Reelin signaling antagonizes β-amyloid at the synapse

Murat S. Durakoglugil[a], Ying Chen[a], Charles L. White[b], Ege T. Kavalali[c], and Joachim Herz[a,c,1]

Departments of [a]Molecular Genetics, [b]Pathology, and [c]Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75390

Communicated by Michael S. Brown, The University of Texas Southwestern Medical Center, Dallas, TX, July 30, 2009 (received for review June 15, 2009)

Abnormal processing of the amyloid precursor protein (APP) and β-amyloid (Aβ) plaque accumulation are defining features of Alzheimer disease (AD), a genetically complex neurodegenerative disease that is characterized by progressive synapse loss and neuronal cell death. Aβ induces synaptic dysfunction in part by altering the endocytosis and trafficking of AMPA and NMDA receptors. Reelin is a neuromodulator that increases glutamatergic neurotransmission by signaling through the postsynaptic ApoE receptors Apoer2 and Vldlr and thereby potently enhances synaptic plasticity. Here we show that Reelin can prevent the suppression of long-term potentiation and NMDA receptors, which is induced by levels of Aβ comparable to those present in an AD-afflicted brain. This reversal is dependent upon the activation of Src family tyrosine kinases. At high concentrations of Aβ peptides, Reelin can no longer overcome the Aβ induced functional suppression and this coincides with a complete blockade of the Reelin-dependent phosphorylation of NR2 subunits. We propose a model in which Aβ, Reelin, and ApoE receptors modulate neurotransmission and thus synaptic stability as opposing regulators of synaptic gain control.

Alzheimer disease | apolipoprotein E | long-term potentiation | NMDA receptor | Aβ oligomer

Alzheimer disease (AD) is a complex genetic neurodegenerative disease that afflicts an increasing fraction of our aging population (1). A characteristic feature of AD is the accumulation of oligomeric and higher-order aggregates of the $A\beta_{1-42}$ form of the amyloid-β (Aβ) peptide, which is physiologically released by sequential proteolytic cleavage from the amyloid-β precursor protein (APP) (2, 3). Abnormal, amyloidogenic Aβ processing and plaque formation progressively lead to synaptic dysfunction, synapse loss, and ultimately neuronal death.

Recent insight into the pathophysiological functions of Aβ came from studies that demonstrated a potent negative impact of Aβ oligomers on synaptic functions that underlie long-term synaptic plasticity (4–6). Incubation of hippocampal neurons and slices with Aβ oligomers leads to intracellular trapping or functional impairment of AMPA and NMDA-type glutamate receptors (7–9), thereby decreasing long-term potentiation (LTP), an enhancement of synaptic strength that is correlated with memory (4, 5, 10–13). Consistent with these in vitro findings, Palop et al. (14, 15) showed that overexpression of mutant APP forms in mice resulted in a global dysregulation of neuronal network activity in vivo.

Reelin is a signaling protein that is produced by interneurons in the brain and that has an effect on synaptic functions that is opposite to that of Aβ oligomers. Reelin addition to hippocampal slices results in enhanced LTP (16) as a result of the activation of Src family tyrosine kinases (SFKs) (17–19), which increase NMDA receptor activity by tyrosine phosphorylation of their NR2 subunits (20, 21). These effects of Reelin are mediated by a pair of homologous cell surface receptors designated apolipoprotein E receptor 2 (Apoer2) and very low-density lipoprotein receptor (Vldlr), which belong to a family of highly conserved endocytic signaling receptors. Reelin binding to both proteins in concert controls neuronal migration during development (22), as well as glutamatergic neurotransmission through differential enhancement of NMDA receptor and AMPA receptor activity in the adult nervous system (20, 21, 23, 24). Thus, Aβ and ApoE receptors converge on common molecular mechanisms, which are essential for synapse formation and maintenance. This raises the possibility that Reelin signaling could prevent or reverse the negative effect of Aβ oligomers on synaptic function.

Here we show that Reelin signaling in excitatory synapses can restore normal synaptic plasticity, which is impaired by concentrations of oligomeric Aβ peptides that lie well within the range present in the brains of AD patients. This reversal requires the ApoE receptor-dependent activation of SFKs. We propose a model in which Aβ and ApoE receptors function in tandem as opposing regulators of synaptic efficacy.

Results

Several independent studies have consistently shown that Aβ oligomers can potently suppress long-term potentiation, an electrophysiological parameter that measures activity-induced synaptic strengthening. By contrast, activation of ApoE receptor signaling by Reelin potently increases long-term potentiation, and mice that lack the Reelin receptors Apoer2 or Vldlr exhibit early LTP defects (16).

We tested whether Reelin was capable of reversing the CA1 field LTP reduction brought on by $A\beta_{1-42}$ oligomers (400 nM) in WT hippocampal slices (Fig. 1*A*, closed circles). When Reelin was present in the perfusion medium (closed triangles), LTP was restored almost to control levels (i.e., no Aβ, no Reelin, open squares). The physical state of the $A\beta_{1-42}$ oligomers used in the experiments throughout this study is shown (Fig. 1*A Inset*), and the potency of our purified Reelin preparations used in these studies, indicated by the ability of different Reelin concentrations to induce Dab1 tyrosine phosphorylation, is shown (Fig. 1*A Lower*). Consistent with the findings in other laboratories, monomeric $A\beta_{1-42}$ (1°) was ineffective. A truncated form of the Aβ peptide consisting only of amino acids 25 to 35 ($A\beta_{25-35}$), which form the minimal fibrillogenic region was also able to potently suppress baseline LTP at consistently lower concentrations (100 nM; Fig. 1*B*, closed circles). This suppression was again fully reversible by co-application of Reelin (closed triangles), indicating that LTP suppression induced by low concentrations of $A\beta_{1-42}$ or $A\beta_{25-35}$ was not caused by a non-specific cytotoxic effect.

We have reported earlier (20) that Reelin increases tyrosine phosphorylation of NR2A as well as NR2B subunits (Fig. 2*A*, lane 2, *Upper* and *Lower*, respectively). NR2 tyrosine phosphorylation increases the expression of NMDA receptors at the cell surface by preventing their endocytosis (9). It also increases their

Author contributions: M.S.D., E.T.K., and J.H. designed research; M.S.D. and Y.C. performed research; C.L.W. contributed new reagents/analytic tools; M.S.D., Y.C., E.T.K., and J.H. analyzed data; and J.H. wrote the paper.

The authors declare no conflicts of interest.

[1]To whom correspondence should be addressed. E-mail: joachim.herz@utsouthwestern.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0908176106/DCSupplemental.

