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Citation: Selcher, Joel C., Weifeng Xu, Jesse E. Hanson, Robert C. Malenka, and Daniel V. Madison. "Glutamate Receptor Subunit GluA1 Is Necessary for Long-Term Potentiation and Synapse Unsilencing, but Not Long-Term Depression in Mouse Hippocampus." Brain Research 1435 (January 2012): 8–14.

As Published: http://dx.doi.org/10.1016/j.brainres.2011.11.029

Publisher: Elsevier

Persistent URL: http://hdl.handle.net/1721.1/102657

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

Published in final edited form as:
Brain Res. 2012 January 30; 1435: 8–14. doi:10.1016/j.brainres.2011.11.029.

Glutamate Receptor Subunit GluA1 is Necessary for Long-term Potentiation and Synapse Unsilencing, but not Long-term Depression in Mouse Hippocampus

Joel C. Selcher[1], **Weifeng Xu**[2], **Jesse E. Hanson**[1], **Robert C. Malenka**[2], and **Daniel V. Madison**[1]

[1]Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305, USA

[2]Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California 94305

Abstract

Receptor subunit composition is believed to play a major role in the synaptic trafficking of AMPA receptors (AMPARs), and thus in activity-dependent synaptic plasticity. To isolate a physiological role of GluA1-containing AMPARs in area CA3 of the hippocampus, pair recordings were performed in organotypic hippocampal slices taken from genetically modified mice lacking the GluA1 subunit. We report here that long-term potentiation (LTP) is impaired not only at active but also at silent synapses when the GluA1 subunit is absent. The GluA1 knockout mice also exhibited reduced AMPAR-mediated evoked currents between pairs of CA3 pyramidal neurons under baseline conditions suggesting a significant role for GluA1-containing AMPARs in regulating basal synaptic transmission. In two independent measures, however, long-term depression (LTD) was unaffected in tissue from these mice. These data provide a further demonstration of the fundamental role that GluA1-containing AMPARs play in activity-dependent increases in synaptic strength but do not support a GluA1-dependent mechanism for reductions in synaptic strength.

Keywords

Glutamate receptor; AMPA receptor; knockout; synaptic plasticity; silent synapse; long-term potentiation; long-term depression; hippocampus

1. INTRODUCTION

Excitatory synaptic transmission in the mammalian brain occurs predominately through the glutamatergic system mediated by postsynaptic AMPA (Alpha-Amino-3-Hydroxy-5-

Corresponding Author: Daniel V. Madison, Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305-5345, USA madison@stanford.edu. Tel: +1-650=725-7563, FAX +1-650-618-2779.
Authors' present addresses: W. Xu: Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. JC Selcher and JE Hanson: Genentech, Inc., South San Francisco, CA 94080, USA.

Methyl-4-Isoxazole Propionic Acid) and NMDA (N-methyl-D-aspartate) receptors. AMPA receptors (AMPARs) carry the postsynaptic current in most cases, while NMDA receptors (NMDARs) do not support current flow at or near resting membrane potential because of voltage-dependent magnesium blockade. NMDARs, which become unblocked during strong postsynaptic depolarizations are necessary for most induction of changes in the strength of synaptic transmission, either in the positive direction (e.g. long-term potentiation, LTP) or in the negative direction (e.g. long-term depression, LTD). These changes in synaptic strength are expressed via alterations in the number of AMPARs in the postsynaptic membrane, where they can detect synaptically-released glutamate, with addition of synaptic AMPARs expressing LTP and their removal expressing LTD (for review see: (Malinow and Malenka, 2002; Shepherd and Huganir, 2007). The cellular mechanisms underlying the dynamic regulation of glutamate receptor expression are incompletely understood, but the subunit composition of AMPARs has been suggested to play a major role (Andrasfalvy et al., 2003; Emond et al., 2010; Passafaro et al., 2001; Piccini and Malinow, 2002; Shi et al., 2001); for reviews, see (Malinow and Malenka, 2002; Shepherd and Huganir, 2007).

