Protection against Aβ-mediated rapid disruption of synaptic plasticity and memory by memantine

Igor Klyubin [a,b,1], Qinwen Wang [c,d,1], Miranda N. Reed [e,f,1], Elaine A. Irving [g], Neil Upton [g], Jacki Hofmeister [i], James P. Cleary [f,h,i,1], Roger Anwyl [b,c,1], Michael J. Rowan [a,b,*,1]

[a] Department of Pharmacology and Therapeutics, Trinity College, Dublin 2, Ireland
[b] Trinity College Institute of Neuroscience, Dublin 2, Ireland
[c] Department of Physiology, Trinity College, Dublin 2, Ireland
[d] Department of Physiology and Pharmacology, Medical School, Ningbo University, Ningbo, China
[e] N. Bud Grossman Center, University of Minnesota, Minneapolis, MN, 55455, USA
[f] Department of Neurology, University of Minnesota, Minneapolis, MN, 55455, USA
[g] Neurosciences CEDD, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, United Kingdom
[h] Department of Psychology, University of Minnesota, Minneapolis, MN, 55455, USA
[i] Geriatric Research, Education and Clinical Center, Veterans Affairs Medical Center, Minneapolis, MN, 55417, USA

Abstract

Soluble amyloid-β protein (Aβ) may cause cognitive impairment in Alzheimer's disease in the absence of significant neurodegeneration. Here, the ability of the NMDA receptor (NMDAR) antagonist memantine to prevent synthetic Aβ-mediated rapid functional deficits in learned behavior and synaptic plasticity was assessed in the rat. *In vitro*, pretreatment with a clinically relevant, NMDAR blocking concentration of memantine partially inhibited the induction of long-term potentiation (LTP) in the dentate gyrus and prevented further inhibition caused by exposure to $A\beta_{1-42}$. Whereas systemic injection with memantine alone inhibited LTP in the CA1 area *in vivo*, a subthreshold dose partially abrogated the inhibition of LTP by intracerebroventricular soluble $A\beta_{1-42}$. Similarly, systemic treatment with memantine alone impaired performance of an operant learning task and a subthreshold dose prevented the $A\beta_{1-42}$-mediated increase in perseveration errors. The acute protection afforded by memantine, albeit in a narrow dose range, against the rapid disruptive effects of soluble $A\beta_{1-42}$ on synaptic plasticity and learned behavior strongly implicate NMDAR-dependent reversible dysfunction of synaptic mechanisms in Aβ-mediated cognitive impairment.
© 2009 Elsevier Inc. All rights reserved.

Keywords: Amyloid-β protein; Memantine; NMDA receptor; Long-term potentiation; Excitatory synaptic transmission; Learning; Memory; Alzheimer's disease

1. Introduction

Initial research on the involvement of amyloid-β protein (Aβ) in the dementia of Alzheimer's disease laid particular emphasis on investigating the putative neurotoxicity of the fibrillar form of Aβ, large quantities of which accumulate as insoluble plaques in the brain as the disease progresses (Lorenzo and Yankner, 1996). Consistent with this hypothesis, pre-aggregated Aβ containing fibrils can cause delayed cognitive effects that are associated with neurodegeneration (Maurice et al., 1996; McDonald et al., 1994; Nitta et al., 1994; Stephan et al., 2001). However, there is a poor correlation between the brain's load of fibrillar Aβ and cognitive status at the time of death for patients with clinical Alzheimer's disease (Roth et al., 1966; Terry, 1996). A much stronger relationship exists between dementia severity and the concentration of soluble Aβ species (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). Growing evidence strongly suggests

---

that soluble oligomers of Aβ may cause cognitive impairment, especially during early stages of the disease when there is little evidence of neurodegeneration (Haass and Selkoe, 2007). In animal studies, Aβ oligomers have been found to cause rapid disruption of learning (Cleary et al., 2005; Lesne et al., 2006) and the synaptic mechanisms underlying memory, including synaptic plasticity (Cullen et al., 1997; Lambert et al., 1998). Indeed, oligomeric Aβ derived from chemical synthesis and derived from human cerebrospinal fluid or brain, or amyloid precursor protein-transfected cells, rapidly and potently inhibit long-term potentiation (LTP) of glutamatergic transmission in brain areas involved in memory formation, including the hippocampus (Hu et al., 2008; Klyubin et al., 2008; Lambert et al., 1998; Shankar et al., 2008; Walsh et al., 2002). The disruption of LTP mechanisms by soluble oligomers of Aβ may underly their rapid cognitive impairing effects (Cleary et al., 2005; Lesne et al., 2006).

Glutamatergic mechanisms have been extensively investigated in Alzheimer's disease (Greenamyre and Young, 1989; Hynd et al., 2004; Lawlor and Davis, 1992). Early research focused on the role of Aβ enhancement of glutamatergic excitotoxicity, including that mediated through N-methyl-D-aspartate glutamate receptors (NMDARs) (Koh et al., 1990; Mattson et al., 1992). Consistent with this, Aβ enhances NMDAR agonist-induced delayed cognitive dysfunction (Dornan et al., 1993; Nakamura et al., 2006). Indeed, Aβ can directly inhibit glutamate uptake by cultured astrocytes and neurons (Fernandez-Tome et al., 2004; Harkany et al., 2000; Harris et al., 1995; Matos et al., 2008), and synaptosomes (Keller et al., 1997), albeit at relatively high concentrations. Aβ has also been found to enhance glutamate release from microglia (Noda et al., 1999), isolated brain nerve terminals (Bobich et al., 2004) and brain slices (Arias et al., 1995; Chin et al., 2007; Kabogo et al., 2008; Puzzo et al., 2008). Recently, soluble oligomers of Aβ were found to rapidly and potently trigger Ca^{2+} influx and oxidative stress in cultured hippocampal neurons that was prevented by NMDAR antagonists (De Felice et al., 2007; Kelly and Ferreira, 2006). Intriguingly, Lacor et al. (2007), De Felice et al. (2007) and Dewachter et al. (2009) provided evidence that NMDARs were closely associated with Aβ oligomer binding sites on these neurons.

Support for an involvement of NMDARs in the cognitive deficits of Alzheimer's disease is provided by the current clinical use of memantine (Raina et al., 2008), which has been found to act as a low-to-moderate affinity open channel uncompetitive inhibitor of NMDARs at therapeutic concentrations (Lipton, 2007; Parsons et al., 2007). Although consistent with the putative involvement of excitotoxic mechanisms, the use of memantine to enhance cognition is somewhat paradoxical, given the requirement for NMDAR-mediated transmission in many forms of learning and synaptic plasticity (Riedel et al., 2003). Clinical doses of memantine are generally well tolerated (Raina et al., 2008) (but see Rammsayer, 2001; Sang et al., 2002; Villoslada et al., 2008) but controversy has arisen as to whether or not neuroprotection against excitotoxicity can be achieved without impairing cognition (Creeley et al., 2006; More et al., 2008). It has been proposed that memantine, unlike many high affinity antagonists (Ikonomidou and Turski, 2002), may prevent excessive or inappropriate activation of NMDARs while leaving physiological NMDAR-mediated transmission unaffected (Lipton, 2007; Parsons et al., 2007). Support for this proposal was obtained in *in vitro* experiments where inhibition of LTP caused by lowering extracellular Mg^{2+} concentration (Frankiewicz and Parsons, 1999) or by exogenous application of NMDA (Zajaczkowski et al., 1997) was prevented by therapeutic concentrations of memantine that did not significantly affect NMDAR-dependent LTP under control conditions (Coan et al., 1989; Frankiewicz et al., 1996; Zorumski and Izumi, 1998).

