.

which subsequently triggers different kinase pathways and increases protein transcription. This process strengthens synapses and increases synaptic density.^{96,97} Long-term depression occurs when there is little stimulation at an established synapse or by asynchronous stimulation of iGluRs. Long-term depression has the opposite effect of long-term potentiation (i.e., reducing synaptic strength and preventing memory formation). Long-term depression can be caused by a reduction in the levels of NMDA and AMPA receptors at the postsynaptic membrane or by repeated weak stimulation of AMPA receptors that does not lead to the depolarization of the postsynaptic membrane and therefore does not fully activate NMDA receptors. This leads to a reduction in the number of neuronal spines on neurons and reduced synaptic activity. A decrease in long-term potentiation and an increase in long-term depression have both been observed in Alzheimer disease pathology.^{96,98}

Many different paradigms have been used to show that amyloid β disrupts long-term potentiation and reduces synaptic plasticity.^{98,99} Cullen and colleagues⁹⁹ reported that intracerebroventricular injections of amyloid β 1–40 and amyloid β 1–42 reduced the duration of high-frequency stimulation-induced long-term potentiation field excitatory postsynaptic potentials in the CA1 region of rat brains. This decrement in long-term potentiation was confirmed in studies using triple transgenic mice expressing mutant human APP, PS and tau, where intraneuronal amyloid β peptide was shown to accumulate with age and correlate with cognitive deficits observed in these mice. The cognitive deficits were observed to occur before amyloid β deposition, thus indicating that soluble amyloid β possibly disrupts normal neuronal function well before plaque formation.¹⁰⁰ When another mouse model, Tg2576 mice, which also expressed mutant human APP and had an increased amyloid β burden, were fed a γ -secretase inhibitor to reduce amyloid β levels, there was a profound restoration of impaired long-term potentiation, confirming that amyloid β does affect synaptic plasticity.¹⁰¹

To understand the mechanisms whereby amyloid β can influence synaptic plasticity, many groups have examined the effects of amyloid β peptide derived from different sources in organotypic hippocampal slice preparations. These studies have shown that naturally occurring amyloid β peptides obtained from spinal fluid¹⁰² or directly from brains of individuals with Alzheimer disease¹⁰³ and naturally secreted amyloid β oligos from cultured cells can affect long-term potentiation and synaptic density.^{104,105} On the basis of these results, it has been suggested that amyloid β dimers and larger aggregates are required for amyloid β -induced inhibition of long-term potentiation and that monomeric amyloid β peptide is not inhibitory.^{102,105} These effects could be reversed by removing amyloid β with monoclonal antibodies directed against the peptide.¹⁰² There is also evidence that specific concentrations of soluble amyloid β oligomers can inhibit long-term potentiation while increasing long-term depression.^{98,104}

Organotypic hippocampal slices from wild-type mice and mice overexpressing mutant APP have also been used extensively to look at the effects of amyloid β on neuron morphology, spine density, long-term potentiation and long-term de-

pression.^{98,105,106} Knafo and colleagues¹⁰⁷ assessed changes in the morphology and density of dendritic spines from the dentate gyrus in mice expressing PS1 and Swedish APP (APP^{sw}), a form of APP with a mutation at the β -cleavage site that increases amyloid β production. These results showed that the proximity of amyloid β plaques to the dendrites affected spine density and shape compared with littermate control mice. Dendrites passing through the plaque had a much lower spine density than dendrites that abutted the plaque but did not pass through it, whereas dendrites that were further removed from the plaque had a similar number of spines as those found in nontransgenic mice. These investigators also assessed the morphology of the spines, and whereas the number of spines was similar between the APP^{sw} transgenic mice and nontransgenic controls in areas where plaques were not present, the number of large spines that are usually associated with memory was much higher in the nontransgenic mice. Thus, APP^{sw} transgenic mice were unable to form synapses as readily as nontransgenic mice, an observation that correlates well with the cognitive deficit found in mice expressing APP/PS1.¹⁰⁷ As the loss of large spines is found throughout the dentate gyrus of the mutant APP transgenic mice, it is likely that soluble forms of amyloid β peptides, rather than amyloid β -containing neuritic plaques, are responsible for this morphological deficit.

These studies have shown that high levels of soluble amyloid β can reduce the number and alter the morphology of dendritic spines in mutant APP transgenic mice and that exogenous amyloid β produces similar results in hippocampal slices and primary neurons. Using an elegant hippocampal slice culture system, Wei and colleagues¹⁰⁸ further elucidated the effects of amyloid β proximity on synaptic plasticity. Neurons in the CA3 region of the hippocampus have long axons that form synapses with the neurons in the CA1 region. When neurons from the CA3 region were transfected with APP, CA1 neurons that were very close to the transfected cells showed reduced spine density, and the remaining spines demonstrated an altered morphology. Transfected CA1 neurons also caused a reduction in spine density in non-transfected neurons that were close to the transfected cells. Spine density and morphological changes were reversed when cultures were treated with a γ -secretase inhibitor, indicating that amyloid β was responsible for reduced synaptic density.

The role of glutamate in amyloid β -dependent spine loss, long-term potentiation inhibition and long-term depression induction has also received considerable attention. The NMDA receptor antagonist AP-5 prevents amyloid β -induced spine loss, thus suggesting that glutamate is essential for amyloid β -induced changes in spine density and morphology.¹⁰⁶ More recently, amyloid β -induced activation of extrasynaptic NR2B has been suggested to play a critical role in this process. When wild-type mouse hippocampal slice cultures were treated with either soluble amyloid β oligomers or the glutamate uptake inhibitor D,L-threo- β -benzyloxyaspartate there was an increase in extrasynaptic NR2B-mediated inhibition of long-term potentiation that was attenuated by the specific NR2B inhibitors

ifenprodil and Ro 25-6981.¹⁰⁴ Furthermore, it was shown that when glutamate levels were reduced by the glutamate scavenger system, glutamic pyruvic transaminase and pyruvate, amyloid β could no longer induce inhibition of long-term potentiation. Taken together, these data suggest that extrasynaptic NR2B activation can possibly mediate amyloid β -induced inhibition of long-term potentiation and that this depends to some extent on the presence of glutamate.

Patch clamp studies on the field excitatory postsynaptic currents after low-frequency stimulation of neurons in the CA1 region of hippocampal slices have shown that soluble amyloid β oligomer-induced long-term depression also depends on glutamate.¹⁰⁸⁻¹¹⁰ When the glutamate scavenger, glutamic pyruvic transaminase, was used in combination with NMDA receptor and mGluR inhibitors, they were able to restore amyloid β -induced long-term depression to normal levels, showing that it was mediated by excessive glutamate stimulation of both NMDA receptor and mGluRs. The glutamate uptake inhibitor D,L-threo- β -benzyloxyaspartate as well as inhibitors of calcineurin and glycogen synthase kinase-3 (GSK-3) were then used to show that amyloid β -induced long-term depression depended on excessive glutamate levels and activation of these enzymes.^{108,110} There is also evidence that amyloid β peptide can alter the levels/efficiency of glutamate transporters, which are known to be decreased in APP transgenic mice and Alzheimer disease brains,^{59,111,112} thus suggesting a role for amyloid β in long-term depression by regulating glutamate clearance.

Amyloid β -mediated neuronal function and glutamate

The significance of amyloid β in synaptic plasticity is well established, but its role in regulating synaptic function is still poorly understood. Low concentrations (i.e., picomolar or nanomolar) of amyloid β 1–40 have been shown to increase viability of cultured neurons,^{15,85} and there is evidence that amyloid β may have a role in normal synaptic function (Box 1).¹²⁷ This is supported by recent data that showed depletion of endogenous amyloid β peptide by using an amyloid β antibody or small interfering RNA against murine APP can reduce long-term potentiation and contextual fear and reference memories in wild-type mice.¹²⁸ There is also evi-

dence that synaptic activity increases amyloid β production both in vitro and in vivo,^{95,105,129} whereas increased concentrations of soluble amyloid β in cells overexpressing APP depressed synaptic activity;¹³⁰ thus it is likely that amyloid β may act as regulator of synaptic activity through a negative feedback loop. This is supported by electrophysiological studies that showed that amplitudes of NMDA and AMPA receptor-mediated excitatory postsynaptic currents were significantly enhanced in APP knockout hippocampal cultured neurons. A similar increase in synaptic transmission was detected when wild-type cultured neurons were treated with the γ -secretase inhibitor DAPT, which decreases levels of amyloid β peptides.¹³¹ Conversely, cultured slices overexpressing APP showed a reduced NMDA receptor-dependent synaptic transmission.¹²⁷ It is thus possible that dysfunction of the negative feedback loop mediated by amyloid β could lead to increased silencing of neurons by reducing synaptic transmission in affected areas of the brain in individuals with Alzheimer disease.