AMPARs are comprised of combinations of four subunits, GluA1–4, each with distinct C-terminal tails and a diverse array of intracellular binding partners (Shepherd and Huganir, 2007). Previous studies, including those of a knockout mouse, deficient in the GluA1 (aka GluR1 or GluR-A) subunit of the AMPA receptor, have demonstrated a requirement for GluA1-containing AMPARs in the expression of LTP (Andrasfalvy et al., 2003; Mack et al., 2001; Shi et al., 2001; Zamanillo et al., 1999), although these results may be dependent on the age of the animal (Jensen et al., 2003) and the induction protocol used (Hoffman et al., 2002). Besides lacking LTP, this mouse also shows alterations in other forms or aspects of plasticity (Andrasfalvy et al., 2003; Erickson et al., 2010; Hardingham and Fox, 2006; McCormack et al., 2006; Phillips et al., 2008), certain learning tasks (Reisel et al., 2002; Sanderson et al., 2008; Schmitt et al., 2003; Schmitt et al., 2004; Schmitt et al., 2005) and behavioral assays (Bannerman et al., 2003; Bannerman et al., 2004; Wiedholz et al., 2008)

In the present study, we re-examine and extend these findings using a paired recording technique that enables the study of synaptic transmission and plasticity at the minimal synaptic connection between two individual CA3 pyramidal neurons (Pavlidis and Madison, 1999). In addition, this technique also permits examination of state-dependent mechanisms underlying changes in synaptic strength (Emond et al., 2010; Montgomery and Madison, 2002; Montgomery and Madison, 2004)

2. RESULTS

2.1

To assess the functional role of the GluA1 subunit of AMPARs in synaptic transmission and plasticity, simultaneous whole cell recordings were performed on pairs of synaptically connected hippocampal CA3 pyramidal neurons in slices prepared from GluA1$^{-/-}$ mice and wild-type littermate controls. The primary utility of recording from such pairs, as compared to other electrophysiological methods, is that it allows for the study of unitary synaptic connections, comprised of one or a very few synapses (Pavlidis & Madison 1999). This allows for the identification of the connection as active (i.e. having AMPAR and NMDAR-mediated synaptic transmission) or silent (i.e. lacking AMPAR-mediated but having NMDAR-mediated transmission). Silent synapses have not been previously examined in GluA1-deficient brain tissue.

In greater than half of the pairs from both knockout and wild-type mice (52.6% for GluA1$^{-/-}$ and 52.2% for WT), the two CA3 pyramidal neurons were connected by “active” synapses, defined by displaying an EPSC in response to an action potential in the

presynaptic cell. Such unitary EPSCs are mediated by AMPARs (Montgomery et al., 2001). We first tested the role of the GluA1 subunit in potentiation of the strength of these active synapses by comparing LTP induced in pairs from wild-type versus GluA1-lacking mouse slices (Fig. 1*A*). Stable LTP was reliably induced in wild-type pairs by holding the postsynaptic cell at a depolarized potential (~0 mV) in voltage-clamp while inducing the presynaptic cell to fire action potentials at 1 Hz for 1 min (LTP between 30–35 min post-induction; 188.7 % of baseline, +/− 29.2, n=11). In slices from the GluA1 knockout, this same pairing protocol produced an initial increase in postsynaptic response in GluA1-lacking pairs, that was significantly attenuated compared to controls and was not long-lasting, decaying back to near baseline within 30 min (112% of baseline, +/− 9.1 at 30–35 min post induction; n=12) (Fig 1A). Thus, very little LTP was present in the absence of the GluA1 AMPA receptor subunit. While we did not explicitly test the role of NMDARs in these experiments, in previous experiments on potentiation using this same pairing induction protocol, all potentiation was prevented by the application of the NMDA antagonist AP5 (50 uM), so we presume that all potentiation in this figure is NMDAR-dependent.

Silent synapses (Charpier et al., 1995; Faber et al., 1991) were identified physiologically as connections that displayed NMDAR-mediated postsynaptic responses (when the postsynaptic cell was depolarized) but had no detectable AMPAR-mediated responses at resting membrane potentials (Isaac et al., 1995; Isaac et al., 1999; Liao et al., 1999). As with active synapses, silent connections can be potentiated by pairing presynaptic action potentials with postsynaptic depolarization (Isaac et al., 1995,1999; Liao et al., 1995, (Montgomery et al., 2001). This synapse “unsilencing” in cell pairs is revealed by the appearance of robust AMPAR-mediated currents evoked in response to subsequent presynaptic stimulation (Fig 1B;(Montgomery et al., 2001). However, in cell pairs connected by silent synapse(s) from a GluA1 $^{-/-}$ mouse, the pairing protocol had no effect. Presynaptic action potentials still produced no AMPAR-mediated EPSCs even though the continued existence of an NMDAR-mediated current was always confirmed (Fig. 1B). Despite the fact that the pairing protocol resulted in unsilencing in 7 of 8 silent wild-type pairs, none of the 4 silent pairs from GluA1-deficient slices could be awakened (Fig. 1*C*). These results further demonstrate a necessity for GluA1 in synaptic potentiation, whether from an active or silent state.

