Alzheimer amyloid-β oligomer bound to postsynaptic prion protein activates Fyn to impair neurons

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Amyloid-beta (Aβ) oligomers are thought to trigger Alzheimer’s disease pathophysiology. Cellular prion protein (PrPc) selectively binds oligomeric Aβ and can mediate Alzheimer’s disease–related phenotypes. We examined the specificity, distribution and signaling of Aβ-PrPc complexes, seeking to understand how they might alter the function of NMDA receptors (NMDARs) in neurons. PrPc is enriched in postsynaptic densities, and Aβ-PrPc interaction leads to Fyn kinase activation. Soluble Aβ assemblies derived from the brains of individuals with Alzheimer’s disease interacted with PrPc to activate Fyn. Aβ engagement of PrPc-Fyn signaling yielded phosphorylation of the NR2B subunit of NMDARs, which was coupled to an initial increase and then a loss of surface NMDARs. Aβ-induced dendritic spine loss and lactate dehydrogenase release required both PrPc and Fyn, and human familial Alzheimer’s disease transgene–induced convulsive seizures did not occur in mice lacking PrPc. These results delineate an Aβ oligomer signal transduction pathway that requires PrPc and Fyn to alter synaptic function, with deleterious consequences in Alzheimer’s disease.

RESULTS

Prion protein is enriched in postsynaptic densities

Aβo binding sites show postsynaptic dendritic localization5,22,33. To the extent that PrPc is relevant to Aβo-driven pathology, it may be concentrated in similar regions. Our initial studies documented colocalization between Aβo binding and PrPc, but did not characterize sites of PrPc enrichment5.

The PSD is distinct ultrastructurally and biochemically. Proteomic analyses using mass spectrometry have shown that PrPc is a component of the PSD34. To examine PrPc enrichment in the PSD, we performed immunoblots on various subcellular fractions (Fig. 1a,b). PrPc was enriched in synaptosomal fractions. Isolation of the PSD from synaptosomes segregated the PSD-95 marker protein fully to the PSD and synaptophysin to the detergent-extractable fraction. Substantial amounts of PrPc co-fractionated with PSD-95, yielding fivefold PrPc enrichment in

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Aβo binding to PrP<sup>C</sup> generates Fyn kinase activation

The nonreceptor tyrosine kinase Fyn is a possible mediator of signal transduction from the Aβo-PrP<sup>C</sup> complex. It is possible to monitor the activation of Src family kinases (SKFs) by measuring the expression of phosphospecific epitopes. We examined cultures for SKF activation after 15-min exposure to Aβ species (Fig. 2 and Supplementary Figs. 1–4). Wild-type cortical neurons at 21 d in vitro (DIV) showed increased levels of pY416-Fyn (SKF) in response to Aβo (Fig. 2a,c). The phospho-specific antibody used in these immunoblots detects pY416 in several SKFs, but kinase-specific immunoprecipitation revealed that PrP<sup>C</sup>-dependent activation was Fyn specific (Supplementary Fig. 1a). Fyn activation was oligomer specific; no activation was detected after exposure to monomeric Aβo (Fig. 2c and Supplementary Fig. 1b) or fibrillary Aβo (Supplementary Fig. 1c,d).

We asked whether PrP<sup>C</sup> is required for the Aβo-induced Fyn activation. Previously, we found that Aβo binding to PrP<sup>C</sup> is blocked by antibody to PrP<sup>C</sup> (6D11)⁵. This antibody also prevented Fyn activation by Aβo (Fig. 2a,c). Notably, in Prp<sup>−/−</sup> cultures, activation of Fyn by Aβo was eliminated (Fig. 2b,c). Although Aβo failed to activate Fyn in Prp<sup>−/−</sup> neurons, Reelin-induced Fyn activation was preserved (Fig. 2d,e). Thus, although PrP<sup>C</sup> accounts for ~50% of Aβo binding sites⁵⁻³⁵, it accounts fully for Fyn signaling.

We also verified that Aβo-induced PrP<sup>C</sup>-dependent Fyn activation in cell lines. Stably transfected CV-1 cells expressing PrP<sup>C</sup> (SCA-7), Fyn or both were generated (Supplementary Fig. 2a). PrP<sup>C</sup> protein per mg of total protein in parental CV-1 cells was less than 5% of the concentration in brain, and the level in PrP<sup>C</sup> expressing CV-1 cells was 50–100% of brain (data not shown). Aβo increased the amount of pY416-SFK twofold exclusively in cells expressing both PrP<sup>C</sup> and Fyn (Supplementary Fig. 2a,b). In N2A neuroblastoma, similar PrP<sup>C</sup>-dependent, Aβo-induced Fyn activation was observed in detergent-insoluble subcellular fractions (Supplementary Fig. 2c,d), although activation was not detected in HEK293T cells. We conclude that Aβo-PrP<sup>C</sup> complexes activate Fyn in many cell types.

Figure 1 Localization of prion protein to the postsynaptic density. (a) The indicated subcellular fractions (20 µg protein) were analyzed by immunoblot with antibodies to PrP<sup>C</sup>, Fyn, PSD-95, synaptophysin and actin. The lower two panels are from a separate preparation. S1, P1 and P2 are sequential brain fractions from differential centrifugation. (b) Quantification of PSD-95, PrP<sup>C</sup> or Fyn levels normalized to actin. Data are mean ± s.e.m. for three independent fractionations from separate animals. (c) Immunohistochemistry of 21 DIV hippocampal neurons stained with antibodies to PSD-95 (red) and PrP<sup>C</sup> (6D11, green). Colocalization is indicated by arrows. Scale bars represent 4 µm.

Aβo may activate PrP<sup>C</sup>-Fyn signaling by altering PrP<sup>C</sup> clustering, inducing PrP<sup>C</sup> conformations or by differential association with other proteins. To examine PrP<sup>C</sup> clustering, we treated primary neurons with antibodies to PrP<sup>C</sup> (6D11 or SAF32), as described for other cells²⁴,²⁵,³⁵. Neither bivalent nor clustered antibodies to PrP<sup>C</sup> activated Fyn in primary neurons (Fig. 2b,i). Thus, PrP<sup>C</sup> clustering alone is not sufficient for Aβo activation of Fyn in neuronal lipid rafts.

Human Alzheimer’s brain contains PrP<sup>C</sup>-binding Aβ species

Aβo has been obtained from multiple sources, including synthetic peptide, transgenic mouse brain, transfected cell lines and the brains of individuals with Alzheimer’s disease¹⁻⁴. To advance pathophysiological understanding, it is of greatest importance to examine the action of Aβo derived from the brains of individuals with Alzheimer’s disease. Notably, Aβo derived from the brains of individuals with Alzheimer’s disease requires PrP<sup>C</sup> to suppress LTP¹⁰,¹⁶. Thus, we assessed PrP<sup>C</sup>-interacting Aβo from human Alzheimer’s disease tissue for Fyn activation (Fig. 3).