One possible mechanism by which excess amyloid β could reduce synaptic transmission is by inducing endocytosis of NMDA and AMPA receptors from the postsynaptic membranes.^{108,113,132,133} This is especially important with respect to AMPA receptors, as trafficking of these receptors to and from the cell surface is very dynamic.¹¹⁹ Hsieh and colleagues¹⁰⁸ showed that amyloid β -induced synaptic depression and dendritic spine loss are dependent on AMPA receptor removal from the postsynaptic membrane. They also suggested that endocytosis of GluR2 drives the removal of AMPA receptors from the cell surface, as cells transfected with a mutant form of GluR2, which is endocytosed more readily than wild-type GluR2, showed a reduction in AMPA as well as NMDA receptor-mediated transmission. Amyloid β has also been reported to regulate NMDA receptor trafficking in cortical neurons derived from mice overexpressing APP^{SW}.¹³³ These mice had the same total number of NMDA receptors, but fewer cell surface NMDA receptors than nontransgenic mice. Exogenous application of amyloid β 1–42 to nontransgenic cortical neurons promoted NMDA receptor endocytosis, indicating that the reduced receptor levels at the cell surface of APP^{SW} mice was due to increased removal rather than disrupted trafficking to the membrane.¹³³

A role for striatal-enriched tyrosine phosphatase (STEP₆₁) has recently been linked to amyloid β -induced synaptic function since it increases the endocytosis of NMDA and AMPA receptors from the postsynaptic membrane.¹³³⁻¹³⁶ The STEP₆₁ is enriched in synapses, suggesting that it has a specialized role in cell signalling. The levels of STEP₆₁ were reported to be increased in 2 mouse models of Alzheimer disease¹³³ and in humans¹³⁵ with Alzheimer disease. There is evidence that amyloid β peptides, delivered using the enriched conditioned media of cells overexpressing APP, can increase synaptic depression by enhancing STEP₆₁ activity via multiple mechanisms.^{133,135,136} First, amyloid β is known to impair the ubiquitin proteasome system,¹³⁷ which can increase STEP₆₁ protein levels in APP transgenic mice and in neuronal cell cultures.¹³⁵ Second, amyloid β can activate α_7 nicotinic receptors or NMDA receptors and increase Ca^{2+} entry into the cell. This

Box 1: The roles of amyloid β peptide in the function and control of the glutamate cycle and glutamatergic neurons

- Inhibit long-term potentiation^{99,104,105,113,114}
- Increase long-term depression^{98,108-110}
- Decrease synaptic density and alter the morphology of dendritic spines^{98,106,107,115}
- Regulate glutamate uptake from the synapse^{50,73,110,116,117}
- Stimulate release of glutamate^{117,118}
- Increase endocytosis of AMPA and NMDA receptors^{108,119}
- Disrupt the postsynaptic density and prevent NMDA and AMPA receptors reaching the cell surface^{120,121}
- Increase tau phosphorylation/cell death¹²²⁻¹²⁶

AMPA = α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA = N -methyl-D-aspartate.

triggers protein phosphatase 2B, which activates STEP₆₁ by dephosphorylation,¹³⁸ leading to increased internalization of the NR1/NR2B receptor complex.¹³³ Third, mGluR 5 activation has also been shown to increase STEP₆₁ translation in rat hippocampal slices and synaptosomes, which leads to extracellular signal-related kinase1/2 (ERK1/2)-dependent dephosphorylation of the GluR2 isoform and subsequent endocytosis of the AMPA receptor.¹³⁶ Taken together these findings indicate that amyloid β can activate and increase protein levels of STEP₆₁, resulting in enhanced dephosphorylation and endocytosis of glutamate receptors and reduced synaptic function.

It has recently been reported that soluble amyloid β 1–40 can disrupt the postsynaptic density (PSD), an electron dense thickening of the membrane comprising a network of proteins, including glutamate receptors, adhesion molecules, scaffolding proteins, cytoskeletal proteins and associated signalling molecules. The PSD, which is involved in maintaining synaptic homeostasis, is regulated by cytoskeletal protein PSD-95 and the master organizing proteins Shank and Homer.^{120,121} There is evidence that PSD-95 and Shank are especially important for ensuring that AMPA and NMDA receptors get to the cell surface and stay at the plasma membrane,^{120,139} and Homer is important in mGluR 1 localization.¹⁴⁰ Amyloid β colocalizes with PSD-95¹¹⁵ and causes a breakdown of the PSD-95, Shank and Homer clusters and a reduction in levels of NMDA and AMPA receptors at the plasma membrane.¹²¹ These observations are supported by the analysis of amyloid β and PSD-95 levels in intact nerve terminals taken from Alzheimer disease and control brains, which showed a 19% decrease in PSD-95 levels and a 132% increase in the amyloid β levels in Alzheimer disease compared with control brains.¹⁴¹ One of the most important signalling proteins that is highly abundant in the PSD is Ca²⁺/CaMKII, which plays a role in the delivery of AMPA receptors to the synapses.^{142,143} There is evidence that mice overexpressing APP not only show reduced synaptic CaMKII and cell surface GluR1 levels, but also exhibit decreased AMPA receptor transmission as observed in amyloid β 1–42 treated neurons.^{144,145} Thus, synaptic function is a closely controlled process requiring the appropriate activation of different types of proteins, including structural proteins controlling the architecture of pre- and postsynapses, kinase and phosphatases that mediate the effects of glutamate and other neurotransmitters. The complexity of amyloid β -induced synaptic dysfunction is evidenced by its ability to affect numerous elements of the synaptic machinery, which can directly influence pathological abnormalities in the brains of individuals with Alzheimer disease.

Glutamate release/uptake and amyloid β peptide

It is now well established that amyloid β peptides are produced constitutively by brain cells, including neurons, and are found in the nanomolar–picomolar range in the cerebrospinal fluid of healthy individuals,^{15,84} thus raising the possibility that these peptides under normal conditions may have a role in the regulation of brain neurotransmitter/

modulator release. We and others have previously reported that amyloid β 1–40/1–42 peptides can inhibit the release of acetylcholine, particularly from hippocampal and cortical regions of the rat brain.^{37,146,147} Additionally, there is evidence that amyloid β -related peptides can modulate the release of noradrenaline,^{148,149} but not of γ -aminobutyric acid,¹⁵⁰ from rat brain slice preparations. As for glutamate release, it has been reported earlier that pretreatment of hippocampal slices with micromolar concentrations of amyloid β 25–35 can potentiate glutamate release.¹⁵⁰ More recently, using an *in vitro* slice preparation, we have shown that nanomolar concentrations of amyloid β 1–40/1–42 peptides without pretreatment can potentiate potassium-evoked glutamate release from the hippocampus and cortex — the areas affected in Alzheimer disease. The effect is specific and appears to be mediated by direct interaction with the glutamatergic terminals.¹¹⁸ This idea is reinforced by the evidence that preincubation of nerve endings with amyloid β 1–42 can potentiate [¹⁴C]glutamic acid release, at least in part, by interacting with an N-type voltage-operated calcium channel.¹⁴⁸ These findings, together with the observation that glutamatergic terminals containing VGLUT1 are localized in close apposition to the APP-positive neurons in the hippocampus, provide an anatomical basis to suggest a role of endogenously derived amyloid β peptides in the presynaptic regulation of glutamate release from selected brain regions. At present, the underlying mechanisms by which amyloid β can potentiate glutamate release remains unclear, but it is of interest to note that nanomolar concentrations of amyloid β 1–40 did not significantly alter glutamate release from the striatum, an area that is relatively spared in Alzheimer disease.¹¹⁸ Whether micromolar concentrations of amyloid β peptide can enhance glutamate release from striatal slices remains to be established. However, these results raise the possibility that preferential vulnerability of hippocampal and cortical neurons could relate, at least in part, to their sensitivity to amyloid β peptides.

In contrast to glutamate release, amyloid β effects on glutamate uptake are somewhat controversial. Some studies have suggested that micromolar concentrations of amyloid β 25–35 can enhance glutamate uptake by astrocytes, which is mediated by increased expression of the glutamate transporter GLAST and enhanced by ERK inhibition, but unaffected by antioxidants.^{151,152} However, nanomolar concentrations of the amyloid β 25–35 peptide were found to increase extracellular glutamate levels by inhibiting its uptake in cultured neurons and astrocytes.^{116,153} Harris and colleagues¹⁵³ proposed that oxidative stress induced by the amyloid β peptide damages glutamate transporters, preventing glutamate uptake, and this proposal is supported by the increased levels of conjugation observed between the reactive oxygen species 4-hydroxy-2-nonenal and GLT-1 in synaptosomal preparations treated with amyloid β peptide and in the Alzheimer disease brain.¹⁵⁴ More recent data have confirmed that amyloid β 1–40 reduces glutamate uptake, and this also suggests that oxidative damage, as well as decreased GLT-1 and GLAST levels, could mediate reduced glutamate clearance from the synapse.⁷³ Interestingly, prolonged exposure of cultured microglia to a 5 μ M concentration of amyloid β 25–35 can increase the

extracellular levels via reverse glutamate transporters.¹⁵⁵ Thus amyloid β peptides can influence extracellular glutamate levels by regulating uptake and/or release of glutamate, which may be relevant in normal physiological and pathological conditions.¹⁵⁵

Glutamate and amyloid β -mediated toxicity

Chronic exposure to amyloid β peptides can induce toxicity in a variety of cell lines and in primary rat and human cultured neurons. The toxicity of the peptide is related to its ability to form insoluble aggregates.^{84,156-158} However, recent evidence suggests that the most detrimental forms of amyloid β peptides are the soluble oligomers and that the insoluble amorphous or fibrillar deposits represent a less harmful inactivated form of the peptide.¹⁵ The mechanisms associated with amyloid β toxicity are not clearly defined, but appear to involve alterations in intracellular calcium, production of free radicals, phosphorylation of tau protein and/or activation of caspase and noncaspase pathways that culminate in programmed cell death.^{25,84,159} Studies on a variety of cell lines and primary cultured neurons suggest that amyloid β toxicity might be mediated either by interaction with a hydroxysteroid dehydrogenase enzyme or by plasma membrane receptors for advanced glycation end products, class A scavenger receptor, p75 neurotrophin receptor, amylin or α_7 nicotinic receptors.^{117,160-164} However, a number of studies have clearly indicated that amyloid β toxicity is mediated, at least in part, by glutamate-mediated excitotoxicity, which involves activation of the NMDA receptors, leading to elevated intracellular Ca²⁺ and consequent stimulation of a cascade of enzymes resulting in cell death.^{28,76,77,158} Amyloid β -induced excitotoxic cell death was first reported in the early 1990s, with initial reports indicating that prolonged exposure of amyloid β peptides, including amyloid β 25–35 and amyloid β 1–38 with glutamate induced greater cell death than exposure to amyloid β or glutamate alone in mouse cortical neuronal cultures.¹²² The role of glutamate receptors in this process has subsequently been shown through the use of glutamate receptor agonists and antagonists.¹²³ When Mattson and colleagues¹²³ exposed human primary neuronal cultures to amyloid β 1–38/25–35 and either NMDA or KA, they showed that glutamate receptor activation increased cell death, possibly through a dysregulation of Ca²⁺ homeostasis, and that the NMDA receptor antagonist APV and the KA receptor antagonist DGG reduced cell death. This is supported by more recent data showing that inactivation of the NMDA receptor by antagonists, such as MK-801, AP5 or memantine, can protect neurons from amyloid β 1–40/1–42 toxicity,¹²⁴⁻¹²⁶ that amyloid β 1–40/1–42-induced neurodegeneration in the adult rat brain is mediated, in part, by activation of the NMDA receptor,^{165,166} and that transgenic mice exhibiting high levels of amyloid β peptide show increased vulnerability to excitotoxicity.^{167,168} Taken together, these studies suggest that glutamate receptor activation may be essential for amyloid β -induced cell death.

