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TARP γ-8 controls hippocampal AMPA receptor number, distribution and synaptic plasticity

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Synaptic plasticity involves activity-dependent trafficking of AMPA-type glutamate receptors. Numerous cytoplasmic scaffolding proteins are postulated to control AMPA receptor trafficking, but the detailed mechanisms remain unclear. Here, we show that the transmembrane AMPA receptor regulatory protein (TARP) γ-8, which is preferentially expressed in the mouse hippocampus, is important for AMPA receptor protein levels and extrasynaptic surface expression. By controlling the number of AMPA receptors, γ-8 is also important in long-term potentiation, but not long-term depression. This study establishes γ-8 as a critical protein for basal AMPA receptor expression and localization at extrasynaptic sites in the hippocampus and raises the possibility that TARP-dependent control of AMPA receptors during synapse development and plasticity may be widespread.

Glutamate mediates most excitatory synaptic transmission in the CNS by activating AMPA- and NMDA-type ionotropic neurotransmitter receptors. AMPA receptors (AMPARs) mediate much of the moment-to-moment transmission, whereas NMDA receptor (NMDAR) activation initiates both long-term potentiation (LTP) and long-term depression (LTD). Synaptic AMPARs, in contrast to NMDARs, are highly mobile, and activity-dependent recruitment of synaptic AMPARs underlies aspects of synaptic plasticity[1–3].

The mechanisms underlying AMPAR trafficking have received considerable attention. AMPARs are heterotetramers derived from subunits GluR1–GluR4 (refs. 4–6), and the cytoplasmic tails of AMPAR subunits interact with numerous postsynaptic scaffolding proteins[7–10]. Many of these proteins, including GRIP/ABP, PICK1 and SAP97, contain PDZ domains, which are protein-protein interaction motifs that bind to the C-terminal tails of specific receptors. Other cytoplasmic proteins, including NSF, AP2 and protein 4.1, bind to more proximal sites in the C-terminal domains of GluR subunits. A number of these interactions, especially those with GluR2, have been implicated in hippocampal synaptic plasticity; however, mice lacking GluR2 and GluR3 have normal LTP and LTD[11].

The first transmembrane protein found to interact with AMPA receptors, stargazin, which is mutated in stargazer mice[12], controls the synaptic targeting and functioning of AMPARs in cerebellar granule cells by multiple mechanisms[13–15]. Stargazin interacts with AMPARs early in the synthetic pathway, ensures their proper maturation and promotes their surface expression[16]. Stargazin also binds to the scaffolding protein PSD-95, and this interaction translocates surface AMPARs to the synapse[15,17]. The defects in AMPAR trafficking in stargazer mice seem to be restricted to cerebellar granule cells, as synaptic transmission in hippocampus is normal[14]. Among a large family of stargazin-related proteins[18,19], a subset (γ-3, γ-4, γ-8), the TARPs, can rescue the AMPAR defects in stargazer cerebellar granule cells[16]. γ-8 has high sequence homology with stargazin[18,19]; however, γ-8 also has two unique domains in the C-terminal tail[20]. TARPs are differentially distributed in the brain[16], raising the possibility that they either function similarly in different brain areas or have distinct properties. However, no direct evidence exists demonstrating a critical role for TARPs in AMPAR trafficking outside of the cerebellum.

To address these issues we investigated the role of the TARP γ-8, which is preferentially expressed in the hippocampus[16]. Overexpressing γ-8 in hippocampal pyramidal cells caused a threefold enhancement in extrasynaptic AMPAR-mediated currents, but no change in synaptic AMPAR currents. Mice deficient in γ-8 protein showed a substantial loss and mislocalization of hippocampal GluR1 and GluR2/3 proteins. These mice also showed a differential regulation of functional AMPARs in extrasynaptic and synaptic pools: extrasynaptic receptors recorded from somatic outside-out patches were essentially depleted, whereas synaptic pools were modestly impaired. Finally, *γ-8*$^{-/-}$ (also known as *Cacng8*$^{-/-}$) mice had impaired synaptic plasticity. This establishes γ-8 as a critical protein for AMPAR expression and distribution in the hippocampus.

RESULTS

γ-8 expression alters functional distribution of AMPARs

To examine the effects of γ-8 in hippocampal pyramidal neurons, we coexpressed γ-8 and green fluorescent protein (GFP) via an internal ribosome entry site (*γ-8-IRES-GFP*) in organotypic slice cultures using a Semliki Forest virus. Simultaneous recordings from GFP-labeled and

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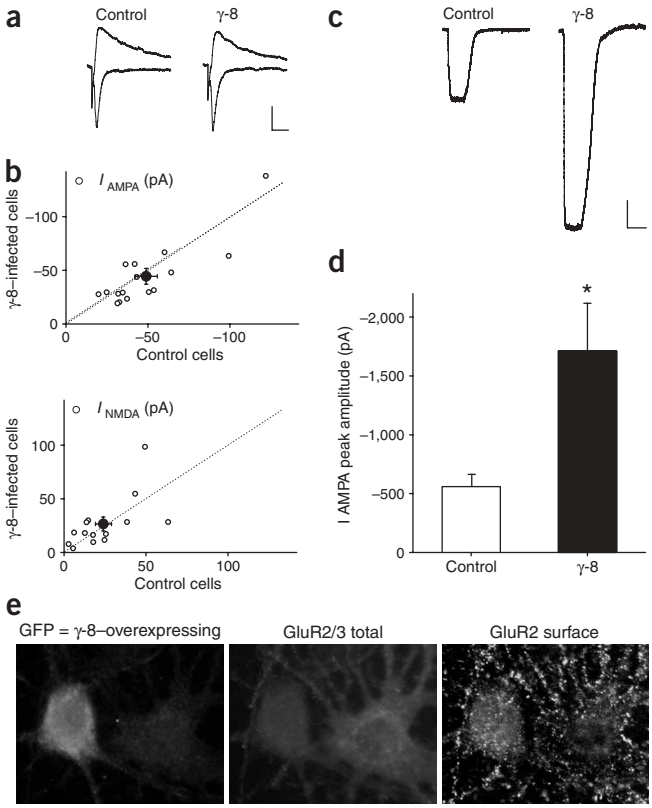

Figure 1 γ-8 expression increases extrasynaptic but not synaptic AMPARs. (**a**) Sample traces of evoked EPSCs recorded simultaneously from a pair of CA1 pyramidal cells at membrane potentials of –70 mV and +40 mV. The recordings are from a *γ-8-IRES-GFP*–infected cell (γ-8) and a neighboring uninfected cell (control). Scale bars: 10 pA, 50 ms. (**b**) Distribution of EPSC amplitudes in pairs of γ-8–infected cells and control cells shows that γ-8 has no effect on either synaptic AMPAR or NMDAR EPSCs ($P = 0.25$, $n = 16$ pairs and $P = 0.63$, $n = 14$ pairs, respectively). Large filled circles represent the average value for all cells. (**c**) Sample traces of AMPA-evoked currents (2 s, 500 μM AMPA + 100 μM cyclothiazide) from outside-out somatic patches in control and *γ-8*–infected pyramidal cells. Scale bar, 200 pA, 2 s. (**d**) γ-8 overexpression markedly increases (~300%) extrasynaptic AMPAR responses ($P = 0.001$, $n = 9$ for control and infected cells). (**e**) Immunofluorescent labeling of rat hippocampal cultured neurons overexpressing γ-8 (GFP-expressing neuron at left) and control neurons (non–GFP expressing neuron at right) shows that in γ-8–overexpressing cells, total somatic GluR2 levels were unchanged, but surface GluR2 levels were increased.

neighboring control neurons compared the size of synaptic AMPAR and NMDAR currents. Recordings from a number of pairs demonstrated that γ-8 overexpression did not influence synaptic transmission (mean peak amplitude I_{AMPA}: control, −49 ± 7 pA; infected, −44 ± 7 pA, $n = 16$ pairs; mean peak amplitude I_{NMDA}: control, 24 ± 5 pA; infected, 26 ± 6 pA, $n = 14$ pairs; **Fig. 1a**,**b**). By contrast, γ-8 caused a threefold enhancement of AMPA-mediated responses in outside-out somatic membrane patches (control, −560 ± 100 pA, $n = 9$; infected, −1,712 ± 407 pA, $n = 9$; **Fig. 1c**,**d**). As excitatory synapses do not contact the soma of pyramidal cells, this finding indicates that γ-8 increases the number of extrasynaptic AMPARs.