We developed a sensitive assay for the detection of Aβ species that interact with PrP<sup>C</sup> (Fig. 3a). The 23–111 Aβ-interacting domain of PrP<sup>C</sup> was purified from recombinant E. coli and immobilized in microtitre plates. Specimens containing Aβo were added, and bound Aβ was detected using antibody to Aβ. Detection of PrP<sup>C</sup>-bound Aβo was robust with N terminus–directed Aβ antibodies (polyclonal 2454 and monoclonal 82E1; Supplementary Fig. 5a) or with an antibody to Aβ that detects only oligomeric species (NU-4, data not shown),
Figure 2 Aβ oligomers activate Fyn kinase. (a) Mouse embryonic day 17 (E17) wild-type (WT) cortical neurons at 21 DIV were treated with 0–3 μM Aβo (monomer equivalent concentration, estimated Aβo 0–30 nM) for 20 min after a 1-h pre-incubation with (right) or without (left) 6D11. Whole-cell lysates were analyzed by immunoblot with antibodies to phospho-SFK (Tyr 416) and Fyn. Actin served as a loading control. (b) Cortical neurons from E17 Pmp22 mice were treated with 0–3 μM Aβo for 15 min after 21 DIV. Lysates were analyzed by immunoblot using antibodies to phospho-SFK (Tyr 416) and Fyn. GAPDH served as a loading control. (c) Quantification of phospho-SFK level in the lysate, normalized to Fyn immunoreactivity (wild type, n = 4; Pmp22, n = 4; mean ± s.e.m.). ***P < 0.001, one-way ANOVA (F = 8.83, degrees of freedom = 1) with Tukey post hoc pairwise comparisons. Data were normalized to 0 μM; the phospho-SFK levels in arbitrary units of fluorescence intensity without normalization at 0 μM for Aβo, Aβo + 6D11, Aβo (6H monomer) and Aβo + Pmp22 were 0.82 ± 0.22, 0.86 ± 0.10, 0.99 ± 0.21 and 0.94 ± 0.02, respectively. (d) Cortical neurons from wild-type or Pmp22 mice after 21 DIV were treated with 5 nM reelin for 25 min. Lysates were analyzed by immunoblot with antibodies to phospho-SFK (Tyr416), Fyn and PrPC. (e) Quantification of phospho-SFK level in the lysates (from d) normalized to Fyn immunoreactivity from three biologically independent experiments. Data are presented as mean ± s.e.m. *P < 0.05, ***P < 0.001, Student’s two-tailed t-test. (f) Cortical neurons from wild-type after 21 DIV were treated with 0–1 μM Aβo. Prior to Aβo exposure, the indicated cultures were pre-treated with 5 mg ml−1 MBCD for 1 h or 0.1 unit of PI-PLC for 10 min. Whole-cell lysates were analyzed by immunoblot with antibodies to phospho-SFK (Tyr416), Fyn and PrPC. (f) Quantification of phospho-SFK level in the lysates (from f) normalized to Fyn immunoreactivity from three independent experiments. Data are presented as mean ± s.e.m., n.s., not significant, P = 0.89 and 0.94, *P < 0.05. (h) Cortical neurons from wild-type mice were treated after 21 DIV with 1 μM Aβo, 10 μg ml−1 antibody to PrPC (6D11 or SAF32) or control IgG (control), followed by adding 5 μg ml−1 mouse antibody to IgG for cross-linking (α-IgG). Whole-cell lysates were analyzed by immunoblot with antibodies to phospho-SFK (Tyr416), Fyn and PrPC. Actin served as a loading control. (i) Quantification of phospho-SFK level normalized to Fyn immunoreactivity from three independent experiments, as in h. Data are presented as mean ± s.e.m. **P < 0.001, *P < 0.05, one-way ANOVA (F = 5.38; degrees of freedom = 7), Tukey post hoc comparisons.

Having detected PrPC-interacting Aβ in the brains of individuals with Alzheimer’s disease, we sought to determine whether these assemblies activate neuronal Fyn (Fig. 3c–e). Extracts from Alzheimer’s disease brains (6 μg protein per ml) stimulated Fyn activation in mouse cortical cultures, but extracts from control brains did not (P < 0.05; Fig. 3c,d). To assess whether this activation was a result of PrPC-interacting species, we pre-absorbed human TBS brain extracts with PrPC-Fc affinity resin. PrPC-Fc resin, but not Fc control resin, prevented Fyn activation (Fig. 3c,e). The Aβ dependence of Fyn activation was assessed with antibody to Aβ (82E1) and abrogation was observed (Fig. 3c,e). As for synthetic Aβo stimulation of Fyn, the signaling induced by Alzheimer’s disease TBS extracts was absent in Pmp22−/− cells and in cells pretreated with the 6D11 antibody to PrPC (Fig. 3c,e). Moreover, the level of PrPC-interacting Aβ species in human brain extracts correlated with the level of Fyn activation (Fig. 3f). Thus, TBS-soluble Aβ derived from the brains of individuals with Alzheimer’s disease stimulates neuronal Fyn via PrPC.

NMDAR subunits phosphorylated by Aβ–PrPC–Fyn signaling

NMDARs are important for synaptic plasticity and Alzheimer’s disease. Intracellular segments of NR2A and NR2B subunits contain tyrosine residues phosphorylated by SFKs. Thus, Aβo–PrPC–mediated Fyn activation may be directed towards NMDAR. We examined total NR2B and pY1472 NR2B levels in neuroblastoma cells expressing Fyn, PrPC or NR2B after exposure to Aβo (Supplementary Fig. 6a). pY1472 NR2B expression was selectively increased by Aβo treatment.

but not with C terminus–directed antibodies, including AB5306 (Supplementary Fig. 5a). This may be a result of the inaccessibility of the Aβ C terminus in oligomeric assemblies under native conditions. Using purified Aβo, the assay has a linear range from 20–14,000 pg (Fig. 3c). The specificity for oligomeric Aβ over monomeric Aβ or fibrillar Aβ exceeded 30-fold (Fig. 3a).

We assessed PrPC-interacting Aβ species in Tris-buffered saline (TBS) homogenates of brain cleared by ultracentrifugation. PrPC-interacting Aβ was detected in transgenic APP/PSen mouse brain, but not in wild-type brain (using synthetic Aβo as a standard: transgenic, 394 ± 72 ng of Aβ per g of brain; wild type, <15, n = 7 brains; mean ± s.e.m., P < 0.001, ANOVA, F = 28.48, degrees of freedom = 1). We measured PrPC-interacting Aβo in TBS-soluble cortical extracts from a cohort of autopsy–confirmed cases of Alzheimer’s disease versus controls. The average level in control brains was below the detection limit, whereas samples from cases of Alzheimer’s disease contained 20.8 ± 2.6 ng of Aβo per g of brain (mean ± s.e.m., P < 0.001; Fig. 3b). This value suggests that a substantial proportion of Aβ in TBS-soluble extracts from Alzheimer’s disease brains interacts with PrPC. ELISA measurement revealed that ~50% of Aβ42 immunoreactivity in TBS-soluble Alzheimer’s disease extracts absorbed to PrP-coated wells (Supplementary Fig. 5b). Urea elution recovered ELISA-detectable Aβ42 selectively from PrP(23–111) wells exposed to Alzheimer’s disease extracts (Supplementary Fig. 5c). Urea allowed >100% recovery of initial Aβ42 ELISA signal (Supplementary Fig. 5c), likely as a result of denaturation of secondary structure in Aβ or associated proteins, which would otherwise limit detection.
in Fyn-overexpressing cells. This increase was blocked by the 6D11 antibody to PrPC. Next, we generated endogenous proteins in cortical cultures exposed to Aβo (Fig. 4a,b). Over 20 min, Aβo induced a dose-dependent fivefold increase in pY1472 NR2B expression. This effect was specific for the oligomeric Aβo, as fresh Aβ had no effect (data not shown). Aβo-induced NR2B phosphorylation was eliminated in Prnp−/− (Fig. 4a,b) and Fyn−/− cultures (Supplementary Fig. 7b) and by the 6D11 antibody to PrPC (Fig. 4a,b). Thus, Aβo-induced phosphorylation of Y1472 in NR2B is a PrPC-Fyn–mediated signaling event.