At present, it is unclear how amyloid β peptides can regulate activation of glutamate receptors, leading to the death of

neurons. There is evidence that amyloid β peptide can bind directly to glutamate receptors, increasing their activity and leading to influx of Ca²⁺ in organotypic slice cultures. Antagonists of NMDA and AMPA receptors can prevent influx of Ca²⁺ and cell death.²⁷ Alternatively, it is possible that amyloid β -related peptides can increase extracellular glutamate levels by potentiating release^{117,118,155} and/or inhibiting the uptake of the neurotransmitter,^{116,153} which can subsequently trigger death of neurons by excitotoxicity. It is therefore likely that a combination of reduced glutamate clearance from the synaptic cleft, increased release of glutamate from neurons and glia, and the subsequent activation of the glutamate receptors contribute to toxicity mediated by amyloid β -related peptides.

Glutamate and amyloid β -mediated tau phosphorylation

A number of earlier studies have suggested a critical role for tau in the genesis of amyloid β toxicity, but the cellular mechanisms by which tau mediates degeneration of neurons remain unclear. Using cultured neurons/cell lines, it has been shown that amyloid β 1–40/1–42 can induce site-specific phosphorylation of tau protein by activating multiple kinases, including ERK1/2, PKC, cyclin-dependent kinases (CDK), Fyn kinase and GSK-3 β . Given the evidence that tau phosphorylation occurs before the loss of neurons and that inhibition of tau phosphorylation can prevent amyloid β -induced neurodegeneration,¹⁶⁹⁻¹⁷² it is likely that increased levels of phosphorylated tau can contribute to the death of neurons by triggering loss of microtubule binding, impaired axonal transport and neuritic dystrophy. A critical role of the phosphorylated tau protein in amyloid β -induced toxicity has been established by the evidence that cells undergoing amyloid β toxicity exhibit increased levels of tau phosphorylation,^{169,170} that inhibition of tau phosphorylation by blocking tau kinases can prevent cell death,^{171,173} and that neurons cultured from tau protein knockout mice are resistant to amyloid β toxicity.¹⁷⁴

Given the importance of glutamate in amyloid β -mediated toxicity, a functional interrelationship between glutamate receptors and tau phosphorylation has long been investigated in a variety of experimental paradigms. We and others have shown that glutamate receptor antagonists can protect neurons against amyloid β 1–40/1–42-mediated toxicity.¹²⁴⁻¹²⁶ This effect appears to be mediated, at least in part, by reversing or decreasing activation of tau kinases, such as ERK1/2 and GSK-3 β , that are involved in the phosphorylation of tau protein rather than altering extracellular glutamate levels or regulation of amyloid β conformation or internalization.¹²⁵ Apart from influencing tau phosphorylation directly, there is evidence that Fyn kinase can regulate glutamatergic NMDA receptor activation following amyloid β treatment by triggering phosphorylation and subsequent interaction of the NR2B subunit of the receptor with PSD-95. The NR2B/PSD-95/Fyn complex that is formed when NMDA receptors are activated perpetuates Ca²⁺ influx, which can activate 2 key tau kinases, GSK-3 β ¹⁷⁵ and CDK5.¹⁷⁶ It is therefore possible that NMDA receptor activation stabilizes the NR2B/PSD-95/Fyn complex,

resulting in a persistent activation of the NMDA receptor channel and increasing tau phosphorylation either by direct phosphorylation of tau via Fyn kinase or by activating other tau kinases, such as GSK-3 β or CDK5.¹⁷⁷ It is also of interest to note that tau is required for the transportation of Fyn kinase to the postsynapse, where it forms a complex with PSD-95 and the NR2B subunit of the NMDA receptor. Disrupting the localization of Fyn kinase or the formation of the NR2B/PSD-95/Fyn complex was found to protect neurons against amyloid β -induced toxicity.¹⁷⁸ Collectively, these results suggest not only increased tau phosphorylation, but also that tau-mediated transportation of Fyn kinase to postsynaptic sites is possibly required for the degeneration of neurons induced by amyloid β -related peptides.

Increased tau phosphorylation may also be a consequence of reduced dephosphorylation of the protein. The serine/threonine phosphatases, such as protein phosphatases 1, 2A, 2B (i.e., calcineurin) and 5, have all been shown to dephosphorylate tau, and the activity of protein phosphatases 1, 2A and 5 is known to be reduced by 20%–30% in brains of individuals with Alzheimer disease.^{179–181} With protein phosphatases 1 and 2A accounting for 90% of serine/threonine phosphatase activity in mammalian cells^{182,183} and tau dephosphorylation predominantly being mediated by protein phosphatase 2A,¹⁷⁹ there is convincing evidence that down-regulation of this phosphatase can lead to increased tau hyperphosphorylation in the brains of individuals with Alzheimer disease. Inhibiting protein phosphatase 2A has been shown to increase tau hyperphosphorylation by reducing its dephosphorylation directly^{184,185} and by increasing the activity of tau kinases, such as CaMKII,¹⁸⁴ GSK-3 β ¹⁸⁶ and ERK1/2.¹⁸⁷ In addition, protein phosphatase 2A has been shown to dephosphorylate tau in an NMDA and KA receptor-dependent manner.^{188–190} When rat cortical neurons were treated for 1 hour with 100 μ M of glutamate or NMDA there was an initial reduction in phosphorylated tau, which was prevented when the cells were cotreated with okadaic acid, a protein phosphatase 1 and 2A inhibitor. This effect was only measured for the first 4 hours after glutamate or NMDA administration, and so the duration of this effect was not determined.¹⁸⁸ This is supported by in vivo studies showing that mice injected with KA exhibit a transient reduction in tau phosphorylation and increase in protein phosphatase 2A activity¹⁹⁰ followed by an increased tau hyperphosphorylation and reduced protein phosphatase 2A activity. Thus, it is likely that glutamate receptor activation may transiently reduce tau phosphorylation, but eventually leads to hyperphosphorylation of the protein, at least in part by reducing protein phosphatase 2A activity. Collectively, these results raise the possibility that reducing tau dephosphorylation may also partly contribute to the increased phosphorylation of tau protein and subsequent degeneration of neurons and/or formation of tangles observed in Alzheimer disease.

More recently, a number of studies have indicated that caspase- and calpain-mediated proteolytic cleavage of tau protein, in addition to enhanced phosphorylation, may have a role in the amyloid β -induced degeneration of neurons. There is evidence that 43–50 kDa truncated tau generated by

caspase-3 tends to assemble more rapidly into filaments than full-length tau and has been detected before the formation of tangles and cell death.^{191–194} Similarly, 17 kDa tau generated by calpain following treatment with amyloid β -related peptides was also detected before enhanced tau phosphorylation or neurite degeneration in cultured neurons. This proteolytic cleavage of tau may lead to neurodegeneration either directly, as reported in various cell lines and neurons, or indirectly by reducing the pool of full-length tau available for binding to microtubules.^{195,196} There is evidence that NMDA receptor-mediated activation of calpain may be involved in triggering the generation of 17 kDa tau fragments, which can lead to the degeneration of neurons, as both NMDA receptor antagonists and calpain inhibitors are found to protect neurons from cell death.¹⁹⁷ Thus, NMDA receptor-regulated tau cleavage and hyperphosphorylation of the protein may represent 2 different mechanisms by which tau can cause cell death.

