Neuron Article

Metabotropic Glutamate Receptor 5 Is a Coreceptor for Alzheimer A β Oligomer Bound to Cellular Prion Protein

Ji Won Um,¹ Adam C. Kaufman,¹ Mikhail Kostylev,¹ Jacqueline K. Heiss,¹ Massimiliano Stagi,¹ Hideyuki Takahashi,¹ Meghan E. Kerrisk,² Alexander Vortmeyer,³ Thomas Wisniewski,⁴ Anthony J. Koleske,² Erik C. Gunther,¹ Haakon B. Nygaard,¹ and Stephen M. Strittmatter^{1,*}

¹Cellular Neuroscience, Neurodegeneration and Repair Program, Departments of Neurology and Neurobiology

²Department of Molecular Biophysics and Biochemistry

³Department of Pathology

Yale University School of Medicine, New Haven, CT 06536, USA

⁴Department of Neurology, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA

*Correspondence: stephen.strittmatter@yale.edu

http://dx.doi.org/10.1016/j.neuron.2013.06.036

SUMMARY

Soluble amyloid- β oligomers (Αβο) trigger Alzheimer's disease (AD) pathophysiology and bind with high affinity to cellular prion protein (PrP^C). At the postsynaptic density (PSD), extracellular Aßo bound to lipid-anchored PrP^C activates intracellular Fyn kinase to disrupt synapses. Here, we screened transmembrane PSD proteins heterologously for the ability to couple A β o-PrP^C with Fyn. Only coexpression of the metabotropic glutamate receptor, mGluR5, allowed PrP^C-bound Aβo to activate Fyn. PrP^C and mGluR5 interact physically, and cytoplasmic Fyn forms a complex with mGluR5. Aβo-PrP^C generates mGluR5-mediated increases of intracellular calcium in Xenopus oocytes and in neurons, and the latter is also driven by human AD brain extracts. In addition, signaling by Aßo-PrP^C-mGluR5 complexes mediates eEF2 phosphorylation and dendritic spine loss. For mice expressing familial AD transgenes, mGluR5 antagonism reverses deficits in learning, memory, and synapse density. Thus, Aβo-PrP^C complexes at the neuronal surface activate mGluR5 to disrupt neuronal function.

INTRODUCTION

Alzheimer's disease (AD) has a distinct pathology with plaques of amyloid- β (A β) and tangles of hyperphosphorylated tau. Rare autosomal dominant AD cases with mutations of amyloid- β precursor protein (APP) or presenilin (PS1 or PS2) provide proof that A β pathways can trigger AD (reviewed in Holtzman et al., 2011). Other APP mutations reduce AD risk (Jonsson et al., 2012). Biomarker studies of late onset AD have shown that A β dysregulation, detected by CSF levels or by PET, is the earliest detectable change, consistent with A β as a trigger (Holtzman et al., 2011).

The mechanism whereby Aβ leads to AD is less clear. Attention has focused on soluble oligomers of AB (ABo) as causing synaptic malfunction and loss of dendritic spines (Shankar et al., 2008). In the only reported genome-wide unbiased screen for Aβo binding sites, we identified PrP^C (Laurén et al., 2009). Aβ binding to PrP^C is high affinity and oligomer-specific (Chen et al., 2010; Laurén et al., 2009). In vivo, PrP^C is not essential for certain Aβ-related phenotypes, but is required for cell death in vitro, for reduced survival of APP/PS1 transgenic lines, for epileptiform discharges, for synapse loss, for serotonin axon degeneration, and for spatial learning and memory deficits (reviewed in Um and Strittmatter, 2013). Critically, the ability of human AD brain-derived Aß species to suppress synaptic plasticity requires PrP^C, and human AD brain contains PrP^C-interacting Aβo and Aβ-PrP^C complexes (Barry et al., 2011; Freir et al., 2011; Um et al., 2012; Zou et al., 2011).

Aβo-PrP^C complexes signal to intracellular Fyn kinase (Larson et al., 2012; Um et al., 2012). PrP^C phenotypes in fish and worms require Fyn (Bizat et al., 2010; Málaga-Trillo et al., 2009), Fyn regulates Glu receptor traffic and plasticity (Grant et al., 1992; Prybylowski et al., 2005), and Fyn interacts with tau (Ittner et al., 2010; Roberson et al., 2011). Both PrP^C and Fyn are enriched in the postsynaptic density (PSD), and Aβo engagement of PrP^C activates Fyn to phosphorylate NMDA receptors (Larson et al., 2012; Um et al., 2012).

The connection from A β o-PrP^C complexes to Fyn cannot be direct, because PrP^C is anchored via glycolipid to the plasma membrane whereas Fyn is cytoplasmic. Because both are enriched in PSDs (Collins et al., 2006; Um et al., 2012), we hypothesized that a transmembrane PSD protein might couple PrP^C with Fyn. The PSD proteome includes 81 transmembrane proteins (Collins et al., 2006; Emes et al., 2008). Here, we screened PSD transmembrane proteins for their ability to couple A β o-PrP^C with Fyn. We identified mGluR5 as linking PrP^C to Fyn. Activation of neuronal Fyn requires both mGluR5 and PrP^C. A β o-PrP^C can drive mGluR5-dependent calcium mobilization and eEF2 phosphorylation. Antagonists of mGluR5 prevent A β o-induced dendritic spine loss and AD transgene learning and memory deficits. These studies define an A β o-PrP^C-mGluR5 complex that leads to impaired neuronal function.



Figure 1. Metabotropic GluR5 Links Aβo/PrP^C Complexes to Fyn

(A) Schematic indicating that Aβo/PrP^C complexes require a coreceptor to activate Fyn in PSDs.

(B) Pie charts show transmembrane proteins (81) among total PSD proteins (651). Transmembrane proteins were subdivided; those that were screened in Aβo/Fyn assays (56, yellow) and those not (25, gray).

(C) Immunoblot of screening for Fyn activation. HEK293T cells were transfected with expression vectors for Fyn, PrP^C, or candidate genes. After treatment with 1 μM Aβo for 15 min, lysates were analyzed by anti-phospho-SFK (Tyr416) or anti-Fyn immunoblot.

(D) The ratio of phospho-SFK to Fyn is plotted from three experiments with A β o. Blue line indicates the mean of all controls. Dark gray is 1 SD, and light gray is 2 SD from control.

RESULTS

Screen for PSD Proteins Mediating A β o/PrP^C Signaling Identifies mGluR5

We considered the 81 known transmembrane PSD proteins as potential mediators (Figures 1A and 1B). We utilized a cell type in which PrP^C and Fyn fail to couple. When PrP^C and Fyn are overexpressed in HEK293T cells, ABo does not activate Fyn, as in neurons (Um et al., 2012). We coexpressed PSD proteins together with PrP^C and exposed the HEK cells to Aβo prior to assessing Fyn activation by anti-phospho-SFK (Src family kinase) immunoblot (Figures 1B-1D). In addition to 56 documented PSD proteins, we included APLP1 and APLP2, due similarity with the PSD protein, APP, and known interaction with PrP^C or Aβo (Bai et al., 2008; Laurén et al., 2009; Schmitt-Ulms et al., 2004). We included the LRRTM family because they organize synapses and modify $A\beta$ levels (Linhoff et al., 2009; Majercak et al., 2006). Of 61 proteins screened, only mGluR1 and mGluR5 increased Fyn activation by >2 SD (Figures 1C and 1D). mGluR5 is reported to coimmunoprecipitate and activate Fyn (Heidinger et al., 2002), to redistribute after Aßo (Renner et al., 2010), to colocalize with A_bo (Renner et al., 2010), and to be required for Aβo suppression of LTP (Rammes et al., 2011; Shankar et al., 2008; Wang et al., 2004).

In the initial screen with A β o, mGluR1 or mGluR5 activity might have been ligand-dependent or independent. Although coexpression of either receptor results in baseline activation of Fyn, only mGluR5 mediates A β o activation (Figures 1E–1G). A β oinduced Fyn activation in transfected HEK cells is PrP^C dependent, as shown previously for neurons (Um et al., 2012), because when mGluR5 is expressed without PrP^C, no A β o regulation of Fyn occurs. In contrast, basal Fyn activity (without A β o) is independent of PrP^C and equal for mGluR1 and mGluR5. Thus, mGluR5 alone has the property of mediating A β o-PrP^C activation of Fyn in HEK cells.

Although EphB2 is not a PSD consensus member, we considered EphB2 as a link between A β o and Fyn because it couples with Fyn during development, and because A β alters EphB2 level (Cissé et al., 2011; Takasu et al., 2002). In HEK, coexpression of EphB2 and Fyn yields kinase activation (Takasu et al., 2002), but EphB2 does not mediate A β o signaling (Figure S1 available online).