Moreover, the roles of PrPC and Fyn are gene dose–dependent, with Aβo–induced NMDAR phosphorylation being reduced in Prnp or Fyn heterozygous neurons (Supplementary Fig. 7a,c).

During the first 15 min of Aβo treatment, NR2B phosphorylation was enhanced, but after 1–3 h, phosphorylation was suppressed (Fig. 4c,d). Aβo is known to increase the level of STEP tyrosine phosphatase23,37. Given that STEP and Fyn counteract one another, we compared the time course of these events relative to NR2B phosphorylation (Fig. 4c,d). At the onset of Aβo action, Fyn was activated and pY1472 NR2B expression was increased, with no change in STEP protein level. Later on, STEP increased while Fyn returned to baseline, with a net decrease in NR2B phosphorylation. Thus, a biphasic effect of Aβo on NR2B is triggered by PrPC engagement.
Surface NMDAR and calcium signaling induced by Aβ-PrPC-Fyn

Phosphorylation of NR2B at Y1472 is known to reduce AP-2–mediated endocytosis. Using cell surface biotinylation, we examined the extent to which NR2B is accessible at the cell surface versus being sequestered by NMDARs and by other channels. Pretreatment with Aβ increased fluorescence signal microscopically (Supplementary Movie 1) and in microtiter wells (Fig. 5g). Pretreatment with Aβ for 15 min generated significantly increased (P < 0.05) NMDA-induced, PrPC-fibril-induced glutamate responses in cortical neurons. NMDA produced elevations of intracellular calcium that were mediated by NMDARs and by other channels. Pretreatment with Aβ receptors were dephosphorylated and internalized, NMDA-induced calcium signals were suppressed (Fig. 5g). In cortical neurons lacking PrPC, Aβ did not alter NMDA responsiveness (Fig. 5g). The Aβ effect was oligomer specific, as neither Aβ monomers nor Aβ fibrils altered NMDA responses (Supplementary Fig. 8d). Bath application of glutamate produced elevations of intracellular calcium that were mediated by NMDARs and other channels. Pretreatment with Aβ for 1 h suppressed glutamate responses in wild-type, but not in Prnp−/− or Fyn−/−, neurons (Supplementary Fig. 8a-c). Thus, Aβ-induced, PrPC- mediated alterations in NMDAR create transient increases and then decreases in neuronal calcium.

We considered whether the transient increase in surface NR2B might lead to a brief period of excitotoxicity. We first examined N2A cells

Changing surface levels of NMDAR are expected to mediate alterations in NMDA-induced calcium levels. We used a calcium-sensitive fluorescent dye to monitor intracellular calcium in cortical neurons. NMDA produced increased fluorescence signal microscopically (Supplementary Movie 1) and in microtiter wells (Fig. 5g). Pretreatment with Aβ for 15 min generated significantly increased (P < 0.05) NMDA-induced, PrPC-fibril-induced glutamate responses in cortical neurons. NMDA produced elevations of intracellular calcium that were mediated by NMDARs and by other channels. Pretreatment with Aβ receptors were dephosphorylated and internalized, NMDA-induced calcium signals were suppressed (Fig. 5g). In cortical neurons lacking PrPC, Aβ did not alter NMDA responsiveness (Fig. 5g). The Aβ effect was oligomer specific, as neither Aβ monomers nor Aβ fibrils altered NMDA responses (Supplementary Fig. 8d). Bath application of glutamate produced elevations of intracellular calcium that were mediated by NMDARs and other channels. Pretreatment with Aβ for 1 h suppressed glutamate responses in wild-type, but not in Prnp−/− or Fyn−/−, neurons (Supplementary Fig. 8a-c). Thus, Aβ-induced, PrPC- mediated alterations in NMDAR create transient increases and then decreases in neuronal calcium.

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with and without overexpression of Fyn and PrP<sup>C</sup>. Combined expression of Fyn and PrP<sup>C</sup> substantially increased the release of cellular lactate dehydrogenase (LDH) induced by a 90-min exposure to Aβo (Supplementary Fig. 6b). To examine the role of endogenous PrPC and Fyn in Aβo neuronal toxicity, we used primary cortical cultures. Brief exposure to Aβo reduced cell viability, and 10% of cellular LDH was released into the medium (Fig. 5h and Supplementary Fig. 8e). A decrease in 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) chemical reduction was not detectable, as it was in the range of variability of the assay (Supplementary Fig. 9a). When Aβo exposure was extended to 72 h, no further LDH release was observed (Supplementary Fig. 9b), indicating that this cell toxicity occurs largely during the initial 90 min of Aβo exposure, when surface NR2B is increased.

To determine whether the correlation of acute Aβo cell toxicity with surface expression of NR2B and Aβ-Fyn signaling is functional, we examined the effects of antibody and genetic blockade. Pretreatment with the 6D11 antibody to PrP<sup>C</sup> prevented the Aβo-induced LDH release (Fig. 5h). A requirement for PrP<sup>C</sup> in Aβo-induced neuronal cell death matches recent reports<sup>15,20</sup>. Genetic deletion of either Fyn or PrP<sup>C</sup> rescued neurons from Aβo (Fig. 5h). Heterozygosity for null alleles of Prnp or Fyn reduced LDH release (Supplementary Fig. 7e). For transheterozygous Prnp<sup>-/-</sup> and Fyn<sup>-/-</sup> neurons, there was no detectable Aβo-induced LDH release during the first 90 min (Supplementary Fig. 7e). The toxicity depended primarily on NMDAR, as LDH release was reduced by d(-)-2-amino-5-phosphonovaleric acid (AP5), and on NR2B-containing receptors, as ifenprodil suppressed LDH release (Fig. 5i). Consistent with excitotoxic, non-apoptotic cell toxicity, there was no increase the amount of in activated caspase 3 (Supplementary Fig. 9c,d). Thus, Aβo requires PrP<sup>C</sup> to induce Fyn activation and subsequent NR2B phosphorylation. This phosphorylation is associated with transient increase in NR2B at the cell surface, with consequent excitotoxicity.

**Aβo destabilization of dendritic spines requires PrP<sup>C</sup>-Fyn**

A hallmark of Alzheimer’s disease is synaptic loss<sup>40</sup>. *In vitro* studies have described dendritic spine loss after acute Aβo exposure<sup>3,21,22</sup>. We sought to determine whether PrP<sup>C</sup> and Fyn are involved with this loss.

**Figure 7** Seizures in transgenic Alzheimer’s disease mice require PrP<sup>C</sup>. (a) Chronic video-EEG recordings were obtained from freely moving wild-type, Prnp<sup>-/-</sup>, APP-PSen and APP-PSen Prnp<sup>-/-</sup> mice. Each mouse was monitored continuously for 72 h. A spontaneous seizure recorded from a transgenic Alzheimer mouse (APP-PSen) is illustrated. This seizure is typical in its initiation by a spike-wave discharge and by the postictal attenuation of cerebral rhythms. L and R indicate left and right hemisphere signals, respectively. (b) Kaplan-Meyer curve showing the latency to first seizure during 72 h of continuous EEG. Of the APP-PSen mice with normal PrP<sup>C</sup> expression, 40% had at least one spontaneous generalized seizure during the recording session. The lack of PrP<sup>C</sup> completely rescued this phenotype in transgenic APP-PSen mice (P = 0.017, log rank test). No seizures were recorded in wild-type and Prnp<sup>-/-</sup> mice (wild type, n = 11; Prnp<sup>-/-</sup>, n = 11; APP-PSen, n = 10; APP-PSen Prnp<sup>-/-</sup>, n = 12). (c) Kaplan-Meyer survival curve of the mouse cohort undergoing EEG. More than 50% of transgenic mice died by 12 months age, and this phenotype was fully reversed by a lack of PrP<sup>C</sup> expression (P < 0.001, log rank test; wild type, n = 46; Prnp<sup>-/-</sup>, n = 23; APP-PSen, n = 36; APP-PSen Prnp<sup>-/-</sup>, n = 31).