Glutamate antagonists in the treatment of Alzheimer disease

Owing to the critical role of glutamate in both amyloid β - and tau-mediated pathology, glutamate receptor antagonists have been viewed as good therapeutic targets for many years. Originally the noncompetitive NMDA receptor antagonists MK-801 and phencyclidine were tried as therapies for Alzheimer disease.^{198,199} These compounds bind to sites within the NMDA receptor channel complex and prevent further Ca^{2+} entry by blocking the pore. Unfortunately, these drugs had a slow onset of action and bound irreversibly to the channel, preventing normal physiologic entry of Ca^{2+} into the cell. This led to severe psychotomimetic side effects, such as hallucinations, ataxia and memory loss, thus precluding their use as therapeutics for Alzheimer disease and also other conditions where NMDA receptors were therapeutic targets.^{198,200} Subsequently, other NMDA receptor antagonists, including amantadine and dextromethorphan, have shown promising results in the treatment of other forms of dementia, but have failed to serve as good therapeutics for Alzheimer disease.^{200,201} Interestingly, memantine, which was first synthesized by Eli Lilly in 1968 as a derivative of amantadine, was found to be a noncompetitive, low to moderate-affinity NMDA receptor antagonist that can prevent pathological activation of the receptor without affecting its physiologic functions.^{201–204} This antagonist exhibits a lower binding affinity than MK-801 and phencyclidine, but has a higher affinity than the endogenous NMDA receptor antagonist, the magnesium ion, which normally blocks the voltage-dependent activation of the NMDA receptor. Further studies indicate that memantine preferentially blocks NMDA receptor activity when the channel is excessively open. Since the NMDA receptor channels are open during normal synaptic activity for only milliseconds, memantine is unable to act or accumulate in the channel, thus selectively sparing normal synaptic activity. However, during prolonged receptor activation, as occurs under prolonged depolarization or excitotoxic conditions, memantine becomes an effective blocker of NMDA receptor activity.^{202,203,205} The pharmacokinetics of memantine

and its ability to protect neurons in a variety of in vitro and in vivo animal models suggest a favourable clinical safety profile of the antagonist in the treatment of neurodegenerative disorders. These results, together with some positive data from human clinical trials, convinced the European Union in 2002 and the U.S. Federal Drug Administration in 2003 to approve memantine for the treatment of moderate-to-severe Alzheimer disease.^{203,205}

Accumulated evidence suggests that memantine can provide protection against a variety of insults, including quinolinic acid-induced toxicity, brain trauma, ischemia and amyloid β 1–40/1–42-mediated neurotoxicity under in vitro and in vivo conditions.^{124,126,166,206} We have recently reported that memantine can significantly protect rat primary cortical cultured neurons against amyloid β 1–42-induced toxicity by attenuating activation of tau kinases (i.e., GSK-3 β and ERK-1/2) and phosphorylation of tau protein.¹²⁵ The drug also reduces amyloid β -induced cleavage of caspase-3 and dephosphorylation of cyclic AMP response element-binding protein, both of which are critical for cell survival. However, memantine did not alter either the conformation or internalization of amyloid β peptide, and it was unable to attenuate amyloid β -induced potentiation of extracellular glutamate levels.¹²⁵ In addition to its effect on toxicity, memantine is able to reverse amyloid β -induced long-term potentiation deficits in mouse hippocampal slice cultures²⁰⁷ and in the CA1 region of the adult rat hippocampus under in vivo conditions.¹¹⁴ Consistent with these results, several studies have demonstrated beneficial effects of memantine on cognitive deficits and Alzheimer disease-related pathology in transgenic mice overexpressing amyloid β peptides.^{208–210} There is evidence that memantine can reduce amyloid β plaque burden and increase synaptic density in the hippocampus of Tg2576 transgenic mice.²¹¹ Triple transgenic mice treated daily with 30 mg/kg of memantine for 3 months also showed improvement in cognitive performance at 3 different age groups, which was accompanied by reduced levels of hyperphosphorylated tau and increased levels of inactive GSK-3 β .²⁰⁸ The triple transgenic mice also showed a reduction in insoluble amyloid β , but an increase in soluble amyloid β levels without any alterations in APP, α -CTF or β -CTF after memantine treatment. Thus, it is likely that the antagonist does not alter APP levels/processing, but changes amyloid β aggregation or clearance in animal models of Alzheimer disease.²⁰⁸ This is somewhat contrary to previous studies that have shown lower APP levels and altered APP processing resulting in a reduction in amyloid β 1–40/1–42 production and/or secretion after memantine treatment under in vivo and in vitro conditions.^{91,212,213} Whether the discrepancy could be due to long-term (30 mg/kg/d memantine for 3 mo)²⁰⁸ versus short-term (i.e., 20 mg/kg/d for only 8 d)⁹¹ treatments with memantine or other factors remains to be established. However, these results, taken together, indicate that memantine is able to attenuate both tau- and amyloid β -mediated pathology along with an effect on the cognitive performance of the animal, validating its therapeutic potential in the treatment of Alzheimer disease pathology.

The clinical effects of memantine have been widely studied,

and a number of trials and meta-analyses have shown beneficial effects of the drug on the global status, cognition, behaviour and function of individuals with moderate to severe Alzheimer disease without any major adverse effects.^{214–216} It has also been shown to improve behavioural disturbances, such as agitation and aggression. Its mechanism of action as an NMDA receptor antagonist differs from the cholinesterase inhibitors approved for the treatment of Alzheimer disease (i.e., donepezil, galantamine and rivastigmine), suggesting a theoretical potential to be used in combination with these drugs. Some clinical trials and other lines of experimental data have suggested that the combination of memantine and cholinesterase inhibitors is safe and might provide additive benefits. The side effect profile of memantine is very different from that of the cholinesterase inhibitors, thus allowing its use in individuals with Alzheimer disease who cannot tolerate cholinesterase inhibitors because of their gastrointestinal or cardiac adverse effects. The data on the efficacy of memantine for individuals with mild to moderate Alzheimer disease is somewhat less compelling, with some studies demonstrating no benefit beyond placebo either as monotherapy or in conjunction with cholinesterase inhibitors.^{204,217,218} Unfortunately, to date, clinical studies have only examined the symptomatic benefits of memantine with a limited dose of the drug. Given its purported mechanism of action as an NMDA receptor antagonist, it is of interest to determine whether higher doses of memantine with acceptable tolerability might have disease-modifying effects, such as protection of neurons against excitotoxicity by attenuating tau phosphorylation, which may provide a rationale for the use of the drug in individuals with mild Alzheimer disease or even those at earlier stages.²⁰⁴ Some recent studies have suggested that second-generation analogues of memantine, nitromemantines, may be more neuroprotective than memantine at a lower dosage in both in vitro and in vivo animal models.²¹⁹ These modified drugs not only share memantine's ability to block NMDA receptors, but can also nitrosylate 1 of 5 cysteines in NMDA receptors. Since S-nitrosylation of the NR2A subunit has been shown to modulate NMDA receptor activity,²⁰³ it is likely that nitromemantines may provide a means to specifically target NMDA receptors without causing dangerous side effects, such as lowering blood pressure, that are associated with other nitrosylating drugs like nitroglycerin. However, more research is needed to establish the efficacy of nitromemantines in protecting neurons in animal models as well as clinical safety for their use in humans with Alzheimer disease.

Acknowledgements: The authors are grateful to CIHR and the Canada Research Chairs program for their support to the research programs.

Competing interests: None declared for T.J. Revett. G.B. Baker declares educational grant support from Pfizer Canada and travel and speaker fees from the Turkish Association for Psychopharmacology. J.H. Jhamandas and S. Kar are supported in part by a CIHR operating grant paid to their institution.

Contributors: T.J. Revett and S. Kar designed the study. T.J. Revett acquired the data. T.J. Revett, G.B. Baker, J.H. Jhamandas and S. Kar analyzed the data and wrote the article. All authors reviewed the article and approved its publication.

