mGlu5 receptors and cellular prion protein mediate amyloid-β-facilitated synaptic long-term depression in vivo

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NMDA-type glutamate receptors (NMDARs) are currently regarded as paramount in the potent and selective disruption of synaptic plasticity by Alzheimer’s disease amyloid β-protein (Aβ). Non-NMDAR mechanisms remain relatively unexplored. Here we describe how Aβ facilitates NMDAR-independent long-term depression of synaptic transmission in the hippocampus in vivo. Synthetic Aβ and Aβ in soluble extracts of Alzheimer’s disease brain usurp endogenous acetylcholine muscarinic receptor-dependent long-term depression, to enable long-term depression that required metabotropic glutamate-5 receptors (mGlu5Rs). We also find that mGlu5Rs are essential for Aβ-mediated inhibition of NMDAR-dependent long-term potentiation in vivo. Blocking Aβ binding to cellular prion protein with antibodies prevents the facilitation of long-term depression. Our findings uncover an overarching role for Aβ-PrPÇ-mGlu5R interplay in mediating both LTD facilitation and LTP inhibition, encompassing NMDAR-mediated processes that were previously considered primary.
Increasing our understanding of how amyloid-β protein (Aβ) causes synaptic dysfunction should provide new means of therapeutically targeting early Alzheimer’s disease (AD)\(^1\). It is now well established that Aβ has rapid, profound and selective disruptive effects on synaptic plasticity of excitatory synaptic transmission in vulnerable brain regions, including the hippocampus\(^2\). In addition to causing strong inhibition of long-term potentiation (LTP), Aβ has been reported to enhance long-term depression (LTD). Most research has focused on the actions of Aβ on forms of LTP and LTD that require NMDA-type glutamate receptors (NMDARs)\(^3\)–\(^6\). Indeed, as NMDAR-dependent LTP is likely to underlie synaptic memory mechanisms\(^7\), the inhibition of this form of LTP by Aβ is highly congruent with the ability of Aβ to impair learning and memory\(^8\),\(^9\). Somewhat similarly, excessive enhancement of LTD that requires NMDARs can cause memory retrieval deficits\(^10\),\(^11\). Remarkably, the disruption of NMDAR-dependent synaptic plasticity by Aβ is itself mediated through NMDARs, in particular, those containing the GluN2B subunit\(^12\)–\(^15\).

In contrast, little is known about how Aβ affects forms of synaptic plasticity that do not require NMDARs. Whereas Aβ potently inhibits acetylcholine-induced LTP\(^16\), NMDAR-independent LTD induced by strong high-frequency conditioning stimulation (HFS) appears to be resistant to disturbance by Aβ\(^17\) in the hippocampus \textit{in vitro}. Recently, Aβ was reported to enable an NMDAR-independent LTD that was blocked by metabotropic glutamate-5 receptor (mGlu5R) antagonists in hippocampal slices\(^5\),\(^8\). Indeed, synaptically evoked activation of mGlu5R or other similar G-protein coupled receptors including M1 muscarinic acetylcholine receptors (mAChRs) can induce LTD that does not require NMDARs\(^11\),\(^18\)–\(^20\). Moreover, mAChR-dependent LTD has been proposed to underlie visual recognition memory in the perirhinal cortex\(^21\) and to provide a neurophysiological basis for preserved memory function in the ageing hippocampus\(^22\). Considering the early vulnerability of cholinergic pathways and related signalling in AD\(^23\),\(^24\), we hypothesize that Aβ would inhibit mAChR-dependent LTD.

Remarkably, \textit{in vivo} exposure to low-dose Aβ facilitated an NMDAR-independent form of LTD but does not appear to affect mAChR-dependent LTD. This Aβ-facilitated LTD is found to be mGlu5R-dependent. Moreover, Aβ-mediated inhibition of LTD is also dependent on metabotropic glutamate-5 receptors (mGlu5Rs), indicating a key overarching role of this glutamate receptor subtype. We also discover that cellular PrP, a receptor for certain synaptotoxins Aβ assemblies\(^25\),\(^26\), is necessary for Aβ to facilitate LTD. These data are strongly congruent with recent molecular evidence that Aβ and cellular prion protein (PrP\(^C\)) form a complex with mGlu5R at the postsynaptic density\(^27\) and thereby disrupt synaptic plasticity.

**Results**

\textit{In vivo} induction of mAChR-dependent LTD. In order to study the effects of Aβ on mAChR-dependent LTD \textit{in vivo}, we
developed a novel induction protocol that makes use of the reported requirement for high-intensity pulses to ensure robust synaptic ACh release during low-frequency conditioning stimulation (LFS) in the neocortex. We found that application of strong LFS, consisting of 900 high-intensity pulses at 1 Hz (LFS-900), in the stratum radiatum of anaesthetized rats triggered synaptic LTD that (i) was stable for ~3 h (Fig. 1a,b), (ii) was readily reversible by HFS (Fig. 1a,b) and (iii) was input specific (Fig. 1c,d).