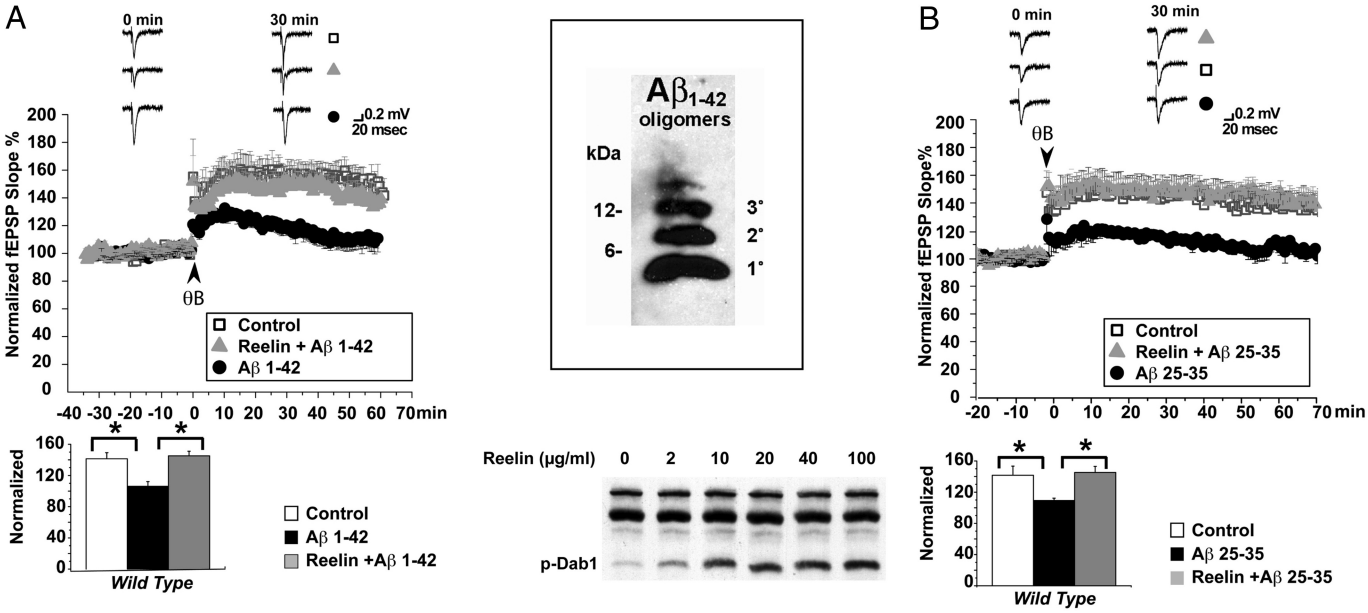

Fig. 1. Reelin rescues Aβ-induced LTP suppression in CA1. (*A*) Suppression of LTP by $A\beta_{1-42}$ is rescued by Reelin in WT hippocampal slices. (*Upper*) LTP was induced by theta burst stimulation (TBS) consisting of 5 trains of 4 pulses at 100 Hz with an inter-burst interval of 20 s at 0 min. Open squares show responses from control slices receiving no treatment ($n = 5$). Solid black circles show responses from slices treated with 400 nM $A\beta_{1-42}$ for 40 to 60 min before TBS. Solid gray triangles show responses of slices from the same animals treated with $A\beta_{1-42}$ in the presence of 5 nM Reelin. Representative fEPSP traces before and 30 min after TBS for the different conditions are shown (*Top*). (Scale bar: 0.2 mV, 20 ms.) (*Lower*) Statistical analysis of average LTP responses between 30 and 35 min after TBS. $A\beta_{1-42}$ significantly attenuates LTP in the absence of Reelin (from 152.99% ± 9.85% to 112.89% ± 6.35%, $n = 5$; $P < 0.05$). LTP reduction by $A\beta_{1-42}$ is prevented in the presence of 5 nM Reelin and no longer significantly different from controls (147.76% ± 9.30%, $n = 5$; $P > 0.05$). Asterisk denotes significance on one-way ANOVA followed by Bonferroni post-test. (*Inset, Right, Top*) Representative immunoblot of synthetic $A\beta_{1-42}$ oligomers used throughout the study. Monomer, dimers, trimers, and tetramers are present. (*Bottom*) Immunoblot of primary cortical neuronal lysates shows induction of Dab1 tyrosine phosphorylation by different concentrations of purified Reelin used throughout the study. (*B*) Suppression of LTP by $A\beta_{25-35}$ is rescued by Reelin in WT hippocampal slices. (*Upper*) LTP was induced by TBS consisting of 5 trains of 4 pulses at 100 Hz with an inter-burst interval of 20 s at 0 min. Open squares show responses from control slices receiving no treatment ($n = 8$). Solid black circles show responses from slices treated with 100 nM $A\beta_{25-35}$ for 40 to 60 min before TBS. Solid gray triangles show responses of slices from the same animals treated with $A\beta_{25-35}$ in the presence of 5 nM Reelin. Representative fEPSP traces before and 30 min after TBS for the different conditions are shown (*Top*). (Scale bar: 0.2 mV, 20 ms.) (*Lower*) Statistical analysis of average LTP responses between 30 and 35 min after TBS. $A\beta_{25-35}$ significantly attenuates LTP in the absence of Reelin (from 141.63% ± 11.75% to 109.21% ± 3.09%, $n = 8$; $P < 0.05$). LTP reduction by $A\beta_{25-35}$ is prevented in the presence of 5 nM Reelin and no longer significantly different from controls (145.26% ± 7.80%, $n = 8$; $P > 0.05$). Asterisk denotes significance on one-way ANOVA followed by post-tests. High resolution traces are shown in the SI.

gating (25). NR2 phosphorylation by Reelin is greatly reduced in the presence of a high concentration of 1 μM $A\beta_{25-35}$ (Fig. 2*A*, lane 4). Similar results were obtained with $A\beta_{1-42}$ (not shown). Because Reelin can no longer efficiently phosphorylate NR2 subunits in the presence of high Aβ peptide levels, we predicted that Reelin would also be unable to prevent the Aβ-mediated LTP suppression at these concentrations. As we had shown earlier (16, 20), Reelin enhances LTP in WT hippocampal slices (closed circles, Fig. 2*B*) over control (i.e., no Reelin, open squares). In the presence of 1 μM $A\beta_{25-35}$, LTP is almost completely abolished (open triangles), and this LTP suppression can no longer be prevented by Reelin (closed diamonds). These results are consistent with the mechanism proposed by Snyder et al. in which Aβ induces the internalization of NMDA receptors (9) and thus renders them inaccessible to Reelin activated SFKs at the cell surface.

We next demonstrated that Reelin is able to compensate for the Aβ-induced reduction of NMDA currents (9) at lower—and thus thought, more physiological—Aβ concentrations (100 nM $A\beta_{25-35}$), and that Reelin does this by increasing NMDA receptor activity directly. To show this, we pharmacologically isolated NMDA receptors in whole-cell recordings from WT hippocampal neurons (Fig. 3*A*). As expected and reported previously (21–23), Reelin application resulted in an immediate increase in NMDA currents, which was compensated by adjusting the gain back to baseline (i.e., baseline adjustment) before Aβ peptide addition (Fig. 3*A*, gray bar). In our hands, at 100 nM, $A\beta_{25-35}$ induced a similar reduction in NMDA currents (Fig. 3*A*, open squares) as had been reported by Snyder et al. (9) for $A\beta_{1-42}$, and this reduction was completely prevented in the presence of Reelin (Fig. 3*A*, filled circles). Thus, Reelin and Aβ actions antagonize each other at the synapse within their normal physiological concentration range. In the presence of low, but not high, levels of Aβ peptides, Reelin can readjust NMDA receptor activity to compensate for the Aβ-induced suppression.

Reelin activates SFKs, mainly Fyn (17–19), through clustering of ApoE receptors (26) and transphosphorylation of Dab1 (22, 27, 28), and this does not occur if both receptors, Apoer2 and Vldlr, are genetically ablated or functionally blocked (19). Fyn is required for LTP induction, but not for baseline synaptic transmission (29). To test whether the activation of SFKs by Reelin is necessary for reversing the Aβ-mediated suppression of NMDA currents, we performed another series of single-cell recordings on hippocampal CA1 neurons treated with Reelin in the presence (Fig. 3*B*, open squares) or absence (Fig. 3*B*, gray triangles) of the SFK inhibitor PP2. In this experiment, we used oligomerized $A\beta_{1-42}$ to induce NMDA receptor-dependent synaptic currents. Consistent with the results shown in Fig. 3*A*, Reelin was equally capable of preventing the $A\beta_{1-42}$-mediated suppression of synaptic NMDA receptor currents, but this functional rescue was prevented when SFK activity was blocked with PP2 (Fig. 3*B*), indicating that Reelin signaling through Apoer2 and Vldlr is necessary (17–19).