References

1. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. *Lancet* 2005;366:2112-7.
2. Guerreiro RJ, Gustafson DR, Hardy J. The genetic architecture of Alzheimer's disease: beyond APP, PSENs and APOE. *Neurobiol Aging*. In press.
3. St George-Hyslop PH, Petit A. Molecular biology and genetics of Alzheimer's disease. *C R Biol* 2005;328:119-30.
4. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* 2009;41:1088-93.
5. Lambert JC, Heath S, Even G, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* 2009;41:1094-9.
6. Seshadri S, Fitzpatrick AL, Ikram MA, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA* 2010;303:1832-40.
7. Rogeava E, Meng Y, Lee JH, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat Genet* 2007;39:168-77.
8. Hollingworth P, Harold D, Jones L, et al. Alzheimer's disease genetics: current knowledge and future challenges. *Int J Geriatr Psychiatry* 2011;26:793-802.
9. Lambert JC, Amouyel P. Genetics of Alzheimer's disease: New evidences for an old hypothesis? *Curr Opin Genet Dev* 2011;21:295-301.
10. Guskiewicz KM, Marshall SW, Bailes J, et al. Association between recurrent concussion and late-life cognitive impairment in retired professional football players. *Neurosurgery* 2005;57:719-26.
11. de la Torre JC. How do heart disease and stroke become risk factors for Alzheimer's disease? *Neurol Res* 2006;28:637-44.
12. Hölscher C. Diabetes as a risk factor for Alzheimer's disease: insulin signalling impairment in the brain as an alternative model of Alzheimer's disease. *Biochem Soc Trans* 2011;39:891-7.
13. Chen J-H, Lin K-P, Chen Y-C. Risk factors for dementia. *J Formos Med Assoc* 2009;108:754-64.
14. Butterfield DA, Pocernich CB. The glutamatergic system and Alzheimer's disease: therapeutic implications. *CNS Drugs* 2003;17:641-52.
15. Selkoe DJ, Schenk D. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol* 2003;43:545-84.
16. Combs B, Voss K, Gamblin TC. Pseudohyperphosphorylation has differential effects on polymerization and function of tau isoforms. *Biochemistry* 2011;50:9446-56.
17. Mi K, Johnson GV. The role of tau phosphorylation in the pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* 2006;3:449-63.
18. Wang JZ, Grundke-Iqbali I, Iqbal K. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. *Eur J Neurosci* 2007;25:59-68.
19. Braak H, Braak E. Evolution of neuronal changes in the course of Alzheimer's disease. *J Neural Transm Suppl* 1998;53:127-40.
20. Chow VW, Mattson MP, Wong PC, et al. An overview of APP processing enzymes and products. *Neuromolecular Med* 2010;12:1-12.
21. LeBlanc AC, Chen HY, Autilio-Gambetti L, et al. Differential APP gene expression in rat cerebral cortex, meninges, and primary astroglial, microglial and neuronal cultures. *FEBS Lett* 1991;292:171-8.
22. Mills J, Reiner PD. Regulation of amyloid precursor protein cleavage. *J Neurochem* 1999;72:443-60.
23. Murphy MP, Levine H. Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers Dis* 2010;19:311-23.
24. Näslund J, Haroutounian S, Mohs R, et al. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA* 2000;283:1571-7.
25. Blurton-Jones M, LaFerla FM. Pathways by which A β facilitates tau pathology. *Curr Alzheimer Res* 2006;3:437-48.
26. Mattson MP. Pathways towards and away from Alzheimer's disease. *Nature* 2004;430:631-9.
27. Alberdi E, Sanchez-Gomez MV, Cavaliere F, et al. Amyloid β oligomers induce Ca $^{2+}$ dysregulation and neuronal death through activation of ionotropic glutamate receptors. *Cell Calcium* 2010;47:264-72.
28. Supnet C, Bezprozvanny I. The dysregulation of intracellular calcium in Alzheimer disease. *Cell Calcium* 2010;47:183-9.
29. Hynd MR, Scott HL, Dodd PR. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochem Int* 2004;45:583-95.
30. Dasilva KA, Shaw JE, McLaurin J. Amyloid- β fibrillogenesis: structural insight and therapeutic intervention. *Exp Neurol* 2010;223:311-21.
31. Morgan D. Immunotherapy for Alzheimer's disease. *J Intern Med* 2011;269:54-63.
32. Pahnke J, Walker LC, Scheffler K, et al. Alzheimer's disease and blood-brain barrier function — Why have anti- β -amyloid therapies failed to prevent dementia progression? *Neurosci Biobehav Rev* 2009;33:1099-108.
33. Samadi H, Sultzer D. Solanezumab for Alzheimer's disease. *Expert Opin Biol Ther* 2011;11:787-98.
34. DeKosky ST, Scheff SW, Styren SD. Structural correlates of cognition in dementia: quantification and assessment of synapse change. *Neurodegeneration* 1996;5:417-21.
35. Francis PT. Glutamatergic systems in Alzheimer's disease. *Int J Geriatr Psychiatry* 2003;18:S15-21.
36. Bell KF, Bennett DA, Cuello AC. Paradoxical upregulation of glutamatergic presynaptic boutons during mild cognitive impairment. *J Neurosci* 2007;27:10810-7.
37. Kar S, Slowikowski SP, Westaway D, et al. Interactions between β -amyloid and central cholinergic neurons: implications for Alzheimer's disease. *J Psychiatry Neurosci* 2004;29:427-41.
38. Morris JC. Challenging assumptions about Alzheimer's disease: mild cognitive impairment and the cholinergic hypothesis. *Ann Neurol* 2002;51:143-4.
39. Bell KFS, Cuello AC. Altered synaptic function in Alzheimer's disease. *Eur J Pharmacol* 2006;545:11-21.
40. Dijk SN, Francis PT, Stratmann GC, et al. Cholinomimetics increase glutamate outflow via an action on the corticostriatal pathway: implications for Alzheimer's disease. *J Neurochem* 1995;65:2165-9.
41. Tsang SWY, Pomakian J, Marshall GA, et al. Disrupted muscarinic M1 receptor signalling correlates with loss of protein kinase C activity and glutamatergic deficit in Alzheimer's disease. *Neurobiol Aging* 2007;28:1381-7.
42. Kowall NW, Beal MF. Glutamate-, glutaminase-, and taurine-immunoreactive neurons develop neurofibrillary tangles in Alzheimer's disease. *Ann Neurol* 1991;29:162-7.
43. Bussière T, Giannakopoulos P, Bouras C, et al. Progressive degeneration of nonphosphorylated neurofilament protein-enriched pyramidal neurons predicts cognitive impairment in Alzheimer's disease: stereologic analysis of prefrontal cortex area 9. *J Comp Neurol* 2003;463:281-302.
44. Fremau RT Jr, Troyer MD, Pahner I, et al. The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron* 2001;31:247-60.
45. Kew JN, Kemp JA. Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology (Berl)* 2005;179:4-29.
46. Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptors in the mammalian central nervous system. *Prog Neurobiol* 1998;54:581-618.
47. Danbolt NC. Glutamate uptake. *Prog Neurobiol* 2001;65:1-105.
48. Ferraguti F, Shigemoto R. Metabotropic glutamate receptors. *Cell Tissue Res* 2006;326:483-504.
49. Gerber U, Gee CE, Benquet P. Metabotropic glutamate receptors: intracellular signalling pathways. *Curr Opin Pharmacol* 2007;7:56-61.
50. Schools GP, Kimelberg HK. mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor mRNAs expressed in hippocampal astrocytes acutely isolated from young rats. *J Neurosci Res* 1999;58:533-43.
51. Vogt KE, Nicoll RA. Glutamate and γ -aminobutyric acid mediate a heterosynaptic depression at mossy fiber synapses in the hippocampus. *Proc Natl Acad Sci U S A* 1999;96:1118-22.
52. Anderson CM, Swanson RA. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* 2000;32:1-14.
53. Storck T, Schulte S, Hofmann K, et al. Structure, expression, and functional analysis of a Na $^{+}$ -dependent glutamate/aspartate transporter from rat brain. *Proc Natl Acad Sci U S A* 1992;89:10955-9.
54. Pines G, Danbolt NC, Bjoras M, et al. Cloning and expression of a rat brain L-glutamate transporter. *Nature* 1992;360:464-7.
55. Shashidharan P, Huntley GW, Meyer T, et al. Neuron-specific human glutamate transporter: molecular cloning, characterization