The present study directly tested the proposal that NMDAR-blocking doses of memantine could prevent the rapid disruption of cognitive mechanisms caused by Aβ at three levels, LTP induction at medial perforant pathway synapses in the dentate gyrus *in vitro*, LTP induction at CA3-to-CA1 synapses *in vivo*, and learned behavior, under the Alternating Lever Cyclic Ratio (ALCR) cognitive test. We also examined the effect of acute treatment with memantine alone over a similar concentration range on LTP and behavior.

2. Materials and methods

2.1. In vitro electrophysiology

Transverse slices of the hippocampus were prepared from male Wistar rats (age 3–4 weeks, weight 40–80 g). The brains were rapidly removed after decapitation and placed in cold oxygenated (95% O_2/5% CO_2) physiological media. Slices (350 μm thick) were cut using an Intracell Plus 1000 and placed in a storage container containing oxygenated medium at room temperature (20–22 °C) for 1 h. The slices were then transferred to a recording chamber for submerged slices and continuously superfused at a rate of 5–6 mL/min at 30–32 °C. The control media contained: (mM) NaCl, 120; KCl, 2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $MgSO_4$, 2.0; $CaCl_2$, 2.0; D-glucose, 10. All solutions contained 100 μM picrotoxin (Sigma) to block $GABA_A$-mediated activity.

Presynaptic stimulation was applied to the medial perforant pathway of the dentate gyrus using a bipolar insulated tungsten wire electrode, and field excitatory postsynaptic potentials (EPSPs) were recorded at a control test frequency of 0.033 Hz from the middle one-third of the molecular layer of the dentate gyrus with a glass microelectrode. The inner blade of the dentate gyrus was used in all studies. In each experiment, an input–output curve (afferent stimulus intensity versus EPSP amplitude) was plotted at the test frequency. For all experiments, the amplitude of the test EPSP was adjusted to one-third of maximum (∼1.2 mV). LTP was evoked by high frequency stimulation (HFS) consisting of 8 trains, each of 8 stimuli at 200 Hz, inter-train interval 2 s, with the stimulation voltage increased during the HFS so as

to evoke an initial EPSP of the train of double the normal test EPSP amplitude. Recordings were analysed using p-CLAMP (Axon Instruments, CA, USA).

2.2. In vivo electrophysiology

In vivo experiments were carried out on urethane (1.5 gm/kg i.p.) anaesthetized male Wistar rats (250–300 g). Body temperature was maintained at 37–37.3 °C. The animal care and experimental protocol was approved by the Department of Health, Republic of Ireland.

Electrodes were made and implanted as described previously (Klyubin et al., 2004). Briefly, twisted-wire bipolar electrodes were constructed from Teflon-coated tungsten wires (62.5 µm inner core diameter, 75 µm external diameter). Single pathway recordings of field EPSPs were made from the stratum radiatum in the CA1 area of the right hippocampal hemisphere in response to stimulation of the ipsilateral Schaffer collateral—commissural pathway. Electrode implantation sites were identified using stereotaxic coordinates relative to bregma, with the recording site located 3.4 mm posterior to bregma and 2.5 mm right of midline, and the stimulating electrode located 4.2 mm posterior to bregma and 3.8 right of midline. The optimal depth of the wire electrodes in the stratum radiatum of the CA1 region of the dorsal hippocampus was determined using electrophysiological criteria and verified post-mortem. Test EPSPs were evoked at a frequency of 0.033 Hz and at a stimulation intensity adjusted to give an EPSP amplitude of 50% of maximum. The HFS protocol for inducing LTP consisted of 10 trains of 20 stimuli, inter-stimulus interval 5 ms (200 Hz), inter-train interval 2 s. The intensity was increased to give an EPSP of 75% of maximum amplitude during the HFS.

To inject samples, a stainless-steel guide cannula (22 gauge, 0.7 mm outer diameter, 13 mm length) was implanted above the right lateral ventricle (1 mm lateral to the midline and 4 mm below the surface of the dura) just prior to electrode implantation. Intracerebroventricular (i.c.v.) injections of 5 µL were made via an internal cannula (28 gauge, 0.36 mm outer diameter). Verification of the placement of the cannula was performed post-mortem by checking the spread of ink dye after i.c.v. injection.

2.3. Behavioral testing

Forty-one male Sprague–Dawley, approximately 120 days old and weighing 300–350 g at the beginning of the experiment, were used. All rats were housed individually with free access to water. Rats were maintained at 90–95% of their free-feeding weights. All experiments were done in accordance with guidelines of the Institutional Animal Care and Use Committee of the Minneapolis Veterans Affairs Medical Center.

Behavioral training and testing was carried out in a two-lever rat test chamber (model E10, Coulbourn Instruments, Inc.) enclosed within a sound-attenuating compartment. Each

station has a house light, two levers, a feeding aperture for pellet delivery situated midway between levers, and stimulus signaling lights above each lever. Food reinforcement consisted of a 45-mg sucrose pellet. An audible pellet-dispenser click signaled food delivery. Experimental sessions were computer controlled, and data were collected automatically (MED PC; Med Associates).

For training, behavioral sessions were conducted five days a week. Rats were first trained to press both levers for food reinforcement. Over approximately 20–30 sessions, the ALCR procedure was introduced and required responses-per-reinforcer criteria were slowly increased toward the final requirements (see below).

The ALCR test has been described in detail previously (Cleary et al., 2005; Richardson et al., 2002). Briefly, under this assay rats must learn a complex sequence of lever-pressing demands in a two-lever experimental chamber. Subjects must alternate to the other lever after pressing one lever enough to satisfy the pressing requirement and getting food reward. The exact number of presses required for each food reward changes, increasing from 2 responses per food pellet up to 56 presses per food pellet, then decreasing back to 2 responses per pellet. Intermediate values are based on the quadratic function, $x^2 - x$. One cycle is an entire ascending and descending sequence of lever press requirements (e.g., 2, 6, 12, 20, 30, 42, 56, 56, 42, 30, 20, 12, 6, and 2 presses per food reward). Six cycles are presented during each daily session. Errors can be of two types. Approach errors are counted when a subject fails to alternate levers after being rewarded or switches from the correct lever to the incorrect lever before completing enough responses to get a reward on the correct lever. Perseveration errors occur when the subject 'perseveres' on the incorrect lever after making an initial approach error. The session ended after 6 cycles were completed or after 2 h. Behavioral sessions were conducted seven days per week during compound testing. Rats received approximately 40 sessions prior to surgery.

2.3.1. Surgery and lateral ventricle cannula implantation

Rats were anesthetized with 60 mg/kg of ketamine and 20 mg/kg xylazine while a 26-gauge cannula was implanted unilaterally in the lateral ventricle. Cannulae were capped with stylets that extended the length of the cannula. Half of the rats received left lateral ventricle cannulae implants and the other half received right ventricle cannulae implants. Rats were allowed to recover for five days following surgery at which point baseline error rates were re-established under ALCR.

2.3.2. Injection schedule, injectates and vehicles

Behavioral sessions were conducted five days per week with all rats receiving all injectates in the same order. Injectates were given approximately every four days. All i.c.v. injections were 10 µL, given to awake freely moving rats, over at least a 3 min period. Under this within-subject design,

all subjects received all injectates. Following i.c.v. Aβ injection, the cannula was capped with a stylet, and the rat was placed in a holding cage for 2 h prior to behavioral assessment under ALCR (30 min for scopolamine). Following surgery, rats were initially injected i.c.v. with saline (0.9%) and saline injections continued periodically throughout the experiment. On non-injection days, rats were subjected to "sham" injections during which the entire injection procedure was performed, but no injectate was administered.