2.2

The average baseline amplitude of the unitary postsynaptic responses in both wild-type and knockout mice varied significantly from pair to pair. Despite this inherent variability, GluA1-lacking CA3 pairs displayed synaptically-evoked EPSCs that were significantly smaller, on average, than normal (Fig. 2*A*). When the responses of each pair were binned according to mean EPSC amplitude, a significantly greater proportion of GluA1 $^{-/-}$ pairs displayed evoked currents that fell within the smallest bin. Presynaptic stimulation in 66.7% of GluA1 $^{-/-}$ pairs (18 of 27 total pairs) elicited postsynaptic responses that averaged less than 10 pA in amplitude compared to only 14.3% of wild-type pairs (5 of 35 total pairs). In addition, the mean and median responses measured in GluA1-lacking pairs were significantly smaller than in their wild-type littermates (GluA1$^{-/-}$: mean EPSC = 12.2 ± 3.0 pA, median EPSC = 6.2 pA; wild-type: mean EPSC = 34.4 ± 6.4 pA, median EPSC = 22.6 pA; $p<0.05$ for both measures). This particular finding confirms that of (Andrasfalvy et al., 2003) and extends it to unitary connections.

To investigate the mechanisms underlying this reduced synaptic response, spontaneous miniature EPSCs were recorded and were also found to have a lower amplitude in the GluA1-knockout tissue (median amplitude: WT: 4.46pA, KO 3.34pA; mean amplitude: WT 8.09pA, KO 4.92pA; the maximum difference between the cumulative distributions, *D*, is: 0.3125 with a corresponding *P* of: 0.037 by the K-S test; Fig. 2*B*). The frequency of

measured miniature (m) EPSC events was also significantly less in the GluA1 $^{-/-}$ pairs than in wild-type pairs (0.94 ± 0.16 Hz versus 0.43 ± 0.03 Hz, respectively; $p<0.05$, data not shown); however, it is unclear if this reflects an actual change in mEPSC frequency or whether the reduction in amplitude has simply pushed the smallest events to a size where they cannot be readily distinguished from baseline noise. In any event, these results were surprising due to the fact that in previous electrophysiological analyses of GluA1 $^{-/-}$ mice (Zamanillo et al., 1999) no alteration in either synaptically-evoked or spontaneous transmission was reported in area CA1 of the hippocampus suggesting that areas CA1 and CA3 may differ in their properties in this regard.

2.3

Having reproduced impaired potentiation (Andrasfalvy et al., 2003; Mack et al., 2001; Shi et al., 2001; Zamanillo et al., 1999), and demonstrated the resistance of silent synapses to awaken in the knockout mice, we next tested whether GluA1 was also required for synaptic depression. It has previously been shown that long-term depression of the synapses connecting two CA3 pyramidal neurons can be induced by a 10-minute application of low-frequency stimulation (LFS, presynaptic action potentials evoked at 1 Hz coupled with slight depolarization of the postsynaptic cell to −55 mV; (Montgomery and Madison, 2002). LFS elicited significant synaptic depression in CA3 pairs from GluA1-deficient mice (Fig. 3, *A* and *B*; 53.8 ± 10.2% of baseline 30–35 min post-LFS, n=5) indistinguishable from that of control mice (Fig. 3*B*, 55.0 ± 16.8% of baseline 30–35 min post-LFS, n=6; $p > 0.05$). Because this was the first demonstration of normal LTD in GluA1 knockout mice, we wanted to eliminate the possibility that this phenomenon was merely a by-product of the cultured slice environment or limited to plasticity within recurrent CA3 synapses. To extend and verify these findings, we tested synaptic plasticity in acute hippocampal slices at Schaffer collateral-CA1 synapses using extracellular field potential recordings. Using two different induction protocols (5 minutes of 3-Hz stimulation or 15 minutes of 1-Hz paired pulse stimulation) applied to slices prepared from adult mice, LTD was induced to a level approximately 20% below baseline in the knockout mice, a level comparable to the wild-type controls (Knockout: *81.5 +/− 5.4% of baseline, n=7*; Wildtype: 82.4 +/− 6/2% of baseline, n=3; ns, $p > 0.05$; Fig. 3C). Together, these results indicate the existence of a GluA1-independent mechanism underlying synaptic depression.

