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RESEARCH ARTICLE | *Cellular and Molecular Properties of Neurons*

GSG1L regulates the strength of AMPA receptor-mediated synaptic transmission but not AMPA receptor kinetics in hippocampal dentate granule neurons

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Mao X, Gu X, Lu W. GSG1L regulates the strength of AMPA receptor-mediated synaptic transmission but not AMPA receptor kinetics in hippocampal dentate granule neurons. *J Neurophysiol* 117: 28–35, 2017. First published October 5, 2016; doi:10.1152/jn.00307.2016.—GSG1L is an AMPA receptor (AMPAR) auxiliary subunit that regulates AMPAR trafficking and function in hippocampal CA1 pyramidal neurons. However, its physiological roles in other types of neurons remain to be characterized. Here, we investigated the role of GSG1L in hippocampal dentate granule cells and found that GSG1L is important for the regulation of synaptic strength but is not critical for the modulation of AMPAR deactivation and desensitization kinetics. These data demonstrate a neuronal type-specific role of GSG1L and suggest that physiological function of AMPAR auxiliary subunits may vary in different types of neurons.

NEW & NOTEWORTHY GSG1L is a newly identified AMPA receptor (AMPAR) auxiliary subunit and plays a unique role in the regulation of AMPAR trafficking and function in hippocampal CA1 pyramidal neurons. However, its role in the regulation of AMPARs in hippocampal dentate granule cells remains to be characterized. The current work reveals that GSG1L regulates strength of AMPAR-mediated synaptic transmission but not the receptor kinetic properties in hippocampal dentate granule neurons.

AMPA receptor; GSG1L; deactivation; desensitization; synapse; hippocampus; outside-out patch

IN THE CENTRAL NERVOUS SYSTEM, fast excitatory synaptic transmission is largely mediated by neurotransmitter glutamate acting primarily on two types of ionotropic glutamate receptors: AMPA receptors (AMPARs) and *N*-methyl-D-aspartate receptors (NMDARs). AMPARs are responsible for basal synaptic transmission at excitatory synapses and provide the majority of depolarizing drive force in postsynaptic neurons. AMPARs are tetraheteromeric assemblies of GluA1-GluA4 subunits and are regulated by a number of transmembrane auxiliary subunits, such as transmembrane AMPAR regulatory proteins (TARPs), cornichon homologs (CNIHs), cysteine-knot AMPAR modulating protein 44 (CKAMP44)/Shisa6, germ cell-specific gene 1-like (GSG1L), porcupine (PORCN), and α/β-hydrolase domain-containing 6 (ABHD6; Erlenhardt et al. 2016; Gu et al. 2016; Haering et al. 2014; Jackson and Nicoll 2011; Khodosevich et al. 2014; Klaassen et al. 2016; McGee et al. 2015; Straub and Tomita 2012; Wei et al. 2016). Accumulating evidence demonstrates that these auxiliary subunits are important in the regulation of the receptor assembly, trafficking, gating, and pharmacology.

A number of studies have shown that AMPAR auxiliary subunits often exhibit cell type-specific function. For example, CNIH2 has been shown to play an important role in AMPAR trafficking to synapses as well as modulates AMPAR kinetic properties in hippocampal CA1 pyramidal neurons (Gill et al. 2011; Herring et al. 2013; Kato et al. 2010a). However, in hippocampal mossy cells in the dentate gyrus, CNIH2 regulates synaptic AMPAR decay kinetics but not amplitude of AMPAR-mediated excitatory postsynaptic currents (EPSCs; Boudkkazi et al. 2014). It has also been reported that CKAMP44 displays distinct functions in hippocampal CA1 pyramidal neurons and dentate granule cells (Khodosevich et al. 2014; von Engelhardt et al. 2010). Recent evidence has shown that GSG1L is a novel AMPAR auxiliary subunit and plays unique roles in the regulation of AMPAR trafficking and channel biophysical properties (Gu et al. 2016; McGee et al. 2015; Schwenk et al. 2012; Shanks et al. 2012). Indeed, GSG1L can suppress single-channel conductance and calcium permeability of recombinant calcium-permeable AMPARs, opposite to the function of TARPs/CNIHs (McGee et al. 2015). In addition, whereas TARPs/CNIHs promote AMPA receptor forward trafficking and slow the receptor kinetics (Haering et al. 2014; Jackson and Nicoll 2011; Straub and Tomita 2012), GSG1L suppresses the delivery of the receptors to synapses and speeds the rate of AMPA receptor deactivation and desensitization in CA1 pyramidal neurons in hippocampal organotypic slice cultures (Gu et al. 2016). However, it remains unknown whether these properties observed in CA1 pyramidal neurons represent the general physiological role of GSG1L or there are cell type-specific functions of GSG1L in the regulation of AMPARs. Here, we combine GSG1L overexpression and gene inactivation in hippocampal dentate granule cells to explore the role of GSG1L in a distinct population of neurons. We find that overexpression of GSG1L profoundly reduced AMPA EPSC and AMPAR-mediated somatic outside-out patch currents, and synaptic transmission is enhanced in GSG1L knockout (KO) dentate granule cells. In contrast,