Consistent with the essential requirement for activation of cholinergic mechanisms in the induction of this form of LTD, LFS-900 failed to induce LTD of synaptic transmission after

![Figure 2](https://example.com/figure2.png)

**Figure 2 | Muscarinic receptor-dependence of LTD in vivo.** (a,b) Systemic injection of scopolamine (0.2 mg kg\(^{-1}\), i.p.), a muscarinic acetylcholine receptor antagonist, completely prevented LFS-induced LTD, whereas application of the nicotinic acetylcholine receptor antagonist mecamylamine (5 mg kg\(^{-1}\), i.p.) did not affect LTD induction. Open triangle, i.p.; hash, intracerebroventricular (i.c.v.). As summarized in (b) the EPSP decreased significantly to 72.0 ± 4.4% in the vehicle control group and the mecamylamine group (67.1 ± 4.9%, n = 4, P < 0.05 compared with Pre, P > 0.05 compared with vehicle) but not in the scopolamine group (96.5 ± 6.4%, n = 6, P > 0.05 compared with Pre, P < 0.05 compared with vehicle); paired \(t\) and one-way ANOVA-Tukey. (c,d) LFS-900-induced LTD was also significantly reduced by treatment with the M1-selective mAChR antagonist pirenzepine (triangle, 50 nmol in 5 μl). As summarized in (d), the EPSP decreased to 67.5 ± 4.5% and 90.4 ± 2.1%, n = 4, in vehicle- and pirenzepine-injected animals, respectively (P < 0.05 compared with Pre and between groups; \(t\)-test). (e,f) Application of LFS-900 before the injection of scopolamine (triangle, 0.2 mg kg\(^{-1}\), i.p.) induced robust LTD (71.7 ± 7.2%, n = 6, P < 0.05 compared with Pre; paired \(t\)). (g,h) The acetylcholinesterase inhibitor donepezil lowered the threshold to induce LTD. (g) The application of weak LFS (bar, LFS-300; 300 high-intensity pulses at 1 Hz) induced a transient synaptic depression in vehicle-injected animals (triangle), whereas the same protocol triggered a robust and stable LTD after acute injection of donepezil (1 mg kg\(^{-1}\), subcutaneously). (h) Veh: 101.8 ± 6.3%; donepezil: 70.5 ± 7.1% at 3 h after LFS. *P < 0.05, \(t\)-test, n = 4 per group. Values are mean ± s.e.m. Calibration bars: vertical, 2 mV; horizontal, 10 ms.
pretreatment with the mAChR antagonist scopolamine (Fig. 2a,b). In contrast, the LTD was not dependent on the activation of nicotinic AChRs, the magnitude of LTD being unaffected by injection of the nicotinic AChR antagonist mecamylamine before LFS-900 (Fig. 2a,b). Consistent with a role for the M1 subtype of mAChR in LTD induction\(^1\), the M1-selective antagonist pirenzepine significantly reduced the magnitude of LTD (Fig. 2c,d). mAChR activation did not appear to be required for LTD maintenance/expression, as injection of scopolamine after the conditioning stimulation, using the same dose that completely prevented LTD induction, did not significantly affect the magnitude of LTD (Fig. 2e,f).

Further evidence that physiological release of ACh is a key factor in LTD induction in vivo was the ability of an agent that enhances the effects of endogenously released ACh, the acetylcholinesterase inhibitor donepezil, to lower the threshold of LTD induction. Thus, we found that a relatively weak LFS conditioning protocol, consisting of 300 high-intensity pulses at 1 Hz (LFS-300) that was at or just below the threshold to induce significant LTD in vehicle-pretreated animals, triggered a large and robust LTD that was stable for at least 3 h in animals pretreated with donepezil (Fig. 2g,h). Moreover, as described below, the induction of this in vivo synaptically evoked mAChR-dependent LTD did not require the activation of NMDA or mGlu5Rs.

Because Aβ can interfere with mAChR-related signalling\(^9\), we went on to examine the ability of Aβ to disrupt this form of LTD.

Aβ enhances an mAChR-independent form of LTD. We investigated the effects of Aβ on synaptically evoked mAChR-dependent LTD in vivo by the injection of Aβ into the lateral cerebral ventricle via a cannula. Initially, we used a soluble synthetic Aβ\(_{1–42}\) preparation that had been centrifuged to remove any fibril aggregates. We chose a dose (160 pmol) of soluble Aβ\(_{1–42}\) that did not affect baseline synaptic transmission but strongly inhibited NMDAR-dependent LTP, as described below and previously\(^3\). To our surprise, in animals pre-injected with soluble Aβ\(_{1–42}\) the application of LFS-900 triggered an LTD that was more stable than the control LTD induced in the absence of Aβ. Thus, LTD induced in the presence of Aβ was stable during the 5-h recording period, whereas control LTD decayed significantly between 3 and 5 h post LFS (Fig. 3a,b). Although we had hypothesized that mAChR-dependent LTD would be inhibited by Aβ, we wondered whether this Aβ-facilitated LTD required mAChRs. We therefore pretreated the rats with scopolamine before Aβ. In contrast to control LTD, which was completely abrogated by the mAChR antagonist (Fig. 2a,b), the time course and magnitude of LTD was only partly reduced by scopolamine in Aβ-treated animals (Fig. 3a,b). These findings indicate that Aβ had enabled an additional LTD that was more stable and independent of mAChRs while at the same time leaving a residual mAChR-dependent LTD relatively unscathed.