3. DISCUSSION

Here, we find impaired AMPAR-mediated transmission and synaptic potentiation in unitary synapses in hippocampal area CA3 of GluA1-deficient mice. The impaired potentiation was seen not only in active connections but also when examining silent connections, which could not be “unsilenced” in the absence of GluA1. The lack of LTP seen in the GluA1-deficient mice using the pair recording technique supports a number of previous studies assessing synaptic plasticity in these mice. In the original article characterizing the GluA1 knockout mice (referred to as GluR-A $^{-/-}$; (Zamanillo et al., 1999), an impairment in tetanus-induced LTP was measured with extracellular field recordings at Schaffer collateral-CA1 synapses. This deficit in LTP was found to be age-dependent, with the ability to induce LTP being intact in mice at P14 (Jensen et al., 2003) but impaired in adult mice (Zamanillo, et al., 1999). This was of initial concern to us, because organotypic slices are prepared from animals aged P5 to P7 and kept only 1 to 2 weeks in vitro before recording. However, the LTP deficit was clearly apparent in these “young” organotypic slices. This may reflect that tissue 'matures' more quickly with regard to LTP in organotypic slices than it does in vivo, or perhaps that there is less age-dependence in CA3-CA3 synapses than in the Schaffer collateral - CA1 synapses studied in the original paper.

Both LTP and silent synapse awakening, are thought to result from insertion of additional AMPARs into the synaptic membrane (Emond et al., 2010; Isaac et al., 1995; Liao et al., 1995; Montgomery et al., 2001; Montgomery and Madison, 2002; Shi et al., 1999), and both are impaired in the GluA1-deficient mice (Andrasfalvy et al., 2003; Zamanillo et al., 1999). Indeed, glutamate synapses in the GluA1 knockout lack the usual synaptic scaling where distal dendritic synapses are stronger than proximal synapses (Andrasfalvy et al., 2003), further suggesting a critical role for the GluA1 subunit in either the trafficking to or stabilization within the postsynaptic membrane. Our data are the first demonstration of a necessary role for the GluA1 subunit in synapse unsilencing.

The baseline amplitude of AMPAR-mediated evoked synaptic transmission in GluA1 KO mice is significantly smaller than in wild-type. There are several possible explanations for this observation. The smaller baseline EPSC amplitude might suggest that such synapses have already “auto-depressed” before recordings were made. If such a mechanism had occurred, it might be apparent in the inability to induce further depression in these synapses, since subsequent depression may be impaired/occluded in the knockout mice. However, we found that LTD was completely intact in GluA1 knockout synapses, suggesting that this is not the case. Alternatively, if GluA1 helps stabilize AMPARs in the synapse (Emond et al., 2010), the lack of GluA1 in the knockouts could lead to fewer AMPARs at the synapse and hence smaller EPSCs, as well as the inability to secure new AMPARs during LTP. An extension of this idea might be that there are separate, specific binding sites, or “slots” in the postsynaptic membrane for AMPA receptor complexes containing GluA1 and those lacking it. The smaller baseline EPSC might simply reflect that only the non-GluA1 “slots” are filled while the GluA1 “slots” remain empty. Since the strength of the synapse would reflect current through both Glu1-containing and lacking receptors, the baseline transmission is smaller. The fact that LTD is intact in the GluA1 $^{-/-}$ mice suggests that regardless of the details of the mechanism, these receptors are not required for LTD, but the inability to fill GluA1 “slots” could account for the impairment of LTP.