Rats were injected i.c.v. with saline (0.9%), scopolamine hydrobromide (2.0 μg, as the salt), and synthetic Aβ42 (32 μM stock) in 10 μL.

Memantine (1–10 mg/kg) was dissolved in saline and administered i.p. in a 1 mL/kg volume. Memantine was administered 45 min prior to the behavioral assessment under ALCR during which time the rat was placed in a holding cage.

2.4. Agents

For the *in vitro* experiments, synthetic Aβ$_{1-42}$ (amyloid-β protein$_{1-42}$) (Bachem) was prepared as a stock solution of 50 μM in ammonium hydroxide (0.1%), stored at −20 °C, and then added to physiological medium immediately prior to each experiment. For the *in vivo* experiments, synthetic Aβ$_{1-42}$ (Biopolymer Laboratory, UCLA Medical School) was re-suspended in ice-cold MilliQ water. An aliquot was removed and centrifuged at $100,000 \times g$ for 3 h, conditions known to pellet fibrils and protofibrils (Klyubin et al., 2004). After centrifugation the supernatant, which had a final concentration of soluble Aβ as determined using the microBCA protein assay (Thermo-Fisher Scientific Life Science Research Products) of 64 μM was stored in small aliquots at −80 °C.

Memantine hydrochloride and scopolamine hydrobromide were purchased from Sigma.

2.5. Statistics

Data are presented as the mean ± S.E.M. For the electrophysiology statistical comparisons were first made using ANOVA followed by two-tailed paired or unpaired Student's *t*-tests. For the behavioral experiments, all rats served as their own control in a within-subject design. Errors for each subject under test injections were compared to the subject's mean error rate derived from 3 contiguous sessions. Paired two-tailed Student's *t*-tests were used to infer differences in errors under test substances, vehicle or sham injections.

3. Results

3.1. The acute inhibitory effect of Aβ on LTP in vitro is attenuated by memantine at a concentration that partially inhibits LTP

Consistent with our previous studies (Wang et al., 2008), acute superfusion of hippocampal slices with Aβ$_{1-42}$

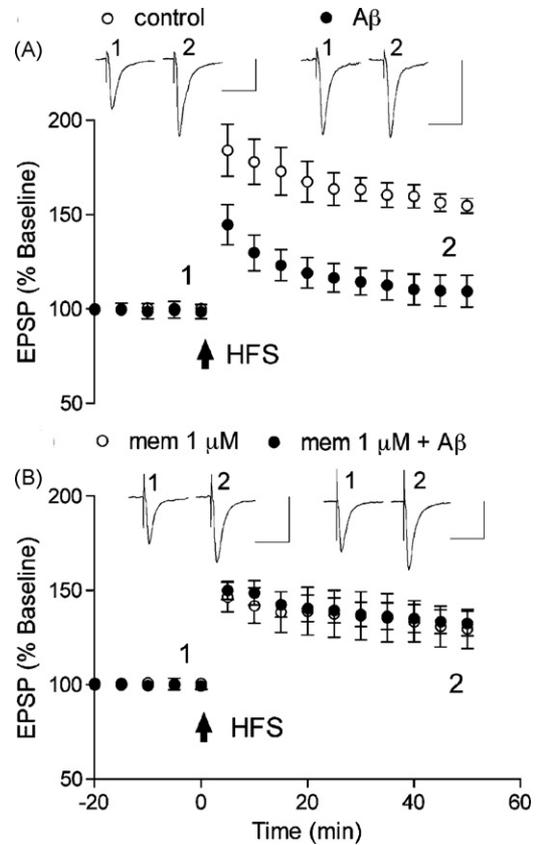

Fig. 1. Protective effect of bath-applied memantine on Aβ-mediated inhibition of LTP in the rat hippocampal slice. (A) In the dentate gyrus of control slices (open circles), high frequency stimulation (HFS, arrow) induced LTP whereas continuous perfusion with synthetic Aβ$_{1-42}$ (500 nM, closed circles), starting 45 min prior to HFS, strongly inhibited induction of LTP. (B) Continuous perfusion with an NMDAR blocking concentration of memantine alone (mem, 1 μM, open circles), starting 2 h prior to HFS, partially inhibited LTP. Pretreatment of slices for 2 h with the same concentration of memantine prevented any further inhibition of LTP by perfusion with 500 nM Aβ$_{1-42}$ (closed circles). Values are the mean ± S.E.M. Insets show typical traces of the field EPSP at the times indicated. Vertical bar 1 mV, horizontal bar 20 ms.

(500 nM) for 45 min strongly inhibited LTP induction by HFS at medial perforant pathway synapses in the dentate gyrus *in vitro* ($109 \pm 3\%$ pre-HFS baseline at 50 min post-HFS, $n = 4$; $P < 0.05$ compared to baseline and compared to control LTP, $154 \pm 4\%$, $n = 5$) (Fig. 1A). In order to study the effect of memantine on the inhibitory effect of Aβ, we chose a concentration of memantine that is reported (Lipton, 2007; Parsons et al., 2007) to be in the NMDAR-blocking and therapeutic range (1 μM). Memantine was pre-applied for 2 h before HFS and was continuously present for the remainder of the experiment. Under these conditions application of memantine alone partially inhibited the induction of LTP ($130 \pm 6\%$, $n = 5$; $P < 0.05$ compared to baseline), the magnitude of LTP being significantly less than in controls ($P < 0.05$) (Fig. 1B). Remarkably, in slices pretreated with this concentration of memantine, superfusion with Aβ$_{1-42}$ did not cause any significant further inhibition of LTP ($133 \pm 3\%$,

$n = 4$; $P < 0.05$ compared to baseline; $P > 0.05$ compared to memantine alone).

3.2. Partial prevention of the acute inhibitory effect of soluble Aβ on LTP in vivo by systemic treatment with a dose of memantine that does not inhibit LTP alone

In order to study the effect of memantine on the acute inhibitory effect of Aβ *in vivo*, we systemically injected a dose (10 mg/kg, i.p.) that has been reported to produce NMDAR-blocking and clinically relevant brain concentrations (Hesselink et al., 1999b; Parsons et al., 2007). When injected alone 40 min prior to HFS, this dose of memantine did not significantly affect LTP at CA3-to-CA1 synapses ($145 \pm 5\%$ baseline, $n = 5$; $P < 0.05$ compared to baseline; $P > 0.05$ compared to control LTP, $145 \pm 8\%$, $n = 5$) whereas injection of twice this dose (20 mg/kg) completely inhibited LTP ($98 \pm 4\%$, $n = 5$; $P > 0.05$ compared to baseline; $P < 0.05$ compared to control) (Fig. 2A). Acute injection of soluble Aβ$_{1–42}$ (40 pmol in 5 μL, i.c.v.) alone 10 min before HFS inhibited LTP ($103 \pm 3\%$ pre-HFS baseline at 2 h post-HFS, $n = 4$; $P > 0.05$ compared to baseline; $P < 0.05$ compared to control LTP, $154 \pm 8\%$, $n = 4$) (Fig. 2B), confirming our previous reports (Klyubin et al., 2004; Wang et al., 2008). However, when 10 mg/kg memantine was injected 30 min before Aβ$_{1–42}$, the inhibitory effect of Aβ$_{1–42}$ was partially prevented ($120 \pm 6\%$, $n = 5$; $P < 0.05$ compared to baseline and compared to control LTP). Increasing the delay between the administration of Aβ$_{1–42}$ and the injection of this dose of memantine to 2 h, similar to the protocol used in the *in vitro* experiments, gave a similar partial protection ($123 \pm 5\%$, $n = 3$, data not shown).