Is the enhancement in surface AMPARs that results from overexpression of γ-8 attributable to the delivery of an intracellular pool of preformed AMPARs, or does γ-8 promote the synthesis of AMPARs that are then delivered to the surface? To examine this issue, we overexpressed γ-8 in dissociated hippocampal neurons and compared the level of surface and total GluR2 levels in γ-8–overexpressing cells and neighboring uninfected cells. We found that the total GluR2 levels were unchanged in γ-8–overexpressing cells (96 ± 8% of control cells, $n = 42$ pairs of neurons from four experiments). However, the ratio of surface-to-total GluR2 levels in the soma of γ-8 overexpressing cells was higher than in neighboring control cells (166 ± 14% of control cells, $n = 42$ pairs of neurons from four experiments; **Fig. 1e**). This suggests that overexpressing γ-8 may enhance the delivery of a preexisting intracellular pool of AMPARs to the plasma membrane.

γ-8 disruption alters AMPAR expression and distribution

We disrupted the *γ-8* gene by homologous recombination in embryonic stem cells (**Fig. 2a**). Southern blot analysis of *Spe*I-digested genomic DNA using the probe schematically depicted in **Figure 2a** was used to ensure proper targeting (**Fig. 2b**). Crossing $\gamma\text{-}8^{+/-}$ mice showed that $\gamma\text{-}8^{-/-}$ mice were born in mendelian ratios, and no gross behavioral or anatomical defects were apparent. Western blot analysis (**Fig. 2c**,**d**)

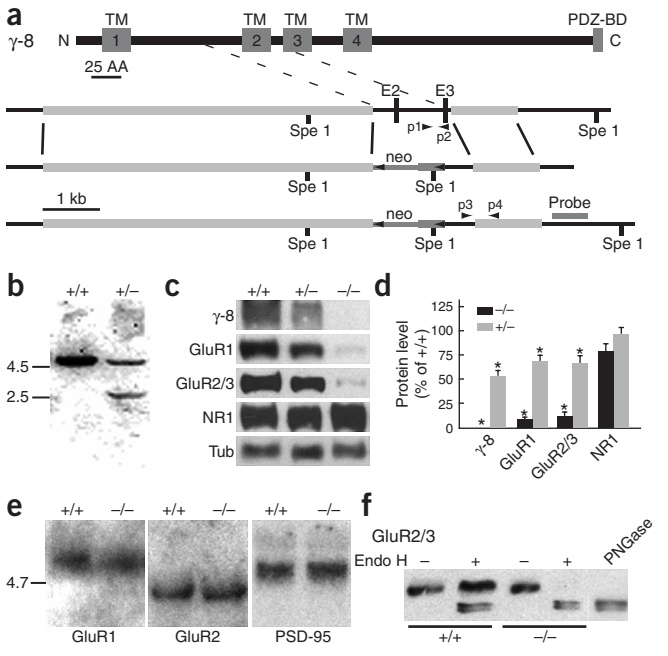

Figure 2 Targeted disruption of γ-8 decreases level of AMPAR proteins. (**a**) Schematic representation of γ-8 protein, genomic locus, targeting vector and targeted allele. Homologous recombination disrupts transmembrane domains 2 and 3 (TM2, TM3). PDZ-BD: PDZ binding domain. E2: exon 2; E3: exon 3. (**b**) Southern blot analysis of *Spe*I-digested genomic DNA using the probe in **a** demonstrates proper targeting. (**c**) Quantitative immunoblot analysis of hippocampal extracts from $\gamma\text{-}8^{+/+}$, $\gamma\text{-}8^{+/-}$ and $\gamma\text{-}8^{-/-}$ mice confirms that the γ-8 protein is not present in $\gamma\text{-}8^{-/-}$ mice. The levels of AMPAR proteins GluR1 and GluR2/3 are reduced in $\gamma\text{-}8^{+/-}$ and $\gamma\text{-}8^{-/-}$mice. The amount of NMDAR subunit NR1 is unaffected. Similar tubulin (Tub) levels confirm equivalent loading. (**d**) AMPAR protein levels are reduced in $\gamma\text{-}8^{+/-}$ and $\gamma\text{-}8^{-/-}$ mice (summary of three experiments). Error bars represent s.e.m. (**e**) Northern blotting indicates that deleting γ-8 does not affect mRNA levels for GluR1, GluR2/3 or PSD-95. (**f**) In the hippocampus of $\gamma\text{-}8^{-/-}$ mice, a large fraction of GluR2/3 remains immature and sensitive to EndoH glycosidase (a longer exposure of this western blot shows a small amount of EndoH-resistant GluR2/3 protein). Glycosylation is removed by the nonspecific *N*-glycosidase, PNGaseF.

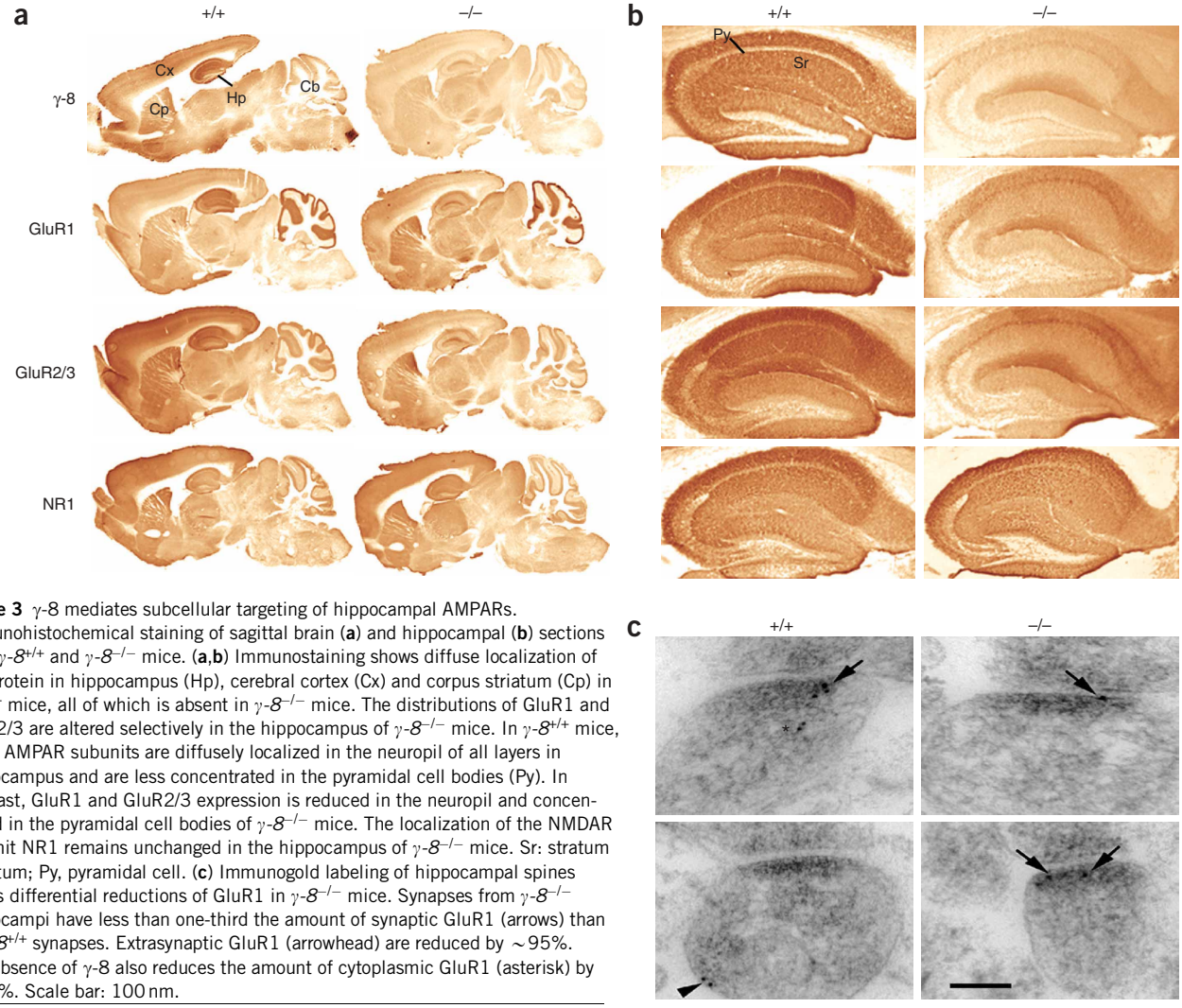

Figure 3 γ-8 mediates subcellular targeting of hippocampal AMPARs. Immunohistochemical staining of sagittal brain (**a**) and hippocampal (**b**) sections from $\gamma\text{-}8^{+/+}$ and $\gamma\text{-}8^{-/-}$ mice. (**a**,**b**) Immunostaining shows diffuse localization of γ-8 protein in hippocampus (Hp), cerebral cortex (Cx) and corpus striatum (Cp) in $\gamma\text{-}8^{+/+}$ mice, all of which is absent in $\gamma\text{-}8^{-/-}$ mice. The distributions of GluR1 and GluR2/3 are altered selectively in the hippocampus of $\gamma\text{-}8^{-/-}$ mice. In $\gamma\text{-}8^{+/+}$ mice, these AMPAR subunits are diffusely localized in the neuropil of all layers in hippocampus and are less concentrated in the pyramidal cell bodies (Py). In contrast, GluR1 and GluR2/3 expression is reduced in the neuropil and concentrated in the pyramidal cell bodies of $\gamma\text{-}8^{-/-}$ mice. The localization of the NMDAR subunit NR1 remains unchanged in the hippocampus of $\gamma\text{-}8^{-/-}$ mice. Sr: stratum radiatum; Py, pyramidal cell. (**c**) Immunogold labeling of hippocampal spines shows differential reductions of GluR1 in $\gamma\text{-}8^{-/-}$ mice. Synapses from $\gamma\text{-}8^{-/-}$ hippocampi have less than one-third the amount of synaptic GluR1 (arrows) than do $\gamma\text{-}8^{+/+}$ synapses. Extrasynaptic GluR1 (arrowhead) are reduced by ~95%. The absence of γ-8 also reduces the amount of cytoplasmic GluR1 (asterisk) by ~80%. Scale bar: 100 nm.

