

Persistent hippocampal CA1 LTP in mice lacking the C-terminal PDZ ligand of GluR1

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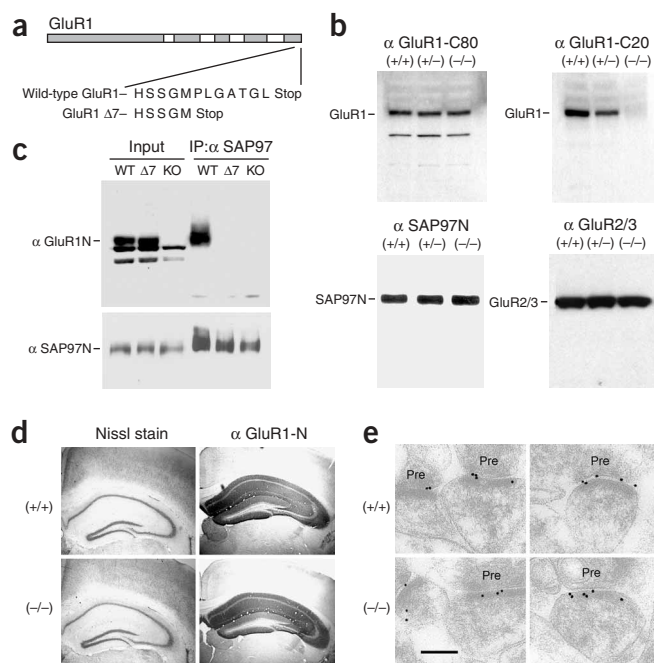
The C-terminal PDZ ligand of the AMPA receptor GluR1 subunit may be important for expression of CA1 hippocampal long-term potentiation. To test this directly *in vivo*, we generated a knock-in mouse lacking the last seven residues of GluR1, comprising the PDZ ligand. This deletion did not affect basal GluR1 synaptic localization, basal synaptic transmission, long-term potentiation or long-term depression, indicating that the ligand is not required for CA1 hippocampal synaptic plasticity.

Recent studies have suggested that the regulated exocytosis and endocytosis of vesicles containing AMPA receptors or the lateral movement of AMPA receptors along the plasma membrane could be an important mechanism regulating the level of synaptic AMPA receptors after the induction of long-term potentiation (LTP) and long-term depression (LTD)¹. A variety of data have indicated that

AMPA receptor interactions with PDZ domain-containing proteins may be an important regulatory step during these cellular processes^{1–7}. A recent study has reported that a single amino acid mutation (T887A) within the PDZ ligand of GluR1 blocks the synaptic delivery of virally overexpressed GluR1 during LTP induction in CA1 region of organotypic hippocampal slices⁸. These results indicate a critical role of the GluR1 PDZ ligand in the membrane insertion of AMPA receptors during activity-dependent processes. To test the role of the GluR1 PDZ ligand in the synaptic targeting of GluR1 and synaptic plasticity *in vivo* directly, we generated a knock-in mouse (GluR1Δ7) that lacks the last seven amino acids of GluR1, which constitute the PDZ ligand (Fig. 1a and Supplementary Fig. 1 online). The GluR1Δ7 mutant mice are viable, breed normally and have no obvious behavioral or developmental phenotypes.

We first examined whether the GluR1 PDZ ligand deletion mutation affected the expression of GluR1, by western blotting using an N-terminal GluR1 antibody. The expression of the mutated GluR1 was normal compared to that in wild-type mice (Fig. 1b), as was the expression of GluR2 and GluR3. Previous studies have shown that GluR1 interacts with the PDZ domain-containing synapse associated protein 97 (SAP97) through its C-terminal PDZ ligand^{9–11}. We therefore measured the expression of SAP97 in the GluR1Δ7 mice and found