3.3. Prevention of the acute disruptive effect of soluble Aβ on learned behavior by systemic treatment with a dose of memantine that does not impair control performance

The Alternating Lever Cyclic Ratio cognitive test was used to study the ability of memantine to alter the acute behavioral effects of Aβ because this test is very sensitive to a rapid disruptive effect of naturally secreted Aβ (Cleary et al., 2005). In animals pre-trained to criterion on the ALCR task, systemic administration of memantine alone, starting 45 min prior to commencing the session, caused a dose-dependent increase in both perseveration and approach errors, with a threshold dose of approximately 3 mg/kg, i.p. (approach errors, $342 \pm 61\%$; perseveration errors, $301 \pm 35\%$, $P < 0.05$ compared to baseline rate; $n = 41$) (Fig. 3A). The lower dose of 1 mg/kg did not significantly affect the number of errors (approach errors, $116 \pm 12\%$; perseveration errors, $125 \pm 17\%$, $P > 0.05$ compared to baseline rate). Injection of soluble Aβ$_{1–42}$ alone i.c.v. (60 pmol in 10 μL) 2 h before commencing testing nearly doubled the number of perseveration errors whereas there was a non-significant increase in the number of approach
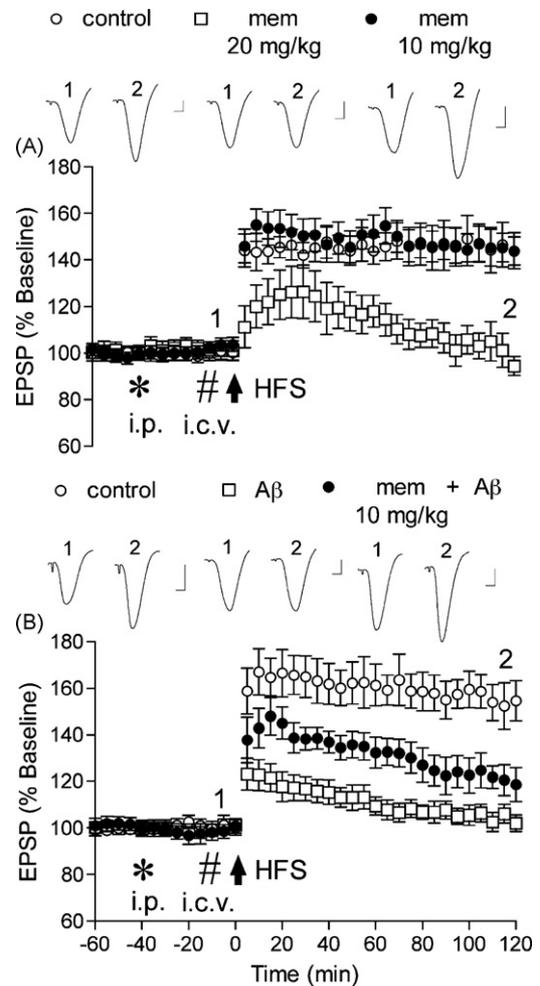

Fig. 2. Protective effect of acute systemic injection of memantine on Aβ-mediated inhibition of LTP *in vivo*. (A) In vehicle-injected control animals HFS induced LTP in the CA1 area of the anesthetized rat hippocampus (open circles). Whereas pre-injection with a dose of 20 mg/kg, i.p. memantine (mem, asterisk, open squares) completely inhibited the induction of LTP by HFS, a dose of 10 mg/kg memantine (asterisk, closed circles) did not significantly affect the magnitude of LTP. (B) Intracerebroventricular (i.c.v.) injection of soluble synthetic Aβ$_{1–42}$ (40 pmol, hash, open squares) 10 min prior to HFS completely inhibited LTP compared to vehicle-injected controls (open circles). In animals pretreated for 30 min with 10 mg/kg memantine (asterisk, closed circles) the injection of Aβ$_{1–42}$ (40 pmol, i.c.v., hash, closed circles) now only partially inhibited LTP. Values are the mean ± S.E.M. Insets show typical traces of field EPSPs recorded at the times indicated. Vertical bar 0.5 mV, horizontal bar 10 ms.

errors (approach errors, $128 \pm 20\%$, $P > 0.05$; perseveration errors, $198 \pm 46\%$, $P < 0.05$) (Fig. 3B). This profile of impairment contrasts with that caused by i.c.v. injection of scopolamine (4 nmol in 10 μL; approach errors, $273 \pm 53\%$, $P < 0.05$; perseveration errors, $384 \pm 76\%$, $P < 0.05$, not shown). Importantly, Aβ$_{1–42}$ failed to significantly increase perseveration errors when the animals also received an injection of the subthreshold dose of memantine (1 mg/kg) (approach errors, $121 \pm 12\%$, $P > 0.05$; perseveration errors, $124 \pm 17\%$, $P > 0.05$).
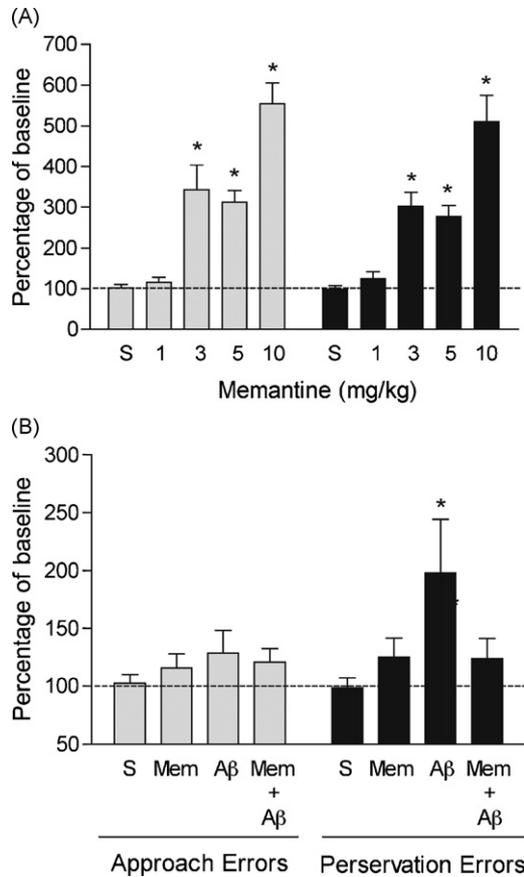
Fig. 3. Protective effect of acute systemic injection of memantine on Aβ-mediated disruption of learned behavior in an operant task. (A) Injection of memantine alone 45 min before commencing the session, dose-dependently increased both approach (grey bars) and perseveration (black bars) errors on the Alternating Lever Cycle Ratio task in the range of 3–10 mg/kg i.p. (B) Injection with soluble synthetic Aβ$_{1-42}$ (60 pmol, i.c.v.) 2 h before commencing the session, significantly increased perseveration errors, but not approach errors, compared to saline, whereas injection with memantine alone (1 mg/kg, i.p.) or memanine + Aβ$_{1-42}$ was not significantly different from saline. *$P < 0.05$. Error bars indicate one S.E.M.