confirmed that γ-8 protein was absent in $\gamma\text{-}8^{-/-}$ mice. We did not find any change in the level of expression of other TARPs (data not shown). Quantitative western blot analysis also showed that GluR1 and GluR2/3 protein levels in the hippocampus were reduced by ~30% in $\gamma\text{-}8^{+/-}$ and ~85% in $\gamma\text{-}8^{-/-}$ mice (**Fig. 2c**,**d**). NMDA receptor (NR1) and tubulin levels were unchanged. Northern blot analysis of hippocampus (**Fig. 2e**) demonstrated that mRNA for GluR1, GluR2 and PSD-95 occurred in normal amounts in $\gamma\text{-}8^{-/-}$ mice (normalization to $\gamma\text{-}8^{+/+}$: 1 ± 0.12 for GluR1, 1.06 ± 0.11 for GluR2, 1.09 ± 0.07 for PSD-95; $n = 3$), implying that γ-8 does not affect the transcription of AMPARs and that the loss of γ-8 is likely to destabilize GluR1 and GluR2/3 proteins.

Many membrane proteins are regulated by quality control mechanisms for exit from the endoplasmic reticulum[21]. Glutamate receptors receive high mannose glycosylation in the endoplasmic reticulum and are later modified with more complex sugars in the Golgi[22,23]. The glycosylation patterns can be distinguished with endoglycoside H (EndoH), which digests the immature high mannose sugars, and PNGaseF, which removes all *N*-linked carbohydrates. In contrast to $\gamma\text{-}8^{+/+}$ mice, much of the GluR2/3 from the hippocampus of $\gamma\text{-}8^{-/-}$ mice was sensitive to EndoH (**Fig. 2f**), suggesting a large pool of immature receptors held in the endoplasmic reticulum. The cleaved band of receptors comigrated with the completely deglycosylated receptors obtained by treatment with PNGaseF.

Immunohistochemical staining (**Fig. 3a**,**b**) also showed that levels of GluR1 and GluR2/3 were greatly reduced in $\gamma\text{-}8^{-/-}$ mice and that the remaining receptors were mislocalized as they were redistributed from the dendrites to the cell body (**Fig. 3a**,**b**). This loss of AMPARs was selective, as the NR1 subunit of NMDARs (**Fig. 3a**,**b**) and PSD-95 (data not shown) were unaltered. To further characterize AMPAR distribution at the subcellular level, we performed immunogold electron microscopy of GluR1 (**Fig. 3c** and **Supplementary Fig. 1**). The synaptic receptor density was decreased by approximately 67% in $\gamma\text{-}8^{-/-}$ mice ($\gamma\text{-}8^{+/+}$, 1.77 ± 0.3, $n = 86$; $\gamma\text{-}8^{-/-}$, 0.56 ± 0.1, $n = 129$, $P = 0.0003$). Furthermore, the number of extrasynaptic receptors was reduced by ~95% ($\gamma\text{-}8^{+/+}$, 0.63 ± 0.14, $n = 85$; $\gamma\text{-}8^{-/-}$, 0.04 ± 0.02, $n = 128$, $P = 0.0001$), and cytoplasmic receptors were reduced in number by ~80% ($\gamma\text{-}8^{+/+}$, 0.96 ± 0.18, $n = 85$; $\gamma\text{-}8^{-/-}$, 0.14 ± 0.04, $n = 128$, $P = 0.00002$).

The pattern of staining of GluR2/3 in $\gamma\text{-}8^{-/-}$ mice is markedly similar to that reported for $GluR1^{-/-}$ (also known as $GluRA^{-/-}$) mice[24]. As AMPAR expression is dependent on the presence of γ-8, we wondered if the amount and distribution of γ-8 might be altered in the $GluR1^{-/-}$ mice. The level of γ-8 protein measured with western

