Intracellular oligomeric amyloid-beta rapidly regulates GluA1 subunit of AMPA receptor in the hippocampus

Daniel J. Whitcomb1,2,*, Ellen L. Hogg1,2,*, Philip Regan1,2,3, Thomas Piers1,2, Priyanka Narayan1, Garry Whitehead1,2, Bryony L. Winters1,2,3, Dong-Hyun Kim1, Eunjoon Kim5, Peter St George-Hyslop5, David Klenerman4, Graham L. Collingridge1,2,3, Jihoon Jo1 & Kwangwook Cho1,2

The acute neurotoxicity of oligomeric forms of amyloid-beta1-42 (Aβ) is implicated in the pathogenesis of Alzheimer’s disease (AD). However, how these oligomers might first impair neuronal function at the onset of pathology is poorly understood. Here we have examined the underlying toxic effects caused by an increase in levels of intracellular Aβ, an event that could be important during the early stages of the disease. We show that oligomerised Aβ induces a rapid enhancement of AMPA receptor-mediated synaptic transmission (EPSCa) when applied intracellularly. This effect is dependent on postsynaptic Ca2+ and PKA. Knockdown of GluA1, but not GluA2, prevents the effect, as does expression of a S845-phosphomutant of GluA1. Significantly, an inhibitor of Ca2+-permeable AMPARs (CP-AMPARs), IEM 1460, reverses the increase in the amplitude of EPSCa. These results suggest that a primary neuronal response to intracellular Aβ oligomers is the rapid synaptic insertion of CP-AMPARs.

Alzheimer’s disease (AD) is defined by two hallmark pathological features: plaques that are composed of insoluble conjugates of the amyloid precursor protein (APP) cleavage product amyloid beta1-42 (Aβ), and tangles, which are mainly composed of hyperphosphorylated tau1. A large number of studies have now established that Aβ causes neurotoxic effects at the synapse, including the dysregulation of synaptic proteins and degeneration of dendritic spines1,2. However, the cellular events that lead to these pathological changes are poorly characterised, which both limits our understanding of the disease and potentially hampers the development of efficacious therapies.

One approach that has been extensively utilised to probe the mechanism of Aβ toxicity is to apply oligomeric forms of the protein acutely to the hippocampus, and study their effects on synaptic transmission and plasticity3–6. Here, Aβ is applied extracellularly, either by injection into the brain or by the perfusion of hippocampal slices, and its toxic effects can take an hour or more to manifest4,5. However, it is unknown whether these noted toxic effects are preceded by other as yet undefined extracellular and intracellular responses to Aβ exposure.

*These authors contributed equally to this work. Correspondence and requests for materials should be addressed to K.C. (email: Kei.Cho@bristol.ac.uk)
Given the extracellular nature of Aβ exposure in these experiments, it is assumed that the toxic effects observed are mediated by a membrane-bound substrate or event, and/or by the internalization of Aβ by affected neurons. Indeed, evidence suggests that plasma membrane receptors serve as substrates for oligomeric Aβ. For instance, both metabotropic glutamate receptors and the prion protein receptor interact with Aβ at the synapse, and these interactions are known to catalyse synaptic dysfunction and cell death. In addition to this, studies now report the capacity for Aβ to form pores in the lipid bilayers of membranes, which can serve as conduits to induce the aberrant entry of Ca2+ into cells. However, there is some uncertainty about the conditions in which membrane receptors and associated events are responsible for the toxic effects, suggesting that additional mechanisms likely also play a role.

A growing number of studies now describe an emerging role of intracellular Aβ accumulation in the pathology of AD. For instance, misprocessed endogenously produced Aβ can accumulate in intracellular compartments as well as the cytosol itself. Several lines of evidence also suggest that extracellular Aβ can translocate into the cytosol from extracellular spaces. Critically, it has been shown that the internalization of Aβ and the presumed increase in the presence of Aβ in intracellular spaces can induce synaptic dysfunction. We have therefore hypothesized that accumulated intracellular Aβ will cause a primary effect on neuronal function. To test this, we have applied Aβ acutely into neurons via a patch electrode and investigated whether intracellular Aβ regulates excitatory synaptic transmission in the CA1-Schaffer collateral synapse in the hippocampus.

**Results**

**Single molecule two-colour fluorescence coincidence detection and analysis of oligomers.** Increasing evidence suggests that small, soluble Aβ oligomers are the driving force in Aβ-mediated toxicity, and their production leads to synaptic dysfunction. Using a protocol whereby synthetic Aβ was aggregated (see Methods), we were able to induce a high population of low-n oligomers, quantified using a single-molecule fluorescence method of confocal two-color coincidence detection (cTCCD) of fluorescently labeled Aβ (Fig. 1a). This protocol generated a heterogeneous preparation of Aβ oligomers, which equated to a 1–5 nM component of oligomers (Fig. 1b, c).