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although GSG1L overexpression can speed up AMPAR kinetics, there is no effect on AMPAR deactivation and desensitization in GSG1L KO granule cells. These data suggest a cell type-specific role for GSG1L in the regulation of AMPARs in the brain.

MATERIALS AND METHODS

Animals. GSG1L knockout rats (Sprague-Dawley) were generated as described before (Gu et al. 2016). Rats or mice (C57BL/6) housing, breeding, and handling protocols were approved by National Institute of Neurological Disorders and Stroke (NINDS) Animal Care and Use Committee (ACUC) at National Institutes of Health (NIH). Rats of both sexes at the age of *postnatal days 13–19* (P13–P19) were used for acute slice electrophysiology. Mice of both sexes at the age of P6–P8 were used for organotypic hippocampal slice cultures.

Plasmids. pCMV6-GSG1L-GFP fusion protein plasmid was purchased from OriGene (MG214180).

Electrophysiology. Transverse 300-μm hippocampal slices were made from P13–P19 wild-type (WT) and GSG1L KOs on a DSK LinearSlicer Vibratome in cutting solution containing (in mM) 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 7 glucose, 1.3 ascorbic acid, and 210 sucrose. Freshly cut slices were placed in a custom-made submersion incubation chamber containing artificial cerebrospinal fluid (ACSF), containing (in mM) 119 NaCl, 2.5 KCl, 26.2 $NaHCO_3$, 1 NaH_2PO_4, 11 glucose, 2.5 $CaCl_2$, and 1.3 $MgSO_4$, and recovered at 32°C for ~30 min. Slices were then maintained in ACSF in the same chamber at room temperature before recording. After 30–60 min of incubation at room temperature, slices were then transferred to a submersion chamber on an upright Olympus BX51WI fluorescence microscope and perfused with normal ACSF and 100 μM picrotoxin saturated with 95% O_2-5% CO_2. The intracellular solution for EPSC and outside-out patch recordings contained (in mM) 135 $CsMeSO_4$, 8 NaCl, 10 HEPES, 0.3 Na_3GTP, 4 MgATP, 0.3 EGTA, 5 QX-314, and 0.1 spermine. Cells were recorded with 3- to 5-MΩ borosilicate glass pipettes. Series resistance was monitored and not compensated, and cells in which series resistance varied by 25% during a recording session were discarded. Recordings were collected with a MultiClamp 700B amplifier (Axon Instruments, Foster City, CA), filtered at 2 kHz, and digitized at 10 kHz. All pharmacological reagents were purchased from Abcam, and other chemicals were purchased from Sigma.

Organotypic hippocampal slice cultures were prepared and transfected as previously described (Lu et al. 2009). Briefly, hippocampi were dissected from P6–P8 wild-type mice (C57BL/6) and transfected biolistically with plasmids 3–4 days after in culture. Slices were cultured for additional 2–5 days before recording. For all recording in slice cultures, ACSF was modified to contain 4 mM $CaCl_2$ and 4 mM $MgSO_4$. For recording evoked EPSCs in organotypic slices, ACSF was also supplemented with 5–20 μM 2-chloroadenosine to dampen epileptiform activity, and $GABA_A$ receptors were blocked by picrotoxin (100 μM). Green fluorescent protein (GFP)-positive neurons in organotypic slice cultures were identified by epifluorescence microscopy.