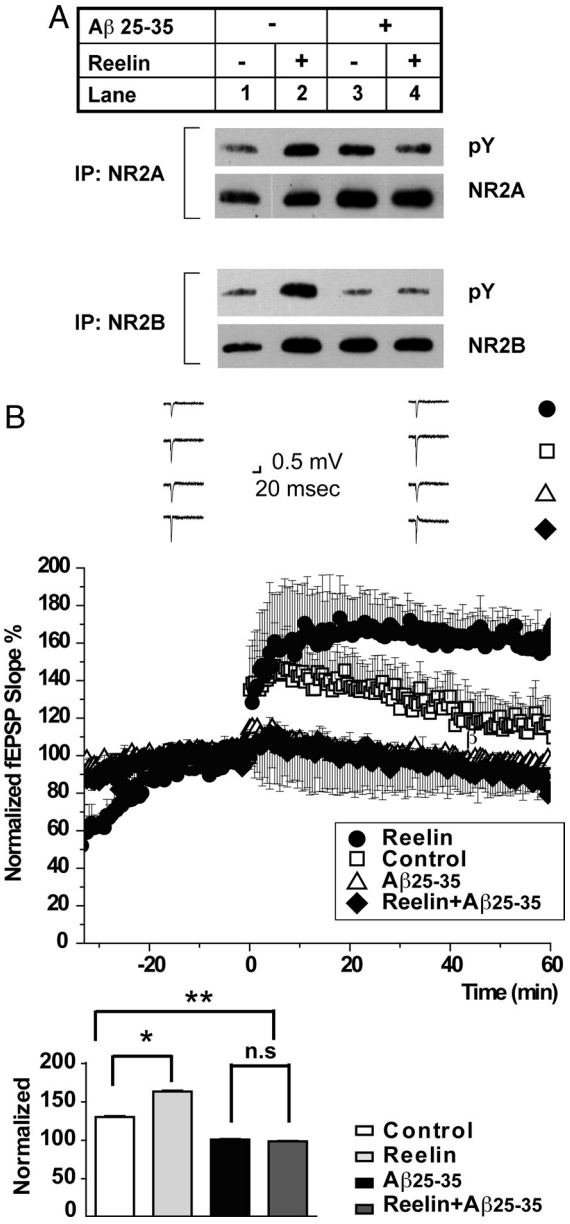

Fig. 2. Opposing effects of Aβ and Reelin on NMDA receptor tyrosine phosphorylation. (*A*) High doses of Aβ peptides prevent NMDA receptor phosphorylation in response to Reelin treatment. Acute WT hippocampal slices were incubated in the presence (*R*) or absence of Reelin (*C*) and $A\beta_{25-35}$. NR2A and NR2B were immunoprecipitated from the homogenized lysates of the slices and analyzed for tyrosine phosphorylation. Total amounts of immunoprecipitated NR2A and NR2B are shown (NR2A, NR2B); one independent experiment is shown ($n = 3$). Tyrosine phosphorylation of NR2A and NR2B in the presence of Aβ and Reelin was not significantly different from control (lane 1) by paired *t* test. (*B*) Reelin enhances LTP in the absence of Aβ, but fails to rescue LTP suppression induced by high concentrations of $A\beta_{25-35}$. (*Upper*) In WT hippocampal slices, Reelin (closed circles) enhances and $A\beta_{25-35}$ (at 1 μM, open triangles) suppresses TBS-induced LTP compared with non-treated control slices (open squares). Reelin fails to restore LTP in the presence of high concentrations of Aβ (1 μM). Application of Reelin alone (closed circles) induces an immediate shift in baseline currents as a result of its direct effect on NMDA and AMPA receptor activity (21). (*Lower*) Statistical analysis of average LTP responses 30 to 35 min after TBS. Control LTP (open bar), 129.30% ± 14.16%, $n = 2$; Reelin (gray bar), 165.65% ± 14.11%, $n = 2$; high-dose $A\beta_{25-35}$ (black closed bar), 99.85% ± 19.19%, $n = 4$; Reelin with high-dose $A\beta_{25-35}$, 97.56% ± 6.83%, $n = 4$. ANOVA was performed, followed by Bonferroni post-test. Control compared with Reelin, $P < 0.05$; control compared with high-dose $A\beta_{25-35}$, $P < 0.05$; high-dose $A\beta_{25-35}$ compared with Reelin and $A\beta_{25-35}$ together, $P > 0.05$. Asterisk denotes significance.

To further eliminate an alternative Reelin signaling-independent mechanism for the observed reversal of the amyloid-induced suppression of field LTP and NMDA receptor activity, we also measured LTP in the presence of the Reelin function blocking CR50 monoclonal antibody (30). If the effect of Reelin were merely mediated by it binding, sequestering, and thus quenching the activity of Aβ oligomers, CR50 should have no effect on the ability of Reelin to restore normal LTP. If, conversely, activation of the Reelin signaling pathway is necessary, as also indicated by the SFK inhibitor results, CR50 should block the effect of Reelin. Fig. 3*C* shows that this is in fact the case. Together, these results exclude a mechanism by which Reelin neutralizes Aβ oligomers extracellularly.

We have shown that activation of ApoE receptors with their natural ligand Reelin can prevent the impairment of synaptic functions caused by Aβ oligomers, which are present in AD but not in normal non-diseased brain (31). We therefore set out to investigate whether Reelin was equally effective in restoring normal synaptic plasticity in hippocampal slices treated with extracts from AD patients containing naturally produced and clinically demonstrated pathogenic Aβ oligomers. Human postmortem cortical brain extracts were prepared as described in *Materials and Methods* and by Shankar et al. (11). Aβ peptides (i.e., monomers, oligomers, and higher-order complexes) were precipitated from equal volumes of lysate, separated on lithium dodecyl sulfate (LDS) gels, and immunoblotted (Fig. 4*A*). Monomers and oligomers derived from synthetic $A\beta_{1-42}$ aggregation were loaded (Fig. 4*A Left*). Cortical extracts from the control (i.e., non-AD) subject (Fig. 4*A Middle*) had amounts of monomeric Aβ (1°) that were comparable to those present in the extracts isolated from the AD case (Fig. 4A *Right*). The control sample, however, did not contain Aβ oligomers and only a trace of higher molecular weight oligomers, in contrast to the AD case extract, which contained readily detectable amounts of dimers as well as abundant multimers. We next compared the effect of control and AD brain extracts on CA1 field LTP (Fig. 4*B*). As reported (11), AD brain extracts (Fig. 4*B*, closed circles) strongly suppressed LTP compared with control (Fig. 4*B*, open squares) and at equivalent concentrations (50 μL extract per mL perfusion medium). Reelin almost completely prevented the LTP defect that was caused by the AD brain extract (Fig. 4*B*, gray triangles). The reversal of this defect by Reelin was almost entirely mediated by its effect on NMDA receptors, rather than AMPA receptors (Fig. 4*C*). When the same experiment was conducted in the presence of the NMDA antagonist AP5, only a small amount of NMDA receptor-independent LTP, comparable in magnitude to the amount of LTP in the presence of Aβ oligomers (Fig. 1 *A* and *B* and Fig. 4*B*), was detected in WT mouse CA1 (Fig. 4*C*, open squares). This residual NMDA receptor-independent LTP was not further reduced by the AD brain extract (Fig. 4*C*, black circles), nor was it increased in the presence of Reelin (Fig. 4*C*, gray triangles), suggesting that the AD brain extract mainly suppresses NMDA receptor activity in hippocampal CA1, and that under these conditions Reelin selectively prevents or restores NMDA, rather than AMPA receptor activity suppressed by Aβ oligomers. Taken together, these results strongly suggest that the Reelin-mediated activation of ApoE receptor signals can protect neurons from the synaptic suppression caused by Aβ oligomers that accumulate in the brains of AD patients and that the synaptic effects of synthetic and naturally produced oligomers are qualitatively equivalent.