4. Discussion

Memantine, at NMDAR blocking and therapeutically relevant doses, reduced the rapid disruptive effects of soluble Aβ$_{1-42}$ on synaptic plasticity and learned behavior. Thus, it attenuated the acute inhibitory effects of Aβ$_{1-42}$ on LTP induction in the hippocampus both *in vitro* and *in vivo*, and the increase in perseveration errors caused by acute treatment with Aβ$_{1-42}$ in an operant learning task. These findings indicate that the disruptive effects of soluble Aβ on memory mechanisms are NMDAR-dependent. However, memantine also had clear disruptive effects on its own over a similar dose-range. It dose-dependently inhibited LTP and increased both the number of perseveration and approach errors. These acute disruptive effects of memantine on synaptic plasticity and behavior are also consistent with its known ability to block NMDARs. The potentially beneficial effects of memantine against Aβ-mediated disruption of LTP and learned behavior combined with its potential disruptive effects over a similar dose range, support the search for agents that more selectively target NMDAR involvement in pathophysiological mechanisms.

The ability of an NMDAR antagonist to attenuate the inhibitory effect of Aβ on LTP at CA3-to-CA1 pyramidal cell and medial perforant path-to-granule cell synapses may appear paradoxical, given the requirement of LTP induction at these synapses for NMDAR activation (Hu et al., 2008; Wu et al., 2001). However, it is known that excessive or inappropriate activation of NMDARs can block LTP (Coan et al., 1989; Zorumski and Izumi, 1998). The protective effect of memantine against inhibition of NMDAR-dependent LTP by Aβ$_{1-42}$ found in the present study both *in vivo* and *in vitro* appears analogous to the reported ability of memantine to prevent the inhibition of LTP due to overactivation of NMDARs caused by lowering extracellular Mg^{2+} concentration (Frankiewicz and Parsons, 1999) or by exogenous application of NMDA (Zajaczkowski et al., 1997) in the CA1 area of hippocampal slices. Since memantine acts as an uncompetitive voltage-dependent inhibitor of NMDARs in the dose range used here (Lipton, 2007; Parsons et al., 2007), the present findings indicate that the rapid disruptive effects of soluble Aβ on LTP involve NMDAR activation. We found evidence that the concentration required to provide protection against Aβ inhibition of LTP was at or near the concentration of memantine that caused partial inhibition of LTP on its own. Although we did not carry out detailed analysis of the dose–response relationship, it appears that memantine may have a stronger inhibitory effect on the induction of LTP in the dentate gyrus than that previously reported for the CA1 area, where a concentration of 1 μM was reported not to affect control LTP (Frankiewicz et al., 1996). In the present *in vivo* studies the memantine concentration in the brain extracellular fluid at the time of the tetanus, based on the findings of Hesselink et al. (1999b), would be expected to be approximately 1 μM after administration of 10 mg/kg, a dose that did not significantly affect control LTP in the CA1 area, consistent with the previous *in vitro* findings of Frankiewicz et al. (1996). A concentration of 1 μM memantine has been reported to occupy approximately 33% of brain NMDAR channels measured by radioligand binding *in vivo* (More et al., 2008) and there is *in vivo* evidence that LTP induction in the dentate gyrus may be more sensitive to inhibition by NMDAR channel blockers than in the CA1 area (Leung and Shen, 1999; Gilbert and Mack, 1990). The ability of memantine to selectively reduce an NMDAR-mediated inhibition of LTP at concentrations that do not affect control LTP may be due to a rapid unblocking of the channel with strong activation, as occurs during high frequency stimulation (Parsons et al., 2007).

An alternative explanation, that memantine may act through non-NMDAR mechanisms, in particular by blocking α7 nicotinic acetylcholine receptors (Aracava et al., 2005; Maskell et al., 2003) but see Parsons et al. (2007), is unlikely. Although Aβ$_{1-42}$ is known to potently bind α7 nicotinic

receptors (Wang et al., 2000), the rapid inhibition of LTP by $A\beta_{1-42}$ was not affected by pharmacological antagonism of $\alpha 7$ nicotinic acetylcholine receptors (Wang et al., 2004).

What might underlie the apparent NMDAR-dependence of the disruptive effects of soluble $A\beta$ on synaptic plasticity? $A\beta$ oligomers may bind to NMDARs or adjacent sites at synapses and thereby promote receptor activation (Cowburn et al., 1997; De Felice et al., 2007; Lacor et al., 2007). Indeed under certain conditions $A\beta$ can selectively enhance NMDAR-mediated currents and synaptic transmission both *in vitro* and *in vivo* (Domingues et al., 2007; Kelly and Ferreira, 2006; Molnar et al., 2004; Szegedi et al., 2005; Wu et al., 1995) but see, for example, Snyder et al. (2005). Alternatively, or in addition, $A\beta$ may increase endogenous glutamate concentration at NMDARs by inhibiting glutamate uptake or promoting the release of glutamate (Harris et al., 1995; Noda et al., 1999). Extrasynaptic NMDARs may preferentially mediate toxic effects of glutamate (Lau and Zukin, 2007; Soriano and Hardingham, 1997; Zhang et al., 2007), and recent evidence indicates that memantine can preferentially block extrasynaptic over synaptic NMDARs (Léveillé et al., 2008; Kotermanski and Johnson, 2009). Interestingly, bath-applied glutamate, which has been reported to preferentially activate extrasynaptic NMDARs (Léveillé et al., 2008), can potentiate $A\beta$ inhibition of LTP (Nakagami and Oda, 2002).

There was a similar narrow separation of the protective and adverse effects of acute memantine treatment in the behavioral studies. Soluble synthetic $A\beta_{1-42}$ had a rapid disruptive effects on learned behavior causing an increase in perseveration errors in the ALCR task, which is consistent with our previous studies with cell-derived $A\beta$ (Cleary et al., 2005; Townsend et al., 2006). However, unlike cell-derived $A\beta$, synthetic $A\beta_{1-42}$ increased the number of perseveration errors without eliciting a significant increase in approach errors in the ALCR task. The lack of change in approach errors may be a dose-related phenomenon due to the reduced sensitivity typically observed under this type of alternation test.

The ALCR task appeared somewhat less sensitive than *in vivo* hippocampal LTP to disruption by synthetic $A\beta_{1-42}$ since, at the doses tested, synthetic $A\beta_{1-42}$ caused complete inhibition of LTP whereas the deficit under the ALCR task was well below the upper limits of sensitivity for this procedure. We previously found that cell-derived oligomers of $A\beta$ were highly disruptive in both of these tests at much lower concentrations (Cleary et al., 2005; Townsend et al., 2006; Walsh et al., 2002). It is not known whether or not performance of the ALCR task is dependent on the hippocampus but we have recently found that spatial working memory in a radial maze, thought to be hippocampal-dependent, is also highly sensitive to the rapid disruptive effects of cell-derived $A\beta$ oligomers (Poling et al., 2008). Because performance of the ALCR task is likely to be dependent on more than one brain area and $A\beta_{1-42}$ disruption of the ALCR task may