All paired recordings involved simultaneous whole cell recordings from one GFP-positive neuron and a neighboring GFP-negative control neuron in hippocampal dentate gyrus region. The stimulus was adjusted to evoke a measurable, monosynaptic EPSC in both cells. AMPA EPSCs were measured at a holding potential of −70 mV, and NMDA EPSCs were measured at +40 mV and at 100 ms after the stimulus, at which point the AMPA EPSC has completely decayed. AMPAR-mediated currents from somatic outside-out patches were recorded at −70 mV by local application of 1 mM glutamate and 100 μM cyclothiazide, in presence of 100 μM D-(−)-2-amino-5-phosphonopentanoic acid (D-APV), 0.5 μM TTX, and 100 μM picrotoxin, for 2 s. Miniature EPSCs (mEPSCs) were acquired in the presence of 0.5–1 μM TTX and 100 μM picrotoxin and were semiautomatically detected by offline analysis using in-house software in IGOR Pro (WaveMetrics) developed in Dr. Roger Nicoll's laboratory at University of California, San Francisco, using an amplitude threshold of 6 pA. All events were visually inspected to ensure they were mEPSCs during analysis, and those non-mEPSC traces were discarded (the recording noise was ~6 pA). For long-term potentiation (LTP) recording at granule cell synapses at the perforant pathway in acute hippocampal slices prepared from WT and KO rats, presynaptic stimulation was applied to the medial perforant pathway with monopolar glass electrodes filled with ACSF. Baseline excitatory postsynaptic currents (EPSCs) were recorded at the frequency of 0.1 Hz and at the holding potential of −70 mV. The amplitude of the baseline EPSC was adjusted to be approximately 50–150 pA. Whole cell LTP was induced within 8 min after rupture of the patch membrane by paring high-frequency stimulation (3 trains of 100 stimuli at 100 Hz, intertrain interval 30 s) with depolarization to 0 mV. After induction, neurons were returned back to −70 mV and were stimulated at 0.1 Hz to record EPSCs.

AMPAR kinetics analysis in hippocampal dentate granule neurons. Recordings for AMPAR kinetics analysis were performed as described before (Gu et al. 2016). The recordings were made with patch pipette internal solution containing (in mM) 135 $CsMeSO_4$, 8 NaCl, 10 HEPES, 0.3 Na_3GTP, 4 MgATP, 0.3 EGTA, 5 QX-314, and 0.1 spermine. L-glutamate (1 mM) was dissolved in extracellular solution containing (in mM) 140 NaCl, 5 KCl, 1.4 $MgCl_2$, 5 EGTA, 10 HEPES, 1 NaH_2PO_4, 10 D-glucose, and 0.01 NBQX, with pH adjusted to 7.4. D-APV (50 μM) and TTX (0.5 μM) were added to the extracellular solution to isolate AMPAR-mediated currents. Fast application/removal of glutamate (every 5 s) was performed using a piezo-controlled fast application system (Siskiyou) with a double-barrel theta glass that enables rapid solution exchange. The glutamate-evoked outside-out patch currents were recorded at −70 mV. Data were collected with a MultiClamp 700B amplifier (Axon Instruments), filtered at 2 kHz, and digitized at 50 kHz.

AMPAR kinetics analysis was performed as described previously (Gu et al. 2016). Briefly, a single weighted decay measure was calculated from the area under the peak-normalized current (Cathala et al. 2005) according to:

$$\tau_{decay} = \frac{1}{I_{peak}} \int_{t_{peak}}^{t_0} I(t)\mathrm{d}t,$$

where t_0 was 60 ms after the peak (Milstein et al. 2007). Curve fitting and data analysis were done with IGOR Pro 6.22A.