**Intracellular infusion of oligomerised Aβ1-42 (Aβ) causes a rapid increase in the AMPAR-mediated EPSC (EPSCA) in CA1 pyramidal neurons.** Since Aβ oligomers are toxic, we were interested in determining the intracellular effects of Aβ oligomers on synaptic function. Neurons were injected with oligomerised Aβ via passive diffusion from the patch pipette, whilst basal synaptic transmission was measured. Aβ oligomers caused a rapid increase in the amplitude of the AMPAR-mediated excitatory postsynaptic current (EPSCA) (181 ± 15%, n = 7, Fig. 2a). In contrast, neither the infusion of non-aggregated, monomeric Aβ nor Aβ oligomers that had been pre-incubated with clusterin, a chaperone that sequesters oligomers, had any significant effect upon EPSCA (81 ± 8%, n = 6, Fig. 2b, and 103 ± 9%, n = 7, Fig. 2c, respectively). The effect of Aβ oligomers was independent of the need to evoke EPSCA, since stopping stimulation for 15 min, shortly after obtaining whole-cell configuration, did not prevent the increase in synaptic transmission (closed circle: 192 ± 26%, n = 6, Fig. 2d). In addition, the effect of Aβ oligomers did not require the activation of NMDA receptors (NMDAR), since EPSCA was enhanced in the presence of the NMDAR antagonist D-AP-5 (147 ± 14%, n = 6, Fig. 2e). This effect was also specific for EPSCA since a pharmacologically-isolated NMDAR-mediated EPSC (EPSCN: holding voltage = −40 mV, 10 μM NBQX perfusion) was unaffected by Aβ oligomer infusion (99 ± 14%, n = 6, Fig. 2f).

**Aβ oligomer-induced increase in EPSCA is dependent on postsynaptic Ca2+ and PKA.** We next investigated the signalling cascades that underlie the rapid action of Aβ oligomers on AMPAR-mediated synaptic transmission (Fig. 3). Changes in postsynaptic Ca2+ levels initiate signal cascades involved in the modulation of synaptic transmission. Therefore we tested whether blockade of postsynaptic Ca2+ mobilisation affects Aβ-mediated EPSCA regulation. The Aβ oligomer-induced increase was dependent on postsynaptic Ca2+, since it was prevented by postsynaptic infusion of the Ca2+ chelator BAPTA (95 ± 12%, n = 7, Fig. 3a), and relied on Ca2+ release from intracellular stores, since bath applied ryanodine also prevents the Aβ-induced EPSCA increase (108 ± 18%, n = 7, Fig. 3b). We were interested in examining the Ca2+-dependent mechanism responsible for these effects, and possible downstream effectors. Ca2+-induced changes in synaptic transmission are known to involve, among other kinases, protein kinase A (PKA). Accordingly, we tested the involvement of PKA in the observed Aβ-induced EPSCA increase. We found that the effect required the activation of PKA, since it was prevented by either Rp-cAMPS, a cyclic AMP analogue that acts as a competitive antagonist of cAMP-induced activation of PKA (97 ± 8%, n = 6, Fig. 3c) or H89, a PKA inhibitor (94 ± 9%, n = 6, Fig. 3d), but not PKC since it was unaffected by the PKC inhibitor Ro 32-0432 (171 ± 7%, n = 6, Fig. 3e) or PKC9-31, a pseudo- substrate of PKC which functions to inhibit the kinase (166 ± 13%, n = 6, Fig. 3f).

Calcium-calmodulin kinase II (CaMKII) is a Ca2+-sensitive kinase that has also been implicated in the regulation of AMPAR expression. We therefore tested the involvement of CaMKII in Aβ-induced EPSCA increase. When cells were infused with Aβ and the CaMKII inhibitor KN-62, we observed an initial increase in EPSCA that rapidly declined (97 ± 16%, n = 7, Fig. 3g).
Figure 1. Generation of lower-\(n\) oligomers of A\(\beta\)42 (A\(\beta\)) (a) A schematic of the principle of single molecule two-colour fluorescence coincidence detection and analysis of oligomers. The protein is labeled with a red or blue fluorophore and aggregated. The sample is then diluted to picomolar concentrations and analysed using single molecule fluorescence. Monomers passing through the probe volume give rise to non-coincident bursts of fluorescence while oligomers give rise to coincident fluorescent bursts, enabling the fraction of oligomers present in the sample to be determined. The intensity of a coincident burst relative to average monomer bursts was determined, allowing the oligomer size to be estimated. (b) Histogram depicting the proportion of monomers and oligomers. (c) Histogram depicting the size distribution of oligomers present in the preparation of A\(\beta\)42 oligomers.
Figure 2. Intracellular infusion of Aβ causes a rapid increase in the AMPAR-mediated EPSC (EPSC_A).
(a) The infusion of 1–5 nM oligomeric Aβ into post-synaptic neurons induces a rapid increase in EPSC_A (n = 7). (b) Monomeric Aβ did not induce an increase in EPSC_A (n = 6). (c) Clusterin (500 nM) prevented the Aβ oligomer-induced facilitation of EPSC_A (n = 6). (d) The increase in EPSC_A is independent of synaptic activity (n = 6). Filled circles depict Aβ infused neurons and open circles depict control neurons. (e) An NMDAR-antagonist, D-AP5 (50 M) has no effect on the Aβ oligomer-induced facilitation of EPSC_A (n = 6). (f) The NMDAR mediated EPSC (EPSC_N) is unaffected by infusion of Aβ oligomers (n = 6). In this (and subsequent figures) graphs plot the mean ± S.E.M. of n experiments.
Figure 3. Aβ oligomer-induced increase in EPSC_A is dependent on Ca^{2+} and PKA. (a) Neurons were infused with Aβ oligomer in the presence of BAPTA (10 mM) in the filling solution. This prevented the Aβ oligomer facilitated increase in EPSC_A (n = 7). (b) Ryanodine infusion via the pipette prevented the Aβ facilitated increase in EPSC_A (n = 7). (c) There was no increase in EPSC_A following preincubation (30 min) with RP-cAMPS (100 μM) (n = 6). (d) H89 infusion via the pipette prevented the Aβ facilitated increase in EPSC_A (n = 6). (e) Ro 32-0432 (10 μM) infusion via the pipette had no effect on the Aβ-mediated increase of EPSC_A (n = 6). (f) PKC 19-31 infusion via the pipette did not prevent the Aβ facilitated increase in EPSC_A (n = 6). (g) KN-62 (10 μM) preincubation (45 min) and infusion via the pipette prevented the sustained Aβ facilitation of EPSC_A (n = 7).
Figure 4. Aβ oligomer-induced expression of CP-AMPARs (a) Aβ failed to increase EPSC_A in GluA1-shRNA transfected cells (n = 7). (b) Aβ oligomers infusion increases EPSC_A in GluA2-shRNA transfected cells (n = 8). (c) Aβ failed to increase EPSC_A in GluA1-S845 phosphomutant transfected cells (n = 7). (d) Bath application of IEM 1460 (100 μM) has no effect on basal transmission EPSC_A (n = 6). (e) The Aβ oligomer-mediated increase in EPSC_A is reduced by bath application of IEM (n = 8).