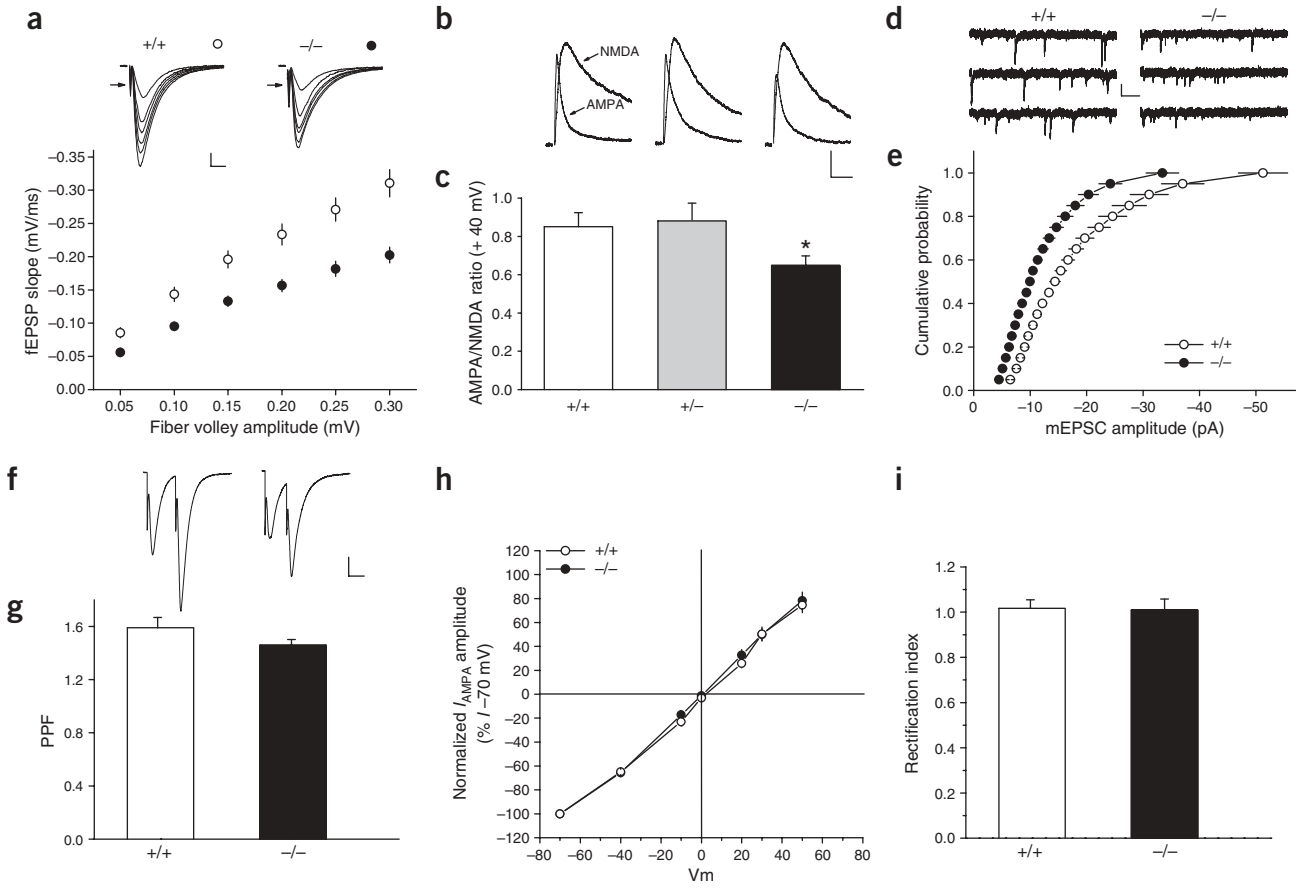

Figure 4 CA1 synaptic AMPAR-mediated responses are impaired in γ-$8^{-/-}$ mice. (**a**) Input-output curves for basal synaptic transmission in hippocampal slices. As illustrated in the sample traces and the graph below, for each input (fiber volley, see arrow), the output (fEPSP) is reduced by ~35% in γ-$8^{-/-}$ slices ($P \leq 0.01$, $n = 22$ for γ-$8^{+/+}$; $n = 27$ for γ-$8^{-/-}$). Scale bar, 0.1 mV, 10 ms. (**b**,**c**) Ratio of AMPA to NMDA current is reduced by ~30% in CA1 pyramidal cells from γ-$8^{-/-}$ mice ($P \leq 0.01$, $n = 21$ for γ-$8^{+/+}$; $n = 20$ for γ-$8^{+/-}$; $n = 35$ for γ-$8^{-/-}$). Sample NMDA and AMPA EPSCs from representative cells are shown in **b** above the respective bars. Calibration: γ-$8^{+/+}$, 12 pA, 50 ms; γ-$8^{+/-}$, 16 pA, 50 ms; γ-$8^{-/-}$, 14 pA, 50 ms. (**d**,**e**) mEPSCs from γ-$8^{-/-}$ CA1 pyramidal cells have reduced amplitude but unchanged frequency. Sample traces are shown in **d**. Scale bar, 10 pA, 0.5 s. **e** shows cumulative frequency distribution of mEPSC amplitudes ($P \leq 0.01$, Kolmogorov-Smirnov test, $n = 14$ for γ-$8^{+/+}$; $n = 24$ for γ-$8^{-/-}$). (**f**,**g**) Paired-pulse facilitation (PPF) does not differ between γ-$8^{-/-}$ ($n = 19$) and γ-$8^{+/+}$ ($n = 19$) cells. **f** shows sample traces above the graph. Scale bar, 25 pA, 25 ms. (**h**) Current-voltage (I/V) plots of evoked AMPA EPSCs show no difference between γ-$8^{-/-}$ ($n = 20$) and γ-$8^{+/+}$ ($n = 14$) cells. (**i**) Rectification indices are similar in both genotypes ($n = 14$ for γ-$8^{+/+}$; $n = 20$ for γ-$8^{-/-}$). Error bars in all graphs represent s.e.m.

blotting was slightly reduced, but not nearly as much as the decrease of GluR1 in γ-$8^{-/-}$ mice (data not shown). Immunohistochemical staining of the *GluR1*$^{-/-}$ hippocampus showed that the remaining GluR2/3 was redistributed to the cell body layer as reported previously[24], but there was no clear change in the pattern of staining for γ-8 (data not shown).

Synaptic and extrasynaptic AMPARs are impaired in γ-$8^{-/-}$ mice

To assess the strength of synaptic transmission, we compared the size of the presynaptic fiber volley (input) with the slope of the EPSP (output) in stratum radiatum (**Fig. 4a**) and found that synaptic transmission was reduced by ~35% in γ-$8^{-/-}$ mice (**Fig. 4a**). To determine whether this reduction in excitatory synaptic transmission was specific to AMPARs, we compared the AMPAR and NMDAR components of the EPSC (**Fig. 4b**). The AMPA/NMDA ratio was reduced by approximately 30% in γ-$8^{-/-}$ mice (**Fig. 4c**), indicating that the defect in synaptic transmission in γ-$8^{-/-}$ mice was limited to the AMPAR component (AMPA/NMDA ratio: γ-$8^{+/+}$, 0.85 ± 0.07, $n = 21$; γ-$8^{+/-}$, 0.88 ± 0.09, $n = 20$; γ-$8^{-/-}$, 0.65 ± 0.05, $n = 35$). As a further test, we compared the stimulus strength used to generate NMDAR EPSCs to the amplitude of these EPSCs in γ-$8^{+/+}$ and γ-$8^{-/-}$ cells. The size of the stimulus required to generate similar-size EPSCs in γ-$8^{+/+}$ and γ-$8^{-/-}$ mice was not significantly different (γ-$8^{+/+}$, I_{NMDA} = 43.6 ± 8.3 pA, stimulus size = 2.7 ± 0.4 arbitrary units, ratio of I_{NMDA}/stimulus size = 22 ± 4.4, $n = 21$; γ-$8^{-/-}$, I_{NMDA} = 60.3 ± 9.1 pA, stimulus size = 3.4 ± 0.3 a.u., ratio of I_{NMDA}/stimulus size = 25.4 ± 6, $n = 35$). The lack of change in the NMDAR EPSC argues against a change in transmitter release. We also found a significant reduction in the size of the AMPAR mEPSCs (**Fig. 4d**,**e**; mEPSC mean peak amplitude: γ-$8^{+/+}$, −18.1 ± 1.3 pA, $n = 14$; γ-$8^{+/-}$, −15.9 ± 1.5 pA, $n = 18$; γ-$8^{-/-}$, −11.9 ± 0.8 pA, $n = 24$). Paired-pulse facilitation, a sensitive measure of changes in the probability of transmitter release, was unchanged (γ-$8^{+/+}$, 1.59 ± 0.08, $n = 19$; γ-$8^{+/-}$, 1.61 ± 0.09, $n = 14$; and γ-$8^{-/-}$ 1.46 ± 0.04, $n = 19$; **Fig. 4f**,**g**). Furthermore, the frequency of mEPSCs, a measure of the probability of transmitter release, was unchanged (1.9 ± 0.4 Hz, $n = 14$ in γ-$8^{+/+}$, 2.4 ± 0.4 Hz, $n = 18$ in γ-$8^{+/-}$ and 1.7 ± 0.5 Hz, $n = 24$ in γ-$8^{-/-}$ cells). Finally, we examined the current/voltage relationship of AMPAR EPSCs to determine if the biophysical property of the

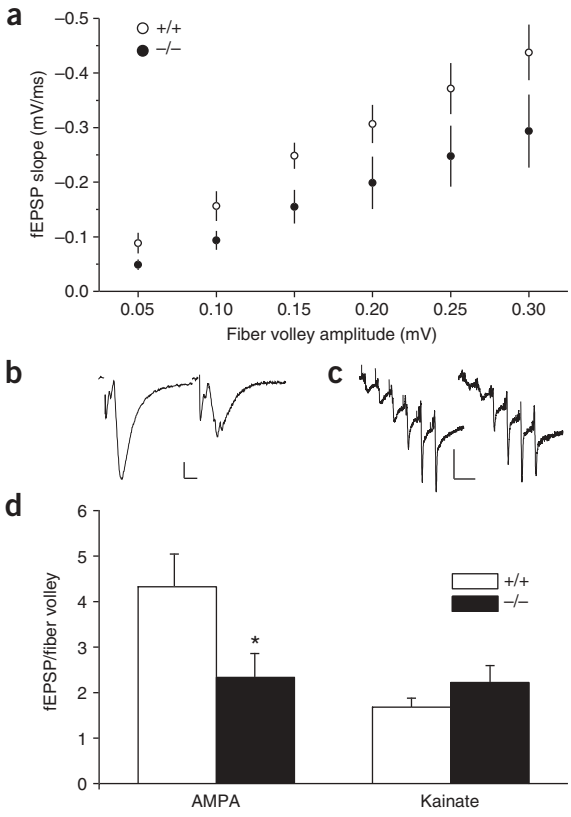

Figure 5 CA3 synaptic AMPAR, but not KAR, responses are impaired in γ-$8^{-/-}$ mice. (**a**) Input-output curves for basal CA3 mossy fiber synaptic transmission in hippocampal slices from γ-$8^{+/+}$ and γ-$8^{-/-}$ mice. The slope of fEPSPs evoked (0.05 Hz) by a range of stimulus intensities is plotted against the amplitude of the corresponding fiber volley. As illustrated, for each input (fiber volley), the output (fEPSP) is reduced by ~50% in slices from γ-$8^{-/-}$ mice ($P \leq 0.01$, $n = 4$ for γ-$8^{+/+}$; $n = 4$ for γ-$8^{-/-}$). AMPAR-mediated mossy fiber fEPSPs (**b**) were evoked by single stimulation (0.2 Hz) in the presence of AP5 (50 μM) and picrotoxin (100 μM) and were abolished by GYKI 53655 (100 μM), whereas KAR-mediated mossy fiber fEPSPs (**c**) were subsequently evoked by repetitive stimulation (six stimuli at 30 Hz given every 5 s) and were abolished by CNQX (50 μM). As illustrated in the sample traces (**b**,**c**) and the summary below (**d**), for a given fiber volley, the AMPAR fEPSP amplitude is reduced by ~50% in slices from γ-$8^{-/-}$ mice, whereas the KAR fEPSP is unaltered ($P \leq 0.01$, $n = 12$ for γ-$8^{+/+}$; $n = 9$ for γ-$8^{-/-}$). Scale bars, 0.1 mV, 10 ms (AMPAR fEPSP) and 0.05 mV, 50 ms (KAR fEPSP). Error bars in all graphs represent s.e.m.

receptors was altered in γ-$8^{-/-}$ cells (**Fig. 4h**). We did not find any change in the rectification index (**Fig. 4i**; $R_I = 1.02 \pm 0.04$, $n = 14$ for γ-$8^{+/+}$, $R_I = 1.01 \pm 0.06$, $n = 11$ for γ-$8^{+/-}$ cells and $R_I = 1.01 \pm 0.05$, $n = 20$ for γ-$8^{-/-}$ cells), suggesting no change in AMPAR subunit composition. Notably, in all experiments performed, we did not observe any difference in basal synaptic transmission between γ-$8^{+/+}$ and γ-$8^{+/-}$ mice.