The normal synaptic depression observed in the GluA1 knockout mice was quite unexpected in light of several recent findings. LTD induction had previously been shown to result in dephosphorylation of GluA1 at two sites, serine-831 and serine-845 (Lee et al., 2000). Furthermore, these phosphorylation events on the GluA1 subunit were reported to be necessary for the expression of LTD, as well as LTP and spatial memories (Lee et al., 2003). Specifically, knock-in mice were generated in which both the S831 and S845 sites in GluA1 were mutated to alanines to prevent phosphorylation of these sites *in vivo* and NMDAR-dependent LTD in area CA1 of the hippocampus was essentially abolished. Although there could be different compensatory mechanisms at work in the GluA1 knockout mice, or these point mutations could possibly act as a dominant negative having a gain-of-function effect of blocking LTD, it is still rather surprising that absolute removal of GluA1 has no effect on synaptic depression whereas a subtle mutation completely eliminates LTD. In addition, infusion of synthetic peptides which disrupt the interaction of GluA2 to specific binding partners (Daw et al., 2000; Emond et al., 2010; Kim et al., 2001) has been shown to interfere with AMPAR internalization and block the expression of LTD. Despite the evidence for a critical role for GluA2/3 in synaptic depression, GluA2- and GluA3-knockout mice as well as GluA2/3 double knockouts all exhibited normal LTD and depotentiation in the CA1 region of the hippocampus (Meng et al., 2003). Furthermore, cells lacking GluA2 and GluA3 exhibit normal endocytosis of AMPARs following activation of NMDARs (Biou et al., 2008). These findings suggested that the GluA1 subunit alone was sufficient for hippocampal synaptic plasticity, although clearly, there is no requirement for it in LTD.

4. EXPERIMENTAL PROCEDURES

The studies and methods of this paper were approved by the Administrative Panel on Laboratory Animal Care (APLAC) of the Stanford University School of Medicine.

Hippocampal organotypic slices from 5- to 7-day-old GluA1$^{-/-}$ mice or wild-type littermates were prepared (Pavlidis and Madison, 1999; Stoppini et al., 1991) and maintained in vitro for 6–14 days before recording. Individual slice cultures were submerged in a recording chamber perfused at 1–2 ml/min with room temperature artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose, pH 7.4, saturated with 95% O_2-5% CO_2. Pyramidal cells in area CA3 were identified using infrared differential interference contrast (IR-DIC) microscopy using a 40X water-immersion objective (Nikon). Whole cell recordings were made using an Axoclamp 1C (for the presynaptic cell) and an Axopatch 2A (for the postsynaptic cell). Events were sampled at 10 kHz and low-pass filtered at 1–2 kHz. Series resistances of voltage-clamp recordings were monitored throughout experiments and recordings where Rs varied by more 30% were rejected (average Rs rr15 MΩ). Electrode resistance was 3–6 MΩ. The electrode internal solutions consisted of (in mM): 120 K gluconate (for the presynaptic cell) or Cs gluconate (for the postsynaptic cell), 40 HEPES, 5 $MgCl_2$, 2 MgATP, 0.3 NaGTP, pH 7.2 with KOH or CsOH. The postsynaptic internal solution also contained 10 μM of a lidocaine derivative (QX-314) to allow the postsynaptic cell to be held at very depolarized potentials without discharging action potentials that would contaminate the recording of NMDAR-dependent EPSCs. Two whole-cell recordings were obtained simultaneously from two CA3 pyramidal neurons, typically with 10–200 μm separating the pre- and postsynaptic cell somata. The presynaptic cell was recorded in current clamp mode and induced to fire single action potentials by brief injection of depolarizing current (20–50 pA for 20 msec) at 0.08–0.2 Hz. Postsynaptic cells were held in voltage clamp at −70 mV unless otherwise noted. In a synaptically-connected pair of CA3 neurons, evoking a presynaptic action potential produced a monosynaptic excitatory postsynaptic current (EPSC). These synaptic responses were blocked completely by bath application of an AMPA receptor antagonist (NBQX, 10 μM) at a holding potential of −70 mV. LTP was induced by holding the postsynaptic cell near 0 mV in voltage-clamp, while inducing presynaptic action potentials at 1Hz for 60 sec. LTD was induced by holding the postsynaptic cell at −55mV in voltage-clamp while inducing presynaptic action potentials at 1 Hz for 10 minutes.

For those LTD experiments conducted in acutely-prepared (i.e. standard, non-organotypic) slices, transverse hippocampal slices (0.35 mm) were prepared from 3- to 7- week-old mice and allowed to recover in a holding chamber for at least 1 h before transfer to the recording chamber. Slices were submerged in ACSF as above (but with 0.1 mM picrotoxin added). We performed extracellular field recordings using a patch pipette filled with ACSF. Schaffer collateral afferents were stimulated using glass pipette electrodes at 0.1 Hz except during the LTD induction protocol. For 3 week-old animals, LTD was induced with 900 single stimulations delivered at 5 Hz. To induce LTD in animals over 4 weeks old, 900 paired pulses with 50 ms interval were delivered at 1 Hz.