Figure 1 Generation of GluR1 mutant mice lacking the last seven amino acids comprising the PDZ ligand. (a) Schematic representation of GluR1 protein and C-terminal amino acid sequence after modification of stop codon. (b) Immunoblot analysis of wild-type (+/+), heterozygous mutant (+/–) and homozygous mutant (–/–) mice. GluR1 with the seven-amino-acid deletion can be detected by an antibody raised against the last 80 amino acids of the GluR1 C terminus (α GluR1-C80) but not by an antibody against the last 20 amino acids of the GluR1 C terminus (α GluR1-C20). α SAP97N, antibody against the N terminus of SAP97; α GluR2/3, antibody against GluR2/3. (c) Co-immunoprecipitation of GluR1 and SAP97 *in vivo*. Membrane fractions of brain homogenate of wild-type (WT), homozygous GluR1Δ7 mutant (Δ7) and GluR1 knockout mice (KO; Supplementary Fig. 4) were solubilized and immunoprecipitated with antibody to SAP97 (α SAP97) and detected with an antibody to the GluR1 N terminus (α GluR1N). Lower panel shows immunoprecipitated SAP97. (d) Gross hippocampal anatomy and localization of GluR1 immunoreactivity in wild-type and GluR1Δ7 mutant mice. Left, Nissl staining; right, GluR1 N-terminal immunocytochemistry. (e) Immunoelectron microscopic labeling of GluR1 receptor localization in wild-type and GluR1Δ7 mutant mice. ‘Pre’ indicates presynaptic terminal. Scale bar, 200 nm. All experiments were done in accordance with the policies of the Animal Care and Use Committee at the Johns Hopkins School of Medicine.



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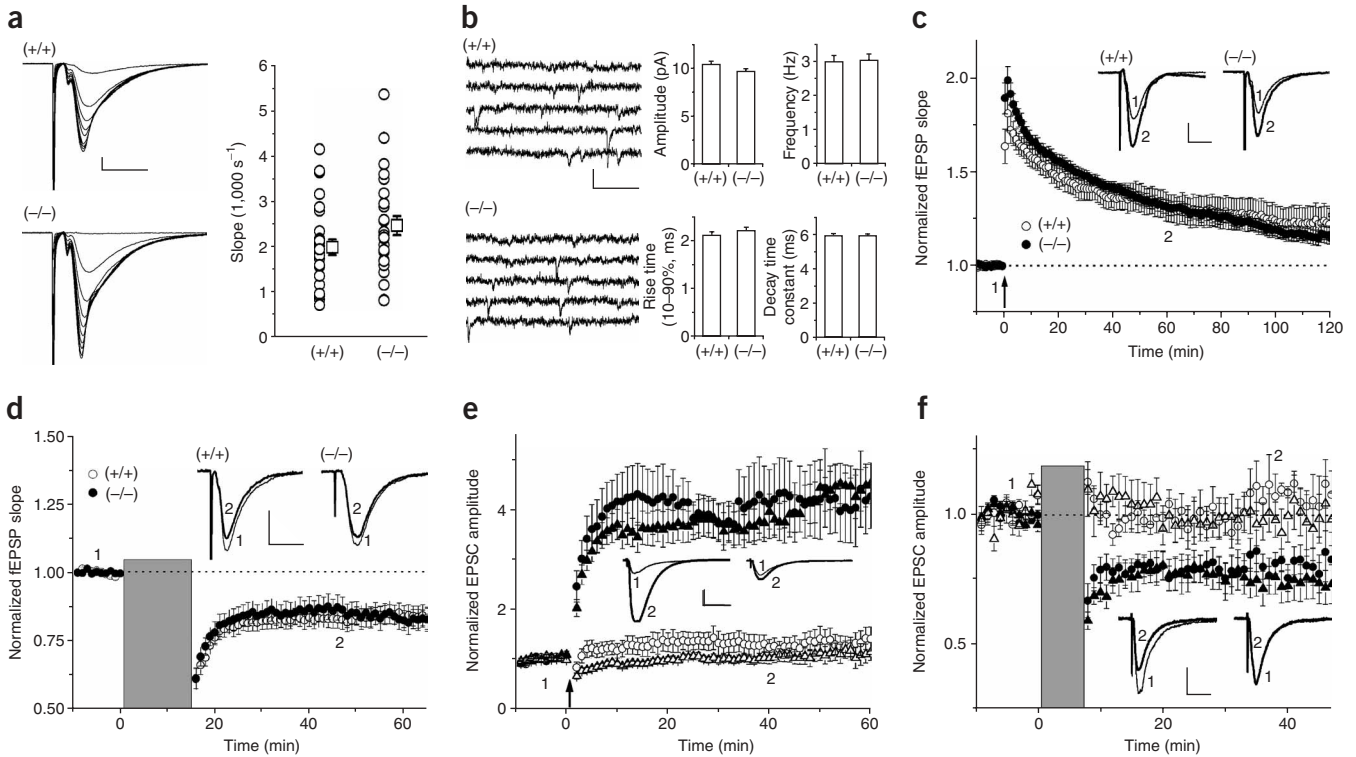