Are the defects in synaptic AMPAR-mediated transmission restricted to CA1 Schaffer collateral synapses? To address this issue, we turned to the CA3 mossy fiber synapse and compared the size of the fiber volley with the AMPAR field EPSP in γ-$8^{+/+}$ and γ-$8^{-/-}$ mice (**Fig. 5a**). As with the synapses in CA1, the AMPAR EPSP was clearly reduced. Although the excitatory synapses in the CA1 region of the hippocampus are typical of most cortical excitatory synapses in that AMPARs and NMDARs are colocalized in the postsynaptic density, hippocampal mossy fiber synapses also express kainate receptors (KARs)[25,26]. Does γ-8 have a role in trafficking KARs? After first comparing the size of the fiber volley with the AMPAR EPSP field (**Fig. 5b**), as previously described, we then added the AMPAR-selective antagonist GYKI 53655 and stimulated the mossy fibers repetitively to evoke a KAR-mediated field EPSP (fEPSP; **Fig. 5c**). We then compared the size of the kainate fEPSP with the original fiber volley. We did not detect any difference between γ-$8^{+/+}$ and γ-$8^{-/-}$ mice (**Fig. 5d**).

Although the defect in synaptic transmission in γ-$8^{-/-}$ mice was significant, the magnitude of the defect was modest compared with the marked loss of AMPAR protein. To address this issue, we recorded in the presence of tetrodotoxin whole-cell responses to bath application of AMPA, which activates AMPARs throughout the cell, including somatic and dendritic extrasynaptic AMPARs, as well as synaptic AMPARs. We found a marked reduction in the size of currents in γ-$8^{+/-}$ mice (-392 ± 57 pA, $n = 9$) and γ-$8^{-/-}$ mice (-156 ± 25 pA, $n = 15$) compared with γ-$8^{+/+}$ mice (-674 ± 84 pA, $n = 14$; **Fig. 6a**). This result suggests that the pool of extrasynaptic receptors is greatly reduced. This possibility was directly tested by pulling outside-out patches from the soma of hippocampal pyramidal cells. Indeed, there was a 90% decrease in AMPAR-mediated responses from γ-$8^{-/-}$ mice (**Fig. 6b**,**c**). Furthermore, as with the whole-cell responses, the responses in γ-$8^{+/-}$ mice (-366.8 ± 70.3 pA, $n = 20$) were intermediate between γ-$8^{+/+}$ (-601.3 ± 72.3 pA, $n = 12$) and γ-$8^{-/-}$ mice (-52.2 ± 9.5 pA, $n = 17$), indicating that the number of extrasynaptic AMPARs is tightly coupled to the number of γ-8 molecules. Although the extrasynaptic responses were obtained from somatic membrane, the immunogold studies suggest that the loss of extrasynaptic receptors also occurs throughout the dendrites. We also examined the responses to NMDA application with both whole-cell recording and outside-out patches and found no difference between γ-$8^{+/+}$ and γ-$8^{-/-}$ mice (whole-cell recording: γ-$8^{+/+}$, -1.0 ± 0.1 nA, $n = 5$; γ-$8^{-/-}$, -1.1 ± 0.1 nA, $n = 5$; outside-out patches: γ-$8^{+/+}$, -31.6 ± 0.6 pA, $n = 10$; γ-$8^{-/-}$, -31.0 ± 0.4 pA, $n = 8$).

All cell types analyzed in the hippocampus of γ-$8^{-/-}$ mice, including pyramidal cells in CA1, interneurons in stratum radiatum and granule cells in dentate gyrus, showed a marked alteration in extrasynaptic AMPAR responses (**Fig. 6d**,**e**; interneurons: γ-$8^{+/+}$, -254 ± 83 pA, $n = 7$; γ-$8^{-/-}$, -36 ± 14 pA, $n = 6$; granule cells: γ-$8^{+/+}$, -302 ± 53 pA, $n = 7$; γ-$8^{-/-}$, -96 ± 29 pA, $n = 6$). In contrast, striatal medium spiny neurons had normal extrasynaptic AMPA responses (**Fig. 6d**,**e**; γ-$8^{+/+}$, -126 ± 22 pA, $n = 7$; γ-$8^{-/-}$, -123 ± 30 pA, $n = 6$), indicating that the effect of γ-8 deletion is specific to the hippocampus.

Is the trafficking of synaptic AMPARs in the γ-$8^{-/-}$ mice due to other TARPs or to a TARP-independent mechanism? To begin to address this question, we have generated double γ-$2^{-/-}\gamma$-$8^{-/-}$ knockout mice. These mice showed a more severe reduction (~50%) in synaptic transmission (AMPA/NMDA ratio γ-$2^{+/+}\gamma$-$8^{+/+}$, 0.87 ± 0.06, $n = 41$; γ-$8^{-/-}$, 0.65 ± 0.05, $n = 35$; γ-$2^{-/-}\gamma$-$8^{-/-}$, 0.44 ± 0.06, $n = 9$), supporting the proposal that at least some of the remaining AMPAR-mediated synaptic transmission in the γ-$8^{-/-}$ mice is due to other hippocampal TARPs.

Long-term potentiation is impaired in γ-$8^{-/-}$ mice

What might be the consequences of the loss of γ-8 on synaptic plasticity? In a blind fashion, we compared the magnitude of LTP induced by brief tetanic stimulation (two 1-s, 100-Hz tetani separated by 20 s), using field potential recordings in the CA1 area of the hippocampus. LTP in γ-$8^{-/-}$ mice was reduced by 75% compared with LTP in γ-$8^{+/+}$ mice (fEPSP slope 30–40 min after tetanization:

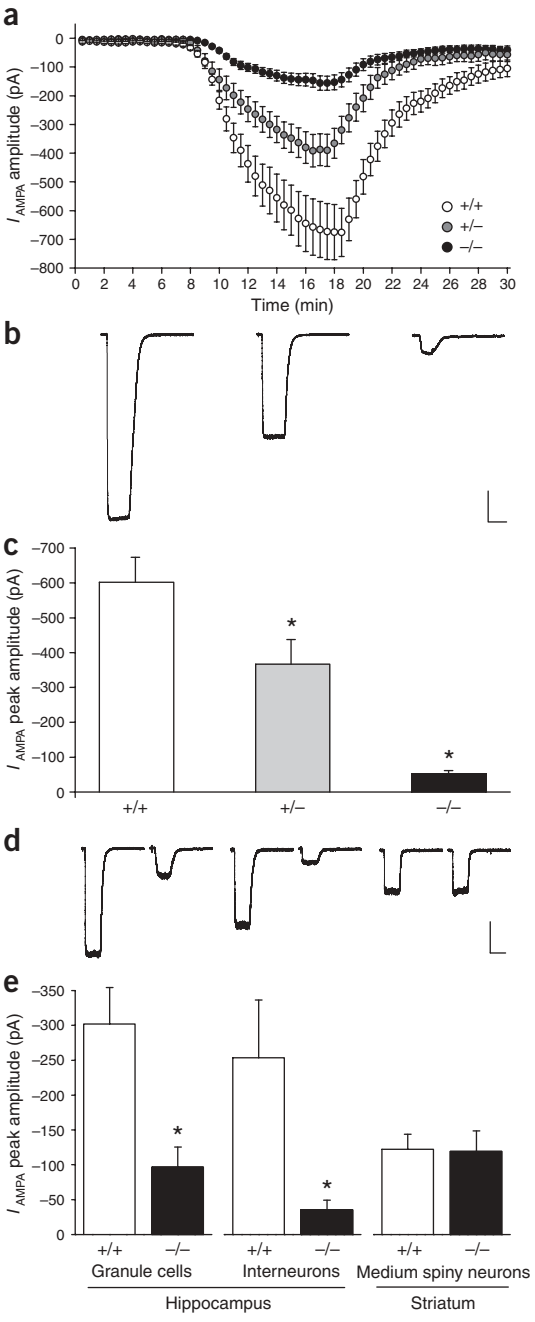

Figure 6 Extrasynaptic AMPAR-mediated responses are severely reduced in hippocampus of γ-8$^{-/-}$ mice. (**a**) Whole-cell currents evoked by bath application of AMPA (10 min, 100 nM + 0.5 μM tetrodotoxin + 10 μM cyclothiazide) were strongly reduced in γ-8$^{-/-}$ pyramidal cells (γ-8$^{-/-}$, $n = 15$ decreasing to 12; γ-8$^{+/-}$ $n = 9$; γ-8$^{-/-}$ $n = 16$ decreasing to 11). (**b**,**c**) AMPA-evoked currents (2 s, 500 μM AMPA + 100 μM cyclothiazide) from outside-out somatic patches decreased markedly (90%) in the number of extrasynaptic AMPARs in γ-8$^{-/-}$ hippocampal pyramidal cells. Responses in γ-8$^{+/-}$ cells ($n = 20$) are intermediate between γ-8$^{+/+}$ ($n = 12$) and γ-8$^{-/-}$ cells ($n = 17$). Sample currents are shown above the respective bars. Calibration, 100 pA, 2s. (**d**,**e**) Hippocampal interneurons in stratum radiatum and granule cells in dentate gyrus from γ-8$^{-/-}$ mice also show a marked alteration in extrasynaptic AMPAR-mediated responses (interneurons: γ-8$^{+/+}$, $n = 7$; γ-8$^{-/-}$, $n = 6$; granule cells: γ-8$^{+/+}$, $n = 7$; γ-8$^{-/-}$, $n = 6$). However, γ-8$^{-/-}$ medium spiny neurons recorded from the striatum have normal extrasynaptic AMPAR-mediated responses (γ-8$^{+/+}$, $n = 7$; γ-8$^{-/-}$, $n = 6$). Scale bar, 100 pA, 2 s. Error bars in all graphs represent s.e.m.

γ-8$^{+/+}$, 140 ± 12%, $n = 10$; γ-8$^{-/-}$, 109 ± 4%, $n = 17$; **Fig. 7a**). Because synaptic AMPAR responses are reduced in γ-8$^{-/-}$ mice, it was possible that unblocking of NMDARs was reduced compared with control slices. To circumvent this, recordings were made with cesium-filled electrodes to facilitate depolarizing the cell, and LTP was induced by 1-min pairing of 2-Hz synaptic stimulation with depolarization of the cell to 0 mV. The defect in LTP was still apparent in these experiments (EPSC amplitude 30–40 min after pairing, γ-8$^{+/+}$, 165 ± 14%, $n = 10$ versus γ-8$^{-/-}$, 118 ± 11%, $n = 14$; **Fig. 7b**), indicating that the defect in LTP in γ-8$^{-/-}$ mice is downstream of NMDAR activation. Notably, in all the experiments performed, we did not observe any difference in LTP between γ-8$^{+/+}$ and γ-8$^{+/-}$ mice. As a control for the effect of deleting γ-8 on NMDAR-dependent LTP, we also examined mossy fiber LTP in CA3, which is independent of NMDARs and is expressed presynaptically[27,28]. Mossy fiber LTP was unaltered in γ-8$^{-/-}$ mice (γ-8$^{+/+}$, 121 ± 5%, $n = 6$; γ-8$^{-/-}$, 125 ± 7%, $n = 9$).

We also examined long-term depression (LTD) in CA1 hippocampal pyramidal cells from γ-8$^{-/-}$ mice. We carried out whole-cell recording and induced LTD by 3-min pairing of 5-Hz synaptic stimulation with depolarization of the cell to –40 mV. We did not find any difference in the magnitude of LTD between γ-8$^{+/+}$ and γ-8$^{-/-}$ cells (**Fig. 7c**).

DISCUSSION

These results indicate that TARP γ-8 regulates AMPAR protein levels and extrasynaptic surface expression. By controlling the number of AMPA receptors, γ-8 has an important role in LTP, which requires the recruitment of AMPARs to synapses. These findings extend previous work on stargazer in cerebellar granule cells by showing that a protein homologous to stargazin strongly regulates AMPAR expression and function in the hippocampus. γ-8 has properties distinct from stargazin, as it preferentially controls hippocampal extrasynaptic AMPAR pools. These findings were unexpected, considering the stargazer mouse phenotype, which shows only modest reduction in cerebellar AMPAR levels and an absence of both synaptic and extrasynaptic AMPARs.

Loss of AMPAR protein in the γ-8$^{-/-}$ hippocampus

The role of γ-8 in AMPAR function in hippocampus showed notable differences from the role of stargazin in the cerebellum. Stargazer mice have a ∼20% reduction in GluR2/3 levels in cerebellum[16]. On the other hand, the primary defect in the γ-8$^{-/-}$ mouse was the loss of AMPAR proteins in the hippocampus (85%). It is noteworthy that this massive loss makes it difficult to determine to what extent the defect in synaptic and extrasynaptic AMPAR-mediated currents actually reflects compromised supply pools of receptors, compromised trafficking mechanisms, or both. Indeed, in addition to the regulation of AMPAR expression, γ-8 may also contribute to the increased delivery of AMPARs to the membrane from an intracellular pool, as suggested by the acute enhancement in the number of surface AMPARs induced by γ-8 overexpression, independent of total AMPAR protein levels. How might assembly with γ-8 regulate AMPAR expression? The normalcy of GluR1 and GluR2 mRNA levels in γ-8$^{-/-}$ hippocampus suggests that the loss of AMPAR proteins may be due to enhanced receptor degradation, although one cannot exclude altered protein synthesis. However, the absence of stargazin induces the unfolded protein response in cerebellar granule cells[29], supporting the idea that TARPs help stabilize AMPAR proteins and prevent their degradation by the proteasome. This by itself could increase the probability of AMPAR membrane insertion. One cannot exclude the possibility that γ-8 might

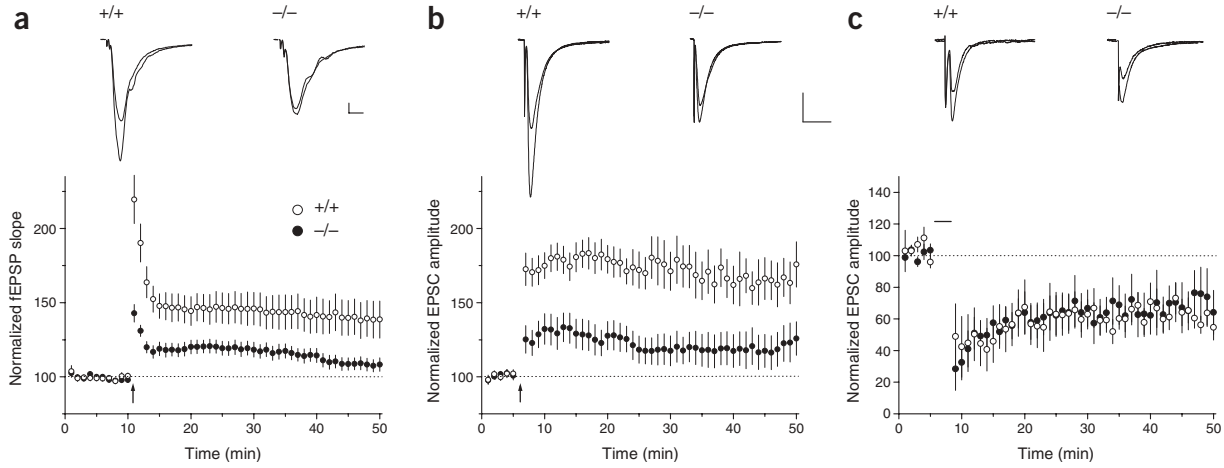

Figure 7 Impairment of LTP, but not LTD, in hippocampal CA1 synapses of γ-$8^{-/-}$ mice. (**a**) Extracellular recordings of fEPSPs in slices before and after tetanic stimulation of Schaffer collaterals (arrow, two trains of 100 Hz for 1 s, 20 s apart). LTP was produced in γ-$8^{+/+}$ slices ($n = 10$) and was significantly ($P \leq 0.05$) reduced in γ-$8^{-/-}$ slices ($n = 17$). Sample traces represent averaged field potentials before and 30–40 min after tetanization. Scale bar, 0.1 mV, 10 ms. Data were obtained in 100 μM picrotoxin and 4 mM Ca^{2+} and Mg^{2+}. (**b**) Whole-cell recordings from CA1 pyramidal cells before and after a pairing protocol (arrow, 2 Hz, 0 mV for 1 min.). LTP was induced in γ-$8^{+/+}$ cells ($n = 14$ decreasing to 9) but was severely decreased in γ-$8^{-/-}$ cells ($P \leq 0.01$, $n = 22$ decreasing to 11). Sample EPSCs recorded before and 30–40 min after pairing are shown above. Scale bar for **b** and **c**: 50 pA, 50 ms. (**c**) Whole-cell recordings from CA1 pyramidal cells before and after a pairing protocol (bar, 5 Hz, −40 mV for 3 min). LTD was induced, and no difference was found between γ-$8^{+/+}$ ($n = 6$) and γ-$8^{-/-}$ cells ($n = 7$). Sample EPSCs recorded before and 30–40 min after pairing are shown above.

also stabilize extrasynaptic and synaptic AMPARs through reduced basal receptor endocytosis, although this seems unlikely for activity-dependent synaptic receptor endocytosis, as LTD is not modified in γ-$8^{-/-}$ mice. The immature endoplasmic reticulum–type glycosylation of AMPARs in the γ-$8^{-/-}$ hippocampus also suggests that γ-8 has a role in AMPAR trafficking early in the biosynthetic pathway. Indeed, incompletely folded or assembled proteins cannot exit the endoplasmic reticulum because of a stringent quality control system[30]. Lack of γ-8 may expose endoplasmic reticulum retention signals on AMPAR subunits, as has been shown for the regulated trafficking of ATP-sensitive K^+ channel complexes[31]. Future studies will be required to determine the detailed molecular mechanisms for γ-8 regulation of AMPAR expression.