be caused by an action at extra-hippocampal sites, it is possible that a lower concentration was reached at these sites after the i.c.v. injection. Interestingly, the relatively low dose of 1 mg/kg memantine prevented the increase in behavioral errors caused by $A\beta_{1-42}$, but a dose ten times higher only had a partial protective effect against $A\beta_{1-42}$-mediated inhibition of LTP. The relatively high potency of memantine against the $A\beta_{1-42}$-elicited increase in perseveration errors may be due to the relatively weak nature of the deficit. In contrast to $A\beta_{1-42}$, cognitive performance under the ALCR task was severely disrupted under doses of memantine that did not appear to affect *in vivo* LTP. Whereas the memantine dose of 10 mg/kg had no significant effect on hippocampal LTP, it caused a marked increase in both perseveration and approach errors in the ALCR task. Acute treatment with memantine alone can impair other types of learning over a somewhat higher dose range to that found here for the ALCR task (Hesselink et al., 1999a; More et al., 2008; Zajaczkowski et al., 1997; Zoladz et al., 2006). It should be noted that the disruptive effect of memantine alone on the ALCR task performance was limited to the day of injection, with no carryover effects on the following days. However, rather than being solely attributable to a direct effect on cognition, deleterious effect of the higher doses of memantine alone may have been influenced by sensorimotor impairment since acute treatment with NMDAR antagonists (Bannerman et al., 2006; Cain et al., 1997; Riedel et al., 2003), including memantine (Hesselink et al., 1999a; Wenk et al., 1995; Zoladz et al., 2006), dose-dependently cause such generalized deficits. In clinical practice patients treated with memantine usually have their oral dosage adjusted gradually upwards over a number of days. Although the final extracellular brain concentration achieved in patients may be similar to that achieved after an acute i.p. injection of 10 mg/kg in rats (approximately 1 μM) many potential unwanted side effects may be minimised. Currently memantine is only approved for the treatment of Moderate-to-Severe AD with ongoing evaluation of studies examining its therapeutic utility at earlier stages of AD.

Overall, the present findings suggest that acute treatment with clinically relevant NMDAR blocking doses of memantine can attenuate the rapid disruption of hippocampal LTP *in vitro* and *in vivo*, and learned behavior deficits under an operant task, caused by exogenous soluble synthetic $A\beta$. However a similar range of doses of memantine alone caused significant functional impairment, supporting the search for more pathophysiologically selective NMDAR antagonists.

Disclosure statement

References

Aracava, Y., Pereira, E.F., Maelicke, A., Albuquerque, E.X., 2005. Memantine blocks alpha7* nicotinic acetylcholine receptors more potently than N-methyl-D-aspartate receptors in rat hippocampal neurons. J. Pharmacol. Exp. Ther. 312, 1195–1205.

Arias, C., Arrieta, I., Tapia, R., 1995. Beta-amyloid peptide fragment 25–35 potentiates the calcium-dependent release of excitatory amino acids from depolarized hippocampal slices. J. Neurosci. Res. 41, 561–566.

Bannerman, D.M., Rawlins, J.N., Good, M.A., 2006. The drugs don't work-or do they? Pharmacological and transgenic studies of the contribution of NMDA and GluR-A-containing AMPA receptors to hippocampal-dependent memory. Psychopharmacology (Berl.) 188, 552–566.

Bobich, J.A., Zheng, Q., Campbell, A., 2004. Incubation of nerve endings with a physiological concentration of Abeta$_{1-42}$ activates CaV2.2 (N-type)-voltage operated calcium channels and acutely increases glutamate and noradrenaline release. J. Alzheimers Dis. 6, 243–255.

Cain, D.P., Saucier, D., Boon, F., 1997. Testing hypotheses of spatial learning: the role of NMDA receptors and NMDA-mediated long-term potentiation. Behav. Brain Res. 84, 179–193.

Chin, J.H., Ma, L., MacTavish, D., Jhamandas, J.H., 2007. Amyloid beta protein modulates glutamate-mediated neurotransmission in the rat basal forebrain: involvement of presynaptic neuronal nicotinic acetylcholine and metabotropic glutamate receptors. J. Neurosci. 27, 9262–9269.

Cleary, J.P., Walsh, D.M., Hofmeister, J.J., Shankar, G.M., Kuskowski, M.A., Selkoe, D.J., Ashe, K.H., 2005. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat. Neurosci. 8, 79–84.

Coan, E.J., Irving, A.J., Collingridge, G.L., 1989. Low-frequency activation of the NMDA receptor system can prevent the induction of LTP. Neurosci. Lett. 105, 205–210.

Cowburn, R.F., Wiehager, B., Trief, E., Li-Li, M., Sundstrom, E., 1997. Effects of beta-amyloid-(25–35) peptides on radioligand binding to excitatory amino acid receptors and voltage-dependent calcium channels: evidence for a selective affinity for the glutamate and glycine recognition sites of the NMDA receptor. Neurochem. Res. 22, 1437–1442.

Creeley, C., Wozniak, D.F., Labruyere, J., Taylor, G.T., Olney, J.W., 2006. Low doses of memantine disrupt memory in adult rats. J. Neurosci. 26, 3923–3932.

Cullen, W.K., Suh, Y.H., Anwyl, R., Rowan, M.J., 1997. Block of LTP in rat hippocampus in vivo by β-amyloid precursor protein fragments. Neuroreport 8, 3213–3217.

Dewachter, I., Filipkowski, R.K., Priller, C., Ris, L., Neyton, J., Croes, S., Terwel, D., Gysemans, M., Devijver, H., Borghgraef, P., Godaux, E., Kaczmarek, L., Herms, J., Van Leuven, F., 2009. Deregulation of NMDA-receptor function and down-stream signaling in APP[V717I] transgenic mice. Neurobiol. Aging 30, 241–256.

De Felice, F.G., Velasco, P.T., Lambert, M.P., Viola, K., Fernandez, S.J., Ferreira, S.T., Klein, W.L., 2007. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J. Biol. Chem. 282, 11590–11601.

Domingues, A., Almeida, S., da Cruz e Silva, E.F., Oliveira, C.R., Rego, A.C., 2007. Toxicity of beta-amyloid in HEK293 cells expressing NR1/NR2A or NR1/NR2B N-methyl-D-aspartate receptor subunits. Neurochem. Int. 50, 872–880.

Dornan, W.A., Kang, D.E., McCampbell, A., Kang, E.E., 1993. Bilateral injections of beta A(25–35)+IBO into the hippocampus disrupts acquisition of spatial learning in the rat. Neuroreport 5, 165–168.

Fernandez-Tome, P., Brera, B., Arevalo, M.A., de Ceballos, M.L., 2004. Beta-amyloid 25–35 inhibits glutamate uptake in cultured neurons and astrocytes: modulation of uptake as a survival mechanism. Neurobiol. Dis. 15, 580–589.

Frankiewicz, T., Potier, B., Bashir, Z.I., Collingridge, G.L., Parsons, C.G., 1996. Effects of memantine and MK-801 on NMDA-induced currents in cultured neurones and on synaptic transmission and LTP in area CA1 of rat hippocampal slices. Br. J. Pharmacol. 117, 689–697.

Frankiewicz, T., Parsons, C.G., 1999. Memantine restores long term potentiation impaired by tonic N-methyl-D-aspartate (NMDA) receptor activation following reduction of Mg^{2+} in hippocampal slices. Neuropharmacology 38, 1253–1259.

Gilbert, M.E., Mack, C.M., 1990. The NMDA antagonist, MK-801, suppresses long-term potentiation, kindling, and kindling-induced potentiation in the perforant path of the unanesthetized rat. Brain Res. 519, 89–96.

Greenamyre, J.T., Young, A.B., 1989. Excitatory amino acids and Alzheimer's disease. Neurobiol. Aging 10, 593–602.

Haass, C., Selkoe, D.J., 2007. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat. Rev. Mol. Cell Biol. 8, 101–112.