Aβ oligomer-induced enhancement of EPSC_A is mediated by the GluA1 subunit of AMPARs. Since homomeric forms of GluA1-AMPARs characteristically display greater conductance than GluA2 containing AMPARs, we hypothesized that infusion of Aβ facilitates EPSC_A through an increase in synaptic homomeric GluA1 AMPARs. Indeed, the activation of PKA can lead to the insertion of GluA1-containing, GluA2-lacking AMPARs, known as Ca_2+ permeable AMPARs (CP-AMPARs). To test this, we knocked down either GluA1 or GluA2 in neurons of organotypic hippocampal slices using biolistic shRNA transfection. GluA1-shRNA transfected cells did not show the rapid effect of Aβ oligomer infusion on EPSC_A (90 ± 7%, n = 7, Fig. 4a). In comparison, a rapid increase of EPSC_A...
was observed in GluA2-shRNA transfected cells (176 ± 18%, n = 8, Fig. 4b). The synaptic expression of GluA1 involves the PKA-dependent phosphorylation of the serine 845 residue (s845) of GluA1. Therefore, we knocked down endogenous GluA1 whilst simultaneously expressing an shRNA resistant form of GluA1 that cannot be phosphorylated at s845 (s845-phosphomutant). Infusion of Aβ oligomers in s845-phosphomutated cells had no effect on EPSC_A (89 ± 8%, n = 7, Fig. 4c).

Collectively, these data suggest that the rapid Aβ oligomer-induced changes in EPSC_A may be due to a PKA-dependent synaptic insertion of CP-AMPARs. To test this directly, we bath-applied IEM 1460 (IEM), a compound that selectively blocks CP-AMPARs. IEM had no effect on EPSC_A when using control pipette solution (99 ± 8%; 30 min after IEM treatment, n = 6, Fig. 4d), which is consistent with a negligible contribution by CP-AMPARs to basal AMPAR-mediated transmission. However, IEM dramatically reduced the EPSC_A following infusion with Aβ oligomers (159 ± 11%; 10 min after infusion of Aβ oligomers; 77 ± 10%; 30 min after the start of IEM treatment, n = 8, Fig. 4e). This suggests that Aβ oligomer infusion causes a rapid increase in the synaptic expression of CP-AMPARs, resulting in the observed facilitation of EPSC_A amplitude.

Using biotinylation assays from hippocampal slices, we found that the surface expression of GluA1 was significantly increased with exogenous Aβ treatment but that there was no change in GluA2/3 expression (Fig. 5A). This suggests that exogenously applied Aβ also induces the insertion of CP-AMPARs. To support these findings, we measured EPSC_A during the extracellular perfusion of Aβ. We found that there was an increase of EPSC_A on application of Aβ (145 ± 7%, n = 6, Fig. 5B), which was prevented when slices were continually perfused with IEM (89 ± 6%, n = 6, Fig. 5C).

Discussion
Here we have revealed a rapid synaptic response to intracellular accumulation of Aβ oligomers. Several lines of evidence suggest that extracellular Aβ oligomers are taken up into neurons where they impair synaptic function. By studying the effects of intracellularly applied Aβ oligomers we have found a rapid action: the insertion of CP-AMPARs via a PKA-dependent phosphorylation of s845 of GluA1. These effects, occurring as a primary response to the emergence of cytosolic Aβ oligomers, could contribute to a key catalyzing mechanism of subsequent aberrant synaptic transmission. This finding therefore highlights a surprising discrepancy in our current understanding of the effects of Aβ on synaptic receptors. Whereas previous studies have shown that Aβ can drive the downregulation of synaptic transmission, in some cases mediated by the internalization of AMPARs and NMDARs, our findings and those of others seem to indicate, in contrast, that Aβ can actually facilitate synaptic transmission, possibly through inducing the expression of receptors. Presumably this is due to different time courses of Aβ-mediated toxic effects (the above studies, for example, range in treatment times from minutes to hours) and/or CP-AMPARs mediated secondary toxic insults.