**Figure 6** PrP<sup>C</sup> and Fyn are required for Aβ-induced dendritic spine loss. (a) Hippocampal neurons of the indicated genotypes were transfected with a Myr-EGFP expression vector and then cultured for 21 d before live imaging. After 1 h, 500 nM Aβo (monomer equivalent, 5 nM estimated oligomer) or vehicle was added and observations were continued for 5 h. Lost dendritic spines after Aβo addition to the wild-type neurons are indicated with arrowheads. Scale bar represents 1 μm. A typical three-dimensional image of such spines is provided in Supplementary Movie 2. (b) Dendritic spines were observed at 15 min intervals as described in a. The percentage of spines lost at the indicated times after 500 nM Aβo addition is indicated for a wild-type culture. Data are presented as mean ± s.e.m. from n = 3 separate cultures. (c) Dendritic spine loss over 5 h is plotted as a function of Aβo addition and genotype. The indicated samples were incubated with 50 μM AP5 during the Aβo exposure. The data are presented as mean ± s.e.m. (wild type, n = 12; Prnp<sup>-/-</sup>, n = 3; Fyn<sup>-/-</sup>, n = 3; AP5, n = 3 independent cultures from separate embryos for each genotype or drug). n.s., not significant (P = 0.44, 0.22 and 0.50) ***P < 0.001, **P < 0.01, *P < 0.05, one-way ANOVA (F = 8.94, degrees of freedom = 7), Tukey post hoc comparisons.

**Figure 5a** Aβo-induced dendritic spine loss.

**Supplementary Figure 8:** (a) Hippocampal neurons of the indicated genotypes were transfected with a Myr-EGFP expression vector and then cultured for 21 d before live imaging. After 1 h, 500 nM Aβo (monomer equivalent, 5 nM estimated oligomer) or vehicle was added and observations were continued for 5 h. Lost dendritic spines after Aβo addition to the wild-type neurons are indicated with arrowheads. Scale bar represents 1 μm. A typical three-dimensional image of such spines is provided in Supplementary Movie 2.
To provide a robust measure for fractional loss, we repeatedly imaged the same dendritic segments expressing membrane-tethered enhanced GFP (EGFP) over a 6-h period (Fig. 6a and Supplementary Movie 2). Under control conditions, dendritic spines were stable, with less than 2% gains or losses. Aβo treatment increased spine loss, without substantial alteration of spine gain. Over 5 h, 10–15% of spines were lost following Aβo treatment, whereas adjacent spines were morphologically stable (Fig. 6). The time course was gradual after an initial lag (Fig. 6b). Although loss was increased fivefold following Aβo treatment, the loss remained minor compared with the variability of spine density, emphasizing the need for time-lapse imaging.

To assess the roles of PrPC and Fyn in Aβo-induced spine loss, we cultured neurons from embryos homozygous for null alleles (Fig. 6a,c). Spine destabilization by Aβo was eliminated in Prnp−/− and Fyn−/− neurons. Thus, Aβo-induced spine loss requires both PrPC and Fyn. We also tested whether Aβo-induced spine loss requires NMDAR. As seen in previous studies with Aβ species derived from individuals with Alzheimer’s disease, the NMDA antagonist AP5 blocked dendritic spine loss by Aβo (Fig. 6c).

**Pmp loss prevented seizures in Alzheimer’s disease mice**

Epileptiform discharges have been observed in transgenic mouse models of Alzheimer’s disease and seizures are more common in Alzheimer’s disease41. We hypothesized that network instability may derive from Aβo-PrPC-Fyn-induced alterations in synapses. We examined epileptiform discharges of >5 s in APPsw/PsEn1ΔE9 transgenic mice with or without PrPC expression at 9–10 months of age (Fig. 7a,b). After implantation of intracranial electrodes, continuous video and electroencephalography (EEG) records were monitored for 72 h. Consistent with previous studies42, 40% of the APP/PsEn transgenic mice had at least one electrographic seizure (Fig. 7b). The majority included an initiating spike and a 20–60 s run of high amplitude epileptiform activity, and were followed by a post-ictal attenuation of cerebral rhythms (Fig. 7a). At the video level, each of the electrographic seizures was accompanied by tonic posturing and myoclonus (Supplementary Movies 3 and 4). The ictal period commonly includes locomotor hyperactivity and the postictal period is marked by hypoactivity. None of the 12 APP/PsEn transgenic lacking PrPC exhibited an electrographic or behavioral seizure during the 72-h period (Fig. 7b). Neither the wild-type nor Prnp−/− mice had electrographic abnormalities during similar monitoring, although single spikes were occasionally observed in all genotypes. Thus, the electrographic phenotype of this Alzheimer’s disease model requires PrPC.

The seizure phenotype may account for the reduced survival of APP/PsEn mice via status epilepticus, or sudden unexpected death in epilepsy. In the cohort studied here, reduced survival for the APP/PsEn genotype was fully rescued by PrP(23–111) (Fig. 7c), as in our previous separate analysis13. De facto, mice monitored by EEG were selected from the two-thirds of the transgenic mice that escaped early death. Considering both phenotypes together, 70% of the APP/PsEn mice suffered either early death or seizures, whereas <4% of the APP/PsEn Prnp−/− mice showed one of these phenotypes.

**DISCUSSION**

Our results describe a Fyn signaling pathway activated by Aβo-PrPC complexes. Pathophysiological relevance is supported by the binding of human Alzheimer’s disease–derived Aβo to PrPC2, enrichment of PrPC in PSDs, PrPC dependence of Fyn activation by Aβo in neurons, and a requirement for both Fyn and PrPC for Aβo-induced changes in NR2B. Short-term activation of this pathway resulted in increases in surface NMDAR and excitotoxicity, followed by dendritic spine and surface receptor loss. The complete rescue of Alzheimer’s disease transgene mortality by PrPC deletion was marked and may have been secondary to the PrPC dependence of epileptiform activity detected by EEG recording. Together, these data document a biochemical pathway downstream of Aβo-PrPC complexes that potentially contributes to Alzheimer’s disease pathogenesis (Supplementary Fig. 10).

**Different Aβ oligomer species and mechanisms**

Aβo derived from synthetic peptide, cell culture, transgenic brains and the brains of individuals with Alzheimer’s disease has been analyzed in various functional and biochemical assays. The Aβ peptide can assume different oligomeric states, each distinct from monomer or fibrillar peptide. The resolution of oligomeric Aβ forms is not defined biophysically, although molecular weight and/or valency clearly differ between preparations. Variable outcomes in functional experiments with different Aβo preparations may be the results of uncharacterized variation between preparations10. We found that Aβo from the brains of individuals with Alzheimer’s disease interacts with PrPC, confirming the pathological relevance of the studies. Suppression of LTP by extracts from the brains of individuals with Alzheimer’s disease has also been reported to require PrPC (refs. 10,16).