Data analysis. All paired recording data were analyzed statistically with a two-tailed paired Student's *t*-test. For all other analyses except the mEPSC cumulative distributions, an unpaired two-tailed *t*-test was used. Kolmogorov-Smirnov test was used for mEPSC cumulative distributions. The data were presented as means ± SE, and statistical significance was defined as $P < 0.05$, 0.01, or 0.001. *P* values ≥0.05 were considered not significant.

RESULTS

Previously, we have explored the role of GSG1L in hippocampal CA1 pyramidal neurons and found that GSG1L plays a unique role in the regulation of AMPAR-mediated synaptic transmission (Gu et al. 2016). In situ hybridization data indicate that GSG1L is broadly expressed in the brain with a higher mRNA expression level in dentate granule cells than in CA1 pyramidal neurons (http://mouse.brain-map.org/) in hippocampus. To examine the role of GSG1L in dentate granule cells, we employed a gene gun-mediated transfection approach to overexpress GSG1L fusion to GFP (GSG1L-GFP) in dentate granule cells in organotypic hippocampal slice cultures. Two to five days after transfection, we performed

simultaneous dual whole cell voltage-clamp recordings to measure AMPAR- and NMDAR-mediated EPSCs (AMPA EPSCs and NMDA EPSCs, respectively) in a GFP-positive cell and a nearby nontransfected control cell. The stimulation electrode was placed in the dendritic regions of granule cells to evoke the EPSCs in both cells. We found that expression of GSG1L-GFP strongly reduced the amplitude of AMPA EPSCs, but there was no significant difference of NMDA EPSCs, indicating that increased expression of GSG1L specifically suppresses AMPAR-mediated synaptic transmission (Fig. 1*A*).

AMPARs are not only localized at synapses, but also highly abundant at extrasynaptic membranes, which have been proposed to serve as a reserve receptor pool to supply AMPARs during synaptic plasticity (Granger et al. 2013; Lu et al. 2009). We thus performed outside-out patch recording to measure somatic extrasynaptic AMPARs in GSG1L-overexpressing dentate granule cells. We pulled outside-out patches from somatic area to avoid the contamination of synaptic AMPARs, as dentate granule cells lack excitatory synaptic input onto the soma. AMPAR-mediated outside-out patch currents were evoked by glutamate (1 mM) in the presence of cyclothiazide (100 μM) to block AMPAR desensitization. In the control, nontransfected granule cells, 1 mM glutamate evoked strong inward currents on somatic outside-out patches (Fig. 1*B*). In contrast, the glutamate-evoked, outside-out patch currents were strongly reduced in granule neurons expressing GSG1L-GFP (Fig. 1*B*). Thus overexpression of GSG1L inhibits the currents mediated by AMPARs on somatic outside-out patches excised from dentate granule cells. Taken together, these overexpression experiments demonstrate that GSG1L is sufficient to suppress the delivery of AMPARs to neuronal surface and synapses in dentate granule cells.

To explore the role of GSG1L further, we characterized basal synaptic transmission onto dentate granule cells in GSG1L knockout (KO) rats that we previously generated (Gu et al. 2016). In acute hippocampal slices, we first measured the ratio of AMPA EPSCs at −70 mV to NMDA EPSCs at +40 mV and found that the ratio was significantly enhanced in dentate granule cells from GSG1L KO rats (Fig. 2*A*), suggesting that AMPAR-mediated synaptic transmission in dentate granule cells is enhanced in GSG1L KOs. There was no change of paired-pulse ratio (PPR), a measure of presynaptic neurotransmitter release probability (Fig. 2*B*), indicating that loss of GSG1L does not change presynaptic glutamate release probability. We also measured AMPAR-mediated miniature EPSCs (mEPSCs) at −70 mV in the presence of tetrodotoxin (TTX) and picrotoxin to block action potentials and $GABA_A$ receptor-mediated synaptic currents, respectively. In GSG1L KO granule cells, mEPSC frequency, but not amplitude, was significantly enhanced (Fig. 2*C*), suggesting a possible increase of functional synapses containing AMPARs in GSG1L KO dentate granule cells. We also examined long-term potentiation (LTP), a cellular model for learning and memory. We found that high-frequency stimulation-induced LTP in WT and KO dentate gyrus granule cells is similar and there is no significant difference between genotypes (Fig. 2*D*). Taken together, these data indicate that genetic deletion of GSG1L enhances AMPAR-mediated synaptic transmission while leaving PPR and LTP unchanged in dentate granule cells.