We wondered whether this Aβ-facilitated additional, mAChR-independent, LTD was due to the ability of Aβ to lower the threshold for LTD induction in vivo. We therefore used the weak LFS conditioning protocol (LFS-300). In addition to our standard soluble Aβ\(_{1–42}\) preparation we also tested a preparation of soluble Aβ\(_{1–42}\) enriched with protofibrils (Fig. 4). We combined the results obtained with the two synthetic Aβ\(_{1–42}\) preparations because there was no quantitative difference in their effects on LTD. The application of weak LFS-300 induced a large and robust...
LTD that was stable for at least 3 h in animals injected with Aβ1–42 (Fig. 5a,b), but not vehicle or a control, reverse sequence peptide Aβ42–1 (Fig. 5a,b). This dose (160 pmol) of Aβ1–42 did not affect baseline synaptic transmission (Fig. 5a,b) and consistent with a relatively selective action of Aβ on the mechanisms underlying LTD induction, the same dose applied after the LFS-300 conditioning stimulation failed to facilitate LTD (Fig. 5c,d). Moreover, the LTD induced by weak LFS-300 in the presence of Aβ, like the additional LTD induced by the strong LFS-900 protocol, was also mACHR-independent, not being blocked by scopolamine pretreatment (Fig. 5e,f).

Although synthetic Aβ is most commonly used in studies of synaptic plasticity disruption, it is important to determine whether similar effects are caused by natural Aβ. The presence of water-soluble SDS-stable Aβ dimer in post-mortem brain extracts is highly correlated with ante-mortem dementia status31 and such Aβ can inhibit LTP and promote LTD in vitro3–5. It is therefore of great interest to assess whether AD brain-derived Aβ can also facilitate LTD induction in vivo. Consequently, we tested the ability of Aβ in water-soluble extracts of two different AD brains to mimic the ability of synthetic Aβ1–42 to lower the threshold for LTD induction in vivo. As can be seen from the Western blot of one of the AD brain extracts (Fig. 6a), Aβ runs on SDS gel predominantly as either monomer or dimer. These water-soluble SDS-stable species include a wide distribution of assemblies when analysed by size exclusion chromatography (SEC), ranging from monomer to ≥70 kDa (ref. 8). Similar to synthetic Aβ, the injection of Aβ-containing AD brain soluble extract enabled the induction of robust and stable LTD by LFS-300 (Fig. 6b,c). Importantly, immunodepletion of Aβ from the AD brain sample abrogated its ability to enable LTD induction. This finding indicates that soluble Aβ is responsible for the lowering of the LTD induction threshold by the AD brain extract. Which SDS-stable Aβ assembly is responsible for the facilitation of LTD by AD TBS brain extract remains to be determined.

Aβ-facilitated LTD is NMDAR-independent. Because Aβ has been reported to promote NMDAR-dependent LTD5–6, we...
HFS-induced de-depression of LTD is NMDAR-dependent. In the light of the contrasting findings regarding the involvement of NMDARs in the disruptive effects of Aβ on LTD (in vivo) and HFS-induced LTD, we also tested the GluN2B-selective competitive antagonist MTEP (6 mg kg$^{-1}$, intraperitoneal (i.p.)) that completely blocks Aβ-facilitated LTD induced by LFS-900 (Fig. 7e,f). Aβ-facilitated LTD induced by LFS-300 is NMDAR-independent.