- and expression in human brain. *Brain Res* 1994;662:245-50.
56. Kashani A, Lepicard E, Poirel O, et al. Loss of VGLUT1 and VGLUT2 in the prefrontal cortex is correlated with cognitive decline in Alzheimer disease. *Neurobiol Aging* 2008;29:1619-30.
 57. Kirvell SL, Esiri M, Francis PT. Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. *J Neurochem* 2006;98:939-50.
 58. Sokolow S, Luu SH, Nandy K, et al. Preferential accumulation of amyloid β in presynaptic glutamatergic terminals (VGLUT1 and VGLUT2) in Alzheimer's disease cortex. *Neurobiol Dis* 2012;45:381-7.
 59. Jacob CP, Koutsilieri E, Bartl J, et al. Alterations in expression of glutamatergic transporters and receptors in sporadic Alzheimer's disease. *J Alzheimers Dis* 2007;11:97-116.
 60. Scott HA, Gebhardt FM, Mitrovic AD, et al. Glutamate transporter variants reduce glutamate uptake in Alzheimer's disease. *Neurobiol Aging* 2011;32:553.e1-11.
 61. Robinson SR. Changes in the cellular distribution of glutamine synthetase in Alzheimer's disease. *J Neurosci Res* 2001;66:972-80.
 62. Hynd MR, Scott HL, Dodd PR. Selective loss of NMDA receptor NR1 subunit isoforms in Alzheimer's disease. *J Neurochem* 2004;89:240-7.
 63. Hynd MR, Scott HL, Dodd PR. Differential expression of N-methyl D-aspartate receptor NR2 isoforms in Alzheimer's disease. *J Neurochem* 2004;90:913-9.
 64. Williams C, Mehrian Shai R, Wu Y, et al. Transcriptome analysis of synaptoneuroosomes identifies neuroplasticity genes overexpressed in incipient Alzheimer's disease. *PLoS ONE* 2009;4:e4936.
 65. Pellegrini-Giampietro DE, Bennett MV, Zukin RS. AMPA/kainate receptor gene expression in normal and Alzheimer's disease hippocampus. *Neuroscience* 1994;61:41-9.
 66. Yasuda RP, Ikonomovic MD, Sheffield R, et al. Reduction of AMPA-selective glutamate receptor subunits in the entorhinal cortex of patients with Alzheimer's disease pathology: a biochemical study. *Brain Res* 1995;678:161-7.
 67. Dewar D, Chalmers DT, Graham DI, et al. Glutamate metabotropic and AMPA binding sites are reduced in Alzheimer's disease: an autoradiographic study of the hippocampus. *Brain Res* 1991;553:58-64.
 68. Aronica E, Dickson DW, Kress Y, et al. Non-plaque dystrophic dendrites in Alzheimer hippocampus: a new pathological structure revealed by glutamate receptor immunocytochemistry. *Neuroscience* 1998;82:979-91.
 69. Albasanz JL, Dalfo E, Ferrer I, et al. Impaired metabotropic glutamate receptor/phospholipase C signaling pathway in the cerebral cortex in Alzheimer's disease and dementia with Lewy bodies correlates with stage of Alzheimer's-disease-related changes. *Neurobiol Dis* 2005;20:685-93.
 70. Lee HG, Ogawa O, Zhu X, et al. Aberrant expression of metabotropic glutamate receptor 2 in the vulnerable neurons of Alzheimer's disease. *Acta Neuropathol* 2004;107:365-71.
 71. Masliah E, Alfors M, DeTeresa R, et al. Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. *Ann Neurol* 1996;40:759-66.
 72. Li S, Mallory M, Alfors M, et al. Glutamate transporter alterations in Alzheimer's disease are possibly associated with abnormal APP expression. *J Neuropathol Exp Neurol* 1997;56:901-11.
 73. Matos M, Augusto E, Oliveira CR, et al. Amyloid- β peptide decreases glutamate uptake in cultured astrocytes: involvement of oxidative stress and mitogen-activated protein kinase cascades. *Neuroscience* 2008;156:898-910.
 74. Parikh MS, Brewer GJ. Mitoenergetic failure in Alzheimer disease. *Am J Physiol Cell Physiol* 2007;292:C8-23.
 75. Greenamyre JT, Maragos WF, Albin RL, et al. Glutamate transmission and toxicity in Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* 1988;12:421-30.
 76. Dong XX, Wang Y, Qin ZH. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol Sin* 2009;30:379-87.
 77. Vosler PS, Brennan CS, Chen J. Calpain-mediated signaling mechanisms in neuronal injury and neurodegeneration. *Mol Neurobiol* 2008;38:78-100.
 78. Ulas J, Weihmuller FB, Brunner LC, et al. Selective increase of NMDA-sensitive glutamate binding in the striatum of Parkinson's disease, Alzheimer's disease, and mixed Parkinson's disease/Alzheimer's disease patients: an autoradiographic study. *J Neurosci* 1994;14:6317-24.
 79. Ikonomovic MD, Mizukami K, Warde D, et al. Distribution of glutamate receptor subunit NMDAR1 in the hippocampus of normal elderly and patients with Alzheimer's disease. *Exp Neurol* 1999;160:194-204.
 80. Panegyres PK, Zafiris-Toufexis K, Kakulas BA. The mRNA of the NR1 subtype of glutamate receptor in Alzheimer's disease. *J Neural Transm* 2002;109:77-89.
 81. Sze C, Bi H, Kleinschmidt-DeMasters BK, et al. N-methyl-D-aspartate receptor subunit proteins and their phosphorylation status are altered selectively in Alzheimer's disease. *J Neurol Sci* 2001;182:151-9.
 82. Allinson TM, Parkin ET, Turner AJ, et al. ADAMs family members as amyloid precursor protein alpha-secretases. *J Neurosci Res* 2003;74:342-52.
 83. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001;81:741-66.
 84. Clippingdale AB, Wade JD, Barrow CJ. The amyloid- β peptide and its role in Alzheimer's disease. *J Pept Sci* 2001;7:227-49.
 85. Plant LD, Boyle JP, Smith IF, et al. The production of amyloid β peptide is a critical requirement for the viability of central neurons. *J Neurosci* 2003;23:5531-5.
 86. Roberson MR, Harrell LE. Cholinergic activity and amyloid precursor protein metabolism. *Brain Res Brain Res Rev* 1997;25:50-69.
 87. Hoey SE, Williams RJ, Perkinton MS. Synaptic NMDA receptor activation stimulates α -secretase amyloid precursor protein processing and inhibits amyloid- β production. *J Neurosci* 2009;29:4442-60.
 88. Lee RK, Jimenez J, Cox AJ, et al. Metabotropic glutamate receptors regulate APP processing in hippocampal neurons and cortical astrocytes derived from fetal rats. *Ann N Y Acad Sci* 1996;777:338-43.
 89. Marcello E, Gardoni F, Mauceri D, et al. Synapse-associated protein-97 mediates alpha-secretase ADAM10 trafficking and promotes its activity. *J Neurosci* 2007;27:1682-91.
 90. da Cruz e Silva OA, Rebelo S, Vieira SI, et al. Enhanced generation of Alzheimer's amyloid- β following chronic exposure to phorbol ester correlates with differential effects on α and ϵ isozymes of protein kinase C. *J Neurochem* 2009;108:319-30.
 91. Bordji K, Becerril-Ortega J, Nicole O, et al. Activation of extrasynaptic, but not synaptic, NMDA receptors modifies amyloid precursor protein expression pattern and increases amyloid- β production. *J Neurosci* 2010;30:15927-42.
 92. Lesné S, Ali C, Gabriel C, et al. NMDA receptor activation inhibits α -secretase and promotes neuronal amyloid- β production. *J Neurosci* 2005;25:9367-77.
 93. Cirrito JR, Kang JE, Lee J, et al. Endocytosis is required for synaptic activity-dependent release of amyloid- β in vivo. *Neuron* 2008;58:42-51.
 94. Kim SH, Fraser PE, Westaway D, et al. Group II metabotropic glutamate receptor stimulation triggers production and release of Alzheimer's amyloid- β 42 from isolated intact nerve terminals. *J Neurosci* 2010;30:3870-5.
 95. Tang BL. Neuronal protein trafficking associated with Alzheimer disease: from APP and BACE1 to glutamate receptors. *Cell Adh Migr* 2009;3:118-28.
 96. Kullmann DM, Lamsa KP. Long-term synaptic plasticity in hippocampal interneurons. *Nat Rev Neurosci* 2007;8:687-99.
 97. Morris RG. Long-term potentiation and memory. *Philos Trans R Soc Lond B Biol Sci* 2003;358:643-7.
 98. Shankar GM, Li S, Mehta TH, et al. Amyloid- β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 2008;14:837-42.
 99. Cullen WK, Suh YH, Anwyl R, et al. Block of LTP in rat hippocampus in vivo by β -amyloid precursor protein fragments. *Neuroreport* 1997;8:3213-7.
 100. Oddo S, Caccamo A, Shepherd JD, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A β and synaptic dysfunction. *Neuron* 2003;39:409-21.
 101. Townsend M, Qu Y, Gray A, et al. Oral treatment with a γ -secretase inhibitor improves long-term potentiation in a mouse model of Alzheimer's disease. *J Pharmacol Exp Ther* 2010;333:110-9.
 102. Klyubin I, Betts V, Welzel AT, et al. Amyloid β protein dimer containing human CSF disrupts synaptic plasticity: prevention by systemic passive immunization. *J Neurosci* 2008;28:4231-7.
 103. Shankar GM, Bloodgood BL, Townsend M, et al. Natural oligomers of the Alzheimer amyloid- β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J Neurosci* 2007;27:2866-75.
 104. Li S, Jin M, Koeglspurger T, et al. Soluble A β oligomers inhibit