Deletion of the GluR1 subunit[24] redistributes GluR2/3 in a manner similar to what we found here for both GluR1 and GluR2/3 in the γ-$8^{-/-}$ mice. However, in contrast to the deletion of γ-8, *GluR1*$^{-/-}$ mice show modest changes in the expression of GluR2, GluR3 and GluR4 (refs. 24,32). The amount of γ-8 protein is only slightly reduced, and its distribution not obviously affected in the *GluR1*$^{-/-}$ mice. We suggest that in the *GluR1*$^{-/-}$ mice there is a great excess of γ-8 compared with the remaining GluR2/3 receptors. Thus, although GluR2/3, which is presumably associated with γ-8, is mislocalized, this represents a small fraction of the total γ-8 pool. This is likely to indicate that γ-8 does not require association with AMPARs for its processing and dendritic trafficking. This is reminiscent of other ionic channel non-pore-forming auxiliary subunits, such as the β1b and β2a subunit of L-type calcium channels, which can be normally expressed and trafficked to the membrane in the absence of the pore-forming subunit α1 (refs. 33–35). Presumably, normal trafficking of these auxiliary subunits is made possible by the lack of both a retention signal from these proteins and an export signal from other proteins.

Differential delivery of limited AMPARs to the synapse

Functional AMPARs are essentially absent from extrasynaptic membranes and synapses in cerebellar granule cells of stargazer mice[13–15], whereas AMPAR synaptic transmission is reduced by only ∼35% in the hippocampus of γ-$8^{-/-}$ mice, despite the marked decrease in AMPAR expression. Whereas stargazin seems to be the only TARP expressed in cerebellar granule cells, hippocampal pyramidal cells express significant amounts of other TARPs (γ-2, γ-3 and γ-4)[15,16]. This redundancy could mask some functions of γ-8 that we did not demonstrate in the present study, such as a direct role in synaptic trafficking. Indeed, it is possible that AMPAR trafficking by other TARPs accounts for the remaining currents in hippocampal γ-$8^{-/-}$ pyramidal cells. γ-$2^{-/-}\gamma$-$8^{-/-}$ mice show a more severe reduction (∼50%) in synaptic transmission, supporting the proposal that other hippocampal TARPs contribute to the synaptic targeting of at least part of the remaining AMPARs in the γ-$8^{-/-}$ mice. Alternatively, it is possible that some residual TARP-independent trafficking of AMPARs occurs in hippocampal pyramidal cells. Whereas the synaptic AMPAR response was reduced by only ∼35%, the extrasynaptic responses were reduced 90% in the γ-$8^{-/-}$ mice. As extrasynaptic AMPARs represent the majority of surface AMPARs, we cannot exclude that the massive decrease in extrasynaptic receptors may in part be secondary to the diminished pool of AMPARs in the cell. In addition, extrasynaptic AMPAR responses are significantly reduced in the γ-$8^{+/-}$ mice and greatly enhanced in neurons overexpressing γ-8. This enhancement occurrs in the absence of obvious changes in synaptic AMPARs. This could be due to a limited number of γ-8–interacting proteins (such as membrane-associated guanylate kinases (MAGUKs)), necessary for the synaptic insertion of AMPARs, or to a sufficient level of endogenous γ-8 to support AMPAR synaptic targeting. These results, which are similar to the results of overexpression of γ-2 (ref. 17), indicate that the number of extrasynaptic AMPARs is tightly regulated by the levels of γ-8.

On the other hand, synaptic AMPARs are largely immune from the marked loss of cytoplasmic and extrasynaptic AMPARs, indicating that pyramidal cells selectively maintain AMPAR-mediated synaptic transmission with very few receptors. *GluR1*$^{-/-}$ mice show a similar loss of extrasynaptic AMPARs with modest[36] or no[24] decreases in

synaptic transmission. We propose that the strong reduction in the total number of AMPARs observed in both the *GluR1*$^{-/-}$ and the γ-*8*$^{-/-}$ mice is responsible for this common phenotype. Indeed, synaptic AMPARs, which represent a small proportion of surface AMPARs, can presumably be maintained by the relatively normal levels of other AMPAR subunits (GluR2, GluR3, GluR4) in the *GluR1*$^{-/-}$ mice and by the few remaining AMPAR subunits (GluR1, GluR2, GluR3, GluR4) that may be associated with other TARPs in the γ-*8*$^{-/-}$ mice.

LTP is impaired in the γ-*8*$^{-/-}$ mouse

The defect in LTP in γ-*8*$^{-/-}$ mice, which occurs downstream of NMDAR activation, has a number of possible explanations. An intriguing possibility is that γ-8 has a direct role in LTP. In support of this conclusion we have recently found that the intracellular C termini of stargazin and other TARPs have multiple phosphorylation sites for CaMKII/protein kinase C (PKC) and that expressing a construct in which the serines are mutated to alanines also blocks LTP[37]. Another possibility is that during LTP, AMPARs are recruited to the synapse either from the extrasynaptic pool by lateral diffusion or from an intracellular pool, and the loss of AMPARs in γ-*8*$^{-/-}$ mice contributes to the defect in LTP. A similar interpretation could explain the loss of LTP in the *GluR1*$^{-/-}$ mice[24].

Unique role for TARPs in regulating ionotropic receptors

Numerous studies have addressed the mechanisms underlying synaptic AMPAR trafficking[1–3,7–10], but a number of issues remain poorly understood. The proposed mechanisms include phosphorylation of the C-terminal region of AMPARs and protein-protein interactions with cytoplasmic scaffolding proteins. Although both mechanisms have been implicated in LTP and LTD, many questions persist. Mice lacking the phosphorylation sites on GluR1 that are involved in synaptic plasticity show normal basal synaptic transmission[38]. This suggests that the activity-dependent trafficking of AMPARs differs from basal receptor delivery. Mice lacking both GluR2 and GluR3 have normal LTP and LTD, but effects on baseline transmission are less clear[11]. That is, the input/output curve for field EPSPs is reduced, but the amplitudes of AMPAR-mediated mEPSCs are normal[11]. Further studies are required to reconcile discrepancies between the mouse knockout studies and the large body of literature showing that subunit-specific AMPAR protein interactions with previously identified cytoplasmic proteins are essential in hippocampal receptor trafficking.

The present results demonstrate a role for γ-8 in maintaining normal levels and distribution of AMPAR protein in hippocampal neurons. The fact that γ-8 overexpression greatly increases the number of surface extrasynaptic receptors, independent of total AMPAR protein levels, suggests that γ-8 has an additional role in AMPAR trafficking after receptor maturation that is distinct from maintaining the levels of AMPAR protein. However, we cannot exclude the possibility that the potential stabilization of AMPAR subunits by γ-8 may increase the probability of their membrane insertion. A general theme derived from studies on voltage-sensitive ion channels indicates that non-pore-forming auxiliary subunits are important for controlling the formation, stabilization, trafficking and gating of the pore forming subunit[39,40]. γ-8, as well as γ-2, are the first proteins found to have an analogous role for any ionotropic receptor[41]. This raises the intriguing possibility that auxiliary proteins may control the expression of other ionotropic receptors. Alternatively, because of the central role AMPAR trafficking plays in plasticity, TARPs may have specifically evolved to assist in the dynamic behavior of this class of receptor. It will be of interest in future studies to determine the trafficking mechanism responsible for controlling the remaining AMPARs in the γ-*8*$^{-/-}$ mice and to determine how TARP-dependent trafficking and the various other proposed trafficking mechanisms work in concert to govern the number of AMPARs present at the synapse.

METHODS

Antibodies. The rabbit polyclonal antibody to γ-8 has been characterized previously[16]. A list of the commercial antibodies used in this study is available in the **Supplementary Methods**.