We sought to determine whether neuronal mGluR5 is required for Aβo-induced Fyn activation. The mGluR5 negative allosteric modulator, MPEP, blocks Aβo-induced Fyn activation in HEK cells (Figure 1E), so we preincubated cortical neurons with MPEP, or the related MTEP, prior to A β o (Figures 1H and 1J). Neither MTEP nor MPEP alters baseline Fyn activity, but both eliminate A β o-induced activation. The mGluR1 antagonist, MPMQ, does not prevent A β o-induced Fyn activation (Figures 1H and 1J). We also cultured *Grm5^{-/-}* cortical neurons and exposed them to A β o at 21DIV (Figures 1I and 1J). Under basal conditions, phospho-Fyn levels were similar to wild-type (WT), but the increase by A β o was eliminated. Thus, mGluR5, as well as PrP^C, is required for this A β o signal transduction.

mGluR5 Does Not Bind A βo but Physically Associates with PrP^{C} and Fyn

With evidence that PrP^{C} , mGluR5, and Fyn participate in A β o signaling, we assessed physical interaction among them. We visualized A β o binding to COS-7 cells expressing mGluR5, PrP^{C} , both, or neither (Figures 2A and 2B). A β o binding to PrP^{C} -expressing cells is not altered by mGluR5, and there is no detectable binding of A β o to mGluR5 without PrP^{C} . PrP^{C} alone accounts for A β o surface binding.

If mGluR5 serves as a bridge between PrP^C and Fyn, then it is predicted to interact physically with both. We confirmed an association of mGluR5 with Fyn (Heidinger et al., 2002), and observed no alteration by PrP^{C} or A_βo (Figure S2A). Both mGluR1 and mGluR5 associate with Fyn, but mGluR8 does not (Figure S2B). In HEK293T cells, PrP^C immunoprecipitates contain mGluR5, regardless of Aβo (Figure 2C). Both mGluR1 and mGluR5, but not mGluR8, coimmunoprecipitate with PrP^C (Figure 2D). We utilized this specificity to examine whether discrete mGluR5 domains are responsible for PrP^C interaction (Figure S2C). Chimeric proteins containing the N-terminal globular domain from one mGluR fused to the transmembrane domains from another mGluR were coexpressed with PrP^C. Each chimera coimmunoprecipitates PrP^C less effectively than mGluR5 (Figure S2D), suggesting that the PrP^C-interacting regions are distributed in the protein.

In brain, mGluR5 and PrP^C are coenriched in detergent-resistant PSD fractions (Figure 2E) (Collins et al., 2006; Um et al., 2012). To assess association of endogenous proteins extractable with nondenaturing detergent, we covalently crosslinked proteins and then precipitated PrP^C. The PrP^C immunoprecipitates contain mGluR5, but not mGluR8, NR2B, or GluR1 (Figure 2F). Immunoprecipitates from $Pmp^{-/-}$ samples do not exhibit mGluR5. Thus, A β o-PrP^C, PrP^C-mGluR5, and mGluR5-Fyn pairwise physical associations are detectable.

(J) Quantification of phospho-SFK level in lysates from (H) and (I) normalized to Fyn. Mean ± SEM, n = 3 experiments. ***p < 0.001; *p < 0.05; ANOVA, Tukey post hoc pairwise comparisons.

See also Figure S1.

⁽E) HEK293T cells were transfected with vectors for Fyn, PrP^{C} , or mGluR5, and treated with 0 or 1 μ M A β o for 15 min. Some cultures were preincubated for 30 min with 100 μ M MPEP prior to A β . Lysates were analyzed by anti-phospho-SFK (Tyr416), anti-Fyn, anti-mGluR5, or anti-PrP^C immunoblot. Actin is loading control. (F) HEK293T cells were transfected with vectors for Fyn, PrP^{C} , or Myc-mGluR1 as indicated. After cell treatment with 0 or 1 μ M A β o for 15 min, lysates were analyzed by anti-phospho-SFK (Tyr416), actin is loading control.

⁽G) Quantification of phospho-SFK level in lysates from (E) and (F) normalized to Fyn. Mean ± SEM, n = 4 experiments. **p < 0.01; *p < 0.05; ANOVA, Tukey post hoc pairwise comparisons.

⁽H) Cortical neurons from E17 WT mice at 21 DIV were treated with 0 or 1 μ M of A β o for 15 min. Indicated samples were treated with 100 μ M MTEP or 10 μ M MPMQ for 30 min prior to A β o. Lysates were subjected to immunoblot with anti-Fyn, or anti-phospho-SFK (Tyr416). Actin is loading control.

⁽I) Cortical neurons from WT or Grm5^{-/-} mice after 21 DIV were treated with 0 or 1 µM Aβo for 15 min. Lysates were analyzed by anti-phospho-SFK (Tyr416), anti-Fyn, or anti-mGluR5 immunoblot. Actin is loading control.



Figure 2. PrP^C Interacts with mGluR5

(A) A60 (250 nM) binding to COS7 cells expressing mGluR5, PrP^C, or coexpressing mGluR5 with PrP^C (top). Protein expression was confirmed by immunofluorescence (bottom). Scale bar represents 50 µm.

(B) Dose response for A β o binding to COS7 cells from experiments as in (A). Mean ± SEM, n = 3 experiments.

(C) HEK293T cells were transfected with vector for either mGluR5 or PrP^C, or cotransfected for mGluR5 and PrP^C. After treatment with 0 or 1 μM Aβo for 1 hr, lysates (input) and anti-PrP^C immunoprecipitates were immunoblotted with either anti-mGluR5 or anti-PrP^C.

(D) HEK293T cells were transfected with vector for PrP^C or cotransfected with different Myc-tagged mGluRs and PrP^C, as indicated. Lysates (input) and anti-Myc immunoprecipitates were immunoblotted with either anti-Myc or anti-PrP^C antibodies.

(E) Indicated fractions (20 µg protein) were prepared and analyzed by immunoblot with anti-PrP^C, anti-mGluR5, anti-EphB2, anti-PSD95, and anti-synaptophysin antibodies.

(F) Brain lysates from WT or *Pmp^{-/-}* mice were crosslinked with the cleavable DTSSP. Whole lysates (5% input) and anti-PrP^C immunoprecipitates were immunoblotted with anti-mGluR5, anti-mGluR8, anti-NR2B, anti-GluR1, or anti-PrP antibodies. Asterisk, Ig light chain.

(G) His-tagged human PrP^C was incubated with DIV21 WT or $Grm5^{-/-}$ neurons for 1 hr. Neurons were then fixed and stained with human-specific anti-PrP^C antibody and Alexa-568 secondary antibody with rhodamine-phalloidin as counterstain. Scale bar represents 10 μ m.

(H) Quantification of His-PrP^C bound to WT or $Grm5^{-/-}$ neurons. His-PrP^C immunofluorescence was measured by ImageJ in segments of primary dendrites 10 μ m from the soma and background-subtracted. Mean ± SEM, n = 3 embryos for each genotype.***p < 0.001; Student's two-tailed t test. See also Figure S2.

To evaluate PrP^C specificity for mGluR5, we applied soluble PrP^C as a ligand to mGluR5-expressing cells. PrP-His binds more avidly to COS-7 cells expressing mGluR5 than to nontransfected or mGluR1-expressing or mGluR8-expressing cells (Figures S2E and S2F). Thus, in this more stringent test of protein interaction, mGluR5, but not mGluR1 or mGluR8, has affinity for PrP^C. We utilized similar conditions to examine soluble PrP^C binding to neurons with a human-specific anti-PrP^C to selectively visualize immunoreactivity of the recombinant ligand (Figures 2G and 2H). Punctate binding of soluble PrP^C along dendrites is visible (Figure 2G). This staining is reduced by 70% in *Grm5^{-/-}* cultures, with the remaining signal similar to



the nonspecific low-affinity level observed in nontransfected COS-7 cells (Figures S2E and S2F). We conclude that mGluR5 contributes significantly as a PrP^C partner on the neuronal surface.

Expression of PrP^{C} Plus mGluR5 Supports A β o-Induced Increase of Intracellular Calcium

Activation of Fyn is one consequence of mGluR5 engagement, but activation of the heterotrimeric GTPases, G_q/G_{11} , with subsequent production of inositol (3,4,5) trisphosphate (IP3) and release of intracellular calcium, is the more prominent pathway (Lüscher and Huber, 2010). We considered whether A β o-PrP^C might activate this pathway by injecting RNA for mGluR5 and PrP^C into *Xenopus laevis* oocytes and applying a two-electrode

Figure 3. Aßo Directly Stimulate mGluR5

(A) Representative traces obtained from oocytes expressing mGluR5 plus PrP^C, or mGluR1 plus PrP^C. Bath application (bars) of A β o (1.5 μ M monomer equivalent, estimated 15 nM oligomer) or Glu (100 μ M) elicits an inward current from mGluR5 plus PrP^C oocyte. Exposure to Glu, but not A β o, elicits a response in oocytes expressing mGluR1 and PrP^C.

(B and C) Quantification of the peak current elicited by glutamate (B) or A β o (C) in oocytes expressing the indicated proteins, with or without pretreatment with 5 μ g/ml anti-PrP^C antibody, 6D11. Data are presented as mean \pm SEM, n = 8–28 oocytes. **p < 0.01; ANOVA, Tukey post hoc pairwise comparisons.

(D) Dose response for A β o-induced current in oocytes expressing mGluR5 and PrP^C. Mean \pm SEM for n = 4–7 oocytes.