The ability of Aβo species to interact with PrP(23–111) distinguishes a subset of peptide with deleterious actions on neurons and synapses. The level of PrP(23–111)-interacting Aβ species in TBS-soluble extracts from the brains of individuals with Alzheimer’s disease is within the range of values determined by previous studies43–45. Measurements of Aβ after immunoprecipitation or immunoblot of TBS-soluble extracts from the brains of individuals with Alzheimer’s disease have yielded values43 very similar to those that we observed for PrP(23–111)–interacting species. This raises the possibility that a substantial fraction of Aβ in the brains of individuals with Alzheimer’s disease is capable of interacting with PrP(23–111). Further analysis of human samples for PrP(23–111)-interacting Aβ species may provide a useful tool for following disease status and/or treatment efficacy.

**Postsynaptic action of Aβo-PrPC complexes**

Synapse loss is among the most prominent and consistent aspects of Alzheimer’s disease. Using immunohistochemical localization and subcellular fractionation, we found that PrPC was concentrated at synapses and enriched in PSD. These findings are consistent with several unbiased proteomic studies examining PrP(23–111) in PSD fractions46,47, and PrP(23–111) is included in a ‘consensus PSD’ data set48. PSD localization is consistent with PrP(23–111) being involved in mediating the local effects of Aβo on synaptic plasticity, dendritic spine retraction and synaptic loss. Indeed, our genetic analysis revealed that both PrP(23–111) and Fyn are required for dendritic spine loss. Previous studies have shown that chronic loss of synaptic markers in transgenic Alzheimer’s disease mice requires PrP(23–111) (refs. 13,14). However, another study found that Aβ-PrPC-induced loss of dendritic spines in culture is independent of PrP(23–111) (ref. 12). The PrPC-negative result may reflect differences in the Aβ preparation. In this regard, the observation that PrPC-Fc recognizes Alzheimer’s disease–derived Aβ species supports the idea that PrPC-dependent pathways are important for Alzheimer’s disease.

Engagement of an Aβo-PrPC-Fyn pathway increased NMDAR phosphorylation and altered receptor localization. The biphasic effect on surface NMDAR was coupled with short-term cell toxicity, followed by a loss of surface receptor. Both acute augmentation and chronic suppression of NMDA responses by Aβo have been reported18,21,48. Future studies of GluR trafficking could delineate the sites of receptor relocalization in the neuron relative to synapses, as well as the timing of Aβo-PrPC-Fyn induced changes in neurotransmission.
Fyn activation by PrPC
Although PrPC and Fyn were associated physically and Aβ engagement of PrPC led to Fyn activation, the two proteins are present on different faces of the plasma membrane. PrPC is present on the extracellular side of the membrane, whereas Fyn is present on the intracellular side, and the two polypeptides therefore cannot be in direct physical contact. Lipid rafts are crucial for signal transduction, and cell-bound Aβo is known to localize to rafts in a Fyn-dependent manner. It is possible that the coalescence of Aβo-PrPC and Fyn in lipid rafts allows signaling without a transmembrane polypeptide partner. However, it seems more plausible that one or more membrane-spanning partners link the two proteins.

Previous reports have described PrPC-dependent signaling in certain cells after antibody cross-linking. We found that clustering of PrPC with antibody to PrPC was not sufficient to induce Fyn activation, as observed with Aβo in primary neurons. This suggests that PrPC conformational changes occur when Aβo binds to it. The Aβo binding domain of PrPC (amino acids 23–111) is thought to be natively disordered, and Aβo may stabilize a specific conformation of the protein, leading to signal transduction through Fyn.

Although acute engagement of PrPC by Aβo activates Fyn, in vivo levels of Fyn are similar in wild-type and Alzheimer’s disease transgenic mice. This is likely related to secondary changes in the chronic state. The biphasic effect of Aβo on NMDAR is consistent with compensatory dysregulation. In this regard, it is notable that the STEP phosphatase counteracts Fyn activation and that STEP protein levels are increased in transgenic Alzheimer’s disease models and Alzheimer’s disease32,37. In culture, early PrPC-dependent Fyn activation was required for the later loss of surface NMDAR, which was correlated with STEP expression. Such compensation may be important in chronic Alzheimer’s disease pathophysiology after acute Aβo-PrPC-Fyn engagement.

Fyn in PrPC-dependent and Alzheimer’s related pathways
Our results suggest that Fyn is important for coupling Aβo and PrPC to changes in neuronal function. There are multiple lines of evidence linking Fyn kinase function to synapse plasticity and dysfunction in Alzheimer’s disease. Fyn is enriched in the PSD and is known to phosphorylate NR2A and NR2B. Mice with loss of Fyn function exhibit early PrPC-dependent Fyn activation was required for the later loss of surface NMDAR, which was correlated with STEP expression. Such compensation may be important in chronic Alzheimer’s disease pathophysiology after acute Aβo-PrPC-Fyn engagement.

Seizures and Alzheimer’s disease
Mice carrying human Alzheimer’s disease transgenes exhibit altered network activity and epilepticiform discharges. We confirmed a similar finding in APPswes/PSen1A9 mice and found that this phenotype was PrPC-dependent and included convulsive seizures. Single spikes without behavioral changes were not a reliable distinguisher between wild-type and APP/PSen transgenic phenotypes in our studies. Given that Fyn gain of function reduces seizure thresholds, Aβo-PrPC-Fyn signaling may explain abnormal EEG patterns. It has been suggested that epilepticiform discharges explain early death in Alzheimer’s disease.
11. Cisse, M. et al. 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).

12. Keshelava, H.W., Nguyen, L.N., Nabavi, S. & Malinow, R. The prion protein as a receptor for amyloid-beta. *Nature* 466, E3–4 (2010).

13. Gimbel, D.A. et al. Memory impairment in transgenic Alzheimer mice requires cellular prion protein. *J. Neurosci.* 30, 6367–6374 (2010).

14. Chung, E. et al. Anti-PrPC monoclonal antibody infusion as a novel treatment for cognitive deficits in an Alzheimer’s disease model mouse. *BMC Neurosci.* 11, 130 (2010).

15. Resenbecker, U.K. et al. The cellular prion protein mediates neurotoxic signalling of beta-sheet-rich conformers independent of prion replication. *EMBO J.* 30, 2057–2070 (2011).

16. Barry, A.E. et al. Alzheimer’s disease brain-derived amyloid-beta–mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. *J. Neurosci.* 31, 7259–7263 (2011).

17. Bate, C. & Williams, A. Amyloid-beta–induced synapse damage is mediated via cross-linkage of cellular prion proteins. *J. Biol. Chem.* 286, 37955–37963 (2011).

18. You, H. et al. Abeta neurotoxicity depends on interactions between copper ions, prion protein and N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA* 109, 1737–1742 (2012).

19. Alier, K., Ma, L., Yang, J., Westaway, D. & Jhamandas, J.H. Abeta inhibition of ionic conductance in mouse basal forebrain neurons is dependent upon the cellular prion protein PrPC. *J. Neurosci.* 31, 16292–16297 (2011).

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

21. Shankar, G.M. et al. Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor–dependent signalling pathway. *J. Neurosci.* 27, 2866–2875 (2007).

22. Lacor, P.N. et al. Abeta oligomer-induced aberrations in synapse composition, shape and density provide a molecular basis for loss of connectivity in Alzheimer’s disease. *J. Neurosci.* 27, 796–807 (2007).