Aβ-facilitated LTD is mGlu5R-dependent. Apart from NMDARs, metabotropic glutamate receptors, in particular the mGlu5R subtype, have been implicated in the synaptic plasticity disrupting actions of Aβ in vitro$^{3,4,36}$. Bearing in mind the apparently differential roles of NMDARs in the effects of Aβ on different forms of synaptic plasticity, next we assessed the involvement of mGlu5R in both Aβ-mediated inhibition of LTP as well as Aβ-facilitated LTD in vivo. Remarkably, systemic administration of the selective mGlu5R antagonist (negative allosteric modulator) MTEP prevented both of these disruptive actions of Aβ without affecting either control LTP or control LTD. Thus, in animals administered with MTEP before intracerebroventricular (i.c.v.) injection of either synthetic or AD brain-derived Aβ the application of LFS-300 failed to induce LTD (Fig. 8a–d). Importantly, the same dose of MTEP had no effect on control LTD induced by LFS-900 (Fig. 8e,f), indicating that whereas Aβ-facilitated LTD is mGlu5R-dependent, this was not the case for the control mAChR-dependent LTD. Somewhat similarly, whereas Aβ$_{1–42}$ strongly inhibited LTP in vehicle-pretreated animals, an identical HFS-triggered robust LTP in animals injected with MTEP followed by Aβ (Fig. 8g,h). These observations underscore the potential importance of non-NMDAR mechanisms in mediating the synaptic plasticity disrupting effects of Aβ in vivo.
findings strongly indicate that Aβ enables LTD induction in vivo with an essential role of mGlu5, bypassing a requirement for activation of muscarinic ACh receptors. Moreover as MTEP prevented Aβ’s effects on both LTP and LTD, mGlu5Rs appear to be more pivotal to the synaptic plasticity disrupting actions of Aβ than NMDARs.

Cellular prion protein mediates Aβ-facilitated LTD. The question arises as to whether or not the facilitation of LTD by Aβ shares other common mechanisms with LTP inhibition by Aβ. Aβ oligomers can bind very potently and selectively to cellular prion protein especially in a region that encompassed the amino-acid sequence 95–105, and thereby mediate inhibition of LTP by synthetic Aβ1–42 (ref. 25). The disease relevance of this finding is underscored by the PrP^C-dependence of the inhibition of LTP by Aβ oligomer-containing soluble extract of AD brain 37. We examined the role of PrP^C in mediating the facilitation of LTD by AD brain Aβ and synthetic Aβ1–42 using monoclonal antibodies to PrP^C. We started with the previously characterized anti-PrP^C antibody 6D11, with an epitope that falls within the amino-acid sequence 93–109 sequence, thereby preventing Aβ1–42 oligomer binding and inhibition of LTP 25. Pretreatment with 6D11 completely prevented the facilitation of LTD by Aβ-containing soluble AD brain extract (Fig. 9a,b). In order to further explore the role of PrP^C, we compared the effect of two other high-affinity anti-PrP^C antibodies (Fig. 9c,d). ICSM18, an antibody directed to helix-1 of PrPC, is known to inhibit Aβ binding to PrP^C and to prevent the LTD disrupting effect of AD brain extracts 37. ICSM41 is an antibody to the structured region of PrP^C with an undefined

Figure 7 | NMDAR antagonists do not affect LTD but prevent LTD reversal. (a,b) LFS-300 (bar) after Aβ1–42 (i.c.v., hash) triggered LTD that was reversed by HFS. The competitive antagonist CPP (open triangle; 10 mg kg⁻¹, i.p.) did not affect Aβ-facilitated LTD but prevented de-depression. (b) Thus LTD at 1.5 h measured 75.9 ± 4.0% (n = 5) and 66.5 ± 5.8% (n = 7) in the vehicle + Aβ group and CPP + Aβ group, respectively (P < 0.05 compared with Pre, one-way ANOVA-Tukey, P > 0.05 between groups; two-way ANOVA followed by unpaired t). The EPSP measured 102.7 ± 5.1% in the vehicle + Aβ group (at 3 h, P > 0.05 compared with Pre and P < 0.05 compared with 1.5 h post LFS) and 73.3 ± 7.6% in the CPP + Aβ group (P > 0.05 compared 1.5 h post LFS, P < 0.05 compared with the vehicle + Aβ group). (c,d) Injection of Ro 25-6981 (open triangle; 6 mg kg⁻¹, i.p.), a negative allosteric modulator of GluN2B-containing NMDARs, did not prevent Aβ1–42 (hash)-facilitated LTD (75.5 ± 6.3% at 3 h, n = 5, P < 0.05 compared with Pre, P > 0.05 compared with 68.6 ± 4.0% in the vehicle + Aβ injection group; t-tests). (e,f) Control LTD, induced by LFS-900 (bar) was also reversed by HFS (arrow). CPP failed to significantly affect control LTD, but blocked de-depression. (f) Thus, LFS-900 induced LTD in controls (71.1 ± 5.3% at 1.5 h, n = 6, P < 0.05 compared with Pre; one-way ANOVA-Tukey) and CPP-injected rats (59.9 ± 8.0%, n = 5, P < 0.05 compared with Pre, P > 0.05 compared with vehicle; two-way ANOVA followed by unpaired t). The EPSP measured 92.9 ± 5.8% at 90 min in controls (P > 0.05 compared with Pre) and 68.0 ± 8.0% in the CPP group (P > 0.05 compared with 1.5 h post LFS, P < 0.05 compared with vehicle). Values are mean ± s.e.m. Calibration: vertical, 2 mV; horizontal, 10 ms.
Figure 8 | mGlu5R-dependence of Aβ-mediated disruption of both LTD and LTP but not control LTD or control LTP. (a,b) Systemic administration of the selective mGlu5R antagonist MTEP (open triangle; 3 mg kg\(^{-1}\), i.p.) completely prevented the induction of LTD by LFS-300 (bar) in animals injected i.c.v. with soluble Aβ\(_{1-42}\) (hash) (68.6 ± 4.0% in the vehicle + Aβ group, n = 5, P < 0.05 compared with Pre, and compared with 98.4 ± 7.6% in the MTEP + Aβ group, n = 5, P > 0.05 compared with Pre; t-tests). (c,d) Similarly, MTEP completely prevented the induction of LTD in animals injected with Aβ-containing AD brain extract. As summarized in (d) the EPSP measured 76.2 ± 3.6% in the vehicle + AD group, n = 4, (P < 0.05 compared with Pre, and compared with 97.4 ± 5.6% in the MTEP + AD group, n = 4, P > 0.05 compared with Pre; t-tests). (e,f) In contrast, the same dose of MTEP that prevented Aβ-facilitated LTD failed to significantly affect control LTD induced by LFS-900 (72.0 ± 4.4% in the vehicle + vehicle group, n = 11, P < 0.05 compared with Pre, and P > 0.05 compared with 80.6 ± 7.2% in the MTEP + vehicle group, n = 5, P < 0.05 compared with Pre; t-tests). (g,h) i.c.v. injection of soluble Aβ\(_{1-42}\) (hash), at the dose that facilitated LTD, blocked LTP completely at 3 h post HFS. Although systemic administration of MTEP (3 mg kg\(^{-1}\)) did not affect HFS-induced control LTP, it prevented Aβ-mediated impairment of LTP. As summarized in (h), HFS induced significant (P < 0.05 compared with Pre; paired t) LTP in the vehicle control group (123.7 ± 4.0%, n = 5), MTEP + vehicle group (131.5 ± 5.2%, n = 4) and MTEP + Aβ group (120.6 ± 3.4%, n = 8), but not in the vehicle + Aβ group (96.2 ± 4.7%, n = 5), which was significantly different from the other groups (one-way ANOVA-Tukey). Values are mean ± s.e.m. Calibration: vertical, 2 mV; horizontal, 10 ms.