- long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. *J Neurosci* 2011;31:6627-38.
105. Selkoe DJ. Soluble oligomers of the amyloid β -protein impair synaptic plasticity and behavior. *Behav Brain Res* 2008;192:106-13.
 106. Wei W, Nguyen LN, Kessels HW, et al. Amyloid β from axons and dendrites reduces local spine number and plasticity. *Nat Neurosci* 2010;13:190-6.
 107. Knafo S, Alonso-Nanclares L, Gonzalez-Soriano J, et al. Widespread changes in dendritic spines in a model of Alzheimer's disease. *Cereb Cortex* 2009;19:586-92.
 108. Hsieh H, Boehm J, Sato C, et al. AMPAR removal underlies A β -induced synaptic depression and dendritic spine loss. *Neuron* 2006; 52:831-43.
 109. Kim JH, Anwyl R, Suh YH, et al. Use-dependent effects of amyloidogenic fragments of β -amyloid precursor protein on synaptic plasticity in rat hippocampus in vivo. *J Neurosci* 2001;21:1327-33.
 110. Li S, Hong S, Shepardson NE, et al. Soluble oligomers of amyloid β protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron* 2009;62:788-801.
 111. Masliah E, Alford M, Mallory M, et al. Abnormal glutamate transport function in mutant amyloid precursor protein transgenic mice. *Exp Neurol* 2000;163:381-7.
 112. Scott HL, Pow DV, Tannenberg AE, et al. Aberrant expression of the glutamate transporter excitatory amino acid transporter 1 (EAAT1) in Alzheimer's disease. *J Neurosci* 2002;22:RC206.
 113. Zhao WQ, Santini F, Breese R, et al. Inhibition of calcineurin-mediated endocytosis and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors prevents amyloid β oligomer-induced synaptic disruption. *J Biol Chem* 2010;285:7619-32.
 114. Klyubin I, Wang Q, Reed MN, et al. Protection against A β -mediated rapid disruption of synaptic plasticity and memory by memantine. *Neurobiol Aging* 2011;32:614-23.
 115. Lacor PN, Buniel MC, Chang L, et al. Synaptic targeting by Alzheimer's-related amyloid β oligomers. *J Neurosci* 2004;24:10191-200.
 116. Fernández-Tomé P, Brera B, Arevalo MA, et al. β -amyloid25-35 inhibits glutamate uptake in cultured neurons and astrocytes: modulation of uptake as a survival mechanism. *Neurobiol Dis* 2004;15: 580-9.
 117. Chin JH, Ma L, MacTavish D, et al. Amyloid β protein modulates glutamate-mediated neurotransmission in the rat basal forebrain: involvement of presynaptic neuronal nicotinic acetylcholine and metabotropic glutamate receptors. *J Neurosci* 2007;27:9262-9.
 118. Kabogo D, Rauw G, Amritraj A, et al. β -amyloid-related peptides potentiate K $^{+}$ -evoked glutamate release from adult rat hippocampal slices. *Neurobiol Aging* 2010;31:1164-72.
 119. Opazo P, Choquet D. A three-step model for the synaptic recruitment of AMPA receptors. *Mol Cell Neurosci* 2011;46:1-8.
 120. Gong Y, Lippa CF. Review: disruption of the postsynaptic density in Alzheimer's disease and other neurodegenerative dementias. *Am J Alzheimers Dis Other Demen* 2010;25:547-55.
 121. Roselli F, Hutzler P, Wegerich Y, et al. Disassembly of shank and homer synaptic clusters is driven by soluble β -amyloid(1-40) through divergent NMDAR-dependent signalling pathways. *PLoS ONE* 2009;4:e6011.
 122. Koh JY, Yang LL, Cotman CW. β -amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. *Brain Res* 1990;533:315-20.
 123. Mattson MP, Cheng B, Davis D, et al. β -Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J Neurosci* 1992;12:376-89.
 124. Floden AM, Li S, Combs CK. β -amyloid-stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor α and NMDA receptors. *J Neurosci* 2005;25:2566-75.
 125. Song MS, Rauw G, Baker GB, et al. Memantine protects rat cortical cultured neurons against β -amyloid-induced toxicity by attenuating tau phosphorylation. *Eur J Neurosci* 2008;28:1989-2002.
 126. Tremblay R, Chakravarthy B, Hewitt K, et al. Transient NMDA receptor inactivation provides long-term protection to cultured cortical neurons from a variety of death signals. *J Neurosci* 2000;20:7183-92.
 127. Kamenetz F, Tomita T, Hsieh H, et al. APP processing and synaptic function. *Neuron* 2003;37:925-37.
 128. Puzzo D, Privitera L, Fa M, et al. Endogenous amyloid β is necessary for hippocampal synaptic plasticity and memory. *Ann Neurol* 2011;69:819-30.
 129. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid- β levels in vivo. *Neuron* 2005;48:913-22.
 130. Ting JT, Kelley BG, Lambert TJ, et al. Amyloid precursor protein overexpression depresses excitatory transmission through both presynaptic and postsynaptic mechanisms. *Proc Natl Acad Sci U S A* 2007;104:353-8.
 131. Priller C, Bauer T, Mittelregger G, et al. Synapse formation and function is modulated by the amyloid precursor protein. *J Neurosci* 2006;26:7212-21.
 132. Kurup P, Zhang Y, Xu J, et al. A β -mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. *J Neurosci* 2010;30:5948-57.
 133. Snyder EM, Nong Y, Almeida CG, et al. Regulation of NMDA receptor trafficking by amyloid- β . *Nat Neurosci* 2005;8:1051-8.
 134. Braithwaite SP, Adkisson M, Leung J, et al. Regulation of NMDA receptor trafficking and function by striatal-enriched tyrosine phosphatase (STEP). *Eur J Neurosci* 2006;23:2847-56.
 135. Kurup P, Zhang Y, Venkitaramani DV, et al. The role of STEP in Alzheimer's disease. *Channels (Austin)* 2010;4:347-50.
 136. Zhang Y, Venkitaramani DV, Gladding CM, et al. The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. *J Neurosci* 2008;28: 10561-6.
 137. Oh S, Hong HS, Hwang E, et al. Amyloid peptide attenuates the proteasome activity in neuronal cells. *Mech Ageing Dev* 2005;126: 1292-9.
 138. Paul S, Nairn AC, Wang P, et al. NMDA-mediated activation of the tyrosine phosphatase STEP regulates the duration of ERK signaling. *Nat Neurosci* 2003;6:34-42.
 139. Ehrlich I, Klein M, Rumpel S, et al. PSD-95 is required for activity-driven synapse stabilization. *Proc Natl Acad Sci U S A* 2007;104:4176-81.
 140. Kammermeier PJ. Surface clustering of metabotropic glutamate receptor 1 induced by long Homer proteins. *BMC Neurosci* 2006;7:1.
 141. Gylys KH, Fein JA, Yang F, et al. Synaptic changes in Alzheimer's disease: increased amyloid-beta and gliosis in surviving terminals is accompanied by decreased PSD-95 fluorescence. *Am J Pathol* 2004;165:1809-17.
 142. Hayashi Y, Shi SH, Esteban JA, et al. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* 2000;287:2262-7.
 143. Poncer JC, Esteban JA, Malinow R. Multiple mechanisms for the potentiation of AMPA receptor-mediated transmission by α -Ca $^{2+}$ /calmodulin-dependent protein kinase II. *J Neurosci* 2002;22:4406-11.
 144. Gu Z, Liu W, Yan Z. β -Amyloid impairs AMPA receptor trafficking and function by reducing Ca $^{2+}$ /calmodulin-dependent protein kinase II synaptic distribution. *J Biol Chem* 2009;284:10639-49.
 145. Zhao D, Watson JB, Xie CW. Amyloid β prevents activation of calcium/calmodulin-dependent protein kinase II and AMPA receptor phosphorylation during hippocampal long-term potentiation. *J Neurophysiol* 2004;92:2853-8.
 146. Farr SA, Banks WA, Uezu K, et al. Antibody to β -amyloid protein increases acetylcholine in the hippocampus of 12 month SAMP8 male mice. *Life Sci* 2003;73:555-62.
 147. Kar S, Seto D, Gaudreau P, et al. β -amyloid-related peptides inhibit potassium-evoked acetylcholine release from rat hippocampal slices. *J Neurosci* 1996;16:1034-40.
 148. Bobich JA, Zheng Q, Campbell A. Incubation of nerve endings with a physiological concentration of A β 1-42 activates CaV2.2(N-Type)-voltage operated calcium channels and acutely increases glutamate and noradrenaline release. *J Alzheimers Dis* 2004;6:243-55.
 149. Li M, Smith CP. β -amyloid1-40 inhibits electrically stimulated release of [3 H]norepinephrine and enhances the internal calcium response to low potassium in rat cortex: prevention with a free radical scavenger. *Brain Res Bull* 1996;39:299-303.
 150. Arias C, Arrieta I, Tapia R. β -Amyloid peptide fragment 25-35 potentiates the calcium-dependent release of excitatory amino acids from depolarized hippocampal slices. *J Neurosci Res* 1995;41:561-6.
 151. Abe K, Misawa M. The extracellular signal-regulated kinase cascade suppresses amyloid β protein-induced promotion of glutamate clearance in cultured rat cortical astrocytes. *Brain Res* 2003; 979:179-87.
 152. Rodriguez-Kern A, Gegelashvili M, Schousboe A, et al. β -amyloid and brain-derived neurotrophic factor, BDNF, up-regulate the expression of glutamate transporter GLT-1/EAAT2 via different signaling