(E) Oocytes expressing mGluR5 and PrP^C were either treated for 1 min with 100 μ M Glu to maximally stimulate a response 3 min prior to testing for an A β o response, or not prestimulated with Glu. Alternatively, some oocytes were preincubated with 100 μ M BAPTA-AM for 60 min. After these pretreatments, the peak inward current was measured in response to 1.5 μ M A β o. Mean \pm SEM, n = 6–12 oocytes. **p < 0.005; ANOVA, Tukey post hoc pairwise comparisons. See also Figure S4.

voltage clamp during bath perfusion of A β o. G protein activation of phospholipase C leads to IP3, calcium release, and opening of an easily detected transmembrane chloride channel in oocytes (Saugstad et al., 1996; Strittmatter et al., 1993). Glu-induced responses of 3,000 nA peak current at -60 mV are detected in oocytes expressing mGluR1 or mGluR5 (Figures 3A and 3B). PrP^C does not alter the Glu responses. Bath application of A β o had no effect on conductances for uninjected oocytes, or oocytes expressing mGluR5 alone or PrP^C alone (Figure 3C). However, in the double-

expressing mGluR5-PrP^C oocytes, A β o produced an inward current of 300–450 nA, 10% of the Glu-induced current. We included only mGluR oocyte batches with Glu responses >500 nA. For preparations with <500 nA responses to Glu, A β o responses of 10% Glu magnitude may be present, but are not prominent. The kinetics and reversal potential for the A β o-induced signal were indistinguishable from that of Glu acting on mGluR5 alone (Figure 3A and not shown).

The specificity of the A β o-induced current of PrP^C-mGluR5 oocytes was examined. Although mGluR1 expression leads to equally strong Glu-induced current (Figures 3A and 3B), there is no detectable A β o-induced current (Figures 3A and 3C). PrP^C lacking the A β o binding domain, PrP Δ 23–111 (Chen et al., 2010; Laurén et al., 2009; Um et al., 2012), fails to support



Figure 4. Aß Species from Human AD Brains or Synthetic Aßo Increase Intracellular Calcium via PrP and mGluR5

(A) Change of intracellular calcium in E17 cortical neurons from WT, $Pmp^{-/-}$, or $Grm5^{-/-}$ mice in response to A β o, vehicle (Veh) or ionomycin (500 nM) was monitored by FLIPR calcium assay. Mean \pm SEM, n = 24–40 wells from three to five embryos per genotype.

(B) Quantification of calcium response induced by A β o or vehicle (Veh) from (A). *p < 0.05, **p < 0.01; ANOVA, Tukey post hoc pairwise comparisons. (C) Quantification of calcium response induced by oligomeric A β (A β o), monomeric A β (A β m), or vehicle (Veh). ***p < 0.001; ANOVA, Tukey post hoc pairwise comparisons.

(D) Intracellular calcium in E17 cortical neurons in response to human AD brain extracts (AD), n = 25, age-matched control brain (Con), n = 19 (1.5 μ g total protein/ml) or ionomycin (500 nM) monitored by FLIPR calcium assay. Mean \pm SEM, ***p < 0.001 by repeated-measures ANOVA for 10 s window after AD brain extract. (E) Quantification of calcium response induced by AD or Con extracts from (C). Each dot is from a different brain. ***p < 0.001 by Student's two-tailed t test.

Aßo-induced signaling through mGluR5 (Figure 3C). The anti-PrP^C antibody, 6D11, binds to residues 95-105 and prevents Aßo interaction (Chung et al., 2010; Laurén et al., 2009; Um et al., 2012). Preincubation with 6D11 blocks Aβo responses, but not Glu responses, in PrP^C-mGluR5 oocytes (Figures 3B and 3C). The A_βo-induced response has an EC₅₀ of 1 μ M monomer equivalent, an estimated 10 nM oligomer concentration (Figure 3D). A characteristic of G protein-mediated responses in Xenopus oocytes is strong desensitization. Maximal Glu stimulation nearly eliminates subsequent responses to Glu for 10-15 min. Consistent with the Aβo-PrP^C-mGluR5 responses sharing this pathway, pretreatment with Glu eliminates the response to subsequent A_βo (Figure 3E). In addition, pretreatment with cell permeable BAPTA-AM to chelate intracellular calcium abrogated the Aβo-induced signal (Figure 3E), as for Glu (Saugstad et al., 1996). Thus, Aβo interaction with a PrP^CmGluR5 complex mobilizes calcium stores. Although mGluR5mediated signaling to Fyn is as robust with $\mathsf{A}\beta\mathsf{o}\text{-}\mathsf{Pr}\mathsf{P}^\mathsf{C}$ as with Glu, signaling to calcium mobilization is substantially less effective for Aβo-PrP^C than with Glu as the mGluR5 ligand, so Aβo does not mimic Glu precisely.

Acute A β o-Induced Calcium Signals in Neuronal Culture Require mGluR5 and PrP^C

We considered whether ABo regulates neuronal calcium signaling through mGluR5 directly and acutely. Chronic Aβo-PrP^C-Fyn signaling can indirectly alter NMDA receptor (NMDAR) trafficking to modulate NMDA-induced calcium responses (Um et al., 2012). We used a calcium-sensitive fluorescent dye to assess direct immediate response to ABo in 21 days in vitro (DIV) cortical cultures. In low-density cultures, there is little direct calcium response to $A\beta o$ under the conditions that modulate NMDAR responses (data not shown; Um et al., 2012). With microscopic imaging, Aßo occasionally induces local calcium transients, but there is no generalization and measurement across a microtiter well does not detect a change (not shown). Higher density cultures exhibit spontaneous synchronized calcium increases (Figure S3A) that depend on network connectivity being suppressed by tetrodotoxin (TTX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), or 2-amino-5-phosphonopentanoic acid (APV) (Figures S3B and S3C) (Dravid and Murray, 2004). Under these conditions, A_bo induces an increase of intracellular calcium (Figure 4A). Averaging multiple wells smoothes random spontaneous signals (Figure S3A), and Aβo-induced responses are apparent (Figure 4A). This response is oligomer specific; no response is detected with monomeric A β (Figure 4C). In either $Grm5^{-/-}$ or $Prnp^{-/-}$ neurons, the spontaneous synchronized calcium signals are indistinguishable from WT (Figures S3D and S3E). However, A β o fails to induce a calcium signal in networks lacking either PrP^C or mGluR5 (Figure 4B).

To assess whether similar responses occur with human autopsy tissue, we utilized Tris-buffered saline (TBS)-soluble extracts from human brain, which we have previously characterized for PrP^{C} -interacting A_β species and for Fyn activation in mouse cultures (Table S1) (Um et al., 2012). In high-density cortical cultures, dialyzed TBS-soluble brain extracts from AD cases generate greater calcium mobilization than do control brain extracts (Figures 4D and 4E; p < 0.001). Moreover, the level of PrP(23–111)-interacting Aß in human brain samples correlates with the magnitude of the calcium response (Figure 4F). Preabsorption with anti-A β antibody removes PrP^C-interacting A β immunoreactivity (Figure S3H) and reduces the calcium response (Figures S3F and S3G). Preabsorption with PrP-Fc, but not control Fc resin, removes PrP^C-interacting species and reduces the calcium response (Figures 4G and S3I). Thus, PrP^C-interacting A β species in human AD brain TBS-soluble extracts stimulate calcium signals in high-density neuronal cultures. These responses require network connectivity, and are blocked by TTX, CNQX, or APV (Figure S4J). The mGluR5 antagonists, MPEP and MTEP, block the AD brain extract response (Figure 4I). In contrast, the mGluR1 antagonist MPMQ does not block the AD extract-induced response (Figure 4I). Furthermore, the ability of human AD brain extract to induce a calcium signal is eliminated in $Grm5^{-/-}$ or $Prnp^{-/-}$ neurons (Figure 4H).

We considered the source of calcium for the AD extractinduced signal and its relationship to Fyn. Thapsigargin (TG) pretreatment prevented signaling, consistent with release from endoplasmic reticulum stores (Figure 4I). In contrast, TG pretreatment did not prevent Fyn activation (Figures 4J and 4K), and inhibition of Fyn with saracatinib did not prevent calcium signaling (Figure 4I). Although Fyn and calcium signaling by AD extracts require both PrP^C and mGluR5, the two mediators appear pharmacologically separable.

Chronic A β o has the potential to desensitize mGluR5 calcium responses. We assessed this in two models: HEK293 cells expressing mGluR5 and PrP^C, and low density cultured neurons. HEK-mGluR5 cells respond to Glu with calcium elevation and there is no effect of A β o preincubation (Figures S4A, S4B, and

See also Figure S3.

⁽F) Correlation between AD extract-induced calcium and PrP(23-111)-interacting A β species is plotted. Each point is from a different brain sample. Pearson coefficient of linear correlation reported with two-tailed p.

⁽G) Calcium response induced by AD extracts preabsorbed with Fc (AD Fc) or PrP^{C} -Fc resin (AD PrP-Fc), or Con extracts preabsorbed with Fc (Con Fc) or PrP^{C} -Fc resin (Con PrP-Fc). *p < 0.05, **p < 0.01; ANOVA, Tukey post hoc pairwise comparisons.