Although ICSM41 binds with similar high affinity to recombinant PrP\(^C\) as ICSM18 (IC\(_{50}\): 0.41 ± 0.04 and 0.3 ± 0.1 nM, respectively, n = 9, mean ± s.e.m.), unlike ICSM18, ICSM41 did not prevent Aβ\(_{1-42}\) protofibril binding to PrP\(^C\) (Fig. 10b,c). Consistent with the differential ability of these two antibodies to prevent Aβ\(_{1-42}\) binding to PrP\(^C\), ICSM18 abrogated the facilitation of LTD by soluble AD brain extract, whereas the same dose of ICSM41 had no effect (Fig. 9c,d). These findings provide strong evidence that PrP\(^C\) is required for the enablement of LTD by the most disease relevant form of soluble Aβ, Aβ from AD brain. We also tested the ability of ICSM18 to prevent the facilitation of LTD by synthetic Aβ\(_{1-42}\). Aβ from water-soluble extracts of AD brain contain a mixture of high- and low-molecular weight components, some of which bind to PrP\(^C\) with high affinity. In the case of synthetic Aβ, protofibrillar...
Figure 9 | Cellular prion protein is necessary for the facilitation of LTD by Aβ. (a,b) Injection of 6D11 (triangle; 20 μg in 10 μl, i.c.v.), an antibody directed to the main binding site of Aβ on PrP, 15 min before the injection of soluble Aβ-containing AD brain extract (hash) prevented the facilitation of LTD (interleaved experiments with vehicle + AD, from Fig. 5). As summarized in (b) at 3 h post LFS the EPSP measured 100.5 ± 7.5%, n = 5, in the 6D11 + AD group (P > 0.05 compared with Pre; P < 0.05 compared with the vehicle + AD group; t-test). (c,d) Whereas ICSM18 (30 μg) prevented the facilitation of LTD by AD brain extract, the same dose of ICSM41 was ineffective. As summarized in (d) the EPSP measured 93.1 ± 2.9%, n = 6, in the ICSM18 + AD group (P > 0.05 compared with Pre and P < 0.05 compared with 68.0 ± 4.2%, n = 4, in the ICSM41 + AD group; t-test). (e,f) ICSM18 (3.75 μg) also prevented the facilitation of LTD by protofibril Aβ1–42. As summarized in (f) the EPSP measured 98.1 ± 3.7% at 3 h post LFS in the ICSM18 + Aβ1–42 group (P > 0.05 compared with Pre, n = 4; and P < 0.05 compared with 73.2 ± 4.5%, n = 4, in the vehicle + Aβ1–42 group; t-test). Values are mean ± s.e.m. Insets show representative EPSP traces at the times indicated. Calibration: vertical, 2 mV; horizontal, 10 ms.