Harkany, T., Abraham, I., Timmerman, W., Laskay, G., Toth, B., Sasvari, M., Konya, C., Sebens, J.B., Korf, J., Nyakas, C., Zarandi, M., Soos, K., Penke, B., Luiten, P.G., 2000. Beta-amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis. Eur. J. Neurosci. 12, 2735–2745.

Harris, M.E., Carney, J.M., Cole, P.S., Hensley, K., Howard, B.J., Martin, L., Bummer, P., Wang, Y., Pedigo Jr., N.W., Butterfield, D.A., 1995. Beta-amyloid peptide-derived, oxygen-dependent free radicals inhibit glutamate uptake in cultured astrocytes: implications for Alzheimer's disease. Neuroreport 6, 1875–1879.

Hesselink, M.B., Smolders, H., De Boer, A.G., Breimer, D.D., Danysz, W., 1999a. Modifications of the behavioral profile of non-competitive NMDA receptor antagonists, memantine, amantadine and (+)MK-801 after chronic administration. Behav. Pharmacol. 10, 85–98.

Hesselink, M.B., Smolders, H., Eilbacher, B., De Boer, A.G., Breimer, D.D., Danysz, W., 1999b. The role of probenecid-sensitive organic acid transport in the pharmacokinetics of N-methyl-D-aspartate receptor antagonists acting at the glycine(B)-site: microdialysis and maximum electroshock seizures studies. J. Pharmacol. Exp. Ther. 290, 543–550.

Hu, N.W., Smith, I.M., Walsh, D.M., Rowan, M.J., 2008. Soluble amyloid-beta peptides potently disrupt hippocampal synaptic plasticity in the absence of cerebrovascular dysfunction in vivo. Brain 131, 2414–2424.

Hynd, M.R., Scott, H.L., Dodd, P.R., 2004. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochem. Int. 45, 583–595.

Ikonomidou, C., Turski, L., 2002. Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? Lancet Neurol. 1, 383–386.

Kabogo, D., Rauw, G., Amritraj, A., Baker, G., Kar, S., 2008. Beta-amyloid-related peptides potentiate K(+)-evoked glutamate release from adult rat hippocampal slices. Neurobiol. Aging, doi:10.1016/j.neurobiolaging.2008.08.009.

Keller, J.N., Pang, Z., Geddes, J.W., Begley, J.G., Germeyer, A., Waeg, G., Mattson, M.P., 1997. Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. J. Neurochem. 69, 273–284.

Kelly, B.L., Ferreira, A., 2006. Beta-amyloid-induced dynamin 1 degradation is mediated by N-methyl-D-aspartate receptors in hippocampal neurons. J. Biol. Chem. 281, 28079–28089.

Klyubin, I., Walsh, D.M., Cullen, W.K., Fadeeva, J.V., Anwyl, R., Selkoe, D.J., Rowan, M.J., 2004. Soluble arctic amyloid beta protein inhibits hippocampal long-term potentiation in vivo. Eur. J. Neurosci. 19, 2839–2846.

Klyubin, I., Betts, V., Welzel, A.T., Blennow, K., Zetterberg, H., Wallin, A., Lemere, C.A., Cullen, W.K., Peng, Y., Wisniewski, T., Selkoe, D.J., Anwyl, R., Walsh, D.M., Rowan, M.J., 2008. Amyloid beta protein dimer-containing human CSF disrupts synaptic plasticity: prevention by systemic passive immunization. J. Neurosci. 28, 423–437.

Koh, J.Y., Yang, L.L., Cotman, C.W., 1990. Beta-amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. Brain Res. 533, 315–320.

Kotermanski, S.E., Johnson, J.W., 2009. Mg^{2+} imparts NMDA receptor subtype selectivity to the Alzheimer's drug memantine. J. Neurosci. 29, 2774–2779.

Lacor, P.N., Buniel, M.C., Furlow, P.W., Clemente, A.S., Velasco, P.T., Wood, M., Viola, K.L., Klein, W.L., 2007. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. J. Neurosci. 27, 796–807.

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A., Klein, W.L., 1998. Diffusible, nonfibrillar ligands derived from A beta(1–42) are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. U.S.A. 95, 6448–6453.

Lau, C.G., Zukin, R.S., 2007. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat. Rev. Neurosci. 8, 413–426.

Lawlor, B.A., Davis, K.L., 1992. Does modulation of glutamatergic function represent a viable therapeutic strategy in Alzheimer's disease? Biol. Psychiatry 31, 337–350.

Lesne, S., Koh, M.T., Kotilinek, L., Kayed, R., Glabe, C.G., Yang, A., Gallagher, M., Ashe, K.H., 2006. A specific amyloid-beta protein assembly in the brain impairs memory. Nature 440, 352–357.

Leung, L.S., Shen, B., 1999. N-methyl-D-aspartate receptor antagonists are less effective in blocking long-term potentiation at apical than basal dendrites in hippocampal CA1 of awake rats. Hippocampus 9, 617–630.

Léveillé, F., El Gaamouch, F., Gouix, E., Lecocq, M., Lobner, D., Nicole, O., Buisson, A., 2008. Neuronal viability is controlled by a functional relation between synaptic and extrasynaptic NMDA receptors. FASEB J. 22, 4258–4271.

Lipton, S.A., 2007. Pathologically activated therapeutics for neuroprotection. Nat. Rev. Neurosci. 8, 803–808.

Lorenzo, A., Yankner, B.A., 1996. Amyloid fibril toxicity in Alzheimer's disease and diabetes. Ann. N. Y. Acad. Sci. 77, 89–95.

Lue, L.F., Kuo, Y.M., Roher, A.E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J.H., Rydel, R.E., Rogers, J., 1999. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am. J. Pathol. 155, 853–862.

Maskell, P.D., Speder, P., Newberry, N.R., Bermudez, I., 2003. Inhibition of human alpha 7 nicotinic acetylcholine receptors by open channel blockers of N-methyl-D-aspartate receptors. Br. J. Pharmacol. 140, 1313–1319.

Matos, M., Augusto, E., Oliveira, C.R., Agostinho, P., 2008. Amyloid-beta peptide decreases glutamate uptake in cultured astrocytes: involvement of oxidative stress and mitogen-activated protein kinase cascades. Neuroscience 156, 898–910.

Mattson, M.P., Cheng, B., Davis, D., Bryant, K., Lieburg, I., Rydel, R.E., 1992. Beta-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J. Neurosci. 12, 376–389.

Maurice, T., Lockhart, B.P., Privat, A., 1996. Amnesia induced in mice by centrally administered beta-amyloid peptides involves cholinergic dysfunction. Brain Res. 706, 181–193.

McDonald, M.P., Dahl, E.E., Overmier, J.B., Mantyh, P., Cleary, J., 1994. Effects of an exogenous beta-amyloid peptide on retention for spatial learning. Behav. Neural. Biol. 62, 60–67.

McLean, C.A., Cherny, R.A., Fraser, F.W., Fuller, S.J., Smith, M.J., Beyreuther, K., Bush, A.I., Masters, C.L., 1999. Soluble pool of A beta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann. Neurol. 46, 860–866.

Molnar, Z., Soos, K., Lengyel, I., Penke, B., Szegedi, V., Budai, D., 2004. Enhancement of NMDA responses by beta-amyloid peptides in the hippocampus in vivo. Neuroreport 15, 1649–1652.