⁽H) Calcium response induced by AD or Con extracts in E17 cortical neurons from WT, $Pmp^{-/-}$ or $Grm5^{-/-}$ mice. Mean ± SEM, n = 3 independent embryos for each genotype. *p < 0.05, **p < 0.01; ANOVA, Tukey post hoc comparisons.

⁽I) Calcium response induced by AD or Con extracts in WT cortical neurons. Neurons were pretreated with 100 μ M MPEP, 100 μ M MTEP, 10 μ M MPMQ, 500 nM saracatinib for 1 hr or 100 nM thapsigargin for 24 hr prior to AD extract. *p < 0.05, **p < 0.01, ***p < 0.001; ANOVA, Tukey post hoc pairwise comparisons to AD extract only samples.

⁽J) WT cortical neurons were treated with 0 or 1 µM Aβo for 15 min. Indicated sample was treated with 100 nM thapsigargin for 24 hr prior to Aβo. Lysates were analyzed by anti-phospho-SFK or anti-Fyn immunoblot.

⁽K) Quantification of phospho-SFK level in the lysate normalized to Fyn from three experiments. Mean ± SEM, *p < 0.05; **p < 0.01; ANOVA, Tukey post hoc pairwise comparisons.

S4F). For HEK cells stably expressing mGluR5 and PrP^C, baseline Glu responses are similar, but A β o preincubation suppresses Glu responses by 50% in independent clones (Figures S4A, S4C, S4D, and S4F). This effect requires the A β o-binding PrP(23–111) domain because clones expressing a truncation mutant fail to bind A β o (Figure S5A), and show no A β o suppression of Glu-induced calcium (Figures S4E and S4F). A β oinduced suppression of mGluR5 responses is also observed in neurons. In low density cultures, there are no spontaneous synchronized calcium oscillations, but the mGluR5 agonist, (*S*)-3,5-dihydroxyphenylglycine (DHPG), results in synchronized oscillations (Figure S4G). Pretreatment with A β o nearly eliminates DHPG-induced oscillations in WT neurons (90% inhibition; Figures S4H and S4I). The A β o suppression of DHPG-induced oscillations is limited to 40% in *Prmp^{-/-}* cultures (Figure S4I).

Multiple mechanisms contribute to mGluR5 desensitization, including protein kinase C, calcium/calmodulin binding, and receptor internalization. We assessed the effect of A β o and the group I mGlu receptor agonist, DHPG, on cell surface mGluR5 levels using biotinylation of live neurons with a cell impermeable reagent (Figures S4J–S4L). At 1 hr, DHPG reduces surface mGluR5 by 20%, as described (Choi et al., 2011). In contrast, A β o treatment generates a PrP^C-dependent 25% increase in surface/total mGluR5 ratios (Figures S4J–S4L). The increase after A β o addition may reflect "trapping" of mGluR5 in relatively immobile complexes (Renner et al., 2010). Despite this difference between A β o and DHPG in mGluR5 trafficking, A β o treatment suppresses mGluR5 signaling (Figures S4H and S4I).

$A\beta o$ Signals through PrP^{C} and mGluR5 to Protein Translation Machinery

Metabotropic GluRs have effects on protein translation (Lüscher and Huber, 2010), as well as calcium release and Fyn. We examined whether A_bo-PrP^C-mGluR5 coupling might alter phosphorylation of eukaryotic elongation factor 2 (eEF2). The mGluR5 agonist, DHPG, drives eEF2-56T phosphorylation (Figure S5A). ABo treatment has a similar effect on eEF2 phosphorylation (Figures 5A, 5B, and S5A-S5C). Mediation of the A_βo effect by mGluR5 is demonstrated by inhibition with MTEP (Figures S5B and S5C). In contrast, the mGluR1 antagonist, MPMQ, does not prevent A_bo-induced eEF2 phosphorylation (Figures S5D and S5E). Genetic analysis with Prnp^{-/-} and Grm5^{-/-} neurons confirms that the ABo effect on eEF2 phosphorylation depends on these proteins (Figures 5A and 5B). A_bo-induced eEF2 phosphorylation is detected in dendrites, and is absent in $Prnp^{-/-}$ and $Grm5^{-/-}$ neurons (Figures 5C and 5D). The addition of both A β o and DHPG produced no greater eEF2 phosphorylation than either ligand alone, consistent with occlusive action (Figures S5F and S5G).

Dendritic translation of Arc is under mGluR5 control, via an eEF2-dependent mechanism (Park et al., 2008). As predicted from the mGluR5-mediated action of A β o on p-eEF2, dendritic Arc immunoreactivity is elevated after 5 min A β o exposure (Figures 5E and 5F) and Arc immunoblot signal increases in brain slices (Figures S5H and S5I).

To extend the AD relevance of these observations, we tested whether human AD extracts generated a similar pattern. Pooled TBS-soluble extracts from AD brain, but not control brain, elevated eEF2 phosphorylation in WT mouse 21 DIV neurons (Figures 5G and 5H). This signaling is not observed in $Grm5^{-/-}$ and $Prnp^{-/-}$ cultures. Thus, A β o-PrP^C complexes signal through mGluR5 to modify Fyn activation, calcium levels, and eEF2 phosphorylation.

We considered how A β o-induced eEF2 phosphorylation relates to Fyn activation and calcium signaling. Saracatinib suppresses basal p-SFK levels and prevents A β o stimulation of Fyn (Figure 5I) and also prevents A β o-induced eEF2 phosphorylation (Figures 5I and 5K). Thapsigargin pretreatment prevents AD extract-induced calcium signaling (Figure 4H) and also prevents eEF2 phosphorylation (Figures 5J and 5K). Thus, eEF2 regulation by A β o requires both Fyn and calcium signaling.

Cellular Effects of Aßo Require mGluR5

Time-lapse imaging of myristoyl-GFP expressing neurons (Figure 6A) has shown that A β o leads to a loss of 10% of dendritic spines over 5 hr by a *Pmp*- and *Fyn*-dependent mechanism (Um et al., 2012). Acute DHPG treatment leads to immature spines, reduced spine volume, and fewer surface AMPA receptors during chemical long-term depression (LTD) (Abu-Elneel et al., 2008; Moult et al., 2006; Vanderklish and Edelman, 2002), whereas chronic DHPG results in decreased spine density (Shinoda et al., 2010). We tested whether mGluR5 blockade prevents A β o-induced spine loss. MPEP treatment does not alter baseline spine stability over 5 hr, but prevents A β o-induced loss (Figures 6A and 6B). Deletion of mGluR5 also prevents A β o-induced spine loss (Figures 6A and 6C). Thus, mGluR5 participates in morphological effects of A β o-PrP^C complexes.

The A β o-PrP^C-Fyn pathway contributes to short-term lactate dehydrogenase (LDH) release from neurons (Um et al., 2012). Either addition of the mGluR5 negative allosteric modulator, MTEP, or deletion of the *Grm5* gene prevents A β o-induced LDH release (Figures 6D and 6E).

mGluR5 Antagonist Reverses Behavioral Deficits in FAD Transgenic Mice

The double transgenic APPswe/PS1∆E9 (APP/PS1) mouse exhibits normal behavior through age 6 months, and then progressively loses learning and memory (Gimbel et al., 2010; Jankowsky et al., 2003). We considered a role for mGluR5. As reported (Lu et al., 1997; Xu et al., 2009), constitutive mGluR5 deletion impairs performance (Figure S6). Treatment of WT mice with high dose MTEP, 40 mg/kg, impairs alertness and electroencephalogram (EEG) amplitude (Figure S6). Thus, we sought to achieve partial inhibition of mGluR5 with 15 mg/kg MTEP, which does not alter EEG rhythm (Figure S6).

First, we treated WT and APP/PS1 mice with vehicle or MTEP and assessed spatial learning in the radial arm water maze (Figure 7A). Mice at 9 months age were randomized to blinded treatment with vehicle or MTEP (15 mg/kg, two times a day for 10 days) for 3 days prior to, and then for 7 days throughout memory testing. Learning is impaired in transgenics relative to WT, but is fully recovered with MTEP. There is a significant interaction of genotype and drug (two-way repeated-measures [RM] ANOVA: APP/PS1 × MTEP interaction, p < 0.01; APP/ PS1, p < 0.01; MTEP, p < 0.01).





Figure 5. eEF2 Phosphorylation Is Enhanced by A β o through PrP and mGluR5 (A) DIV21 cortical neurons from WT, $Prmp^{-/-}$, or

 $Grm5^{-/-}$ mice were treated with 0 or 1 μM A βo for 5 min. Lysates were analyzed by anti-phospho-eEF2, or anti-eEF2 immunoblot. GAPDH is loading control.

(B) Phospho-eEF2 level in the lysate normalized to eEF2. WT, n = 3; $\textit{Prnp}^{-/-}$, n = 3; $\textit{Grm5}^{-/-}$, n = 3. Mean \pm SEM, **p < 0.01; Student's two-tailed t test.

(C) Immunohistology of DIV21 WT, $Prnp^{-/-}$, or $Grm5^{-/-}$ cortical neurons stained with antiphospho-eEF2 and anti-MAP2 antibodies after exposure of 0 or 1 μ M A β o. Scale bar represents 10 μ m.