- pathways utilizing transcription factor NF-kappaB. *Neurochem Int* 2003;43:363-70.
153. Harris ME, Wang Y, Pedigo NW Jr, et al. Amyloid β peptide (25-35) inhibits Na⁺-dependent glutamate uptake in rat hippocampal astrocyte cultures. *J Neurochem* 1996;67:277-86.
 154. Lauderback CM, Hackett JM, Huang FF, et al. The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of A β 1-42. *J Neurochem* 2001;78:413-6.
 155. Noda M, Nakanishi H, Akaike N. Glutamate release from microglia via glutamate transporter is enhanced by amyloid- β peptide. *Neuroscience* 1999;92:1465-74.
 156. Pike CJ, Burdick D, Walencewicz AJ, et al. Neurodegeneration induced by β -amyloid peptides in vitro: the role of peptide assembly state. *J Neurosci* 1993;13:1676-87.
 157. Yankner BA. Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron* 1996;16:921-32.
 158. Smith WW, Gorospe M, Kusiak JW. Signaling mechanisms underlying A β toxicity: potential therapeutic targets for Alzheimer's disease. *CNS Neurol Disord Drug Targets* 2006;5:355-61.
 159. Roth KA. Caspases, apoptosis, and Alzheimer disease: causation, correlation, and confusion. *J Neuropathol Exp Neurol* 2001;60:829-38.
 160. El Khoury J, Hickman SE, Thomas CA, et al. Scavenger receptor-mediated adhesion of microglia to β -amyloid fibrils. *Nature* 1996;382:716-9.
 161. Kuner P, Schubel R, Hertel C. β -amyloid binds to p57NTR and activates NF κ B in human neuroblastoma cells. *J Neurosci Res* 1998;54:798-804.
 162. Wang HY, Lee DH, Davis CB, et al. Amyloid peptide A β (1-42) binds selectively and with pM affinity to $\alpha 7$ nicotinic acetylcholine receptors. *J Neurochem* 2000;75:1155-61.
 163. Yan SD, Chen X, Fu J, et al. RAGE and amyloid- β peptide neurotoxicity in Alzheimer's disease. *Nature* 1996;382:685-91.
 164. Jhamandas JH, MacTavish D. Antagonist of the amylin receptor blocks β -amyloid toxicity in rat cholinergic basal forebrain neurons. *J Neurosci* 2004;24:5579-84.
 165. Harkany T, Abraham I, Timmerman W, et al. β -amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis. *Eur J Neurosci* 2000;12:2735-45.
 166. Miguel-Hidalgo JJ, Alvarez XA, Cababelos R, et al. Neuroprotection by memantine against neurodegeneration induced by β -amyloid (1-40). *Brain Res* 2002;958:210-21.
 167. Guo Q, Fu W, Sopher BL, et al. Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. *Nat Med* 1999;5:101-6.
 168. Moechars D, Lorent K, Van Leuven F. Premature death in transgenic mice that overexpress a mutant amyloid precursor protein is preceded by severe neurodegeneration and apoptosis. *Neuroscience* 1999;91:819-30.
 169. Alvarez G, Munoz-Montano JR, Satrustegui J, et al. Regulation of tau phosphorylation and protection against β -amyloid-induced neurodegeneration by lithium. Possible implications for Alzheimer's disease. *Bipolar Disord* 2002;4:153-65.
 170. Brion JP, Anderton BH, Authelet M, et al. Neurofibrillary tangles and tau phosphorylation. *Biochem Soc Symp* 2001;(67):81-8.
 171. Ferrer I, Gomez-Isla T, Puig B, et al. Current advances on different kinases involved in tau phosphorylation, and implications in Alzheimer's disease and tauopathies. *Curr Alzheimer Res* 2005;2:3-18.
 172. Zheng WH, Bastianetto S, Mennicken F, et al. Amyloid β peptide induces tau phosphorylation and loss of cholinergic neurons in rat primary septal cultures. *Neuroscience* 2002;115:201-11.
 173. Stoothoff WH, Johnson GV. Tau phosphorylation: physiological and pathological consequences. *Biochim Biophys Acta* 2005;1739:280-97.
 174. Rapoport M, Dawson HN, Binder LI, et al. Tau is essential to β -amyloid-induced neurotoxicity. *Proc Natl Acad Sci U S A* 2002;99:6364-9.
 175. Lesort M, Jope RS, Johnson GV. Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3 β and Fyn tyrosine kinase. *J Neurochem* 1999;72:576-84.
 176. Hernandez P, Lee G, Sjoberg M, et al. Tau phosphorylation by cdk5 and Fyn in response to amyloid peptide A β (25-35): involvement of lipid rafts. *J Alzheimers Dis* 2009;16:149-56.
 177. Lee G, Thangavel R, Sharma VM, et al. Phosphorylation of tau by fyn: implications for Alzheimer's disease. *J Neurosci* 2004;24:2304-12.
 178. Ittner LM, Ke YD, Delerue F, et al. Dendritic function of tau mediates amyloid- β toxicity in Alzheimer's disease mouse models. *Cell* 2010;142:387-97.
 179. Liu F, Grundke-Iqbali I, Iqbali K, et al. Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur J Neurosci* 2005;22:1942-50.
 180. Liu F, Iqbali K, Grundke-Iqbali I, et al. Dephosphorylation of tau by protein phosphatase 5: impairment in Alzheimer's disease. *J Biol Chem* 2005;280:1790-6.
 181. Gong CX, Shaikh S, Wang JZ, et al. Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain. *J Neurochem* 1995;65:732-8.
 182. Sontag E, Luangpirom A, Hladik C, et al. Altered expression levels of the protein phosphatase 2A ABalpahC enzyme are associated with Alzheimer disease pathology. *J Neuropathol Exp Neurol* 2004;63:287-301.
 183. Vogelsberg-Ragaglia V, Schuck T, Trojanowski JQ, et al. PP2A mRNA expression is quantitatively decreased in Alzheimer's disease hippocampus. *Exp Neurol* 2001;168:402-12.
 184. Benneceb M, Gong CX, Grundke-Iqbali I, et al. Inhibition of PP-2A upregulates CaMKII in rat forebrain and induces hyperphosphorylation of tau at Ser 262/356. *FEBS Lett* 2001;490:15-22.
 185. Gong CX, Lidsky T, Wegiel J, et al. Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease. *J Biol Chem* 2000;275:5535-44.
 186. Qian W, Shi J, Yin X, et al. PP2A regulates tau phosphorylation directly and also indirectly via activating GSK-3 β . *J Alzheimers Dis* 2010;19:1221-9.
 187. Pei JJ, Gong CX, An WL, et al. Okadaic-acid-induced inhibition of protein phosphatase 2A produces activation of mitogen-activated protein kinases ERK1/2, MEK1/2, and p70 S6, similar to that in Alzheimer's disease. *Am J Pathol* 2003;163:845-58.
 188. Adamec E, Mercken M, Beermann ML, et al. Acute rise in the concentration of free cytoplasmic calcium leads to dephosphorylation of the microtubule-associated protein tau. *Brain Res* 1997;757:93-101.
 189. Fleming LM, Johnson GV. Modulation of the phosphorylation state of tau in situ: the roles of calcium and cyclic AMP. *Biochem J* 1995;309:41-7.
 190. Liang Z, Liu F, Iqbali K, et al. Dysregulation of tau phosphorylation in mouse brain during excitotoxic damage. *J Alzheimers Dis* 2009;17:531-9.
 191. Cotman CW, Poon WW, Rissman RA, et al. The role of caspase cleavage of tau in Alzheimer disease neuropathology. *J Neuropathol Exp Neurol* 2005;64:104-12.
 192. Gamblin TC, Chen F, Zambrano A, et al. Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci U S A* 2003;100:10032-7.
 193. Rissman RA, Poon WW, Blurton-Jones M, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest* 2004;114:121-30.
 194. Liu MC, Kobeissy F, Zheng W, et al. Dual vulnerability of tau to calpains and caspase-3 proteolysis under neurotoxic and neurodegenerative conditions. *ASN Neuro* 2011;3:e00051.
 195. Park SY, Ferreira A. The generation of a 17 kDa neurotoxic fragment: an alternative mechanism by which tau mediates β amyloid-induced neurodegeneration. *J Neurosci* 2005;25:5365-75.
 196. Raynaud F, Marcilhac A. Implication of calpain in neuronal apoptosis. A possible regulation of Alzheimer's disease. *FEBS J* 2006;273:3437-43.
 197. Amadoro G, Ciotti MT, Costanzi M, et al. NMDA receptor mediates tau-induced neurotoxicity by calpain and ERK/MAPK activation. *Proc Natl Acad Sci U S A* 2006;103:2892-7.
 198. Kornhuber J, Weller M. Psychotogenicity and N-methyl-D-aspartate receptor antagonism: implications for neuroprotective pharmaceutical therapy. *Biol Psychiatry* 1997;41:135-44.
 199. Doraiswamy PM. The role of the N-methyl-D-aspartate receptor in Alzheimer's disease: therapeutic potential. *Curr Neurol Neurosci Rep* 2003;3:373-8.
 200. Farlow MR. NMDA receptor antagonists. A new therapeutic approach for Alzheimer's disease. *Geriatrics* 2004;59:22-7.
 201. Sonkusare SK, Kaul CL, Ramarao P. Dementia of Alzheimer's disease and other neurodegenerative disorders — memantine, a new hope. *Pharmacol Res* 2005;51:1-17.
 202. Wenk GL, Parsons CG, Danysz W. Potential role of N-methyl-D-

- aspartate receptors as executors of neurodegeneration resulting from diverse insults: focus on memantine. *Behav Pharmacol* 2006;17:411-24.
203. Lipton SA, Chen HS. Paradigm shift in NMDA receptor drug development. *Expert Opin Ther Targets* 2005;9:427-9.
 204. Herrmann N, Li A, Lanctot K. Memantine in dementia: a review of the current evidence. *Expert Opin Pharmacother* 2011;12:787-800.
 205. Parsons CG, Gilling K. Memantine as an example of a fast, voltage-dependent, open channel N-methyl-D-aspartate receptor blocker. *Methods Mol Biol* 2007;403:15-36.
 206. Rao VL, Dogan A, Todd KG, et al. Neuroprotection by memantine, a non-competitive NMDA receptor antagonist after traumatic brain injury in rats. *Brain Res* 2001;911:96-100.
 207. Rammes G, Hasenjager A, Sroka-Saidi K, et al. Therapeutic significance of NR2B-containing NMDA receptors and mGluR5 metabotropic glutamate receptors in mediating the synaptotoxic effects of β -amyloid oligomers on long-term potentiation (LTP) in murine hippocampal slices. *Neuropharmacology* 2011;60:982-90.
 208. Martinez-Coria H, Green KN, Billings LM, et al. Memantine improves cognition and reduces Alzheimer's-like neuropathology in transgenic mice. *Am J Pathol* 2010;176:870-80.
 209. Minkeviciene R, Banerjee P, Tanila H. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. *J Pharmacol Exp Ther* 2004;311:677-82.
 210. Van Dam D, De Deyn PP. Cognitive evaluation of disease-modifying efficacy of galantamine and memantine in the APP23 model. *Eur Neuropsychopharmacol* 2006;16:59-69.
 211. Dong H, Yuede CM, Coughlan C, et al. Effects of memantine on neuronal structure and conditioned fear in the Tg2576 mouse model of Alzheimer's disease. *Neuropsychopharmacology* 2008;33:3226-36.
 212. Alley GM, Bailey JA, Chen D, et al. Memantine lowers amyloid- β peptide levels in neuronal cultures and in APP/PS1 transgenic mice. *J Neurosci Res* 2010;88:143-54.
 213. Ray B, Banerjee PK, Greig NH, et al. Memantine treatment decreases levels of secreted Alzheimer's amyloid precursor protein (APP) and A β peptide in the human neuroblastoma cells. *Neurosci Lett* 2010;470:1-5.
 214. Reisberg B, Doody R, Stoffler A, et al. Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med* 2003;348:1333-41.
 215. van Dyck CH, Tariot PN, Meyers B, et al. A 24-week randomized, controlled trial of memantine in patients with moderate-to-severe Alzheimer disease. *Alzheimer Dis Assoc Disord* 2007;21:136-43.
 216. Winblad B, Jones RW, Wirth Y, et al. Memantine in moderate to severe Alzheimer's disease: a meta-analysis of randomised clinical trials. *Dement Geriatr Cogn Disord* 2007;24:20-7.
 217. Bakchine S, Loft H. Memantine treatment in patients with mild to moderate Alzheimer's disease: results of a randomised, double-blind, placebo-controlled 6-month study. *J Alzheimers Dis* 2007;11:471-9.
 218. Schneider LS, Dagerman KS, Higgins JP, et al. Lack of evidence for the efficacy of memantine in mild Alzheimer disease. *Arch Neurol* 2011;68:991-8.
 219. Lipton SA. Pathologically activated therapeutics for neuroprotection. *Nat Rev Neurosci* 2007;8:803-8.



Journal of Psychiatry & Neuroscience

Change of address

We require 6 to 8 weeks' notice to ensure uninterrupted service. Please send your current mailing label, new address and the effective date of change to:

CMA Member Service Centre

1870 Alta Vista Dr.
 Ottawa ON K1G 6R7
 tel 888 855-2555 or 613 731-8610 x2307
 fax 613 236-8864
 cmamsc@cma.ca