The mechanisms regulating the trafficking of GluA1-containing AMPARs have previously been characterized, and generally converge on C-terminus phosphorylation events. One canonical mechanism is the phosphorylation of the s845 residue on GluA1, priming its expression at the synapse. Our finding that the expression of S845A, a mutant form of GluA1 which cannot be phosphorylated at s845, blocks the Aβ-induced enhancement of EPSC_A, suggests that Aβ operates this rapid effect via a regulated physiological mechanism; the PKA-mediated phosphorylation of GluA1-s845. CaMKII has previously been implicated in AMPAR regulation and synaptic transmission. Consistent with this role, we found that inhibiting CaMKII blocks the Aβ-induced enhancement of EPSC_A. Interestingly, we observed a delayed effect under these conditions; whilst there was an initial increase in EPSC_A, this rapidly declined. This might be explained by previously reported roles for CaMKII in the synaptic stabilization of AMPARs. Therefore, PKA and CaMKII may act in concert in this mechanism, promoting the expression and then stabilization of synaptic AMPARs, respectively. Together, these data raise the interesting question as to whether Aβ might actually operate physiologically to regulate synaptic glutamate receptor expression, and whether its aberrant cytosolic presence leads to a dysregulated physiological process. Clearly, more work is required to further understand a possible non-pathological role of Aβ.

CP-AMPARs are expressed at an early postnatal age and are replaced with GluA2-containing Ca2+-impermeable AMPARs during development. CP-AMPARs are critically involved in physiological and pathological plasticity in the mature synapse. Furthermore, growing evidence suggests CP-AMPARs prime neurodegenerative diseases including stroke, ischaemia and amyotrophic lateral sclerosis. We found that blocking CP-AMPARs prior to exposure to exogenous Aβ prevented the facilitation of synaptic transmission. Therefore, our findings support the hypothesis that progressive Aβ-mediated CP-AMPAR expression is a pivotal catalyst for the onset of pathology.

The early accumulation of intracellular Aβ has been shown to be neurotoxic and transgenic models have shown it to be sufficient for cognitive impairments prior to the increase in extracellular Aβ. Indeed, the accumulation of intracellular Aβ has previously been shown to be prevalent in the brains of AD patients, and this is thought to be one of the earliest events in the pathology, preceding Aβ plaques and neurofibrillary tangles. A recent report has shown that the infusion and accumulation of Aβ into neurons can have significant impairing effects on synaptic function. Accounting for these findings and our data, the accumulation of intracellular Aβ will likely prove to be a catalyzing event in the pathogenesis of the disease. Given that the primary response to an increase in intracellular Aβ...
Figure 5. Exogenous application of Aβ induces GluA1 surface expression. (a) Aβ treatment caused an increase in the surface expression of GluA1, but not GluA2/3 as shown through a biotinylation assay. (b) Exogenous application of Aβ caused an increase in EPSC_A (n = 6), (c) which was prevented when slices were perfused with IEM (n = 6).
appears to be the expression of CP-AMPARs at the synapse, targeting CP-AMPARs may provide a means of restoring synaptic function in AD.

**Methods**

**Amyloid-β preparation.** The amyloid-β 1-42 peptide (Aβ; Millipore, UK) was first dissolved at a concentration of 1 mg/ml in 100% HFIP (1,1,1,3,3,3-hexafluoro-2-propanol [Sigma-Aldrich]). This solution was incubated at room temperature for 1 h with occasional vortexing. Next, the solution was sonicated for 10 min in a water bath sonicator. The solution was then dried under a gentle stream of nitrogen gas. 100% DMSO was then used to resuspend the peptide, which was then incubated at room temperature for 12 h with occasional vortexing. This solution was finally aliquoted into smaller volumes and stored at −80°C. For a working solution, D-PBS (Invitrogen, UK) was added to the peptide stock solution and incubated for 2 h at room temperature to allow for peptide aggregation. To prepare monomeric Aβ, the same procedure outlined above was followed, with the exception of the 2 h room temperature aggregation step.

**Electrophysiology.** All animal experiments were carried out in accordance with the UK Scientific Procedures Act, 1986 and associated guidelines. The methods were carried out in accordance with the approved guidelines. All experimental protocols were approved by the University of Bristol Animal Welfare & Ethical Review Body. Acute hippocampal slices were prepared from 26- to 32- day-old male Wistar rats. Animals were sacrificed by dislocation of the neck and then decapitated. The brain was rapidly removed and placed in ice-cold artificial CSF (aCSF) containing (in mM): 124 NaCl, 3 KCl, 26 NaHCO_3, 1.25 NaH_2PO_4, 2 CaCl_2, 1 MgSO_4, 10 D-glucose, and 0.1 picrotoxin (bubbled with 95% O_2/ 5% CO_2). Transverse hippocampal slices (400μm thick) were prepared using a McIlwain tissue chopper (Mickle Laboratory Engineering Co. Ltd., Gomshall, UK). Hippocampal slices were stored in aCSF (~30 °C) flowing at 2 ml/min. Stimulating electrodes were placed in the CA2 (Schaffer Collateral pathway). Single stimuli (constant voltage) were delivered to the Schaffer Collateral input at 30 sec intervals (0.016 Hz).