23. Snyder, E.M. et al. Regulation of NDMA receptor trafficking by amyloid-beta. *Nat. Neurosci.* 8, 1051–1058 (2005).

24. Pantera, B. et al. PrPC activation induces neurite outgrowth and differentiation in PC12 cells: role for caveolin-1 in the signal transduction pathway. *J. Neurochem.* 110, 194–207 (2009).

25. Mouillet-Richard, S. et al. Cellular prion protein signaling in serotonergic neuronal cells. *Ann. NY Acad. Sci.* 1096, 106–119 (2007).

26. Williamson, R., Usardi, A., Hanger, D.P. & Anderton, B.H. Membrane-bound beta-amyloid oligomers are recruited into lipid rafts by a fyn-dependent mechanism. *FASEB J.* 22, 1552–1559 (2008).

27. Málaga-Trillo, E. et al. Regulation of embryonic cell adhesion by the prion protein. *PLoS Biol.* 7, e55 (2009).

28. Bizut, N. et al. Neuron dysfunction is induced by prion protein with an insertional mutation via a Fyn kinase and reversed by srcinu activation in Caenorhabditis elegans. *J. Neurosci.* 30, 5394–5403 (2010).

29. Suzuki, T. & Okumura-Noji, K. NMDA receptor subunits epsilon 1 (NR2A) and epsilon 2 (NR2B) are substrates for Fyn in the postsynaptic density fraction isolated from the rat brain. *Biochem. Biophys. Res. Commun.* 216, 582–588 (1995).

30. Grant, S.G. et al. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* 258, 1903–1910 (1992).

31. Nakazawa, T. et al. Characterization of Fyn-mediated tyrosine phosphorylation sites on GluR epsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor. *J. Biol. Chem.* 276, 6993–6999 (2001).

32. Chin, J. et al. Fyn kinase induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer’s disease. *J. Neurosci.* 25, 9694–9703 (2005).

33. Rennert, M. et al. Deleterious effects of amyloid beta oligomers acting as an extracellular scaffold for mGluR5. *Neuron* 66, 739–754 (2010).

34. Collins, M.O. et al. Molecular characterization and comparison of the components and multiprotein complexes in the postsynaptic proteome. *J. Neurochem.* 97 Suppl 1, 16–23 (2006).

35. Stucumer, C.A. et al. PrPC capping in T cells promotes its association with the lipid raft proteins reggie-1 and reggie-2 and leads to signal transduction. *FASEB J.* 18, 1731–1733 (2004).

36. Saltar, M.W. & Kalia, L.V. Src kinases: a hub for NMDA receptor regulation. *Nat. Rev. Neurosci.* 5, 317–328 (2004).

37. Zhang, Y. et al. Genetic reduction of striatal-enriched tyrosine phosphatase (STEP) reverses cognitive and cellular deficits in an Alzheimer’s disease mouse model. *Proc. Natl. Acad. Sci. USA* 107, 19014–19019 (2010).

38. Pybylowiok, K. et al. The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. *Neuron* 47, 845–857 (2005).

39. Chen, P., Gu, Z., Liu, W. & Yan, Z. Glycogen synthase kinase 3 regulates N-methyl-D-aspartate receptor channel trafficking and function in cortical neurons. *Mol. Pharmacol.* 72, 40–51 (2007).

40. Scheff, S.W., DeKosky, S.T. & Price, D.A. Quantitative assessment of cortical synaptic density in Alzheimer’s disease. *Neurobiol. Aging* 11, 29–37 (1990).

41. Palop, J.J. et al. Aberrant excitatory neuronal activity and compensatory remodeling of inhibit cerebellar circuits in mouse models of Alzheimer’s disease. *Neuron* 55, 697–711 (2007).

42. Minkeviciene, R. et al. Amyloid beta–induced neuronal hyperexcitability triggers progressive epilepsy. *J. Neurosci.* 29, 3453–3462 (2009).

43. McDonald, J.M. et al. The presence of sodium dodecyl sulphate-stable Abeta dimers is strongly associated with Alzheimer-type dementia. *Brain* 133, 1328–1341 (2010).

44. Steinerman, J.R. et al. Distinct pools of beta-amyloid in Alzheimer disease–affected brain: a clinicopathologic study. *Arch. Neurol.* 65, 906–912 (2008).

45. Kuo, Y.M. et al. Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. *J. Biol. Chem.* 271, 4077–4081 (1996).

46. Peng, J. et al. Semiquantitative proteomic analysis of rat forebrain postsynaptic density fractions by mass spectrometry. *J. Biol. Chem.* 279, 21003–21011 (2004).

47. Yoshimura, Y. et al. Molecular constituents of the postsynaptic density fraction revealed by proteomic analysis using multidimensional liquid chromatography tandem mass spectrometry. *J. Neurochem.* 88, 759–768 (2004).

48. Li, S. et al. Soluble Abeta oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. *J. Neurosci.* 31, 6627–6638 (2011).

49. Kojima, N., Ishibashi, H., Obata, K. & Kandel, E.R. Higher seizure susceptibility and enhanced tyrosine phosphorylation of N-methyl-D-aspartate receptor subunit 2B in fyn transgenic mice. *Learn. Mem.* 5, 429–445 (1998).

50. Ittner, L.M. et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer’s disease mouse models. *Cell* 142, 387–397 (2010).
ONLINE METHODS

Mice. Wild-type, Prnp+/+, APP/PSen and APP/PSen Prnp+/- mice on the C57Bl/J background were as described53. Fyn+/- mice52 were obtained from Jackson Laboratories. Both males and females were used in approximately equal numbers and none were excluded. All experiments were approved by the Institutional Animal Care and Use Committee of Yale University.

Aji peptide. Aji2 oligomer, monomer and fibrillar preparations have been characterized5. Concentrations are in monomer equivalents. Oligomeric preparations with 1 μM total Aji2 peptide contain about 10 nM oligomeric species5.

Recombinant human PrP(23–111). PrP(23–111) protein was produced by modification of previous procedures52. DNA encoding amino acids 23–111 of human PrPsc was cloned into pRSETa vector with an N-terminal extension encoding a hexa-histidine tag and thrombin cleavage site. Plasmid-transformed BL21(DE3) E. coli (Agilent) were cultured overnight without induction and then diluted 1:100 in ZYM-5052 auto-inducing medium and grown for 16 h at 37 °C. Bacteria were lysed in Buffer G (6 M guanidine HCl, 100 mM Na2HPO4, 10 mM Tris-HCl, pH 8) and then centrifuged at 100,000 g for 1 h. The supernatant was applied to Ni-NTA resin. To refold bound protein, a 20–100% stepwise gradient of Buffer B (100 mM Na2HPO4, 10 mM Tris-HCl, 10 mM imidazole, pH 8) in Buffer G was applied. After washing the resin, bound protein was eluted with 100 mM Na2HPO4, 10 mM Tris-HCl and 500 mM imidazole (pH 5.8) and dialyzed against 10 mM Na2HPO4 (pH 5.8) and then against water. Final yields were 30–40 mg of protein per L of culture. Protein was stable at 4 °C for >2 months.

Generation of PrPsc and Fyn stable cell lines. PrPsc-expressing CV-1 cells were isolated by clonal selection of a co-transfected neomycin resistance gene in G418. A clone with PrPsc immunoblot levels comparable to brain was propagated as SCA-7. Recombinant lentiviral particles expressing Fyn (pLEX-JRed, Open Biosystems) were transduced into CV-1 and SCA-7 cells. Cells stably expressing Fyn were selected with puromycin and confirmed by Fyn immunoblot. Cells were maintained in 25 μg ml–1 puromycin.