Discussion

Reelin is an evolutionarily highly conserved signaling protein that controls neuronal migration and positioning during brain development. In the adult brain, Reelin is expressed mainly by GABAergic interneurons, and we and others have shown that Reelin regulates the activity of excitatory synapses (16, 20, 21,

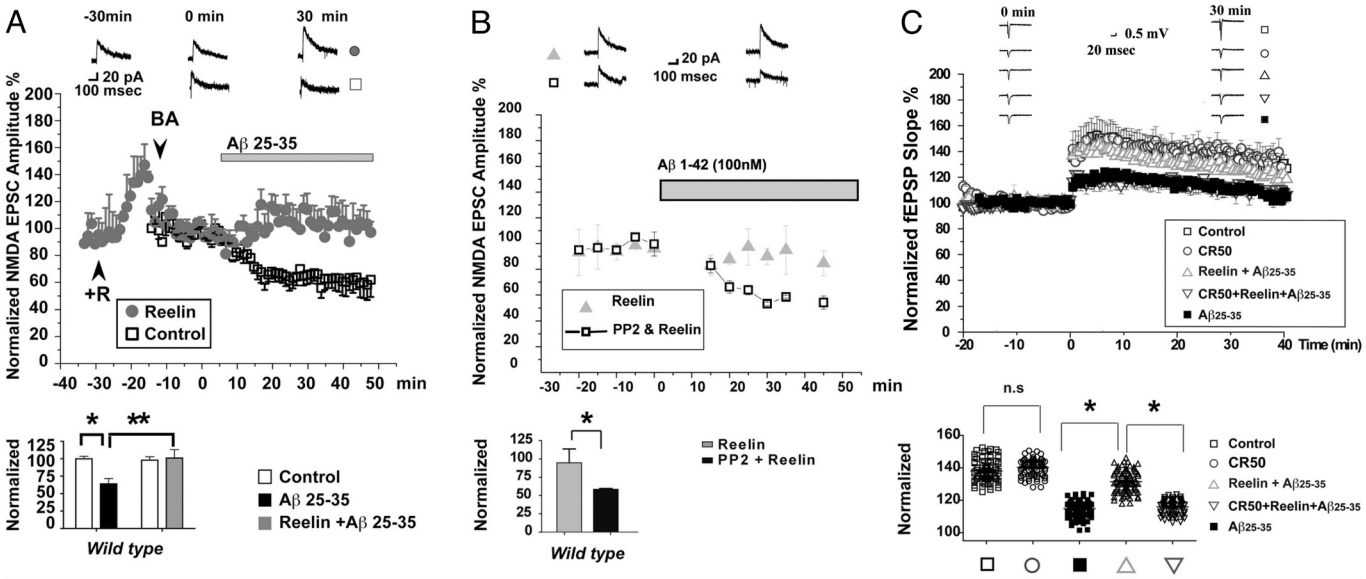

Fig. 3. Reelin rescues Aβ-induced suppression of NMDA currents in mouse hippocampus by activating SFKs. (*A*) Reelin prevents $A\beta_{25-35}$-induced suppression of NMDA currents. (*Upper*) Normalized average amplitudes of evoked NMDA peak currents at +30 mV in single-cell recordings. Open squares show responses from control slices treated with 100 nM $A\beta_{25-35}$ in the absence of Reelin; solid gray circles show responses in the presence of Aβ and 5 nM Reelin. Gray bar indicates the timing of Aβ application. Reelin was applied 25 to 30 min before Aβ perfusion. After stabilization, baseline levels were readjusted (by reducing the gain of the amplifier) to ''pre-Reelin'' levels and monitored for at least 10 min before Aβ application. The time of Aβ application is 0 min. (*Top*) Traces show representative whole-cell recordings 30 min before and after Reelin and Aβ treatment. (Scale bar, 20 pA, 100 ms.) (*Lower*) Statistical analysis comparing extent of NMDA excitatory postsynaptic current (EPSC) amplitude suppression 30 min after Aβ application in the absence and presence of Reelin. Aβ significantly suppresses NMDA currents (64% ± 7.70% of control, $n = 6$; $P < 0.01$, 2-way ANOVA). Reelin prevents this effect (101.07% ± 11.98%, $n = 6$; $P > 0.05$, 2-way ANOVA followed by post-test). Asterisks denote significance. (*B*) SFK activation is required for the prevention of $A\beta_{1-42}$-induced NMDA receptor suppression by Reelin. (*Upper*) Normalized average amplitudes of evoked NMDA peak currents at +30 mV in single-cell recordings. Solid gray triangles show responses from control slices treated with 5 nM Reelin. Open squares show responses from slices treated with PP2 (10 μM) and Reelin. (*Top*) Traces show representative whole-cell recordings 10 min before and 30 min after $A\beta_{1-42}$ application. (Scale bar, 50 pA, 100 ms.) (*Lower*) Statistical analysis of NMDA receptor-dependent EPSC amplitude suppression. No significant change (94.85% ± 18.84% of control) in NMDA currents was seen when 100 nM $A\beta_{1-42}$ was applied in the presence of 5 nM Reelin. When the SFK inhibitor PP2 (10 μM) was added to the perfusate, application of $A\beta_{1-42}$ to slices from the same animals caused a significant suppression of the NMDA currents (58.62% ± 1.05% of control, $n = 4$; $P < 0.05$, unpaired *t* test). (*C*) The function-blocking CR50 antibody prevents Reelin rescue of LTP suppression by Aβ. TBS LTP was induced as described in Fig. 1. In the presence of Reelin alone, LTP is enhanced (16). $A\beta_{25-35}$ oligomers diminish LTP, and this is almost completely prevented by Reelin. Preincubation of Reelin with a threefold molar access of the function blocking CR50 antibody (30) prevents this. CR50 alone has no effect. High resolution traces are shown in the SI.

23). Here, we have demonstrated that Reelin prevents Aβ oligomer-induced suppression of synaptic NMDA receptors. Activation of SFKs by Reelin, which requires the functional ApoE receptors Apoer2 and Vldlr (17–19), is necessary for neutralizing the Aβ-mediated suppression (Fig. 3*B*).

The function of the CNS is dependent upon the finely tuned integration of multiple signals that continuously regulate the activity of its synapses. Some of these mechanisms involve the amyloid precursor protein APP and its product Aβ, as well as ApoE receptors and their ligand Reelin (24). Hsieh et al. (7) and Kamenetz et al. (8) have shown that γ-secretase mediated processing of APP, resulting in the release of Aβ, causes synaptic suppression through AMPA receptor removal, and that in turn synaptic activity increases APP processing through a mechanism that requires NMDA receptors. $A\beta_{1-42}$ has been reported to cause the dephosphorylation of NMDA receptors by activating tyrosine phosphatases, thereby increasing NMDA receptor endocytosis (9). Application of Reelin enhances tyrosine phosphorylation of NR2A and NR2B subunits (Fig. 2*A* and ref. 20). We thus propose a model in which NMDA receptor activity is modulated in opposite directions by the 2 endogenous proteins, Aβ and Reelin (Fig. 5). In this model, Aβ activates tyrosine phosphatases causing NMDA receptor endocytosis and synaptic dysfunction (9), whereas Reelin activates SFKs (17–19), which serves to retain NMDA receptors at the neuronal surface but can also further increase their activity through a mechanism that involves tyrosine phosphorylation of NR2 subunits (25).