(D) Phospoho-eEF2 intensity normalized to MAP2 signal. WT, n = 3; $Prnp^{-/-}$, n = 3; $Grm5^{-/-}$, n = 3. Eight to ten images were analyzed per experiment. Mean \pm SEM, ***p < 0.001; Student's two-tailed t test.

(E) Immunohistology of DIV 21 WT neurons stained with anti-Arc antibody after 1 μ M A β o for 5 min. Scale bar represents 5 μ m.

(F) Arc intensity in dendrites after 1 μM A βo or 100 μM DHPG for 5 min. For dendritic Arc levels, average pixel intensity was measured in secondary dendrites 10 μm from first branch point. WT, n = 6. Mean \pm SEM, **p < 0.01, *p < 0.05; ANOVA, Tukey post hoc pairwise comparisons.

(G) DIV21 cortical neurons from WT, $Grm5^{-/-}$, or $Prmp^{-/-}$ mice were treated with human control or AD brain extracts (30 µg total protein/ml) for 5 min. Lysates were analyzed by anti-phospho-eEF2 or anti-eEF2 immunoblot.

(H) Phospho-eEF2 level normalized to eEF2 from (G). WT, n = 6; $Grm5^{-/-}$, n = 4; $Prmp^{-/-}$, n = 3. Mean ± SEM, *p < 0.05; **p < 0.01; ***p < 0.001; ANOVA, Tukey post hoc pairwise comparisons. (I and J) WT cortical neurons were treated with 0 or 1 μ M A β o for 15 min. Indicated samples were treated with 500 nM saracatinib for 1 hr (I) or 100 nM thapsigargin (TG) for 24 hr (J) prior to A β o. Lysates were analyzed by anti-phospho-SFK, anti-Fyn, anti-phospho-eEF2, or anti-eEF2 immunoblot. GAPDH is loading control.

(K) Phospho-eEF2 level normalized to eEF2 from three experiments. Mean \pm SEM, *p < 0.05; ANOVA, Tukey post hoc pairwise comparisons. See also Figure S5.

We extended this finding with alternative memory tests and additional cohorts. Without MTEP treatment, APP/PS1 mice at 9 months age are unable to distinguish novel and familiar objects, whereas WT mice exhibit novel object preference (Figure 7B; p < 0.001 for WT). Exploration of two novel objects is not different between genotypes during acclimation (not shown). MTEP-treated APP/PS1 mice recover a novel object preference (Figure 7B; p < 0.001 for both WT and APP/PS1 with MTEP).

A separate cohort of APP/PS1 was tested in the Morris water maze. Without treatment, the APP/PS1 mice show greater latencies to locate a hidden platform relative to WT across learning trials (Figure 7C; RM-ANOVA, p < 0.001), and spend less time in the target quadrant during a probe trial for memory 24 hr later (Figure 7D; ANOVA p < 0.001). In contrast, MTEPtreated APP/PS1 mice are indistinguishable from untreated WT or MTEP-treated WT mice in learning and memory (Figures 7C and 7D), but are different from untreated APP/PS1 (Figures 7C and 7D; p < 0.001). There is a significant interaction of genotype and drug (two-way RM-ANOVA in Figure 7C for APP/ PS1 × MTEP interaction, p < 0.001; two-way ANOVA in Figure 7D, p < 0.001).

We also administered MTEP to 3XTg mice expressing mutant APP, PS1, and Tau (Oddo et al., 2003). At 8–9 months, these mice perform normally in the Morris water maze (not shown), but are impaired in novel object recognition (Figure 7E). After randomization to MTEP or vehicle, the 3XTg mice were assessed



Figure 6. Aβo-Induced Toxicity Is Blocked by mGluR5 Antagonists

(Å) Hippocampal neurons (21 DIV) expressing myristoyl-GFP from WT or $Grm5^{-/-}$ mice were imaged for 6 hr with or without MPEP (100 μ M). A β o (500 nM monomer equivalent) or vehicle was added at 1 hr. Note loss of several spines after A β o addition in the WT neurons. Scale bar represents 1 μ m.

(B and C) Dendritic spine loss over 5 hr is plotted as a function of A_βo and MPEP (B) or *Grm5* genotype (C). Mean \pm SEM, n = 3–5 cultures from separate mice of each genotype. *p < 0.05; **p < 0.01; ANOVA, Tukey post hoc pairwise comparisons.

(D) Neurons at DIV21 from WT mice were treated with 0 or 1 μ M A β o for 2 hr. Some cultures were treated with 100 μ M MTEP for 1 hr prior to A β o. Cell toxicity was determined by LDH release. Mean \pm SEM, n = 3 experiments. **** $\rho < 0.001$; ANOVA, Tukey post hoc pairwise comparisons. (E) WT or $Grm5^{-/-}$ cortical neurons were treated with 0 or 1 μ M A β o for 2 hr. Toxicity was determined by LDH release. Mean \pm SEM, n = 4 experiments. *** $\rho < 0.01$; ANOVA, Tukey post hoc pairwise comparisons.

WT and APP/PS1 transgenic mice at 10 months age were treated for 10 days with MTEP, 15 mg/kg two times a day. As expected, control APP/PS1 mice exhibit a 25%-30% decrease in area occupied by presynaptic synaptophysin and postsynaptic PSD-95 immunoreactivity in the dentate gyrus (Figures 8A-8C). The loss of stained synaptic area was fully rescued by a 10-day course of MTEP (Figures 8D and 8E). For WT mice, MTEP did not alter synaptic density. We also assessed synaptic density ultrastructurally, identifying synaptic profiles by the presence of a postsynaptic density and presynaptic vesicles (Figure 8F). Synapse density in transgenic dentate gyrus increased by 20% with MTEP treatment (Figure 8G).

DISCUSSION

This study delineates a direct role for mGluR5 in A β o-related pathophysiology. Of transmembrane PSD proteins, only mGluR5 supports coupling of A β o-PrP^C

for novel object recognition (Figure 7G). MTEP-treated 3XTg mice show a novel object preference (p < 0.01), but vehicle-treated mice do not. Thus, MTEP reverses memory deficits in two transgenic AD mice.

We considered whether improved memory with MTEP is correlated with a reversal of synaptic loss. A separate cohort of to Fyn activation. Intracellular calcium and protein translation are also linked to $A\beta o$ -PrP^C engagement via mGluR5. An mGluR5 dependence of signaling is observed for TBS-soluble extracts of AD brain as well as synthetic A βo , emphasizing the disease relevance. A coreceptor role for mGluR5 is required for dendritic spine loss and transgenic memory impairment.





Figure 7. mGluR5 Antagonist Reverses Learning and Memory Deficits in AD Mouse Models

(A) Spatial learning is plotted as the number of errors in finding a hidden platform in a radial arm water maze at age 9 months. Mean ± SEM for vehicle-treated C57BL/6, n = 11; MTEP-treated C57BL/6. n = 12: vehicle-treated APP/PS1. n = 10: MTEP-treated APP/PS1, n = 10. Performance differed across the last 15 swims by genotype and treatment (two-way RM-ANOVA APP/PS1, p < 0.001; MTEP, p < 0.001). There was an interaction between genotype and treatment (two-way RM-ANOVA APP/PS1 × MTEP p < 0.001). By Tukey post hoc pairwise comparisons across trials, the vehicle-treated APP/PS1 group differed from each of the other groups (p < 0.001), whereas none of the other groups differed from each other (p > 0.05). For indicated trial blocks, the vehicletreated APP/PS1 group differed from each of the other groups (*p < 0.05; **p < 0.01; ***p < 0.001), whereas none of the other groups differed from each other (p > 0.05).

(B) Time spent with a novel object for the same mice as (A): vehicle C57BL/6, n = 11; MTEP C57BL/6, n = 12; vehicle APP/PS1, n = 9; MTEP APP/PS1, n = 9. After being acclimated to an object, vehicle APP/PS1 mice showed no preference for a novel object (two-tailed Student's t test p > 0.05). The other three groups showed preference for a novel object (two-tailed Student's t test p < 0.001).

(C) Spatial learning is plotted as latency to find a hidden platform in a Morris water maze at age 9 months in a cohort different from (A). Mean \pm SEM for untreated (naive) C57BL/6, n = 32; MTEP-treated C57BL/6, n = 13; untreated APP/PS1, n = 38; MTEP-treated APP/PS1, n = 17. Performance differed across the last 16 swims by genotype and treatment (two-way RM-ANOVA for APP/PS1, p < 0.001; for MTEP, p < 0.001). There was an interaction between genotype and treatment (two-way RM-ANOVA, APP/PS1 × MTEP p < 0.001). By Tukey post hoc pairwise comparisons across trials, the vehicle-treated APP/PS1 group differed

from each of the other groups (***p < 0.001), whereas none of the other groups differed from each other (p > 0.05). For specific trial blocks, the untreated APP/PS1 group differed from each of the other groups (*p < 0.05; ***p < 0.001), whereas none of the other groups differed from each other (p > 0.05).