**Hippocampal Slice Culture and Whole-Cell Patch Recording.** Organotypic hippocampal slice cultures were prepared from 6–8 days old Wistar rats. Rats were decapitated and brains were rapidly removed and placed in cold cutting solution that contained (mM) sucrose, 238; KCl, 2.5; NaHCO_3, 26; NaH_2PO_4, 1; D-glucose, 11; MgCl_2, 5 and CaCl_2, 1. Hippocampal slices (350μm) were cut using a McIlwain tissue chopper, and cultured on semi-permeable membrane inserts (Millipore Corporation, Bedford, MA, USA) in a six-well plate containing culture medium (78.8% minimum essential medium, 20% heat-inactivated horse serum, 30 mM HEPES, 26 mM D-glucose, 5.8 mM NaHCO_3, 2 mM CaCl_2, 2 mM MgSO_4, 70μM Ascorbic Acid, 0.1% 1 mg/ml Insulin, pH adjusted to 7.3 and 320–330 mOsm). Slices were cultured for 7–9 days in vitro (DIV) with a change of medium every 2 days, without antibiotics. Neurons were transfected using a biolistic gene gun (Helios Gene-gun system, Bio Rad, U.S.A.) at DIV 3–4 (100 μg DNA; 90% of the construct to test; 10% pEGFP-C1). Electrophysiological recordings were performed at 3–4 days after transfection. Recordings were carried out in solution containing (mM) NaCl, 119; KCl, 2.5; NaHCO_3, 26; NaH_2PO_4, 1; D-glucose, 11; CaCl_2, 4; MgCl_2, 4; picrotoxin, 0.02; 2-chloroadenosine,0.01 and gassed with 5% CO_2/95% O_2.

**GluA2 shRNA.** The following complementary oligonucleotide sequences were annealed and ligated into the EcoRI / Apal sites of the pSilencer v1.0 vector (Ambicom): (Forward) 5'-GAACCTGGCAGGTACCGCTTCAAGAGAAGCCGGTTACCTGCAGGTCTTTTTTTTT-3' and (Reverse) 5'-AATTAAAAAGAAGCTGGGTAACGGGTTCTCTTGGAAAGCCGGTACCTGGCCA GTTACGGCC-3'. The plasmid was then amplified in DH5α competent cells and the purified DNA was qualitatively analysed and sequenced to determine satisfactory plasmid ligation.

**GluA2 shRNA.** The following complementary oligonucleotide sequences were annealed and ligated into the EcoRI / Apal sites of the pSilencer v1.0 vector (Ambicom): (Forward) 5'-CCATCGAAAGTGCTTGGGTAACCGGTACCGCTTCAAGAGAAGCCGGTTACCTGCAGGTCTTTTTTTTTTTTTTTTTTTT3-3' and (Reverse) 5'-AATTAAAAAAGAAGCTGGGTAACGGGTTCTCTTGGAAAGCCGGTACCTGGCCA GTTACGGCC-3'. The plasmid was
then amplified in DH5α competent cells and the purified DNA was qualitatively analysed and sequenced to determine satisfactory plasmid ligation.

**GluA1 S845 mutant.** The GluA1 S845A construct was a generous gift from Jeff Bernhardt. Briefly, site-directed mutagenesis was performed with Chameleon (Stratagene) on the prRK5_GluA1i construct mutating serine 845 residue to alanine.

**Slice biotinylation and NeutraAviBid pull-down.** Surface biotinylation of acute slices was performed as described previously with some modifications. Briefly, slices were initially washed twice in aCSF and subsequently incubated in aCSF containing 1 mg/ml Sulfo-NHS-SS-Biotin (Thermo Scientific, Rockford, USA) for 45 min at 4 °C to allow for labelling of all surface membrane proteins. Excess biotin was removed by washes in aCSF containing NH4Cl. Tissue was then homogenised in lysis buffer containing 25 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 10 mM NaF and a cocktail of protease inhibitors (Sigma, St Louis, USA) and incubated for 30 min prior to centrifugation at 1,000 g to remove cellular debris. The total protein concentration was determined using the Pierce BCA kit. Subsequently, 100 μl of StreptaAvidin beads (Upstate, USA) were added to 500 μg of protein lysate and placed on a rotator at 4 °C for 2 hr. Samples were then washed five times in lysis buffer; beads were pulled-down by gentle centrifugation. Bound proteins were eluted by heating at 90 °C for 5 min prior to gel loading.

**Statistical Analyses.** Data were analyzed from one slice per rat (i.e., n = number of slices = number of rats). Data pooled across slices are expressed as the mean ± s.e.m. Significance (p < 0.05) was tested using two-tailed t-tests. For electrophysiology experiments, mean ± s.e.m. data from the 40 min time-point are described.