Reelin, ApoE receptors, and Aβ oligomers are all present at synapses (4, 20, 32). Nevertheless, co-localization of Reelin and amyloid at the same synapse is actually not necessary for explaining the powerful effect of Reelin on preventing synaptic dysfunction. For example, the data in Fig. 3*A* clearly show that extracellular Reelin potently increases synaptic (i.e., measured as evoked responses) NMDA receptor activity and that this prevents the suppression induced by subsequently added amyloid oligomers. Although it is likely that Reelin activates the ApoE receptor-dependent signaling pathway directly at the synapse (20), it is possible that Reelin is also activating NMDA receptors extra-synaptically and that part of the effect of Reelin on antagonizing amyloid-induced synaptic dysfunction is caused by altering the synaptic dwell time of NMDA receptors (33), and not exclusively by preventing NMDA receptor endocytosis.

Our findings may have direct implications for the molecular mechanisms that underlie the pathogenesis of AD. The seminal findings by the Roses group showed that ApoE genotype predisposes to late-onset AD (34–36). ApoE isoforms differentially impair LTP in the mouse, and ApoE4 knock-in mice are particularly sensitive to LTP suppression by low concentrations of $A\beta_{1-42}$ (37). These independent findings are consistent with our proposed model (Fig. 5) in which Reelin signaling through ApoE receptors counteracts the synaptic suppression induced by Aβ peptides. It is possible that these synaptic ApoE receptor functions could be differentially modulated by ApoE isoforms.

In summary, our findings show that ApoE receptor-dependent postsynaptic signals evoked by Reelin, a signaling

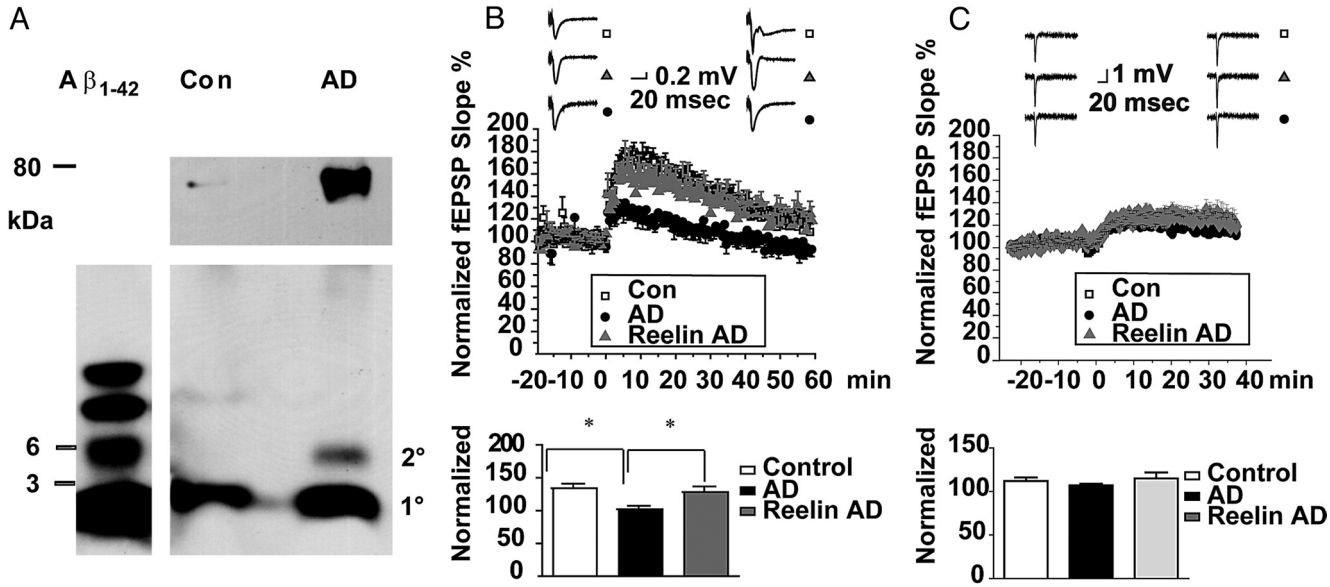

Fig. 4. Reelin prevents LTP suppression induced by cortical AD brain extract. (*A*) Human cortical brain extracts from a normal subject (*Con*, control) and a clinically and histopathologically confirmed AD case were prepared as described in *Materials and Methods* and by Shankar et al. (11). Aβ monomers, oligomers, and high molecular weight complexes (approximately 80 kDa) were immunoprecipitated and separated by LDS PAGE. Monomers and oligomers (dimers, trimers, and tetramers) derived from synthetic $A\beta_{1-42}$ are shown (*Left*). Control brain extract (*Middle*) contains monomeric Aβ, but no detectable oligomers and only a trace amount of higher-order aggregates (*Upper*). By contrast, AD brain extract contains comparable amounts of monomeric Aβ, but Aβ dimers and higher-order multimers were also present. (*B*) (*Top*) AD brain extract (50 μL per mL perfusate, closed circles) potently suppresses LTP, compared with equivalent extracts from control brain (50 μL per mL perfusate, open squares). Reelin almost completely prevents LTP suppression by AD brain extract (gray triangles). (*Lower*) Statistical analysis of average LTP responses between 30 and 35 min after TBS. Control brain extract (open bar), 133.99% ± 7.14%, $n = 4$; AD brain extract (closed bar), 101.86% ± 5.42%, $n = 8$; AD brain extract plus Reelin (gray bar), 128.3% ± 8.42%, $n = 8$. (ANOVA followed by Bonferroni post-test.) Control compared with AD extract, $P < 0.05$; AD extract compared with AD extract plus Reelin, $P < 0.05$. Control extract compared with AD extract plus Reelin, $P > 0.05$. Asterisk denotes significance. (*C*) (*Top*) AD brain extract failed to suppresses NMDA receptor-independent LTP in the presence of NMDA receptor antagonist D-AP5 (50 μM, closed circles) compared with control brain extract (open squares), and Reelin did not alter NMDA receptor-independent LTP in the presence of AD brain extract (gray triangles). (*Lower*) Statistical analysis of average LTP response. Control (open bar), 160.49% ± 14.19%, $n = 7$; AD extract (closed bar), 115.79% ± 6.53%, $n = 12$; AD extract plus Reelin (gray bar), 156.84% ± 12.33%, $n = 11$. (ANOVA followed by Bonferroni post-test.) Control versus AD extract, $P < 0.05$; AD extract versus AD extract plus Reelin, $P < 0.05$. Control extract versus AD extract plus Reelin, $P > 0.05$. Asterisk denotes significance. High resolution traces are shown in the SI.

protein that is secreted by interneurons, prevent the synaptic suppression that is induced by Aβ oligomers. Thus, Aβ and ApoE receptors form a signaling network that may serve to integrate and modulate synaptic activity. Disruption of this balance by overproduction of Aβ or by inhibition of ApoE receptors would be predicted to disturb synaptic plasticity (ref.