Discussion

We describe here for the first time the in vivo induction by synaptic stimulation of an mAChR-dependent homosynaptic LTD. The induction of mAChR-dependent LTD does not require NMDA or mGlu5R activation. Moreover, both chemically synthesized and human brain-derived Aβ enhanced synthetically induced LTD in vivo. Remarkably, in Aβ-treated animals the additional LTD does not require mAChRs, leaving mAChR-dependent LTD relatively intact. However, like mAChR-dependent LTD, the Aβ-facilitated LTD is NMDAR-independent. We found evidence that mGlu5R activation usurps the requirement for mAChRs to enable LTD induction via a process dependent on PrP. Furthermore, Aβ-mediated inhibition of LTP also requires mGlu5R and PrP, placing Aβ–PrP–mGlu5R interactions central to the synaptic plasticity disrupting actions of Aβ in vivo.

LTD that requires mAChR activation has been proposed to be essential for certain forms of learning1, and the preservation of mAChR-dependent hippocampal LTD as animals age may be critical for maintaining cognitive performance22. The apparent assemblies bind most avidly to PrP (ref. 26) (see also Fig. 10a). We tested an eightfold lower dose of ICSM18 in this study because we found that ICSM18 bound to N2A cells, which express glycosylated mature PrP, with an approximately eightfold higher affinity than ICSM41 (XC50 4 ± 1 and 33 ± 7 nM, respectively) (Fig. 10d). We found that this dose of ICSM18 completely abrogated the facilitation of LTD by protofibril Aβ1–42 (Fig. 9e,f). On the basis of the present and our previous37 findings, PrP appears to be a key site of binding and action for Aβ-mediated disruption of both NMDAR-dependent and independent synaptic plasticity in vivo.
Figure 10 | Characterization of the interactions between Aβ and PrP\(\text{C}\) and anti-PrP\(\text{C}\) antibodies. (a) Both A\(\text{B}\)\(_{1–42}\) and biotinylated A\(\text{B}\)\(_{1–42}\) protofibrils bind recombinant PrP\(\text{C}\) at low nanomolar concentrations (\(n = 3 \pm \text{s.e.m.}\)). (b,c) Unlike ICSM18, ICSM41 did not prevent A\(\text{B}\)\(_{1–42}\) protofibril binding to PrP\(\text{C}\) (\(b, n = 3; c, n = 9, \text{mean} \pm \text{s.e.m.}\)). *\(P < 0.05\) compared to control IgG (BRIC222), Kruskal–Wallis one-way ANOVA with Dunn’s multiple comparison test. (d) FACS analysis revealed that ICSM18 bound to N2A cells, which express glycosylated mature PrP\(\text{C}\), with an approximately eightfold higher affinity than ICSM41 (XC\(_{50}\) 4 ± 1nM and 33 ± 7 nM, respectively, \(n = 4\), mean ± s.e.m.).

dearth of studies of mACHR-dependent LTD in vivo may be owing to difficulties in optimizing suitable synaptic stimulation protocols. The present approach utilizes the insights gained from investigations of mACHR-dependent LTD in slices of cerebral cortex.\(^{28}\) Currently used in vitro synaptic stimulation protocols to induce mACHR-dependent LTD at CA3-to-CA1 synapses have been reported to induce an LTD that is at least partly inhibited by mACHR antagonists\(^{19}\). The present finding that synaptic conditioning stimulation can induce LTD that is completely blocked by scopolamine provides strong evidence that mACHR-dependent LTD that lasts for over 5 h can be induced by endogenously released ACh in vivo and therefore supports its proposed role in synaptic information storage.

Because previous reports had indicated that in vitro, Aβ strongly impairs mACHR-mediated signalling\(^{29}\) that may underlie mACHR-dependent LTD in the cerebral cortex\(^{28}\), we predicted that mACHR-dependent LTD in the hippocampus would be inhibited by Aβ in vivo. To our surprise, Aβ enabled additional LTD while at the same time leaving a scopolamine sensitive component of LTD relatively unscathed. It was apparent that Aβ usurped mACHR-dependent LTD by lowering the synaptic stimulation threshold to induce another form of LTD that was mACHR-independent. The mechanisms of the additional LTD, however, appear to be at least partly shared with mACHR-dependent LTD, as the initial phase of the control LTD was partly occluded by the Aβ-enabled LTD.