Figure 2 Basal and activity-dependent synaptic properties of CA1 pyramidal neurons from GluR1 Δ 7 mice. **(a)** Left, sample fEPSP traces evoked with incremental stimulus strength. Scale: 200 μ V, 10 ms. Right, plot of input-output slopes from slices from both wild-type (+/+) and mutant (-/-) mice. Two-tailed *t*-test ($\alpha = 0.05$) showed that slopes from the mutant mice are not significantly different from those from wild-type mice (wild-type, 1.98 ± 0.17 ; mutant, 2.46 ± 0.21 , $P = 0.06$). Open squares indicate mean values and error bars s.e.m. **(b)** Analysis of mEPSC from 3-month-old mice shows no significant difference in mEPSC amplitude, mEPSC frequency, rise time (10–90%) or decay time constant between wild-type and mutant mice. Sample traces are shown at left. Scale: 10 pA, 125 ms. **(c)** Single theta burst–induced LTP shows no significant difference 60 min after LTP induction between wild-type and mutant mice (3 months old). Arrow indicates the time when single theta burst was applied. Inset, sample average traces recorded at time 1 and 2; scale: 200 μ V, 10 ms. **(d)** Low-frequency stimulus (LFS, 1 Hz, 900 pulses)-induced LTD shows normal depression 30 min after LFS in mutant mice. Insets, sample average traces recorded at times 1 and 2 (scale: 300 μ V, 10 ms). Gray box indicates the period when LFS was applied. **(e)** Pairing-induced whole-cell LTP shows similar potentiation 40 min after pairing between wild-type and mutant mice. Arrow indicates the time when pairing stimuli were applied. Filled symbols: LTP path; open symbols: non-LTP path. Circles: wild-type mice; triangles: mutant mice. Insets, sample average traces recorded at time 1 and 2 from mutant mice. Left traces are from LTP path and right traces are from non-LTP path (scale: 200 μ A, 10 ms). **(f)** Pairing-induced whole-cell LTD also shows no significant difference in depression 30 min after pairing between wild-type and mutant mice (3 to 6 weeks old). Gray box indicates the period when pairing stimuli were applied. Filled symbols: LTD path; open symbols: non-LTD path. Circles: wild-type mice; triangles: mutant mice. Insets, sample average traces recorded at time 1 and 2 from mutant mice. Left traces are from LTD path and right traces are from non-LTD path (scale: 100 μ A, 10 ms).

that it was similar to that in wild-type mice (**Fig. 1b**). To examine the interaction of GluR1 and SAP97 in wild-type and GluR1 Δ 7 mutant mice, we carried out co-immunoprecipitation experiments, using GluR1 knockout mouse brain samples as a negative control. The interaction of GluR1 with SAP97 *in vivo* was abolished in GluR1 Δ 7 mutant mice (**Fig. 1c**). These results strongly indicate that the GluR1 Δ 7 mutation severely diminishes GluR1 binding to PDZ domains in mutant mice.