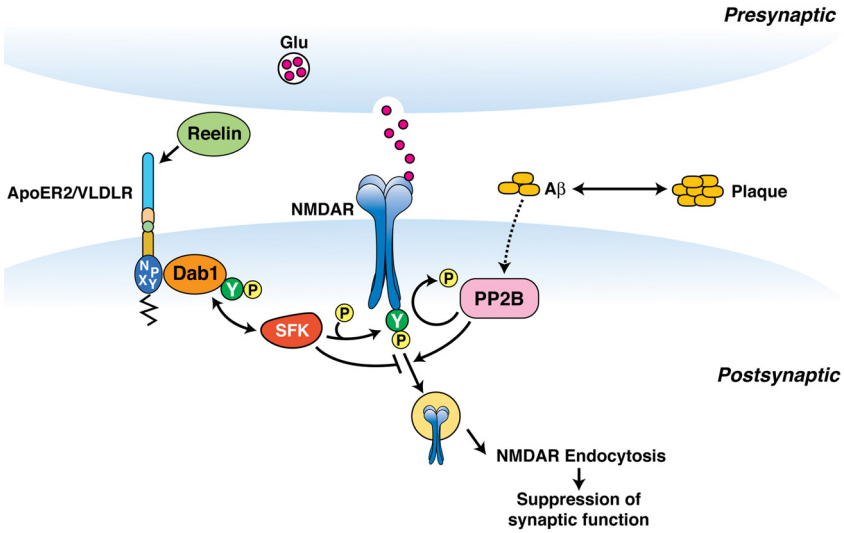

Fig. 5. Model of the regulation of synaptic functions by Reelin. Aβ activates the striatal-enriched protein tyrosine phosphatase, leading to dephosphorylation of tyrosine residues on NMDA receptors. Dephosphorylation of the NR2B subunit correlates with increased NMDA receptor endocytosis and suppression of its synaptic function (9). Reelin activates Src family tyrosine kinases (*SFK*) and enhances tyrosine phosphorylation of NR2A and NR2B subunits. Reelin signaling may prevent Aβ-induced NMDA receptor endocytosis and SFK activation by Reelin restores NMDA receptor activity. We propose that Reelin signaling through the ApoE receptors Apoer2 and Vldlr counteracts the suppressive effect of Aβ at the synapse and that this is important for the maintenance of normal synaptic function.

20 and the present study) and result in synapse and network dysfunction (6, 14, 15).

Materials and Methods

WT C57BL/6 husbandry and housing conditions are described in the *SI Text*. All procedures were performed in accordance with the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals of the University of Texas Southwestern Medical Center at Dallas. Amyloid-β_{1-42} was obtained from Biosource and amyloid-β_{25-35} was obtained from Tocris.

Preparation and Quality Control of Protein Reagents. Preparation of Aβ oligomers is described in the *SI Text*. Reelin was produced and purified and Dab1 tyrosine phosphorylation was determined as described (38).

NMDA Receptor Phosphorylation Assays. Hippocampal slices were processed for detection of NMDA receptor phosphorylation as described in the *SI Text*. NR2A and NR2B subunits were immunoprecipitated from the lysate and tyrosine phosphorylation was detected by Western blot analysis as described previously (23).

Whole-Cell Patch-Clamp Recording. Hippocampal slices were prepared from 9- to 14-d-old mice. Single-cell recordings were performed as described in detail in the *SI Text*. For all recordings, cells were rejected if the input resistance decreased to less than 100 MΩ or the access resistance changed by more than 20%.

Extracellular LTP Recordings. Hippocampal slices were prepared from 2- to 3-month-old mice. LTP was induced and recorded as described in detail in the *SI Text*. Data pooled across slices were expressed as mean $\pm$ SEM and effects of conditioning stimulation were measured after 35 to 40 min of induction of LTP. High resolution traces are shown in Fig. S1.

NMDA Receptor-Independent LTP. LTP was induced in the presence of the NMDA receptor antagonist D-AP5 (50 μM) with two 1-s trains, 200 Hz tetanic stimulation. Control and AD brain extracts were applied at 60 μL per mL perfusate 30 to 40 min before LTP induction and were present throughout the post-tetanic 40-min data collection period. High resolution traces are shown in Fig. S1.

Human Brain Extracts. Frozen human AD and non-AD cortical brain samples (2–4 g) were provided by the University of Texas Southwestern Alzheimer's Disease Center and processed as described in the *SI Text*. Immunoprecipitation of Aβ oligomers and Western blotting were performed as described previously (11). Control and AD brain extracts were compared at 5, 25, and 50 μL per mL perfusate and yielded results that were quantitatively and qualitatively consistent with those reported by Shankar et al. (11). All experiments were performed independently at least 4 times.

ACKNOWLEDGMENTS. We thank Hui-Chuan Reyna, Wen-Ling Niu, and Priscilla Rodriguez for technical assistance; Mike Brown, Joe Goldstein, and Tiina Kotti for critical and constructive comments; and Drs. Nakajima and Mikoshiba for sharing CR50 antibody. This work was supported by grants from the National Institutes of Health, the American Health Assistance Foundation, and the Wolfgang-Paul Program of the Humboldt Foundation.

1. Hardy J (2006) A hundred years of Alzheimer's disease research. *Neuron* 52:3–13.
2. De Strooper B (2007) Loss-of-function presenilin mutations in Alzheimer disease. Talking Point on the role of presenilin mutations in Alzheimer disease *EMBO Rep* 8:141–146.
3. Selkoe DJ (2000) The origins of Alzheimer disease: a is for amyloid. *JAMA* 283:1615–1617.
4. Lacor PN, et al. (2004) Synaptic targeting by Alzheimer's-related amyloid β oligomers. *J Neurosci* 24:10191–10200.
5. Lambert MP, et al. (1998) Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA* 95:6448–6453.
6. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* 298:789–791.
7. Hsieh H, et al. (2006) AMPAR removal underlies Aβ-induced synaptic depression and dendritic spine loss. *Neuron* 52:831–843.
8. Kamenetz F, et al. (2003) APP processing and synaptic function. *Neuron* 37:925–937.
9. Snyder EM, et al. (2005) Regulation of NMDA receptor trafficking by amyloid-β. *Nat Neurosci* 8:1051–1058.
10. Shankar GM, et al. (2007) Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J Neurosci* 27:2866–2875.
11. Shankar GM, et al. (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14:837–842.
12. Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006) Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: A potent role for trimers. *J Physiol* 572:477–492.
13. Walsh DM, et al. (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416:535–539.
14. Palop JJ, Chin J, Mucke L (2006) A network dysfunction perspective on neurodegenerative diseases. *Nature* 443:768–773.
15. Palop JJ, et al. (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* 55:697–711.
16. Weeber EJ, et al. (2002) Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J Biol Chem* 277:39944–39952.
17. Arnaud L, Ballif BA, Cooper JA (2003) Regulation of protein tyrosine kinase signaling by substrate degradation during brain development. *Mol Cell Biol* 23:9293–9302.
18. Ballif BA, Arnaud L, Cooper JA (2003) Tyrosine phosphorylation of Disabled-1 is essential for Reelin-stimulated activation of Akt and Src family kinases. *Brain Res Mol Brain Res* 117:152–159.
19. Bock HH, Herz J (2003) Reelin activates SRC family tyrosine kinases in neurons. *Curr Biol* 13:18–26.
20. Beffert U, et al. (2005) Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. *Neuron* 47:567–579.
21. Qiu S, Zhao LF, Korwek KM, Weeber EJ (2006) Differential reelin-induced enhancement of NMDA and AMPA receptor activity in the adult hippocampus. *J Neurosci* 26:12943–12955.
22. Trommsdorff M, et al. (1999) Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97:689–701.
23. Chen Y, et al. (2005) Reelin modulates NMDA receptor activity in cortical neurons. *J Neurosci* 25:8209–8216.
24. Herz J, Chen Y (2006) Reelin, lipoprotein receptors and synaptic plasticity. *Nat Rev Neurosci* 7:850–859.
25. Salter MW, Kalia LV (2004) Src kinases: A hub for NMDA receptor regulation *Nat Rev Neurosci* 5:317–328.
26. Strasser V, et al. (2004) Receptor clustering is involved in Reelin signaling *Mol Cell Biol* 24:1378–1386.
27. D'Arcangelo G, et al. (1999) Reelin is a ligand for lipoprotein receptors. *Neuron* 24:471–479.
28. Hiesberger T, et al. (1999) Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 24:481–489.
29. Grant SG, et al. (1992) Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* 258:1903–1910.
30. Nakajima K, Mikoshiba K, Miyata T, Kudo C, Ogawa M (1997) Disruption of hippocampal development in vivo by CR-50 mAb against reelin. *Proc Natl Acad Sci USA* 94:8196–8201.
31. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid β-peptide. *Nat Rev Mol Cell Biol* 8:101–112.
32. Groc L, et al. (2007) NMDA receptor surface trafficking and synaptic subunit composition are developmentally regulated by the extracellular matrix protein Reelin. *J Neurosci* 27:10165–10175.
33. Campo CG, Sinagra M, Verrier D, Manzoni OJ, Chavis P (2009) Reelin secreted by GABAergic neurons regulates glutamate receptor homeostasis. *PLoS One* 4:e5505.
34. Corder EH, et al. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261:921–923.
35. Schmechel DE, et al. (1993) Increased amyloid β-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci USA* 90:9649–9653.
36. Strittmatter WJ, et al. (1993) Apolipoprotein E: High-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA* 90:1977–1981.
37. Trommer BL, et al. (2005) ApoE isoform-specific effects on LTP: Blockade by oligomeric amyloid-β1–42. *Neurobiol Dis* 18:75–82.
38. Beffert U, et al. (2002) Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3β. *J Biol Chem* 277:49958–49964.