Particularly surprising was the apparent lack of involvement of NMDARs in the facilitation of LTD by Aβ, especially in view of the presumed essential role of NMDARs in the relatively selective binding of Aβ oligomers to synapses\(^{42}\). Moreover, antagonists of GluN2B subunits prevent Aβ-mediated facilitation of NMDAR-dependent LTD\(^{5,6}\) and inhibition of NMDAR-dependent LTP\(^{12–15}\). These findings have led to the elucidation of a key role of GluN2B subunits in mediating the synaptic plasticity disrupting actions of Aβ and have been extended to include many other deleterious effects of Aβ\(^{40,42}\). However, based on the present results, targeting GluN2B is unlikely to prove to be an effective therapeutic strategy on its own and underlines the need to also explore non-NMDAR mechanisms.

Further undermining the putative primacy of NMDARs in the synaptic actions of Aβ was the finding that Aβ did not significantly affect NMDAR-dependent de-depression. This is all the more remarkable considering that Aβ strongly inhibited NMDAR-dependent LTD at these same synapses using the same HFS induction protocol. Previous research has found that the persistent reversal of LTD by conditioning stimulus requires the recruitment of different signalling pathways to those usually necessary for LTD induction\(^{4,45}\). Thus the lack of inhibition of NMDAR-dependent de-depression at these synapses indicates that the inhibition of LTD by Aβ is not due to the dependence of LTD on NMDARs. Furthermore, the present findings indicate that pharmacological inhibition of NMDARs may prevent potentially physiological reversal of LTD and leave any deleterious effects of Aβ-facilitated NMDAR-independent LTD unopposed.

The present findings underscore a much more central role for the mGlu5R in mediating the synaptic plasticity disrupting effects of Aβ and suggest that the lowering of the threshold for LTD and inhibition of LTD are two sides of one coin. Our finding that Aβ-facilitated LTD, like Aβ-mediated inhibition of LTD, is blocked by antibodies that prevent Aβ binding to PrP\(\text{C}\) provides an explanation for the pivotal role of mGlu5Rs. Previous research\(^{46}\) has revealed that Aβ acts as an extracellular scaffold to promote the inappropriate synaptic mobilization and activation of mGlu5R on cultured neurons. The membrane binding of Aβ is prevented by both anti-mGlu5R and anti-PrP\(\text{C}\) antibodies in a non-additive manner\(^{46}\), consistent with the key role of PrP\(\text{C}\) in the binding of the Aβ oligomer to plasmalemma\(^{25}\). The aberrant clustering of mGlu5R at synapses by Aβ by binding to PrP\(\text{C}\) may trigger disruptive signalling activity that can enable LTD and inhibit LTD induction. Very recently direct evidence that PrP\(\text{C}\) mediates multiple effects of Aβ oligomers, including dendritic spine loss in cultured neurons, by a direct physical linkage of PrP\(\text{C}\) with mGlu5Rs at or near the postsynaptic membrane was reported\(^{27}\). If the formation of Aβ–PrP\(\text{C}\)–mGlu5R complexes is primary, then the requirement for NMDARs that contain GluN2B subunits in the inhibition of LTD by Aβ is likely to be a downstream consequence. Indeed, mGlu5Rs provide a
transduction link in the Aβ–PrP–complex-mediated membrane coupling to NR2B subunits via activation of the tyrosine kinase Fyn11. In addition to Fyn, an Aβ–PrP–mGlu5R-mediated dysregulation of intracellular Ca2+, eukaryotic elongation factor 2 and Arc27 may contribute to synaptic plasticity disruption11 by Aβ in vivo.

Overall, the present research provides strong evidence that an Aβ–PrP–mGlu5R triad is critical for synaptic plasticity disruption, enabling an NMDA-independent LTD to usurp mACHR-dependent LTD and inhibit NMDA-dependent LTP. Selectively targeting this Aβ–PrP–mGlu5R triad offers many possible means of preventing dysfunction of critical brain plasticity mechanisms in early AD.

Methods

Animals and surgery. Adult (250–350 g, 8–11 weeks old) male Wistar rats (Bioresources Unit, Trinity College, Dublin) were used in all experiments. The animals were housed under a 12-h light-dark cycle at room temperature (19–22 °C). Before the surgery, animals were anesthetized with urethane (1.5–1.6 mg/kg, i.p.). Lignocaine (10 mg, 1% adrenaline, subcutaneously) was injected over the area of the skull, where electrodes and screws were to be implanted. The body temperature of the rats was maintained at 37–38 °C with a feedback-controlled heating blanket. The animal care and experimental protocol were approved by the Department of Health, Republic of Ireland.

Cannula implantation. In order to inject drugs or Aβ into the brain, a stainless-steel cannula (22 gauge, 0.7 mm outer diameter) was implanted above the right lateral ventricle (1 mm lateral to the midline and 4 mm below the surface of the dura), i.e., sites that were internal cannula (28 gauge, 0.36 mm outer diameter). The solutions were injected in a 5 μl volume over a 3-min period or 10 μl volume over a 6-min period. Verification of the placement of cannula was performed post mortem by checking the spread of ink dye after i.c.v. injection.