On- and off-line data analysis was performed using custom software developed in our laboratory written in the Labview (National Instruments) programming environment (application written by Eric Schiable and Paul Pavlidis). Spontaneous miniature EPSCs were measured in the presence of 1 μM TTX and 20 μM bicucculine and analyzed using the Mini Analysis Program by Synaptosoft Inc. (version 5.0.1).

Highlights

- AMPA receptor subunit composition plays a major role in trafficking of those receptors in and out of the synaptic membrane and as a result, synaptic plasticity.
- We confirm that active excitatory synapses from hippocampus of a GluA1 AMPA receptor subunit null mouse, cannot undergo long-term potentiation, and show for the first time that silent synapses cannot be activated in this tissue as well.
- The amplitude of quantal excitatory postsynaptic currents is decreased in this knockout tissue.
- We also show for the first time that long-term depression is induced normally in this knockout tissue.

ABBREVIATIONS LIST

AMPA, AMPAR	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid (receptor)
NMDA, NMDAR	N-methyl-D-aspartate (receptor)
LTP	long-term potentiation
LTD	long-term depression
GluA1 (GluA2,3)	AMPAR receptor subunit type 1 (type 2,3)
LFS	low-frequency stimulation
ACSF	artificial cerebro-spinal fluid
GluA1$^{-/-}$	knockout mouse (or tissue) with genetic deletion of GluA1
WT	wild type animal or tissue
EPSC	excitatory postsynaptic current
NBQX	(2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione) (AMPAR antagonist)

Acknowledgments

This work was supported by grants from the National Institute of Mental Health;MH065541 (to DVM), MH086403 and MH063394 (to RCM), MH080310 (to WX), The G. Harold and Leila Y. Mathers Charitable Foundation (to DVM), and by NIH 5T32 NS07280 (Epilepsy Training Grant, David A. Prince, PI) We thank Peter Seeburg at the Max Planck Institute for Medical Research, University of Heidelberg for the generous gift of the GluA1 $^{-/-}$ mouse and members of the Madison lab, Malenka lab and Craig Garner and his lab for helpful discussions.

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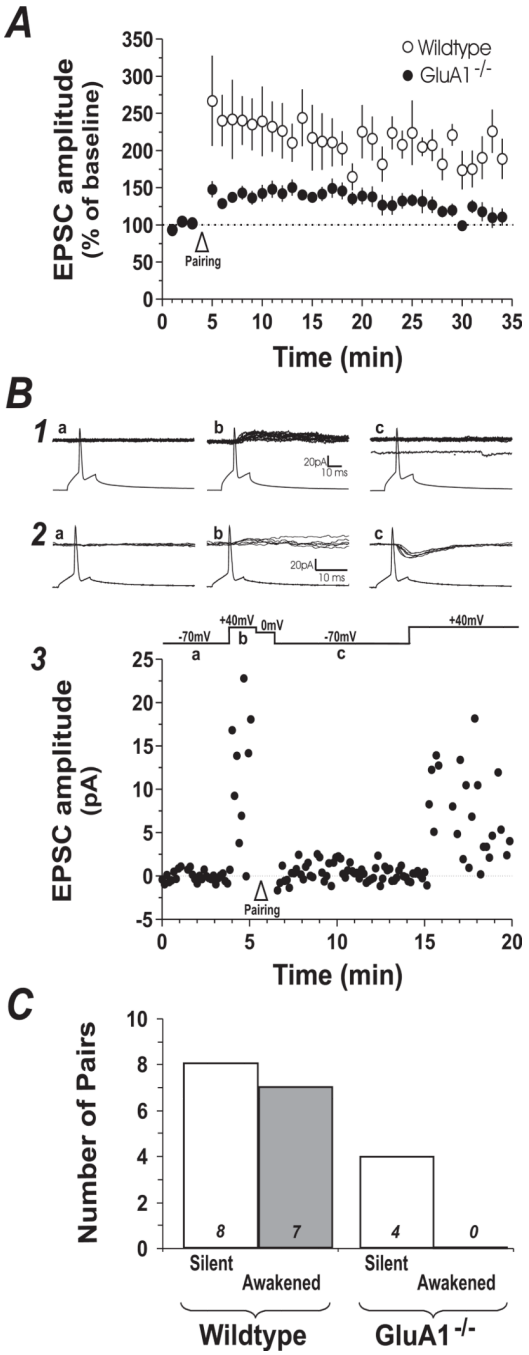