**References**

1. Spires-Jones, T. L. & Hyman, B.T. The intersection of amyloid beta and tau at synapses in Alzheimer’s disease. *Neuron* 82, 756–771 (2014).

2. Crimins, J. I., Pooler, A., Polydoro, M., Luebke, J. I. & Spires-Jones, T. L. The intersection of amyloid beta and tau in glutamatergic synaptic dysfunctions and collapse in Alzheimer’s disease. *Ageing Res Rev* 12, 757–763 (2013).

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

4. Wang, Q., Walsh, D. M., Rowan, M. J., Selkoe, D. J. & Anwyl, R. 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 metabolotropic glutamate receptor type 5. *J Neurosci* 24, 3370–3378 (2004).

5. Jo, J. et al. Abeta(1–42) inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3beta. *Nat Neurosci* 14, 545–547 (2011).

6. Walsh, D. M. et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416, 535–539 (2002).

7. Dinamarca, M. C., Rios, J. A. & Inestrosa N. C. Postsynaptic Receptors for Amyloid-beta Oligomers as Mediators of Neuronal Damage in Alzheimer’s Disease. *Point Physiol* 3, 464 (2012).

8. Lauren, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W. & Strittmatter, S. M. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457, 1128–1132 (2009).

9. Chen, S., Yadav, S. P. & Surewicz, W. K. Interaction between human prion protein and amyloid-beta (Abeta) oligomers: role of N-terminal residues. *J Biol Chem* 285, 26377–26383 (2010).

10. Larson, M. et al. The complex PrP(C)-Fyn couples human oligomeric Abeta with pathological tau changes in Alzheimer’s disease. *J Neurosci* 32, 16857–16871a (2012).

11. Kudo, W. et al. Cellular prion protein is essential for oligomeric amyloid-beta-induced neuronal cell death. *Hum Mol Genet* 21, 1138–1144 (2012).

12. Um, J. W. et al. Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer abeta oligomer bound to cellular prion protein. *Neuron* 79, 887–902 (2013).

13. Nicoll, A. J. et al. Amyloid-beta nanotubes are associated with prion protein-dependent synaptotoxicity. *Nat Commun* 4, 2416 (2013).

14. Lin, H., Bhatia, R. & Lal, R. Amyloid beta protein forms ion channels: implications for Alzheimer’s disease pathophysiology. *FASEB J* 15, 2433–2444 (2001).

15. Demuro, A., Mina, E., Kayed, R., Milton, S. C., Parker, I & Glabe, C. G. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem* 280, 17294–17300 (2005).

16. Jang, H. et al. Truncated beta-amyloid peptide channels provide an alternative mechanism for Alzheimer’s Disease and Down syndrome. *Proc Natl Acad Sci U S A* 107, 6538–6543 (2010).

17. Lin, H. & Arispe, N. I. Single-cell screening of cytosolic [Ca] reveals cell-selective action by the Alzheimer’s Abeta peptide ion channel. *Cell Stress Chaperones*, 20, 333–342 (2015).

18. Kessels, H. W., Nguyen, L. N., Nabavi, S. & Malinow, R. The prion protein as a receptor for amyloid-beta. *Nature* 466, E3–4; discussion E4–5 (2010).

19. Balducci, C. et al. Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein. *Proc Natl Acad Sci U S A* 107, 2295–2300 (2010).

20. Cisse, M., Sanchez, P.E., Kim, D.H., Ho., K., Yu., GQ. & Mucke, L. Ablation of cellular prion protein does not ameliorate abnormal neural network activity or cognitive dysfunction in the J20 line of human amyloid precursor protein transgenic mice. *J Neurosci* 31, 10427–10431 (2011).

21. LaFerla, F. M., Green, K. N. & Oddo, S. Intracellular amyloid-beta in Alzheimer’s disease. *Nat Rev Neurosci* 8, 499–509 (2007).

22. Mohamed, A. & Posse de Chaves, E. Abeta internalization by neurons and glia. *Int J Alzheimers Dis* 2011, 127984 (2011).
23. Yazawa, H et al. Beta amyloid peptide (Abeta42) is internalized via the G-protein-coupled receptor FPR1 and forms fibrillar aggregates in macrophages. *FASEB J* 15, 2454–2462 (2001).

24. Nagele, R. G., D’Andrea, M. R., Anderson, W. J. & Wang, H. Y. Intracellular accumulation of beta-amyloid (1–42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer’s disease. *Neuroscience* 110, 199–211 (2002).

25. Clifford, P. M. et al. Abeta peptides can enter the brain through a defective blood-brain barrier and bind selectively to neurons. *Brain Res* 1142, 223–236 (2007).

26. Yankner, B. A., Duffy, L. K. & Kirschner, D. A. Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. *Science* 250, 279–282 (1990).

27. Takadera, T., Sakura, N., Mohri, T. & Hashimoto, T. Toxic effect of a beta-amyloid peptide (beta 22–35) on the hippocampal neuron and its prevention. *Neurosci Lett* 161, 41–44 (1993).

28. Narayanan, P. et al. The extracellular chaperone clusterin sequesters oligomeric forms of the amyloid-beta(1–40) peptide. *Nat Struct Mol Biol* 19, 79–83 (2012).