More, L., Gravius, A., Nagel, J., Valastro, B., Greco, S., Danysz, W., 2008. Therapeutically relevant plasma concentrations of memantine produce significant L-N-methyl-D-aspartate receptor occupation and do not impair learning in rats. Behav. Pharmacol. 19, 724–734.

Nakagami, Y., Oda, T., 2002. Glutamate exacerbates amyloid beta$_{1-42}$-induced impairment of long-term potentiation in rat hippocampal slices. Jpn. J. Pharmacol. 88, 223–226.

Nakamura, S., Murayama, N., Noshita, T., Katsuragi, R., Ohno, T., 2006. Cognitive dysfunction induced by sequential injection of amyloid-beta and ibotenate into the bilateral hippocampus; protection by memantine and MK-801. Eur. J. Pharmacol. 548, 115–122.

Nitta, A., Itoh, A., Hasegawa, T., Nabeshima, T., 1994. Beta-amyloid protein-induced Alzheimer's disease animal model. Neurosci. Lett. 170, 63–66.

Noda, M., Nakanishi, H., Akaike, N., 1999. Glutamate release from microglia via glutamate transporter is enhanced by amyloid-beta peptide. Neuroscience 92, 1465–1474.

Parsons, C.G., Stoffler, A., Danysz, W., 2007. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system—too little activation is bad, too much is even worse. Neuropharmacology 53, 699–723.

Poling, A., Morgan-Paisley, K., Panos, J.J., Kim, E.M., O'Hare, E., Cleary, J.P., Lesne, S., Ashe, K.H., Porritt, M., Baker, L.E., 2008. Oligomers of the amyloid-beta protein disrupt working memory: confirmation with two behavioral procedures. Behav. Brain Res. 193, 230–234.

Puzzo, D., Privitera, L., Leznik, E., Fa, M., Staniszewski, A., Palmeri, A., Arancio, O., 2008. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. J. Neurosci. 28, 14537–14545.

Raina, P., Santaguida, P., Ismaila, A., Patterson, C., Cowan, D., Levine, M., Booker, L., Oremus, M., 2008. Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. Ann. Intern. Med. 148, 379–397.

Rammsayer, T.H., 2001. Effects of pharmacologically induced changes in NMDA-receptor activity on long-term memory in humans. Learn Mem. 8, 20–25.

Richardson, R.L., Kim, E.M., Shephard, R.A., Gardiner, T., Cleary, J., O'Hare, E., 2002. Behavioural and histopathological analyses of ibuprofen treatment on the effect of aggregated Abeta(1–42) injections in the rat. Brain Res. 954, 1–10.

Riedel, G., Platt, B., Micheau, J., 2003. Glutamate receptor function in learning and memory. Behav. Brain Res. 140, 1–47.

Roth, M., Tomlinson, B.E., Blessed, G., 1966. Correlation between scores for dementia and counts of 'senile plaques' in cerebral grey matter of elderly subjects. Nature 209, 109–110.

Sang, C.N., Booher, S., Gilron, I., Parada, S., Max, M.B., 2002. Dextromethorphan and memantine in painful diabetic neuropathy and postherpetic neuralgia: efficacy and dose–response trials. Anesthesiology 96, 1053–1061.

Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., Brett, F.M., Farrell, M.A., Rowan, M.J., Lemere, C.A., Regan, C.M., Walsh, D.M., Sabatini, B.L., Selkoe, D.J., 2008. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat. Med. 14, 837–842.

Snyder, E.M., Nong, Y., Almeida, C.G., Paul, S., Moran, T., Choi, E.Y., Nairn, A.C., Salter, M.W., Lombroso, P.J., Gouras, G.K., Greengard, P., 2005. Regulation of NMDA receptor trafficking by amyloid-beta. Nat. Neurosci. 8, 1051–1058.

Soriano, F.X., Hardingham, G.E., 1997. Compartmentalized NMDA receptor signalling to survival and death. J. Physiol. 584, 381–387.

Stephan, A., Laroche, S., Davis, S., 2001. Generation of aggregated beta-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. J. Neurosci. 21, 5703–5714.

Szegedi, V., Juhasz, G., Budai, D., Penke, B., 2005. Divergent effects of Abeta1–42 on ionotropic glutamate receptor-mediated responses in CA1 neurons in vivo. Brain Res. 1062, 120–126.

Terry, R.D., 1996. The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. J. Neuropathol. Exp. Neurol. 55, 1023–1025.

Townsend, M., Cleary, J.P., Mehta, T., Hofmeister, J., Lesne, S., O'Hare, E., Walsh, D.M., Selkoe, D.J., 2006. Orally available compound prevents deficits in memory caused by the Alzheimer amyloid-beta oligomers. Ann. Neurol. 60, 668–676.

Villoslada, P., Arrondo, G., Sepulcre, J., Alegre, M., Artieda, J., 2008. Memantine induces reversible neurologic impairment in patients with MS. Neurology, doi:10.1212/01.wnl.0000342388.73185.80.

Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe, D.J., 2002. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.

Wang, H.Y., Lee, D.H., D'Andrea, M.R., Peterson, P.A., Shank, R.P., Reitz, A.B., 2000. Beta-amyloid(1–42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. J. Biol. Chem. 275, 5626–5632.

Wang, J., Dickson, D.W., Trojanowski, J.Q., Lee, V.M., 1999. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. Exp. Neurol. 158, 328–337.

Wang, Q., Walsh, D.M., Rowan, M.J., Selkoe, D.J., Anwyl, R., 2004. Block of long-term potentiation by naturally secreted and synthetic amyloid beta-peptide in hippocampal slices is mediated via activation of the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5. J. Neurosci. 24, 3370–3378.

Wang, Q., Klyubin, I., Wright, S., Griswold-Prenner, I., Rowan, M.J., Anwyl, R., 2008. Alphav integrins mediate beta-amyloid induced inhibition of long-term potentiation. Neurobiol. Aging 29, 1485–1493.

Wenk, G.L., Danysz, W., Mobley, S.L., 1995. MK-801, memantine and amantadine show neuroprotective activity in the nucleus basalis magnocellularis. Eur. J. Pharmacol. 293, 267–270.

Wu, J., Anwyl, R., Rowan, M.J., 1995. Beta-amyloid selectively augments NMDA receptor-mediated synaptic transmission in rat hippocampus. Neuroreport 6, 2409–2413.

Wu, J., Rush, A., Rowan, M.J., Anwyl, R., 2001. NMDA receptor- and metabotropic glutamate receptor-dependent synaptic plasticity induced by high frequency stimulation in the rat dentate gyrus in vitro. J. Physiol. 533, 745–755.

Zajaczkowski, W., Frankiewicz, T., Parsons, C.G., Danysz, W., 1997. Uncompetitive NMDA receptor antagonists attenuate NMDA-induced impairment of passive avoidance learning and LTP. Neuropharmacology 36, 961–971.

Zhang, S.J., Steijaert, M.N., Lau, D., Schutz, G., Delucinge-Vivier, C., Descombes, P., Bading, H., 2007. Decoding NMDA receptor signaling: identification of genomic programs specifying neuronal survival and death. Neuron 53, 549–562.

Zoladz, P.R., Campbell, A.M., Park, C.R., Schaefer, D., Danysz, W., Diamond, D.M., 2006. Enhancement of long-term spatial memory in adult rats by the noncompetitive NMDA receptor antagonists, memantine and neramexane. Pharmacol. Biochem. Behav. 85, 298–306.

Zorumski, C.F., Izumi, Y., 1998. Modulation of LTP induction by NMDA receptor activation and nitric oxide release. Prog. Brain Res. 118, 173–182.