Cell line cultures and preparation of cell lysates. HEK293, CV-1 and N2A cells were rinsed with ice-cold phosphate-buffered saline (PBS) and solubilized in 20 mM Tris (pH 7.4), 1.0% Triton X-100 (vol/vol), 0.1% SDS (wt/vol), 150 mM NaCl, 10% glycerol (vol/vol), complete protease inhibitor cocktail (Roche) and phosphatase inhibitor (Roche). To separate cell lysates into soluble and insoluble fractions, we centrifuged material at 20,000 g for 20 min at 4 °C. Supernatants from the initial fractionation were saved as the soluble fraction. The pellets were rinsed with ice-cold phosphate-buffered saline and solubilized, we centrifuged material at 20,000 g for 20 min at 4 °C. Supernatants from the initial fractionation were saved as the soluble fraction. The pellets were washed once with PBS before re-extraction with 2% SDS as insoluble fraction.

Primary neuronal cultures. Rat E18 and mouse E17 cortical neurons were cultured for 21 d in Neurobasal-A media with B-27, 0.5 mM l-glutamine, penicillin and streptomycin (all from Invitrogen) on plates coated with poly-L-lysine.

Immunoblots. Protein was electrophoresed through 4–20% Tris-glycine or 10–20% Tris-tricine gels (Bio-Rad), transferred to nitrocellulose membranes (Bio-Rad) and blocked (Rockland MB-070-010). Membranes were incubated overnight at 4 °C with primary antibodies to PrPsc (6D11, Covance 39810-500, 1:1000), phospho-SFk (Cell Signaling Technology #2101, 1:1000), Fyn (Cell Signaling Technology #4203, 1:1000), Src (Cell Signaling Technology #2110, 1:1000), Lyn (Santa Cruz Biotechnology sc-28883, 1:1000), c-Yes (Santa Cruz Biotechnology sc-28795, 1:1000), c-Src (Cigma C3956, 1:1000), GAPDH (Sigma-Aldrich G8795, 1:20,000), phospho-NR2B (pY1472; Sigma M2442, 1:1000), NR2B (BD Transduction Laboratories #610416, 1:500), PSD-95 (Cell Signaling Technology #2507, 1:1000), synapto-physin (Cell Signaling Technology #4329, 1:1000), 6E10 (Covance SIG-39300, 1:1000), 82E1 (IBL, 1:100), cleaved caspase-3 (Cell Signaling Technology #9661, 1:1000), caspase-3 (Cell Signaling Technology #9665, 1:1000), Aβ (Cell Signaling Technology #2454, 1:1000). Secondary antibodies were then applied for 1 h at 23 °C (Ori-dy goat or donkey antibody to mouse or rabbit IRDye 680 or 800) and visualized with the Licor Odyssey system.

Immunoprecipitation. We incubated 1 μg of antibody overnight at 4 °C with 1 mg of lysate protein. We then incubated 30 μl of a 1:1 suspension of protein A-Sepharose (Amersham) with sample for 2 h at 4 °C. The resin was washed and immunocomplexes were resolved by SDS-PAGE and immunoblotted.

Immunocytochemistry. CV-1–derived cells were fixed with formaldehyde and permeabilized with Triton X-100. Slides were blocked, and incubated at 4 °C with antibody to phospho-SFk (1:100) and PrPsc (6D11, Covance, 1:300 dilution). Alexa Fluor–488 goat antibody to mouse or Alexa Fluor–568 goat antibody to rabbit were used to detect bound antibodies.

Dissociated hippocampal neurons were prepared from E17–18 C57BL/6 mice. At 21 DIV, cultures were fixed in paraformaldehyde, permeabilized and blocked. Immunostaining was performed with antibodies to PrPsc (6D11, Covance, 1:100) and PSD-95 (Invitrogen #51-6900, 1:100) followed by Alexa-Fluor secondary antibodies. Images were acquired on a Zeiss LSM 510 META laser-scanning confocal microscope using a 63× water objective, with channels scanned separately and a pinhole set to 1.1 μm.

LDH release. LDH release into culture medium was measured with the CytoTox Detection Kit (Roche). Total LDH was determined by lysing all cells with 2% Triton X-100, and experimental values were expressed as a percentage of the total LDH. Absorbance was measured at 490 nm using a VictorX3 Multiplate Label Plate Reader (PerkinElmer).

MTT assay. Cell viability was monitored by the conversion of 3(4,5-dimethylthia-zol-2)-2,5 diphenyl tetrazolium bromide to colored formazan product (MTT, Roche). MTT labeling reagent (final concentration of 0.5 mg ml–1) was added to neurons and incubated for 4 h at 37 °C. Solubilization solution was added plates were incubated for 16 h at 37 °C. Colored formazan products were detected as absorbance at 550 nm in a VictorX3 Multiplate Label Plate Reader.

Cell-surface biotinylation. Rat E18 and mouse E17 cortical neurons at 21 DIV were untreated or treated with Aβo for various times, placed on ice and rinsed in cold PBS. Then, neurons were incubated with 1.5 mg ml–1 sulfo-NHS-LC-biotin (Thermo Scientific) in PBS for 30 min at 4C. Cells were rinsed to remove unbound biotin, and extracted with 1% Triton X-100, 0.1% SDS, complete protease inhibitor cocktail (Roche) and phosphatase inhibitor (Roche). Biotinylated proteins were isolated with NeutRavidin agarose (Pierce), separated by SDS-PAGE and analyzed by immunoblotting.

NMDAR trafficking. Wild-type or Prnp+/- DIV cortical neurons were transfected with expression vector or NR2B-GFP (kindly provided by Z. Yan, State University of New York) using calcium phosphate (Clontech). After 3 d, neurons were treated with 0–1 μM Aβo for 0–60 min. Neurons were placed on ice, and then incubated with Alexa Fluor 555–conjugated antibody to GFP (Molecular Probes, A31851) for 30 min. After three washes with PBS, the cells were fixed with 4% paraformaldehyde (wt/vol) and 4% sucrose (wt/vol) in PBS for 15 min, and then washed three times with PBS. Images were acquired on a Zeiss LSM 510 META laser-scanning confocal microscope with a 63× objective, as above. The relative intensity (surface/total) normalized to the untreated condition was measured by MATLAB software. Values were collected from at least four fields in each culture condition.

Subcellular fractionation of brain tissue. Rat forebrains were homogenized in ice-cold 5 mM Na-HEPES (pH 7.4), 1 mM MgCl2, 0.5 mM CaCl2, complete protease inhibitor cocktail (Roche), phosphatase inhibitor (Roche) with a glass/ Teflon pestle. Extract was spun at 1,400g for 10 min. Supernatant was centrifuged at 13,800g for 10 min to collect the pellet. The pellet was resuspended in Buffer B (0.32 M sucrose, 6 mM TrisHCl (pH 8.0), complete protease inhibitor cocktail (Roche), phosphatase inhibitor (Roche)). The suspension was loaded onto a discontinuous sucrose gradient (0.85 M, 1 M, 1.15 M sucrose solution in 6 mM TrisHCl, pH 8.0), followed by centrifugation for 2 h at 82,500g. The synaptosomal fraction between 1 M and 1.15 M sucrose was collected and adjusted to 4 ml with Buffer B. Equal volume of 6 mM TrisHCl (pH 8.1), and 1% Triton X-100 was added and incubated for 15 min. The suspension was spun at 32,800g for 20 min. The resulting pellet was extracted again with 6 mM TrisHCl (pH 8.1) and 0.5% Triton X-100 for 15 min, and spun again at 201,000g for 1 h. The resulting pellet was analyzed as the PSD.