(D) Performance during a 60 s probe trial, 24 hr after learning, where time spent in the target quadrant was measured. Random chance is 25%. Mean \pm SEM for the groups in (C). Target quadrant time differed by genotype and treatment (two-way ANOVA APP/PS1, p < 0.001; MTEP, p < 0.01). There was an interaction between genotype and treatment (ANOVA, APP/PS1 × MTEP p < 0.001). By Tukey post hoc, the untreated APP/PS1 group differed from others (p < 0.001), whereas none of the other groups differed from each other (p > 0.05).

(E) WT and 3XTg mice of the same genetic background at 8–9 months of age were tested for novel object recognition. WT mice show preference the novel object (two-tailed Student's t test p < 0.05), but 3XTg mice show no preference (p > 0.05). Mean \pm SEM.

(F) The effect of MTEP administration on object recognition is tested in 3XTg mice at age 8 months. Mean \pm SEM for vehicle 3XTg, n = 12; MTEP 3XTg, n = 8. Mice that received MTEP had a significant preference for the novel object (two-tailed Student's t test p < 0.01) whereas vehicle 3XTg mice showed no preference (two-tailed Student's t test p > 0.05).

See also Figure S6.

Together, these findings delineate mGluR5 activation as a critical step in $A\beta o$ signal transduction with potential for therapeutic intervention.

Dysregulation of mGluR5 Signaling in AD

Several previous studies have indirectly implicated mGluR5 in A β o signaling and in AD. A β o alters mGluR5 trafficking in neu-

rons, with reduced diffusion, clustering, aberrant activation, and neurotoxicity (Renner et al., 2010). The results here provide a PrP^{C} -based mechanism for these findings and for downstream signaling. A β o from synthetic, cellular, and human AD brain sources suppresses LTP and enhances LTD. These actions are mimicked by mGluR5 agonists and inhibited by mGluR5 antagonists (Rammes et al., 2011; Shankar et al., 2008; Wang et al.,



Figure 8. Synaptic Markers Recover After 10-Day Treatment With mGluR5 Antagonist

WT and APP/PS1 mice at 10 months of age were treated with 15 mg/kg of MTEP, or saline, by intraperitoneal injection twice a day for 10 days and then sacrificed for histological analysis.

(A–C) The molecular layer of the dentate gyrus of the indicated groups was stained with anti-PSD-95 antibody and imaged with a confocal microscope and a 60× objective lens. Scale bar represents 6 μ m.

(D) Fractional area of immunoreactive puncta for PSD-95 from images as in (A–C). p < 0.05, ANOVA with post hoc pairwise Fisher's least significant difference (LSD). Mean \pm SEM, n = 5 mice per group with three images per mouse.

(E) Fractional area of immunoreactive puncta for synaptophysin. p < 0.05, ANOVA with post hoc pairwise LSD. Mean \pm SEM, for n = 5 mice per group with three images per animal.

(F) Ultrastructure of the molecular layer of the dentate gyrus from a vehicle APP/PS1 mouse. Arrows point to synapses scored in (G). Magnification is 20,500 ×. Scale bar represents 1 µm.

(G) Synapses were counted in a 25 μ m² area in drug-treated and saline-treated samples. p < 0.05, two-tailed Student's t test. Mean ± SEM, n = 5 mice per group with 30 images per mouse.

2004). In human AD, mGluR ligand binding is decreased in brain relative to controls and the loss is correlated with disease progression (Albasanz et al., 2005). Proteins titrated by mGluRs, eEF-2, Arc, and p70 S6 kinase are dysregulated in AD brain (An et al., 2003; Li et al., 2005; Wu et al., 2011).

Signaling Downstream of mGluR5

Canonical mGluR5 signaling couples to G_q/G_{11} GTPases that activate phospholipase C to produce IP3 and release calcium stores (Lüscher and Huber, 2010). mGluR5 also modulates plasma membrane potassium, calcium, and transient receptor

potential channels. Src family tyrosine kinases, including Fyn, have been implicated in linking to NMDA-R (Heidinger et al., 2002; Nicodemo et al., 2010). The proline rich tyrosine kinase 2 (Pyk2) participates in Src/Fyn interaction with mGluR signaling (Heidinger et al., 2002; Nicodemo et al., 2010). The calcium/ calmodulin-dependent eEF2 kinase (eEF2K) is bound to mGluR5 in the basal state, but is released during activation to phosphorylate eEF2 (Lüscher and Huber, 2010). Phospho-eEF2 reduces global translation, but allows increased Arc/Arg3.1 expression (Park et al., 2008). The Homer family plays a role in mGluR signaling, interacting with receptor and eEF2K (Hu et al., 2010; Lüscher and Huber, 2010; Ronesi et al., 2012). Homer interactions with SHANK contribute to PSD localization, specific isoforms have roles in homeostatic scaling.

We show that A β o-PrP^C complexes lead to several mGluR5 outputs. Fyn activation by A β o in cortical neurons requires mGluR5 genetically and pharmacologically. Fyn is implicated in A β o-induced dysregulation of NMDA-R trafficking and activation (Um et al., 2012). Because Fyn binds directly to Tau (Ittner et al., 2010; Lee et al., 2004), this may have implications for AD beyond dysregulation of GluRs.

The A β o-PrP^C-mGluR5 complex also activates phospholipase C, as detected by monitoring calcium-activated chloride channels in oocytes. The ability of A β o or human AD brain TBS-soluble extract to increase calcium in cortical neurons requires mGluR5 and PrP^C. The calcium increase in neurons may occur by the IP3 pathway and also by regulation of NMDA-Rs. Fyn activation by A β o-PrP^C is as strong as that by Glu, whereas calcium mobilization appears to be an order of magnitude less effective for A β o-PrP^C than for Glu. Divergence in A β o-PrP^C-mGluR5 signaling requires further study.

Protein translation plays a major role in mGluR5 signaling (Lüscher and Huber, 2010). The phosphorylation of eEF2 is increased by $A\beta$ o-PrP^C as much as by mGluR5 agonist. Therefore dysregulation of translation may contribute to synaptic dysfunction in AD. Arc is one protein target of mGluR5 signaling that is upregulated by $A\beta$ o acutely. Calcium and Fyn are independent mediators, which appear to cooperate in eEF2 phosphorylation.

We show that mGluR5 antagonists prevent A β o-induced spine loss from hippocampal neurons in vitro and in vivo. Critically, MTEP reverses memory deficits in transgenic AD models. Multiple signaling pathways from A β o-PrP^C-mGluR5 complexes are likely to participate. For spine loss in vitro, Fyn is required (Um et al., 2012), but other mGluR5 signaling components may contribute. Protein translation, calcium release, and Fyn kinase are each known to participate in plasticity, learning, and memory.

Feedback of mGluR5 Signaling on A β Levels

The mGluR5 pathway may also feedback on APP/A β metabolism to exacerbate AD. Specifically, mGluR5 agonism elevates Arc, which enhances A β production by participating in APP and PS1 colocalization within endocytic vesicles (Wu et al., 2011). Shared pathways between AD and Fragile X have been reported (Sokol et al., 2011). The FMRP protein normally represses APP translation. Transgenic mice with both APP transgenes and loss of FMRP have enhanced phenotypes, including audiogenic seizures, which are treatable with MPEP.

mGluR Specificity and Localization

Of mGluR receptors, only mGluR1 and mGluR5 interact with Fyn and PrP^C. Only mGluR5 mediates Aβo-induced stimulation of Fyn and calcium signaling in oocytes. *Grm5* gene deletion and mGluR5-specific compounds reverse Aβo phenotypes, including Fyn activation, neuronal calcium mobilization, eEF2 phosphorylation, spine loss, LDH release, and memory deficits. The mGluR1-specific antagonist, MPMQ, does not block. Thus, mGluR5 appears to be specifically involved in Aβo-PrP^C action.

PrP^C, mGluR5, and Fyn have all been localized to the PSD by subcellular fractionation. For PrP^C and Fyn, high-resolution in situ protein localization in brain has not been reported. For mGluR5, imaging confirms a postsynaptic localization and indicates that mGluR5 is dynamically located at the PSD periphery (Lujan et al., 1996). Dynamic regulation of mGluR5 localization by Aβo has been observed (Renner et al., 2010).

Short-Term Activation versus Longer-Term Desensitization

Although ionotropic receptors function rapidly, metabotropic receptors are slow and show prominent desensitization. A β o levels are highly unlikely to fluctuate on the time scale of synaptic transmission, so A β o-PrP^C complexes may engage mGluR5 and elicit a degree of desensitization that prevents responsiveness to cyclic changes in Glu. Thus, mGluR5 may be dysregulated by acute activation and chronic desensitization.

Alternate Ligands and Accessory Ectodomains for mGluRs

Activation of mGluR5 by Aßo-PrP^C complexes expands the repertoire of metabotropic glutamate receptors. Certain other G protein coupled receptors (GPCRs) respond to more than one ligand, and interact extracellularly with accessory subunits or coreceptors. Melanocortin receptors recognize both melanocortin agonists and Agouti/AgRP antagonists with specificity modified by MRAPs (Breit et al., 2011). Calcitonin and related receptors have ligand preference altered by transmembrane RAMPs (Hay et al., 2006). Noncanonical signaling by Hedgehogs and Wnts through Smoothened and Frizzleds to heterotrimeric G proteins depends on ligand interaction with Patched and LRP5/6, respectively (Angers and Moon, 2009; Robbins et al., 2012). Ectodomain accessory proteins for mGluRs have not been recognized previously, so PrP^C is unique. The only previously known endogenous ligand for mGluR5 is Glu, so the action of Aβo-PrP^C is distinct from precedent. Our findings raise the possibility that mGluR5 may be regulated physiologically by molecules other than Glu.