29. McClade-McCullough, E., Yamamoto, H., Tan, S. E., Brickey, D. A. & Soderling, T. R. Phosphorylation and regulation of glutamate receptors by calcium/calmodulin-dependent kinase II. *Nature* 362, 640–643 (1993).

30. Lledo, P. M., Hjelmstad, G. O., Mukherji, S., Soderling, T. R., Malenka, R. C. & Nicoll, R. A. Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proc Natl Acad Sci U S A* 92, 11175–11179 (1995).

31. Mulkey, R. M., Endo, S., Shenolikar, S. & Malenka, R. C. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* 369, 486–488 (1994).

32. Frey, U., Huang, Y. Y. & Kandel, E. R. Effects of Camp simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 260, 1661–1664 (1993).

33. Nayak, A., Zastrow, D. J., Lickteig, R., Zahniser, N. R., Browning, M. D. Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. *Nature* 394, 680–683 (1998).

34. Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Poncer, J. L. & Malinow, R. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for Glur1 and PDZ domain interaction. *Science* 287, 2262–2267 (2000).

35. Wang, J. H. & Kelly, P. T. Postsynaptic injection of CA2+/-CaM induces synaptic potentiation requiring CaMKII and PKC activity. *Neuron* 15, 443–452 (1995).

36. Clem, R. L., Anggono, V. & Huganir, R. L. PICK1 regulates incorporation of calcium-permeable AMPA receptors during cortical synaptic strengthening. *J Neurosci* 30, 6360–6366 (2010).

37. Swanson, G.T. & Kamboj, S. K., Cull-Candy, SG. Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. *J Neurosci* 17, 58–69 (1997).

38. Man, H. Y., Sekine-Aizawa, Y. & Huganir, R. L. Regulation of [alpha]-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking through PKA phosphorylation of the Glu receptor 1 subunit. *Proc Natl Acad Sci U S A* 104, 3579–3584 (2007).

39. Roche, K. W., O’Brien, R. J., Mannen, A. L., Bernhardt, J. & Huganir, R. L. Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* 16, 1179–1188 (1996).

40. He, K., Song, L., Cummings, L. W., Goldman, J., Huganir, R. L. & Lee, H. K. Stabilization of Ca2+/-permeable AMPA receptors at presynaptic sites by GluR1-S845 phosphorylation. *Proc Natl Acad Sci U S A* 106, 20033–20038 (2009).

41. Samoilova, M. V., Buldakov, S. L., Vorobjev, V. S., Sharovona, I. N. & Magazanik, L. G. The open channel blocking drug, IEM-1460, reveals functionally distinct alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors in rat brain neurons. *Nature Neuroscience* 9, 261–268 (1999).

42. Almeida, C. G. et al. Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiol Dis* 20, 187–190 (2005).

43. Hisieh, H. et al. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* 52, 831–843 (2006).

44. Gu, Z., Liu, W. & Yan, Z. [beta]-Amyloid impairs AMPA receptor trafficking and function by reducing Ca2+/-calmodulin-dependent protein kinase II synaptic distribution. *J Biol Chem* 284, 10639–10649 (2009).

45. Monfort, P. & Felipo, V. Amyloid-beta impairs, and ibuprofen restores, the cGMP pathway, synaptic expression of AMPA receptors and long-term potentiation in the hippocampus. *J Alzheimers Dis* 22, 795–809 (2010).

46. Hartley, DM et al. Protobrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J Neurosci* 19, 8876–8884 (1999).

47. Tozaki, H. et al. The inhibitory and facilitatory actions of amyloid-beta peptides on nicotinic ACh receptors and AMPA receptors. *Biochem Biophys Res Commun* 294, 42–44 (2002).

48. Ye, C., Walsh, D. M., Selkoe, D. J. & Hartley, D. M. Amyloid beta-protein induced electrophysiological changes are dependent on aggregation state: N-methyl-D-aspartate (NMDA) versus non-NMDA receptor/channel activation. *Neurosci Lett* 366, 320–325 (2004).

49. Wang, D., Govindasaih, G., Liu, R., De Arcangelis, V., Cox, C. L. & Xiang, Y. K. Binding of amyloid beta peptide to beta2 adrenergic receptor induces PKA-dependent AMPA receptor hyperactivity. *FASEB J* 24, 3511–3521 (2010).

50. Carriedo, S. G., Yin, H. Z., Sensi, S. L. & Weiss, J. H. Rapid Ca2+ entry through Ca2+/-permeable AMPA/Kainate channels triggers marked intracellular Ca2+ rises and consequent oxygen radical production. *J Neurosci* 18, 7727–7738 (1998).

51. Sensi, S. L., Yin, H. Z., Carriedo, S. G., Rao, S. S. & Weiss, J. H. Preferential Zn2+ influx through Ca2+/-permeable AMPA/kainate channels triggers prolonged mitochondrial superoxide production. *Proc Natl Acad Sci U S A* 96, 2414–2419 (1999).

52. Oh, M. C., Derkach, V. A. & Guire, E. S., Soderling, T. R. Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. *J Biol Chem* 281, 752–758 (2006).