Next, we examined the distribution of GluR1 in hippocampal sections by immunostaining using an antibody against the N terminus of GluR1 (**Fig. 1d**). Mutant mice show a pattern of immunostaining similar to that in wild-type mice. To analyze the localization of the mutant GluR1 subunit at synapses, we used immunogold electron microscopic methods with two different antibodies against the GluR1 N terminus (**Fig. 1e**). Three independent analyses, each with two pairs of animals, showed no significant difference in the distribution of GluR1 in wild-type and homozygous mutant mice (GluR1 Δ 7), either in the average number of gold particles per synapse (wild-type, 0.41 ± 0.028 , $n = 846$ synapses; mutant, 0.37 ± 0.025 , $n = 846$ synapses; two-tailed *t*-test, $P = 0.352$) or in the average number of gold particles

per labeled synapse (wild-type, 1.47 ± 0.062 , $n = 233$ labeled synapses; mutant, 1.44 ± 0.052 , $n = 217$ labeled synapses; $P = 0.714$; **Fig. 1e**). This suggested that there are no major changes in the GluR1 synaptic trafficking in the mutant mice. We also analyzed the extrasynaptic distribution of GluR1 and found no obvious difference in the number of gold particles per postsynaptic process between wild-type and mutant mice (wild-type, 0.11 ± 0.03 , total 983 counts; mutant, 0.14 ± 0.03 , total 1,120 counts; $P = 0.46$).

To check the basal synaptic transmission properties, we first examined the evoked synaptic input-output relationship in acute hippocampal slices. To do this, we first plotted the rising slope of field excitatory postsynaptic potential (fEPSP) against the amplitude of presynaptic fiber volley potential with ten incremental input strengths per slice (not shown). We then calculated the slope of the plot (fEPSP slope vs. fiber volley potential amplitude) of each slice by linear regression (wild-type, $n = 28$; mutant, $n = 26$ slices; **Supplementary Methods and Fig. 2a**). The result showed that mutant mice have a postsynaptic response range similar to that in wild-type mice, indicating that there was no significant difference in synaptic input-output relationship. Next, to test the role of GluR1 PDZ ligand on the basal

synaptic AMPA receptor (AMPA) trafficking, we measured AMPAR-mediated miniature excitatory postsynaptic current (mEPSC) in the presence of 1 μ M tetrodotoxin and 100 μ M AP5 (Fig. 2b). The results showed no significant difference in the amplitude (in pA: wild-type, 10.42 ± 0.28 ; mutant, 9.72 ± 0.22 , $P = 0.06$), frequency (in Hz: wild-type, 2.99 ± 0.19 ; mutant, 3.02 ± 0.19 , $P = 0.89$), rise time (10–90% of peak amplitude, in ms: wild-type, 2.11 ± 0.08 ; mutant, 2.21 ± 0.07 , $P = 0.34$) or decay time constant (in ms: wild-type, 5.89 ± 0.13 ; mutant, 5.90 ± 0.11 , $P = 0.97$) of mEPSC between wild-type ($n = 30$) and mutant mice ($n = 26$), suggesting no major perturbation in basal synaptic AMPAR trafficking in CA1 pyramidal neurons of GluR1 Δ 7 mutant mice. This result is consistent with the immunoelectron microscopy data that showed no changes in the number of synaptic GluR1 in the mutant mice. Furthermore, the whole-cell current and voltage relationship of both AMPAR- and NMDA receptor (NMDAR)-mediated currents in the mutant mice were similar to those in wild-type mice (Supplementary Fig. 2). These results suggest that removal of GluR1 PDZ ligand does not affect the basal AMPAR and NMDAR trafficking at synapses. To test whether the mutation had any effect on presynaptic vesicle release properties, we measured paired-pulse facilitation, but we did not find any significant change in mutant mice (Supplementary Fig. 2).