Targeting mGluR5 for AD Therapy

The delineation of an A β o-PrP^C-mGluR5-Fyn pathway provides potential targets for AD intervention. Antibodies that block A β o binding to PrP^C reverse memory deficits in transgenic AD mice (Chung et al., 2010), and we show that a mGluR5 negative allosteric modulator has a similar effect. However, full mGluR5 antagonism may have deleterious effects on neuronal function and impairment of baseline attention (Lüscher and Huber, 2010; Simonyi et al., 2010). Deficits of contextual fear conditioning and inhibitory learning are observed in the absence of mGluR5 (Xu et al., 2009), and mGluR5 function may contribute to healthy brain aging (Lee et al., 2005; Ménard and Quirion, 2012; Nicolle et al., 1999). Optimal intervention may therefore be designed to prevent $A\beta$ o-PrP^C activation of mGluR5, without modifying Glu activation of mGluR5.

EXPERIMENTAL PROCEDURES

Mouse Strains

All animal studies were conducted with approval of the Yale Institutional Animal Care and Use Committee. The mouse strains have been described previously (Gimbel et al., 2010; Jankowsky et al., 2003; Lu et al., 1997; Oddo et al., 2003).

Cell Culture and Biochemistry

Standard procedures were utilized, including the assessment of intracellular calcium level in neuronal culture (Um et al., 2012) and voltage clamp recording from *X. laevis* oocytes (Laurén et al., 2009; Strittmatter et al., 1993). Freshfrozen postmortem human prefrontal cortex from the brains of AD patients were obtained, as approved by Institutional Review Board collected at New York University and at Yale. Particulate components were removed from TBS homogenates by centrifugation at 100,000 × g for 30 min.

MTEP Treatment and Memory Testing of Transgenic Mice

Mice were randomized to treatment groups and the experimenter was unaware of treatment status throughout behavioral testing. Procedures for Morris water maze testing have been described (Gimbel et al., 2010).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one table, and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.06.036.

ACKNOWLEDGMENTS

We thank Yiguang Fu and Stefano Sodi for excellent technical support. We thank Xinran Liu of the Yale Center for Cell Imaging for advice on synapse ultrastructure analysis. S.M.S. is a cofounder of Axerion Therapeutics, seeking to develop NgR- and PrP-based therapeutics. H.B.N. is an Ellison Medical Foundation AFAR Postdoctoral Fellow and S.M.S. is a member of the Kavli Institute for Neuroscience at Yale University. We acknowledge support from the National Institutes of Health to S.M.S., A.J.K., and T.W., and from the Falk Medical Research Trust and the Alzheimer's Association to S.M.S.

Accepted: June 17, 2013 Published: September 4, 2013

REFERENCES

Abu-Elneel, K., Ochiishi, T., Medina, M., Remedi, M., Gastaldi, L., Caceres, A., and Kosik, K.S. (2008). A delta-catenin signaling pathway leading to dendritic protrusions. J. Biol. Chem. 283, 32781–32791.

Albasanz, J.L., Dalfó, E., Ferrer, I., and Martín, M. (2005). Impaired metabotropic glutamate receptor/phospholipase C signaling pathway in the cerebral cortex in Alzheimer's disease and dementia with Lewy bodies correlates with stage of Alzheimer's-disease-related changes. Neurobiol. Dis. *20*, 685–693.

An, W.L., Cowburn, R.F., Li, L., Braak, H., Alafuzoff, I., Iqbal, K., Iqbal, I.G., Winblad, B., and Pei, J.J. (2003). Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. Am. J. Pathol. *163*, 591–607.

Angers, S., and Moon, R.T. (2009). Proximal events in Wnt signal transduction. Nat. Rev. Mol. Cell Biol. *10*, 468–477.

Barry, A.E., Klyubin, I., Mc Donald, J.M., Mably, A.J., Farrell, M.A., Scott, M., Walsh, D.M., and Rowan, M.J. (2011). Alzheimer's disease brain-derived amyloid-β-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. J. Neurosci. *31*, 7259–7263.

Bizat, N., Peyrin, J.M., Haïk, S., Cochois, V., Beaudry, P., Laplanche, J.L., and Néri, C. (2010). Neuron dysfunction is induced by prion protein with an insertional mutation via a Fyn kinase and reversed by sirtuin activation in Caenorhabditis elegans. J. Neurosci. *30*, 5394–5403.

Breit, A., Büch, T.R., Boekhoff, I., Solinski, H.J., Damm, E., and Gudermann, T. (2011). Alternative G protein coupling and biased agonism: new insights into melanocortin-4 receptor signalling. Mol. Cell. Endocrinol. *331*, 232–240.

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

Choi, K.Y., Chung, S., and Roche, K.W. (2011). Differential binding of calmodulin to group I metabotropic glutamate receptors regulates receptor trafficking and signaling. J. Neurosci. *31*, 5921–5930.

Chung, E., Ji, Y., Sun, Y., Kascsak, R.J., Kascsak, R.B., Mehta, P.D., Strittmatter, S.M., and Wisniewski, T. (2010). Anti-PrPC monoclonal antibody infusion as a novel treatment for cognitive deficits in an Alzheimer's disease model mouse. BMC Neurosci. *11*, 130.

Cissé, M., Halabisky, B., Harris, J., Devidze, N., Dubal, D.B., Sun, B., Orr, A., Lotz, G., Kim, D.H., Hamto, P., et al. (2011). Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. Nature *469*, 47–52.

Collins, M.O., Husi, H., Yu, L., Brandon, J.M., Anderson, C.N., Blackstock, W.P., Choudhary, J.S., and Grant, S.G. (2006). Molecular characterization and comparison of the components and multiprotein complexes in the post-synaptic proteome. J. Neurochem. *97*(*Suppl 1*), 16–23.

Dravid, S.M., and Murray, T.F. (2004). Spontaneous synchronized calcium oscillations in neocortical neurons in the presence of physiological [Mg(2+)]: involvement of AMPA/kainate and metabotropic glutamate receptors. Brain Res. *1006*, 8–17.

Emes, R.D., Pocklington, A.J., Anderson, C.N., Bayes, A., Collins, M.O., Vickers, C.A., Croning, M.D., Malik, B.R., Choudhary, J.S., Armstrong, J.D., and Grant, S.G. (2008). Evolutionary expansion and anatomical specialization of synapse proteome complexity. Nat. Neurosci. *11*, 799–806.

Freir, D.B., Nicoll, A.J., Klyubin, I., Panico, S., Mc Donald, J.M., Risse, E., Asante, E.A., Farrow, M.A., Sessions, R.B., Saibil, H.R., et al. (2011). Interaction between prion protein and toxic amyloid β assemblies can be therapeutically targeted at multiple sites. Nat Commun. 2, 336.

Gimbel, D.A., Nygaard, H.B., Coffey, E.E., Gunther, E.C., Laurén, J., Gimbel, Z.A., and Strittmatter, S.M. (2010). Memory impairment in transgenic Alzheimer mice requires cellular prion protein. J. Neurosci. *30*, 6367–6374.

Grant, S.G., O'Dell, T.J., Karl, K.A., Stein, P.L., Soriano, P., and Kandel, E.R. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. Science *258*, 1903–1910.

Hay, D.L., Poyner, D.R., and Sexton, P.M. (2006). GPCR modulation by RAMPs. Pharmacol. Ther. *109*, 173–197.

Heidinger, V., Manzerra, P., Wang, X.Q., Strasser, U., Yu, S.P., Choi, D.W., and Behrens, M.M. (2002). Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current: mediation through the Pyk2/Src-family kinase pathway in cortical neurons. J. Neurosci. 22, 5452–5461.

Holtzman, D.M., Morris, J.C., and Goate, A.M. (2011). Alzheimer's disease: the challenge of the second century. Sci. Transl. Med. 3, sr1.

Hu, J.H., Park, J.M., Park, S., Xiao, B., Dehoff, M.H., Kim, S., Hayashi, T., Schwarz, M.K., Huganir, R.L., Seeburg, P.H., et al. (2010). Homeostatic scaling requires group I mGluR activation mediated by Homer1a. Neuron *68*, 1128–1142.

Ittner, L.M., Ke, Y.D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wölfing, H., Chieng, B.C., Christie, M.J., Napier, I.A., et al. (2010). Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell *142*, 387–397.

Jankowsky, J.L., Xu, G., Fromholt, D., Gonzales, V., and Borchelt, D.R. (2003). Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. J. Neuropathol. Exp. Neurol. *62*, 1220–1227.

Jonsson, T., Atwal, J.K., Steinberg, S., Snaedal, J., Jonsson, P.V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., et al. (2012). A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature 488, 96–99.