53. Lee, H. K., Takamiya, K., He, K., Song, L. & Huganir, R. L. Specific roles of AMPA receptor subunit GluR1 (GluA1) phosphorylation sites in regulating synaptic plasticity in the CA1 region of hippocampus. *J Neurophysiol* 103, 479–489 (2010).

54. He, K., Lee, A., Song, L., Kanold, P. O. & Lee, H. K. AMPA receptor subunit GluR1 (GluA1) serine-845 site is involved in synaptic depression but not in spine shrinkage associated with chemical long-term depression. *J Neurophysiol* 105, 1897–1907 (2011).

55. Opazo, P., Sainllos, M. & Choquet, D. Regulation of AMPA receptor surface diffusion by PSD-95 slots. *Curr Opin Neurobiol* 22, 453–460 (2012).

56. Pickard, L., Noel, J., Henley, J. M., Collingridge, G. L. & Molnar, E. Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. *J Neurosci* 20, 7922–7931 (2000).

57. Kumar, S. S., Bacci, A., Kharazia, V. & Huguenard, J. R. A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. *J Neurosci* 22, 3005–3015 (2002).

58. Brill, J. & Huguenard, J. R. Sequential changes in AMPA receptor targeting in the developing neocortical excitatory circuit. *J Neurosci* 26, 13918–13928 (2006).

59. Cull-Candy, S., Kelly, L. & Farrant, M. Regulation of Ca2+/-permeable AMPA receptors: synaptic plasticity and beyond. *Curr Opin Neurobiol* 16, 288–297 (2006).
60. Derkach, V. A., Oh, M. C., Guire, E. S. & Soderling, T. R. Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci 8, 101–113 (2007).
61. Feldmeyer, D. et al. Neurological dysfunctions in mice expressing different levels of the Q/R site-unedited AMPAR subunit GluK-B. Nat Neurosci 2, 57–64 (1999).
62. Liu, B. et al. Ischemic insults direct glutamate receptor subunit 2-lacking AMPA receptors to synaptic sites. J Neurosci 26, 5309–5319 (2006).
63. Spatling, J. M, Klein, D. M., Singh, P & Meaney, D. F. Calcium-permeable AMPA receptors appear in cortical neurons after traumatic mechanical injury and contribute to neuronal fate. J Neurotrauma 25, 1207–1216 (2008).
64. Kwak, S. & Weiss, J. H. Calcium-permeable AMPA channels in neurodegenerative disease and ischemia. Curr Opin Neurobiol 16, 281–287 (2006).
65. Zhang, Y., McLaughlin, R., Goodyear, C., LeBlanc, A. Selective cytotoxicity of intracellular amyloid beta peptide1–42 through p53 and Bax in cultured primary human neurons. J Cell Biol 156, 519–529 (2002).
66. Oddo, S., Caccamo, A., Kitazawa, M., Tseng, BP, LaFerla, F. M. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer’s disease. Neurobiol Aging 24, 1063–1070 (2003).
67. Gouras, G. K. et al. Testosterone reduces neuronal secretion of Alzheimer’s beta-amyloid peptides. Proc Natl Acad Sci U S A 97, 1202–1205 (2000).
68. Wegiel, J. et al. Intraneuronal Abeta immunoreactivity is not a predictor of brain amyloidosis-beta or neurofibrillary degeneration. Acta Neuropathol 113, 389–402 (2007).
69. Aoki, M., Volkmann, I., Tjernberg, L. O., Winblad, B. & Bogdanovic, N. Amyloid beta-peptide levels in laser capture microdissected cornu ammonis 1 pyramidal neurons of Alzheimer’s brain. Neuroreport 19, 1085–1089 (2008).
70. D’Andrea, M. R., Nagele, R.G., Wang, H. Y., Peterson, F. A. & Lee, D. H. Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer’s disease. Histopathology 38, 120–134 (2001).
71. Ripoli, C. et al. Intracellular accumulation of amyloid-beta (Abeta) protein plays a major role in Abeta-induced alterations of glutamatergic synaptic transmission and plasticity. J Neurosci 34, 12893–12903 (2014).
72. Thomas-Crusells, J., Vieira, A., Saarma, M. & Rivera, C. A novel method for monitoring surface membrane trafficking on hippocampal acute slice preparation. J Neurosci Methods 125, 159–166 (2003).

Acknowledgements
This study was supported by UK Wellcome Trust-MRC Neurodegenerative Disease Initiative Programme to K.C., G.L.C., D.K. and P.G.H. The Korea-UK Alzheimer’s disease Research Consortium grant to D-H.K. (Korean Ministry of Health and Welfare). E.L.H. and P.R. were supported by MRC PhD studentships. K.C. was supported by the Wolfson Research Merit Award and Royal Society, London.

Author Contributions
The study was conceived and designed by K.C. Electrophysiological studies were conducted by D.J.W., E.L.H., D-H.K., J.J. and biochemical assays were conducted by T.P., G.W. and P.N. The manuscript was written by D.J.W., E.J.K., P.G.H., D.K., G.L.C. and K.C.

Additional Information
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Whitcomb, D. J. et al. Intracellular oligomeric amyloid-beta rapidly regulates GluA1 subunit of AMPA receptor in the hippocampus. Sci. Rep. 5, 10934; doi: 10.1038/srep10934 (2015).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/