FIG. 1. Synaptic potentiation is impaired in both active and silent CA3 pairs from GluA1 knockout mice

A, Summary data showing significantly attenuated LTP in CA3 pairs having active (i.e. non-silent) synaptic connections, taken from knockout mice (●, $n = 12$ pairs) compared to wild-type mice (❍, $n = 11$ pairs). LTP was induced by a pairing protocol in which presynaptic stimulation at 1 Hz is coupled with postsynaptic depolarization to ~0 mV for 1 min. Values are mean ± s.e.m. *B1*, Example of a pair of GluA1$^{-/-}$ neurons connected entirely by silent synapses. This pair shows no AMPAR-mediated current at $V_H = -70$ mV (***a***); however, there is a clear, evoked NMDAR-mediated current at $V_H = +40$ mV (***b***). In this GluA1$^{-/-}$ pair, there is still no AMPA current present following a pairing protocol which reliably

awakens silent synapses in wild-type slices (***c***). *B2*, The identical experiment as in *B1*, except performed on a pair from wild-type tissue. *B3*, A graph of a different representative experiment in this series on a pair of synapses connected by silent synapses. The labels ***a–c*** in the graph indicate the phase of the experiment where the traces in *B1* and *B2* were taken. *C*, Summary data showing the proportion of silent synapses from wild-type and knockout slices that can be awakened using the pairing protocol. The white bars are the total number of silent synapses tests, and the gray bar is the subset of that total that was awakened by the potentiating protocol. The number of pairs in each of these groups is inset in the bars. Eight of 67 total wild-type pairs and 4 of 57 total GluA1-lacking pairs were connected by silent synapses. All silent synapses were from different animals.

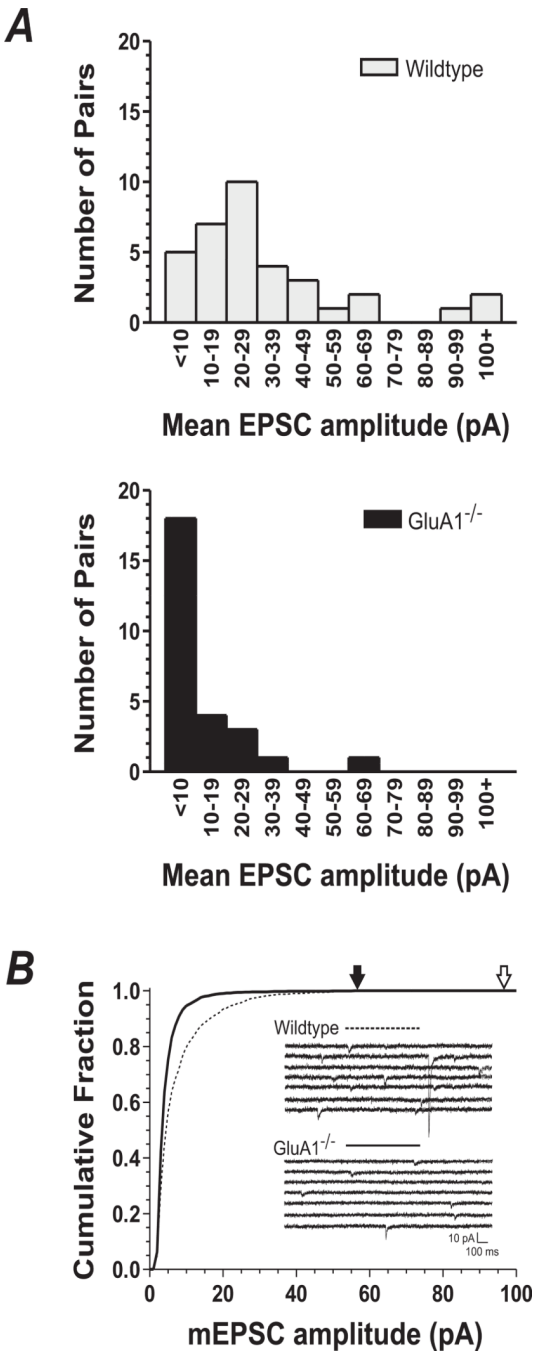

FIG. 2. Reduced synaptic transmission in GluA1-lacking slices

A, Distribution of the amplitudes of synaptic currents evoked by single presynaptic action potentials in synaptically coupled pairs of cells, from wild-type (*top histogram. gray bars*) and GluA1$^{-/-}$ (*bottom histogram, black bars*) pairs. Each CA3 pair was slotted into a 10-pA bin according to the mean amplitude of its evoked EPSC. *B*, Cumulative plot of spontaneous miniature EPSC amplitudes recorded from pyramidal neurons in area CA3 of slices from wild-type (---) and GluA1 knockout (—) mice. Arrows indicate the maximum spontaneous EPSC amplitude measured for knockout (*closed arrow*) and wild-type (*open arrow*) slices. Inset are exemplar raw traces of mEPSCs from both wild-type and knockout slices. Because of the low frequency of mEPSCs, we did not show 'blank' traces having no events, but