Knockout mice. Experimental procedures were in accordance with the animal welfare guidelines of the University of California, San Francisco. To generate knockout mice, embryonic stem cells were injected into mouse blastocytes, and the embryos were implanted into surrogate mothers. Chimeric mice were identified by coat color and mated to C57/B16 mice. Further details on how the knockout mice were made are available in **Supplementary Methods**.

Northern and Southern analysis. Hippocampal RNAs from mice were purified using TRIZOL (Invitrogen). Equal amounts of total RNA were separated on a 1% agarose, 1.3% paraformaldehyde gel followed by transfer to nylon membranes and ultraviolet crosslinking. Further details are available in the **Supplementary Methods**.

Immunoblotting. Hippocampi from mouse siblings (P35–P45) were homogenized in 320 mM sucrose buffer and then sonicated in 2% final SDS. Equal amounts of protein were separated on 8% polyacrylamide gels followed by transfer to PVDF membranes. Proteins were detected by immunoblotting using the HRP-ECL kit from Amersham. Further details are available in the **Supplementary Methods**.

Immunohistochemistry. Anesthetized mice were transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 5 minutes. Further details on the preparation of brain sections are available in the **Supplementary Methods**.

Postembedding immunogold labeling. For postembedding immunogold labeling, parasagittal sections of the hippocampus were cryoprotected, frozen, infiltrated with Lowicryl HM 20 and polymerized with ultraviolet light. Thin sections were immunolabelled using 10 nm immunogold (Ted Pella). Details on how the data were scored are available in the **Supplementary Methods**.

Immunofluorescent assay of GluR2 protein. Staining for GluR2 was performed on rat hippocampal dissociated neuronal cultures 2–3 weeks after plating. Further details of the staining of surface and intracellular GluR2 is provided in the **Supplementary Methods**.

Electrophysiology and viral infection. Rat hippocampal slice cultures[17] and acute mouse hippocampal slices[42] were prepared and recorded from as described previously. Further details are available in the **Supplementary Methods**.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Malinow, R. & Malenka, R.C. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126 (2002).
2. Song, I. & Huganir, R.L. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci.* **25**, 578–588 (2002).
3. Bredt, D.S. & Nicoll, R.A. AMPA receptor trafficking at excitatory synapses. *Neuron* **40**, 361–379 (2003).
4. Dingledine, R., Borges, K., Bowie, D. & Traynelis, S.F. The glutamate receptor ion channels. *Pharmacol. Rev.* **51**, 7–61 (1999).
5. Hollmann, M. & Heinemann, S. Cloned glutamate receptors. *Annu. Rev. Neurosci.* **17**, 31–108 (1994).
6. Wisden, W. & Seeburg, P.H. Mammalian ionotropic glutamate receptors. *Curr. Opin. Neurobiol.* **3**, 291–298 (1993).
7. Barry, M.F. & Ziff, E.B. Receptor trafficking and the plasticity of excitatory synapses. *Curr. Opin. Neurobiol.* **12**, 279–286 (2002).
8. Garner, C.C., Nash, J. & Huganir, R.L. PDZ domains in synapse assembly and signalling. *Trends Cell Biol.* **10**, 274–280 (2000).
9. Scannevin, R.H. & Huganir, R.L. Postsynaptic organization and regulation of excitatory synapses. *Nat. Rev. Neurosci.* **1**, 133–141 (2000).
10. Sheng, M. Molecular organization of the postsynaptic specialization. *Proc. Natl. Acad. Sci. USA* **98**, 7058–7061 (2001).
11. Meng, Y., Zhang, Y. & Jia, Z. Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3. *Neuron* **39**, 163–176 (2003).
12. Letts, V.A. *et al.* The mouse stargazer gene encodes a neuronal Ca^{2+}-channel gamma subunit. *Nat. Genet.* **19**, 340–347 (1998).
13. Chen, L., Bao, S., Qiao, X. & Thompson, R.F. Impaired cerebellar synapse maturation in waggler, a mutant mouse with a disrupted neuronal calcium channel gamma subunit. *Proc. Natl. Acad. Sci. USA* **96**, 12132–12137 (1999).
14. Hashimoto, K. *et al.* Impairment of AMPA receptor function in cerebellar granule cells of ataxic mutant mouse stargazer. *J. Neurosci.* **19**, 6027–6036 (1999).
15. Chen, L. *et al.* Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408**, 936–943 (2000).
16. Tomita, S. *et al.* Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. *J. Cell Biol.* **161**, 805–816 (2003).
17. Schnell, E. *et al.* Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc. Natl. Acad. Sci. USA* **99**, 13902–13907 (2002).
18. Klugbauer, N. *et al.* A family of gamma-like calcium channel subunits. *FEBS Lett.* **470**, 189–197 (2000).
19. Burgess, D.L., Gefrides, L.A., Foreman, P.J. & Noebels, J.L. A cluster of three novel Ca2+ channel gamma subunit genes on chromosome 19q13.4: evolution and expression profile of the gamma subunit gene family. *Genomics* **71**, 339–350 (2001).
20. Chu, P.J., Robertson, H.M. & Best, P.M. Calcium channel gamma subunits provide insights into the evolution of this gene family. *Gene* **280**, 37–48 (2001).
21. Hurtley, S.M. & Helenius, A. Protein oligomerization in the endoplasmic reticulum. *Annu. Rev. Cell Biol.* **5**, 277–307 (1989).
22. Hollmann, M., Maron, C. & Heinemann, S. N-glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluR1. *Neuron* **13**, 1331–1343 (1994).
23. Sans, N. *et al.* Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway. *J. Neurosci.* **21**, 7506–7516 (2001).
24. Zamanillo, D. *et al.* Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science* **284**, 1805–1811 (1999).
25. Castillo, P.E., Malenka, R.C. & Nicoll, R.A. Kainate receptors mediate a slow post-synaptic current in hippocampal CA3 neurons. *Nature* **388**, 182–186 (1997).
26. Vignes, M. & Collingridge, G.L. The synaptic activation of kainate receptors. *Nature* **388**, 179–182 (1997).
27. Nicoll, R.A. & Malenka, R.C. Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* **377**, 115–118 (1995).
28. Henze, D.A., Urban, N.N. & Barrionuevo, G. The multifarious hippocampal mossy fiber pathway: a review. *Neuroscience* **98**, 407–427 (2000).
29. Vandenberghe, W., Nicoll, R.A. & Bredt, D.S. Interaction with the unfolded protein response reveals a role for stargazin in biosynthetic AMPA receptor transport. *J. Neurosci.* **25**, 1095–1102 (2005).
30. Ellgaard, L. & Helenius, A. Quality control in the endoplasmic reticulum. *Nat. Rev. Mol. Cell Biol.* **4**, 181–191 (2003).
31. Zerangue, N., Schwappach, B., Jan, Y.N. & Jan, L.Y. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron* **22**, 537–548 (1999).
32. Jensen, V. *et al.* A juvenile form of postsynaptic hippocampal long-term potentiation in mice deficient for the AMPA receptor subunit GluR-A. *J. Physiol. (Lond.)* **553**, 843–856 (2003).
33. Chien, A.J. *et al.* Roles of a membrane-localized beta subunit in the formation and targeting of functional L-type Ca^{2+} channels. *J. Biol. Chem.* **270**, 30036–30044 (1995).
34. Brice, N.L. *et al.* Importance of the different beta subunits in the membrane expression of the alpha1A and alpha2 calcium channel subunits: studies using a depolarization-sensitive alpha1A antibody. *Eur. J. Neurosci.* **9**, 749–759 (1997).
35. Bogdanov, Y. *et al.* Acidic motif responsible for plasma membrane association of the voltage-dependent calcium channel beta1b subunit. *Eur. J. Neurosci.* **12**, 894–902 (2000).
36. Andrasfalvy, B.K., Smith, M.A., Borchardt, T., Sprengel, R. & Magee, J.C. Impaired regulation of synaptic strength in hippocampal neurons from GluR1-deficient mice. *J. Physiol. (Lond.)* **552**, 35–45 (2003).
37. Tomita, S., Stein, V., Stocker, T.J., Nicoll, R.A. & Bredt, D.S. Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. *Neuron* **45**, 269–277 (2005).
38. Lee, H.K. *et al.* Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* **112**, 631–643 (2003).
39. Catterall, W.A. Structure and regulation of voltage-gated Ca2+ channels. *Annu. Rev. Cell Dev. Biol.* **16**, 521–555 (2000).
40. Arikkath, J. & Campbell, K.P. Auxiliary subunits: essential components of the voltage-gated calcium channel complex. *Curr. Opin. Neurobiol.* **13**, 298–307 (2003).
41. Tomita, S. *et al.* Stargazin modulates AMPA receptor gating and trafficking by distinct domains. *Nature* **435**, 1052–1058 (2005).
42. Fremeau, R.T. *et al.* Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites. *Science* **304**, 1815–1819 (2004).