Larson, M., Sherman, M.A., Amar, F., Nuvolone, M., Schneider, J.A., Bennett, D.A., Aguzzi, A., and Lesné, S.E. (2012). The complex PrP(c)-Fyn couples human oligomeric A β with pathological tau changes in Alzheimer's disease. J. Neurosci. *32*, 16857–71a.

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

Lee, G., Thangavel, R., Sharma, V.M., Litersky, J.M., Bhaskar, K., Fang, S.M., Do, L.H., Andreadis, A., Van Hoesen, G., and Ksiezak-Reding, H. (2004). Phosphorylation of tau by fyn: implications for Alzheimer's disease. J. Neurosci. *24*, 2304–2312.

Lee, H.K., Min, S.S., Gallagher, M., and Kirkwood, A. (2005). NMDA receptorindependent long-term depression correlates with successful aging in rats. Nat. Neurosci. *8*, 1657–1659.

Li, X., Alafuzoff, I., Soininen, H., Winblad, B., and Pei, J.J. (2005). Levels of mTOR and its downstream targets 4E-BP1, eEF2, and eEF2 kinase in relationships with tau in Alzheimer's disease brain. FEBS J. *272*, 4211–4220.

Linhoff, M.W., Laurén, J., Cassidy, R.M., Dobie, F.A., Takahashi, H., Nygaard, H.B., Airaksinen, M.S., Strittmatter, S.M., and Craig, A.M. (2009). An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers. Neuron *61*, 734–749.

Lu, Y.M., Jia, Z., Janus, C., Henderson, J.T., Gerlai, R., Wojtowicz, J.M., and Roder, J.C. (1997). Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J. Neurosci. *17*, 5196–5205.

Lujan, R., Nusser, Z., Roberts, J.D., Shigemoto, R., and Somogyi, P. (1996). Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur. J. Neurosci. *8*, 1488–1500.

Lüscher, C., and Huber, K.M. (2010). Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron *65*, 445–459.

Majercak, J., Ray, W.J., Espeseth, A., Simon, A., Shi, X.P., Wolffe, C., Getty, K., Marine, S., Stec, E., Ferrer, M., et al. (2006). LRRTM3 promotes processing of amyloid-precursor protein by BACE1 and is a positional candidate gene for late-onset Alzheimer's disease. Proc. Natl. Acad. Sci. USA *103*, 17967–17972.

Málaga-Trillo, E., Solis, G.P., Schrock, Y., Geiss, C., Luncz, L., Thomanetz, V., and Stuermer, C.A. (2009). Regulation of embryonic cell adhesion by the prion protein. PLoS Biol. *7*, e55.

Ménard, C., and Quirion, R. (2012). Successful cognitive aging in rats: a role for mGluR5 glutamate receptors, homer 1 proteins and downstream signaling pathways. PLoS ONE 7, e28666.

Moult, P.R., Gladding, C.M., Sanderson, T.M., Fitzjohn, S.M., Bashir, Z.I., Molnar, E., and Collingridge, G.L. (2006). Tyrosine phosphatases regulate AMPA receptor trafficking during metabotropic glutamate receptor-mediated long-term depression. J. Neurosci. *26*, 2544–2554.

Nicodemo, A.A., Pampillo, M., Ferreira, L.T., Dale, L.B., Cregan, T., Ribeiro, F.M., and Ferguson, S.S. (2010). Pyk2 uncouples metabotropic glutamate receptor G protein signaling but facilitates ERK1/2 activation. Mol. Brain *3*, 4. Nicolle, M.M., Colombo, P.J., Gallagher, M., and McKinney, M. (1999). Metabotropic glutamate receptor-mediated hippocampal phosphoinositide

turnover is blunted in spatial learning-impaired aged rats. J. Neurosci. 19, 9604–9610.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y., and LaFerla, F.M. (2003). Tripletransgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron *39*, 409–421.

Park, S., Park, J.M., Kim, S., Kim, J.A., Shepherd, J.D., Smith-Hicks, C.L., Chowdhury, S., Kaufmann, W., Kuhl, D., Ryazanov, A.G., et al. (2008). Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. Neuron *59*, 70–83.

Prybylowski, K., Chang, K., Sans, N., Kan, L., Vicini, S., and Wenthold, R.J. (2005). The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. Neuron *47*, 845–857.

Rammes, G., Hasenjäger, A., Sroka-Saidi, K., Deussing, J.M., and Parsons, C.G. (2011). Therapeutic significance of NR2B-containing NMDA receptors and mGluR5 metabotropic glutamate receptors in mediating the synaptotoxic effects of β -amyloid oligomers on long-term potentiation (LTP) in murine hippocampal slices. Neuropharmacology *60*, 982–990.

Renner, M., Lacor, P.N., Velasco, P.T., Xu, J., Contractor, A., Klein, W.L., and Triller, A. (2010). Deleterious effects of amyloid beta oligomers acting as an extracellular scaffold for mGluR5. Neuron *66*, 739–754.

Robbins, D.J., Fei, D.L., and Riobo, N.A. (2012). The Hedgehog signal transduction network. Sci. Signal. *5*, re6.

Roberson, E.D., Halabisky, B., Yoo, J.W., Yao, J., Chin, J., Yan, F., Wu, T., Hamto, P., Devidze, N., Yu, G.Q., et al. (2011). Amyloid- β /Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. J. Neurosci. *31*, 700–711.

Ronesi, J.A., Collins, K.A., Hays, S.A., Tsai, N.P., Guo, W., Birnbaum, S.G., Hu, J.H., Worley, P.F., Gibson, J.R., and Huber, K.M. (2012). Disrupted Homer scaffolds mediate abnormal mGluR5 function in a mouse model of fragile X syndrome. Nat. Neurosci. *15*, 431–440.

Saugstad, J.A., Segerson, T.P., and Westbrook, G.L. (1996). Metabotropic glutamate receptors activate G-protein-coupled inwardly rectifying potassium channels in Xenopus oocytes. J. Neurosci. *16*, 5979–5985.

Schmitt-Ulms, G., Hansen, K., Liu, J., Cowdrey, C., Yang, J., DeArmond, S.J., Cohen, F.E., Prusiner, S.B., and Baldwin, M.A. (2004). Time-controlled transcardiac perfusion cross-linking for the study of protein interactions in complex tissues. Nat. Biotechnol. *22*, 724–731.

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

Shinoda, Y., Tanaka, T., Tominaga-Yoshino, K., and Ogura, A. (2010). Persistent synapse loss induced by repetitive LTD in developing rat hippocampal neurons. PLoS ONE *5*, e10390.

Simonyi, A., Schachtman, T.R., and Christoffersen, G.R. (2010). Metabotropic glutamate receptor subtype 5 antagonism in learning and memory. Eur. J. Pharmacol. *639*, 17–25.

Sokol, D.K., Maloney, B., Long, J.M., Ray, B., and Lahiri, D.K. (2011). Autism, Alzheimer disease, and fragile X: APP, FMRP, and mGluR5 are molecular links. Neurology *76*, 1344–1352.

Strittmatter, S.M., Cannon, S.C., Ross, E.M., Higashijima, T., and Fishman, M.C. (1993). GAP-43 augments G protein-coupled receptor transduction in Xenopus laevis oocytes. Proc. Natl. Acad. Sci. USA *90*, 5327–5331.

Takasu, M.A., Dalva, M.B., Zigmond, R.E., and Greenberg, M.E. (2002). Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. Science *295*, 491–495.

Um, J.W., and Strittmatter, S.M. (2013). Amyloid- β induced signaling by cellular prion protein and Fyn kinase in Alzheimer disease. Prion 7, 37–41.

Um, J.W., Nygaard, H.B., Heiss, J.K., Kostylev, M.A., Stagi, M., Vortmeyer, A., Wisniewski, T., Gunther, E.C., and Strittmatter, S.M. (2012). Alzheimer amyloid- β oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. Nat. Neurosci. *15*, 1227–1235.

Vanderklish, P.W., and Edelman, G.M. (2002). Dendritic spines elongate after stimulation of group 1 metabotropic glutamate receptors in cultured hippocampal neurons. Proc. Natl. Acad. Sci. USA *99*, 1639–1644.

Wang, Q., Walsh, D.M., Rowan, M.J., Selkoe, D.J., and Anwyl, R. (2004). Block of long-term potentiation by naturally secreted and synthetic amyloid betapeptide in hippocampal slices is mediated via activation of the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5. J. Neurosci. *24*, 3370–3378. Wu, J., Petralia, R.S., Kurushima, H., Patel, H., Jung, M.Y., Volk, L., Chowdhury, S., Shepherd, J.D., Dehoff, M., Li, Y., et al. (2011). Arc/Arg3.1 regulates an endosomal pathway essential for activity-dependent β -amyloid generation. Cell *147*, 615–628.

Xu, J., Zhu, Y., Contractor, A., and Heinemann, S.F. (2009). mGluR5 has a critical role in inhibitory learning. J. Neurosci. *29*, 3676–3684.

Zou, W.Q., Xiao, X., Yuan, J., Puoti, G., Fujioka, H., Wang, X., Richardson, S., Zhou, X., Zou, R., Li, S., et al. (2011). Amyloid-beta42 interacts mainly with insoluble prion protein in the Alzheimer brain. J. Biol. Chem. *286*, 15095–15105.