Supporting Information

Durakoglugil et al. 10.1073/pnas.0908176106

SI Text

WT 129xC57BL/6 hybrid male and female mice were bred in house and were maintained on a 12-h light/12-h dark cycle. Animals were fed a standard rodent chow diet (Diet 7001; Harlan Teklad) and water ad libitum. All procedures were performed in accordance with the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals of the University of Texas Southwestern Medical Center at Dallas. Amyloid-β_{1-42} was obtained from Biosource, dissolved in water, and diluted to 1 mg/mL in PBS solution, followed by incubation at 37 °C for 2 d before use. Amyloid-β_{25-35} was obtained from Tocris, dissolved in water as a 10 mM stock solution, and kept in aliquots at −20 °C until use. Upon thawing, aliquots were diluted to 100 μM and incubated at 37 °C for 1 h before dilution to the final working concentration (100 nM). Aβ_{25-35} preparations were qualitatively and quantitatively highly consistent, and most experiments were thus performed using this shorter peptide. Reelin was produced and purified as described (2).

NMDA Receptor Phosphorylation Assays. Hippocampal slices were incubated in oxygenated artificial cerebrospinal fluid in the presence or absence of Reelin (≈5 nM) for 10 min before and throughout Aβ treatment (1 μM, 1 h). Slices were then homogenized in 900 μL immunoprecipitation buffer (containing 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 1% Nonidet P-40) supplemented with protease and phosphatase inhibitor cocktails (Sigma). NR2A and NR2B subunits were immunoprecipitated from the lysate and tyrosine phosphorylation was detected by Western blot analysis as described previously (3).

Surface Biotinylation. Primary cortical neurons were prepared from embryonic d 16 embryos from timed pregnant mice as described previously (3). At 12 to 13 d in vitro, the neurons were incubated in the presence or absence of Reelin (≈5 nM) for 10 min before and throughout Aβ treatment (1 h). Neurons were washed by cold PBS solution and then incubated in PBS solution containing 1.5 mg/mL sulfo-NHS-LC-biotin (Pierce) for 30 min at 4 °C. Neurons were rinsed in cold PBS solution followed by cold PBS solution containing 1 mM glycine. Then, 300 μL lysis buffer (PBS solution with 0.1% SDS and 1% Triton X-100 and protease inhibitor mixture; Sigma) was added. After 30 min incubation at 4 °C, lysis buffer was collected and centrifuged at 14,000 rpm for 15 min. Eighty percent of the cell lysate was incubated with 50 μL NeutrAvidin agarose (Pierce) at 4 °C for 1 h. Agarose pellets were washed 3 times in washing buffer (500 mM NaCl, 15 mM Tris-HCl, 0.5% Triton X-100, pH 8). Proteins were eluted from agarose beads with SDS sample buffer.

Whole-Cell Patch-Clamp Recording. Hippocampal slices were prepared from 9- to 14-d-old mice. Animals were anesthetized with isoflurane and decapitated and the brain was quickly removed and placed in an ice-slush high sucrose solution (225 mM sucrose, 2.95 mM KCl, 1.25 mM NaH_2PO_4, 1.25 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 10 mM $MgSO_4$, 10 mM glucose, 1 mM ascorbic acid). Transverse 350-μm sections were cut using a Leica VT 1000 S vibratome (Leica). The slices were then transferred to the incubation solution (124 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM D-glucose, 2 mM $CaCl_2$, 1 mM $MgCl_2$) and kept at room temperature for at least 1 h before transfer to the recording chamber.

The recording chamber was kept at 30 °C with a laminar flow rate of 2 to 3 mL/min. NMDA-mediated EPSCs were pharmacologically isolated using 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione to block AMPA receptors and 50 μM picrotoxin to block GABA receptors in incubation solution containing reduced $MgCl_2$ content (0.3 mM $MgCl_2$ instead of 1 mM to partially relieve the $Mg2^+$ block on NMDA receptors). The CA3 region was removed from all slices to prevent the recurrent activity. For stimulation, Pt/Ir cluster electrodes were used (CE2B55; FHC) and placed into stratum radiatum close to the cell being recorded. Stimulus intensity was adjusted to get a one-third maximal response at approximately 100 pA, and pulses were given through an isolated pulse stimulator (model 2100; A-M Systems). The patch electrodes (3–5 MΩ) were filled with CsCl-based intracellular solution [120 mM CsCl, 10 mM Hepes, 1 mM $CaCl_2$, 5 mM tetraethylammonium chloride, 1 mM EGTA, 4 mM ATP, 0.3 mM GTP, 10 mM creatine phosphate, 5 μM lidocaine N-ethyl bromide (QX 314), pH 7.3, 300 mOsmol]. Access resistance was less than 15 MΩ and was left uncompensated.

Signals were recorded using a Multiclamp 700B amplifier (Axon Instruments) and digitized using Digidata 1322A (Axon Instruments) at 10 kHz, filtered and recorded using Clampex 9.2 software (Axon Instruments) at 5 kHz, and analyzed offline with Clampfit. Histograms were calculated as the average of normalized data 30 to 35 min after either Aβ or Reelin application.

To rule out any plasticity changes induced by repeated evoked responses, patch-clamp experiments with Aβ_{1-42} peptide (Fig. 3*C* in the main text) were performed in normal Mg-containing solution (1 mM) and cells were held at -60 mV between the +30 mV (2 sec) recordings. Cells were stimulated with different time intervals (20, 30, and 60 sec), no recording was taken during the first 10 min of Aβ application, and responses were averaged every 5 min for plotting.

For all recordings, cells were rejected if the input resistance decreased to less than 100 MΩ or the access resistance changed by more than 20%.

Extracellular LTP Recordings. Hippocampal slices were prepared from 2- to 3-month-old mice. The brain was quickly removed and placed in an ice-slush/high sucrose Cutting Solution containing 225 mM sucrose, 2.95 mM KCl, 1.25 mM NaH_2PO_4, 1.25 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 10 mM $MgSO_4$, and 10 mM glucose. Transverse 400-μm sections were cut using a vibratome. Slices were then transferred to the incubation chamber containing artificial cerebrospinal fluid (124 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM D-glucose, 2 mM $CaCl_2$, 1 mM $MgCl_2$) and kept at 30 °C for at least 1 h before switching to the recording chamber.