Human brain fractionation. Fresh-frozen post-mortem human pre-frontal cortex from the brains of Alzheimer’s disease patients or control subjects were...
obtained from pre-existing collections, as approved by the Yale Institutional Review Board. Clinical diagnoses were confirmed histologically by examination of adjacent tissue in paraffin sections for abundant Aβ plaques and neurofibrillary tangles, Braak stage VI. Demographic and case details are provided in Supplementary Figure 5d.

For PrP-C affinity resin absorption, TBS-soluble extracts were cleaved overnight at 4 °C with Protein A sepharose beads (GE Healthcare, 1 μg/well) eluted from PrP(23–111) using 30 mM Na2CO3 and 80 mM NaHCO3 (pH 9.6) was added to 384-well Black MaxiSorp ELISA plates (Thermo Scientific). After 2 h at 23 °C, plates were washed with PBS containing 0.05% Tween-20 (PBS-T) and blocked with 5% BSA (wt/vol) in PBS-T (100 μl per well) overnight at 4 °C. After washing again, samples (50 μl) were applied and incubated for 1 h at 23 °C. All the samples included added BSA at 0.5%. The plates were then washed and 20 μl per well of rabbit antibody to Aβ (2454, Cell Signaling Technology, 1:1,000 in PBS-T, 0.5% BSA) was added for 1 h. After another wash, plates were incubated with 20 μl per well of biotinylated donkey antibody to rabbit (Jackson Immunoresearch, 1:1000 in PBS-T, 0.5% BSA) for 30 min, washed three times with PBS-T and then incubated with 20 μl of Europium-conjugated streptavidin (1:1,000 in DELFIA Assay Buffer, PerkinElmer). After a final 4× PBS-T wash, 20 μl of DELFIA Enhancement Solution (PerkinElmer) was added to the wells and time-resolved Europium fluorescence was measured using Victor 3 plate reader (PerkinElmer). Background fluorescence values from uncoated wells were subtracted from the corresponding values of PrP23–111-coated wells.

For PrP(23–111) depletion of Aβ from TBS-soluble extracts from the brains of individuals with Alzheimer’s disease, samples were incubated in PrP(23–111)-coated wells or uncoated control wells in 96-well BSA-blocked MaxiSorp plates (Nunc 80040LE 9093) for 3 h at 23 °C. Unbound material was recovered for ELISA. The plates were washed four times with PBS-T and bound Aβ was eluted from PrP(23–111) using 30 μl per well of 10 M urea. The original and recovered extracts, as well as the urea elutions, were assayed for Aβ42 using a commercially available kit (Invitrogen KHB3441). Elution fractions were diluted to 1 M urea before ELISA.

Imaging of dendritic spine stability. Hippocampi were dissected from E17–19 mouse embryos and digested with papain (37 °C, 5% CO2 for 30 min), and then washed with 1× HBSS. The neurons were transfected with myristoyl-EGFP expression vector using an Amaxa Nucleofector, and plated on poly-d-lysine-coated (100 μg ml−1) glass at 100,000 cells per well in 8-well plates (Lab-Tek Chambered Coverslip 155411). Wild-type controls were plated on half of the 8-well dish. Culture medium was Neurobasal A supplemented with penicillin/streptomycin, 1 mM sodium pyruvate, 2 mM glutamine and B27 supplement. Between 19–23 DIV, hippocampal neurons were observed with a 100× objective on a Nikon Eclipse Ti spinning disk confocal microscope using a 488-nm laser. Approximately 25 fixed locations per 8-well dish were imaged on an automated stage every 15 min for 6 h in a 10-μm z stack at 0.1-μm intervals. For the first h, neurons were untreated. After 1 h, neurons were treated with 500 nM Aβo or F12 vehicle and imaged for 5 h. In some experiments, 50 μM AP5 or vehicle were added immediately before Aβo.

Dendritic spine persistence or creation was assessed in consecutive images of the dendritic segments using ImageJ software without knowledge of genotype or treatment. For each embryo, at least four segments with >30 spines were monitored, and statistics were calculated based on variability between separate embryo cultures. Rare images showed dendritic segments with widespread blebbing and retraction; these were excluded from the analysis.

Cellular Aβ oligomer binding assay. Aβo binding was performed as described5 with slight modifications. Briefly, transfected COS7 cells were treated with biotinylated Aβo at 4 °C for 1 h. After washing, cells were fixed, incubated at 65 °C for 2 h and incubated with alkaline phosphatase–conjugated neutravidin. Bound phosphatase was visualized by 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium reaction and quantified with ImageJ.

Measurement of intracellular calcium. Cortical neurons (21 DIV) from wild-type, Prnp−/− or Fyn−/− mouse embryos were incubated in 96-well plates and treated with 0–1 μM Aβo for 0–60 min plus a calcium dye (FLIPR Calcium 4 assay kit, Molecular Devices). Plate fluorescence (λex = 485 nm, λem = 535 nm) was measured in a VictorX3 Multilabel Plate Reader (PerkinElmer) with or without 50 μM NMDA, 100 μM glutamate or 500 nM ionomycin.

EEG surgeries and analysis. We studied 9–10-month-old mice of the following genotypes: wild-type (C57Bl6), n (11), Prnp−/− (n = 11), APP/PSen (n = 10), and APP/PSen Prnp−/− (n = 12). Mice were anesthetized with isoflurane and mounted in a stereotaxic frame (Kopf). A midline incision was made, and two bilateral burr holes were drilled anterolateral and posterolateral to bregma. Four pre-soldered intracranial screw electrodes (Pinnacle Technology #8403) were inserted, and secured with dental cement (A-M Systems #526000). Electrode wires were soldered to a 6-pin surface mount connector (Pinnacle Technology #8235-SM). Mice were allowed to recover for 7 d before EEG.

Mice were recorded using video-EEG monitoring (Pinnacle Systems #8200-KI-SE3, #8236). EEGs were sampled at 400 Hz with 100× preamplifier gain. Each mouse underwent 72 h of continuous recording. EEG traces were scored manually by an investigator unaware of genotype. A seizure was defined as the abrupt onset of evolving spike-and-wave discharge lasting >5 s, followed by a period of post-ictal attenuation of cerebral rhythms. The recorded seizures were each 20–60 s in duration. There was no requirement for bilateral involvement, but all recorded seizures were generalized. Each seizure was correlated with the video recording and each was accompanied by convulsive behavior. Four of ten APP-PSen had ≥1 seizure over 72 h. Two mice had 1 seizure in this period, the third mouse had 1 seizure per d, and the fourth mouse had an average of 1–2 seizures per d (a total of 5 in 3 d).

There were also single spikes and 3–5-s spike wave discharges that were not analyzed here. Subjectively, spike wave discharges were specific to APP/PSen mice, and greatly reduced by Prnp deletion.

Statistical analyses. Statistical comparisons included one-way ANOVA and repeated-measures ANOVA with post hoc Tukey pairwise comparisons, using SPSS or Prism statistical software. Survival data were analyzed with the logrank test (Mantel-Cox) with SPSS software.

51. Stein, P.L., Lee, H.M., Rich, S. & Soriano, P. pp59fyn mutant mice display differential signaling in thymocytes and peripheral T cells. Cell 70, 741–750 (1992).
52. Zahn, R., von Schnoetter, C. & Wutrich, K. Human prion proteins expressed in Escherichia coli and purified by high-affinity column refolding. FEBS Lett. 417, 400–404 (1997).