instead showed consecutive traces that contained at least one mEPSC. In the wild-type recording, the 7 traces shown occurred over a 14 trace span, and in knockout over a 34 trace span.

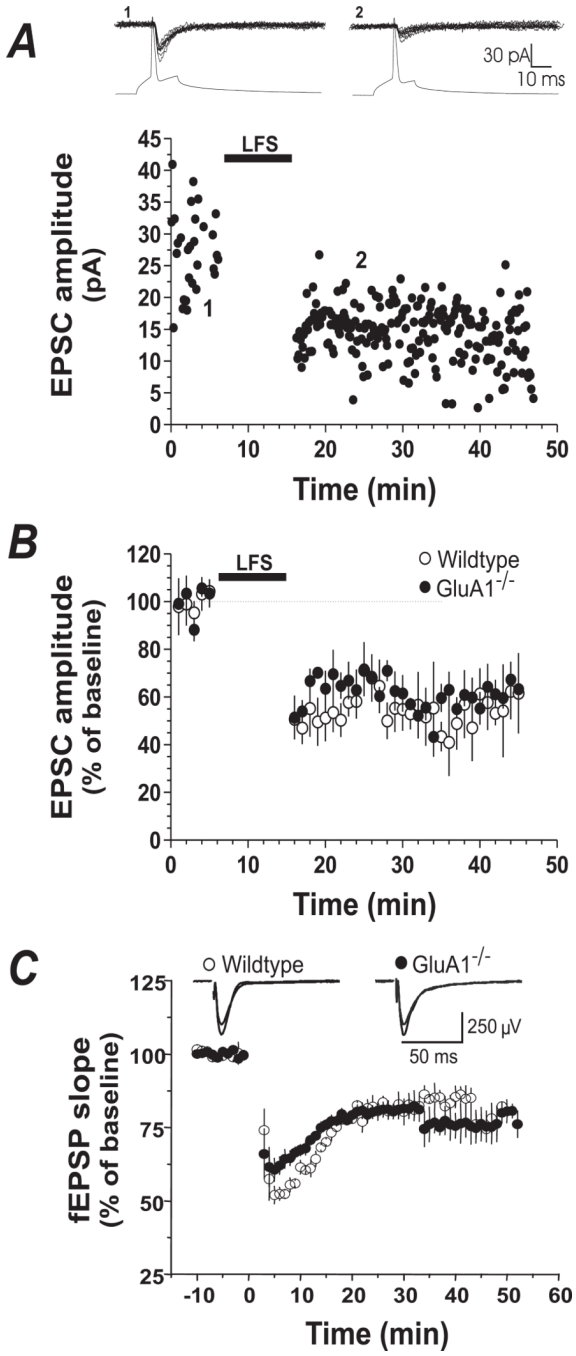

FIG. 3. Normal long term-depression expressed in GluA1-deficient mice

A and *B*, Whole cell recordings from pairs of CA3 pyramidal neurons in organotypic hippocampal slices. *A*, LTD induced by 10 min of LFS (presynaptic stimulation at 1 Hz coupled with slight postsynaptic depolarization to $V_H = -55$ mV) in an individual pair recording experiment from a GluA1 $^{-/-}$ mouse. Inset: example sweeps shown overlaid from before (***1***) and after (***2***) LFS. One example presynaptic action potential is shown for each. *B*, Pooled data showing similar levels of LTD in CA3 pairs from wild-type (❍, *n* = 6 pairs) and knockout (●, *n* = 5 pairs) mice. Values are mean ± s.e.m. *C*, Field recordings assessing LTD at CA3-CA1 synapses. Averaged LTD data from wild-type (❍, 3 slices taken from 2 mice)

and knockout (●, 7/3) mice. Values are mean ± s.e.m. Representative traces are inset in the graph.