Slices were then transferred into a submerged recording chamber and kept at 30 °C at a flow rate of 2 to 3 mL/min. For stimulation concentric bipolar electrodes were used (CBBRC75; FHC). Stimulus intensity was set at 30% to 50% of maximum response (≈1 mV) and delivered thorough an isolated pulse stimulator (model 2100; A-M Systems). Control and experimental levels of LTP were measured on slices prepared from the same hippocampus or litter-mates. A theta burst (TBS; train of 4 pulses at 100 Hz repeated 10 times with 200-ms intervals; repeated 5 times at 10-s intervals) was used as a conditioning stimulus. Purified Reelin (≈5 nM) was added to the recording chamber between 10 and 30 min before TBS, which was applied after a stable baseline had been achieved. Data were recorded

using a Multiclamp 700B amplifier (Axon Instruments) and digitized using Digidata 1322A, (Axon Instruments) at 10 KHz and filtered at 6 KHz and recorded using Clampfit 10.0 Software (Axon Instruments) with a custom-written algorithm and analyzed offline with Clampfit. Data pooled across slices were expressed as the mean ± SEM, and effects of conditioning stimulation were measured after 35 to 40 min of induction of LTP.

NMDA Receptor-Independent LTP. LTP was induced in the presence of NMDA receptor antagonist D-AP5 (50 μM) with two 1-s trains, 200 Hz tetanic stimulation. Control and AD brain extracts were applied at 60 μL per mL perfusate 30 to 40 min before LTP induction and were present throughout the post-tetanic 40-min data collection period.

Human Brain Extracts. Frozen human AD and non-AD cortical brain samples (2–4 g) were provided by the University of Texas Southwestern Alzheimer's Disease Center and homogenized in 4 mL/g of tissue Tris-buffered saline solution (20 mM Tris-HCl, 150 mM NaCl, pH 7.4). Immunoprecipitation of Aβ oligomers and Western blotting were performed as described previously (1). Briefly, Aβ peptides and complexes were immunoprecipitated from 500 μL cleared homogenate with 4 μg monoclonal antibody 82E1 (Immuno-Biological Laboratories) and separated on non-reducing 4% to 12% Bis-Tris gels in LDS sample buffer. Proteins and peptides were transferred to 0.2-μm pore size nitrocellulose membranes (Invitrogen) and immunoblotted with 5 μg/mL Aβ monoclonal antibody 4G8 (Covance). Control and AD patient brain extracts were compared at 5, 25, and 50 μL per mL perfusate and yielded results that were quantitatively and qualitatively consistent with those reported by Shankar et al. (1). All experiments were performed independently at least 4 times.

Extracellular Recordings. In Figs. S1–S4, black traces are control traces and red traces show traces after TBS; different treatments are stated above the traces. Fig. S1 *A* and *B* were recorded in a submerged recording chamber (160 pulses for TBS) whereas Figs. S2*B*, S3*C*, and S4*C* were recorded in an interface chamber (200 pulses for TBS). Experiments shown in Fig. S4*B* were recorded using both an interface and a submerged recording chamber with equivalent results. Fig. S4*C* was recorded in the presence of the NMDA receptor antagonist D-AP5 (50 μM). NMDA receptor-independent LTP was induced using a 200-Hz stimulation protocol. Stimulus artifacts were manually deleted from all of the traces.

Patch-Clamp Recordings (Fig. S3 *A* and *B*). Black traces show control responses, green traces show traces after either Aβ_{25-35} or Aβ_{1-42} treatments, and the blue trace shows Reelin treatment after stimulus adjustment before Aβ_{25-35} treatment.

1. Shankar GM, et al. (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14:837–842.
2. Beffert U, et al. (2002) Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3β. *J Biol Chem* 277:49958–49964.
3. Chen Y, et al. (2005) Reelin modulates NMDA receptor activity in cortical neurons. *J Neurosci* 25:8209–8216.

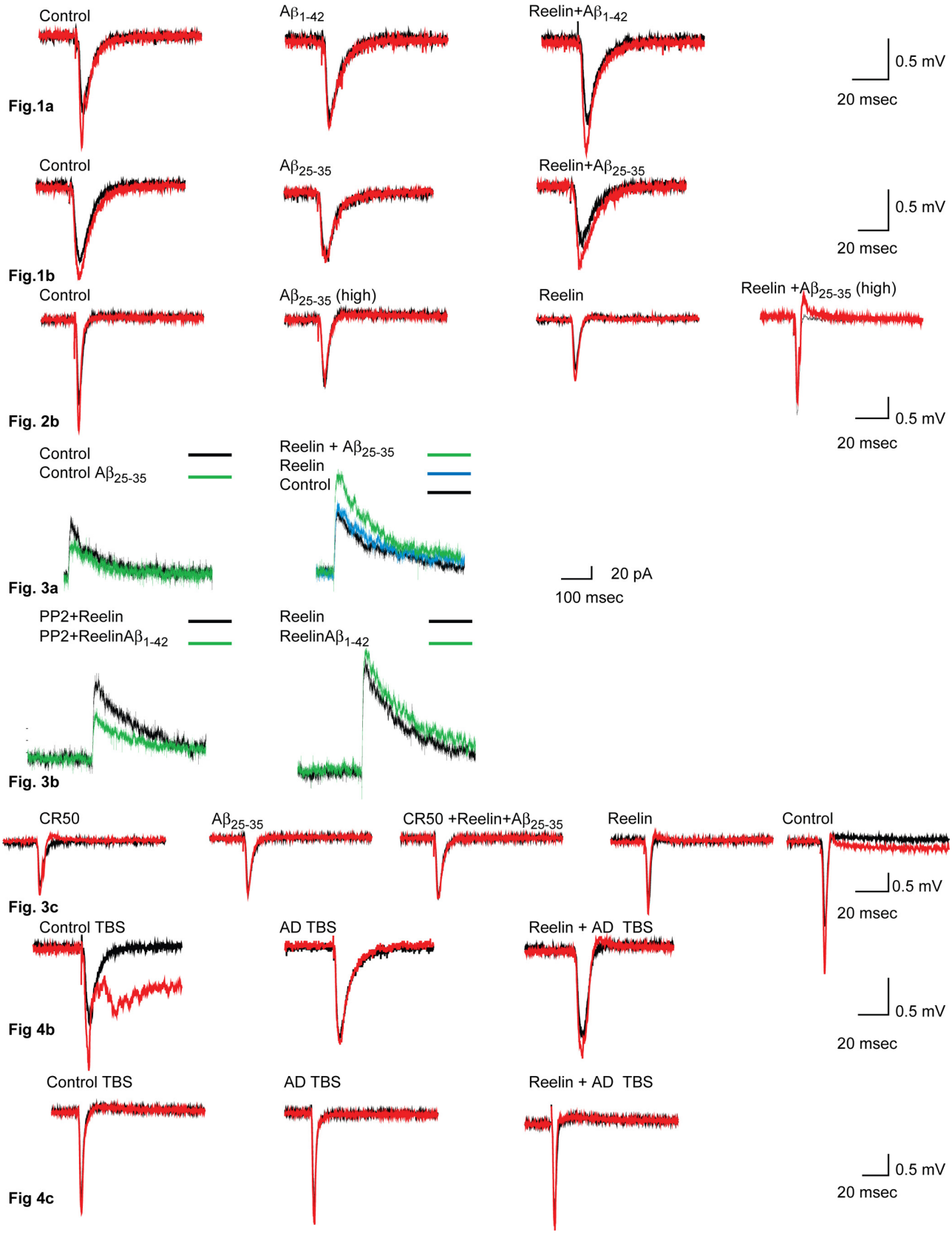

Fig. S1. Higher resolution and magnification of EPSP traces.