Electrode implantation. Monopolar recording electrodes were constructed from Teflon-coated tungsten wires (75 μm inner core diameter, 112 μm external diameter) and twisted bipolar stimulating electrodes were constructed from Teflon-coated tungsten wires (50 μm inner core diameter, 75 μm external diameter) separately27. Field excitatory postsynaptic potentials (EPSPs) were recorded from the stratum radiatum in the CA1 area of the right hippocampus in response to stimulation of the ipsilateral Schaffer collateral-commissural pathway. Electrode implantation sites were identified using stereotaxic coordinates relative to bregma, with the site located at 3.2 mm posterior to bregma and 2.5 mm lateral to midline, and stimulating site 4.2 mm posterior to bregma and 3.8 mm lateral to midline. In some animals, another stimulating electrode was implanted at a site located 2.5 mm posterior to bregma and 2.2 mm lateral to the midline. The final placement of electrodes was optimized by using electrophysiological criteria and confirmed via post-mortem analysis.

Electrophysiology. Test EPSPs were evoked by a single square-wave pulse (0.2 ms duration) at a frequency of 0.033 Hz and an intensity that triggered a 50% maximum EPSP response. LTD was induced using 1 Hz LFS consisting of 900 pulses (0.2 ms duration). During the LFS the intensity was raised to trigger EPSPs of 95% maximum amplitude. A relatively weak LFS protocol, used to study the Aβ-mediated facilitation of LTD, consisted of 300 pulses (0.2 ms duration) at 1 Hz, with an intensity that evoked 95% maximum amplitude. LTP was induced using 200 Hz HFS consisting of one set of 10 trains of 20 pulses (inter-train interval of 2 s). The stimulation intensity was raised to trigger EPSPs of 75% maximum during the HFS. None of the conditioning stimulation protocols elicited any detectable abnormal changes in background EEG, which was recorded from the hippocampus throughout the experiments.

Compounds and antibodies. Scopolamine (Sigma), mecamylamine (Sigma), (R5)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP, Ascent Scientific, Weston-Super-Mare, UK) and 3-(2-methyl-1,3-thiazol-4-yl)methylpyridine hydrochloride (MTEP hydrochloride, Ascent Scientific) were prepared in distilled water and distilled with saline to the required concentration. Pirenzepine (Ascent Scientific) was prepared in distilled water. (2R,5S)−/2-(4-hydroxyphenyl)-l-phenylalanine (DLP) was dissolved in DMSO (dimethylsulphoxide) and diluted in saline. The following monoclonal antibodies, prepared in phosphate-buffered saline (PBS), were used in this study: 6D11 (Covance, # SIG-39810); ICSM18, ICSM41 and BRICC22 (D-Gen, UK, # ICSM18, ICSM41 and BRICC22).

Synthetic Aβ. We made two main different preparations of synthetic Aβ, soluble and protofibril Aβ42. Our standard, soluble Aβ1–42 (Bachem or Biopolymer Laboratory, UCLA Medical School) was prepared as a stock solution of 64 μM in mild alkali (0.1% ammonium hydroxide) in milliQ water (Millipore Corporation, DePuy, PA). Fifty micro litres of 1 μM human hPrP–C176/C176 with 0.1% sodium carbonate, pH 9.6) was bound to medium binding 96-well plates (Greiner) with shaking at 400 r.p.m. for 1 h at 37 °C, washed with 3 × 300 μl of PBS (0.05% Tween-20), blocked with 300 μl Superblock (Thermo Scientific) with shaking at 400 r.p.m. at 37 °C for 1 h and washed with 3 × 300 μl of PBS (0.05% Tween-20). Fifty microlitres of Aβ1–42 protofibrils were incubated in PBS (0.1% Tween-20, 0.1% BSA) for 1 h at 25 °C with shaking at 400 r.p.m. and washed with 3 × 300 μl of PBS (0.1% Tween-20). Aβ was detected using 50 μl of 1 μg/ml 6E10 (Covance,
tions3,6,10,12. No data were excluded, and control experiments were interleaved.

Antigenic antibodies on A
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Author contributions

N.-W.H. and M.J.R. conceived the study; N.-W.H., A.J.N., D.M.W. and M.J.R. designed the research; N.-W.H. and D.Z. performed and analysed electrophysiology experiments; A.J.N. prepared the differentially aggregated Aβ and characterized it and the ICSM antibodies A.J.M. isolated and characterized human brain extracts; T.O.M. characterized the standard soluble Aβ1-42 preparation; S.A.P. performed FACS experiments; C.T. performed EM analysis; N.-W.H., A.J.N., J.C., D.M.W. and M.J.R. wrote the paper.

Additional information

Competing financial interests: D.M.W. is a member of the scientific advisory board of CogRx and Alzinova as well as a consultant to Eisai Co. J.C. is a director and shareholder of D-Gen Limited, an academic spin-out company in the field of prion diagnosis, decontamination and therapeutics. The remaining authors declare no competing financial interests.

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