To investigate the role of the GluR1 PDZ ligand in synaptic plasticity, we first examined LTP induced by single theta burst in the CA1 dendritic region of hippocampal slices (Fig. 2c). We observed LTP in slices from the GluR1 Δ 7 mutant mice, and we did not find any significant change in the magnitude of LTP for up to 2 h of recording as compared with wild-type slices (wild-type, $131 \pm 8\%$, $n = 14$; mutant, $132 \pm 4\%$, $n = 12$, measured 60 minute after theta burst and normalized to the baseline, $P = 0.59$). Although not significant ($P = 0.251$, 2 h after LTP induction), there was a slight difference in the apparent decay of LTP in the mutant mice, suggesting that there may be a small difference in the late phase of LTP in the GluR1 Δ 7 mutant mice. Induction of LTD (induced by 1 Hz, 900 pulses) in the mutant mice was also similar to induction in wild-type mice (Fig. 2d, wild-type, $83 \pm 3\%$, $n = 18$; mutant, $87 \pm 5\%$, $n = 13$, 30 min after LFS, $P = 0.11$). To further confirm the field LTP data, we induced whole-cell LTP by a pairing protocol from CA1 pyramidal neurons. We found no significant difference in the magnitude of LTP between the wild type and mutants in 3-week-old to 6-month-old mice. Figure 2e shows the LTP data from 6-month-old male mouse pairs (wild-type, $417 \pm 71\%$, $n = 11$; knockout, $378 \pm 36\%$, $n = 9$, 40 min after whole-cell LTP, $P = 0.68$). The pairing-induced LTP was NMDAR-dependent and CaMKII-dependent, as AP5 or CaMKII inhibitory peptide could block LTP induction in both wild-type and mutant mice (Supplementary Fig. 3). We also tested pairing-induced LTD and found no significant difference 30 min after pairing (Fig. 2f, wild-type, $81 \pm 4\%$, $n = 11$; mutant, $76 \pm 7\%$, $n = 11$, $P = 0.22$). De-depression after LTD induction was also induced normally compared to wild-type mice (Supplementary Fig. 2). These results show that removal of the GluR1 PDZ ligand has no obvious effect on the induction of activity-dependent synaptic plasticity such as LTP and LTD.

In another approach, we tried to acutely inhibit the function of the endogenous GluR1 PDZ ligand in wild-type mice by perfusing a peptide corresponding to the GluR1 PDZ ligand (15 amino acids, 200 μ M) into the cell and then waiting 30 min before LTP induction. The results show that postsynaptic perfusion of the GluR1 PDZ ligand peptide had no effect on the induction of pairing-induced LTP in rat hippocampal CA1 pyramidal neurons (Supplementary Fig. 3). This data is in contrast with a previous report that showed that overexpression of GFP-tagged constructs containing the whole C-terminal domain of GluR1 (80 amino acids) blocks LTP induction in CA1 pyramidal neurons in slice culture¹². However, the experimental conditions for this study are quite different from the peptide perfusion experiments: the whole C terminus, which contains many other protein-protein interaction sites, was overexpressed in the previous experiments, and the GFP-tagged C-terminal domain was expressed for 24 h, whereas our peptide perfusion experiments were acute experiments.

Taken together, these results indicate that NMDAR-dependent LTP can be induced in the absence of the GluR1 PDZ ligand *in vivo*. This is in contrast to previous results using overexpression of GluR1 PDZ ligand point mutants (T887A) in hippocampal slice culture, which implicate the GluR1 PDZ ligand as a critical factor for CA1 LTP expression⁸. The reason for the discrepancy between these studies is not clear. It is possible that the point mutation of the GluR1 PDZ ligand has different functional effects compared with a complete deletion of the PDZ ligand, or alternatively, the properties of the trafficking of overexpressed GluR1 in organotypic cultures may be different from those seen *in vivo*. However, our studies clearly show that deletion of the GluR1 PDZ ligand has little effect on activity-dependent synaptic plasticity and basal synaptic targeting of GluR1 *in vivo*.

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

ACKNOWLEDGMENTS

This work was supported by US National Institutes of Health grant NS36715 and the Howard Hughes Medical Institute.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the Nature Neuroscience website for details).

Received 1 February; accepted 24 June 2005

Published online at <http://www.nature.com/natureneuroscience/>

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