The data presented thus far suggest that GSG1L might play a similar role in the regulation of the strength of AMPAR-mediated synaptic transmission in hippocampal CA1 neurons and dentate granule cells. As an auxiliary subunit for AMPARs, GSG1L also modulates AMPAR biophysical properties in both heterologous cells and neurons (Gu et al. 2016; McGee et al. 2015; Schwenk et al. 2012; Shanks et al. 2012). To study the role of GSG1L in the regulation of AMPAR kinetic properties in dentate granule cells, we measured deactivation and desensitization properties of AMPARs from somatic outside-out patches excised from granule cells through a piezo-controlled fast solution application system with a double-barrel theta glass pipette that enables rapid solution exchange (Jones et al. 2001). The AMPAR deactivation or desensitization kinetics were measured by calculating weighted

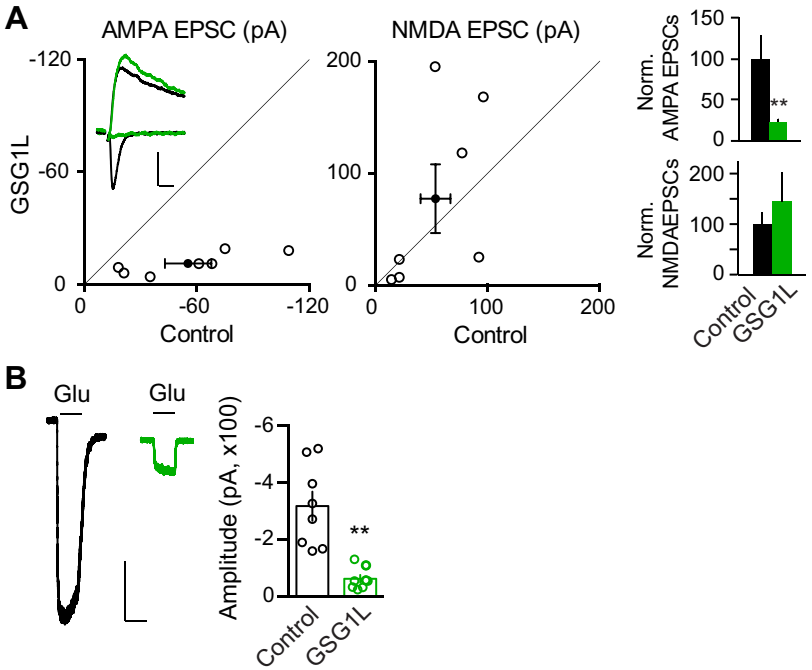

Fig. 1. Overexpression of GSG1L strongly reduced AMPA EPSCs in hippocampal dentate granule cells. *A*: overexpression of GSG1L (AMPA: control, −55.3 ± 12.4 pA; GSG1L, −11.1 ± 2.1 pA; $n = 7$; $^{**}P < 0.01$; NMDA: control, 53.4 ± 13.4 pA; GSG1L, 77.3 ± 30.7 pA; $n = 7$; $P = 0.39$) in cultured organotypic hippocampal slices significantly reduced AMPA but not NMDA EPSCs in dentate granule cells. AMPA EPSCs were recorded at −70 mV, and NMDA EPSCs were recorded at +40 mV in the presence of 100 μM picrotoxin. Scale bar: 50 pA and 20 ms. Norm., normal. *B*: overexpression of GSG1L strongly reduced AMPAR-mediated outside-out patch currents recorded at −70 mV in the presence of 100 μM cyclothiazide (control, −316.7 ± 59.6 pA; $n = 7$; GSG1L, −62.7 ± 15.6 pA; $n = 7$; $^{**}P < 0.01$) in dentate granule neurons in cultured organotypic hippocampal slices. Scale bar: 100 pA and 2 s. Glu, glutamate. All data were presented as means ± SE.

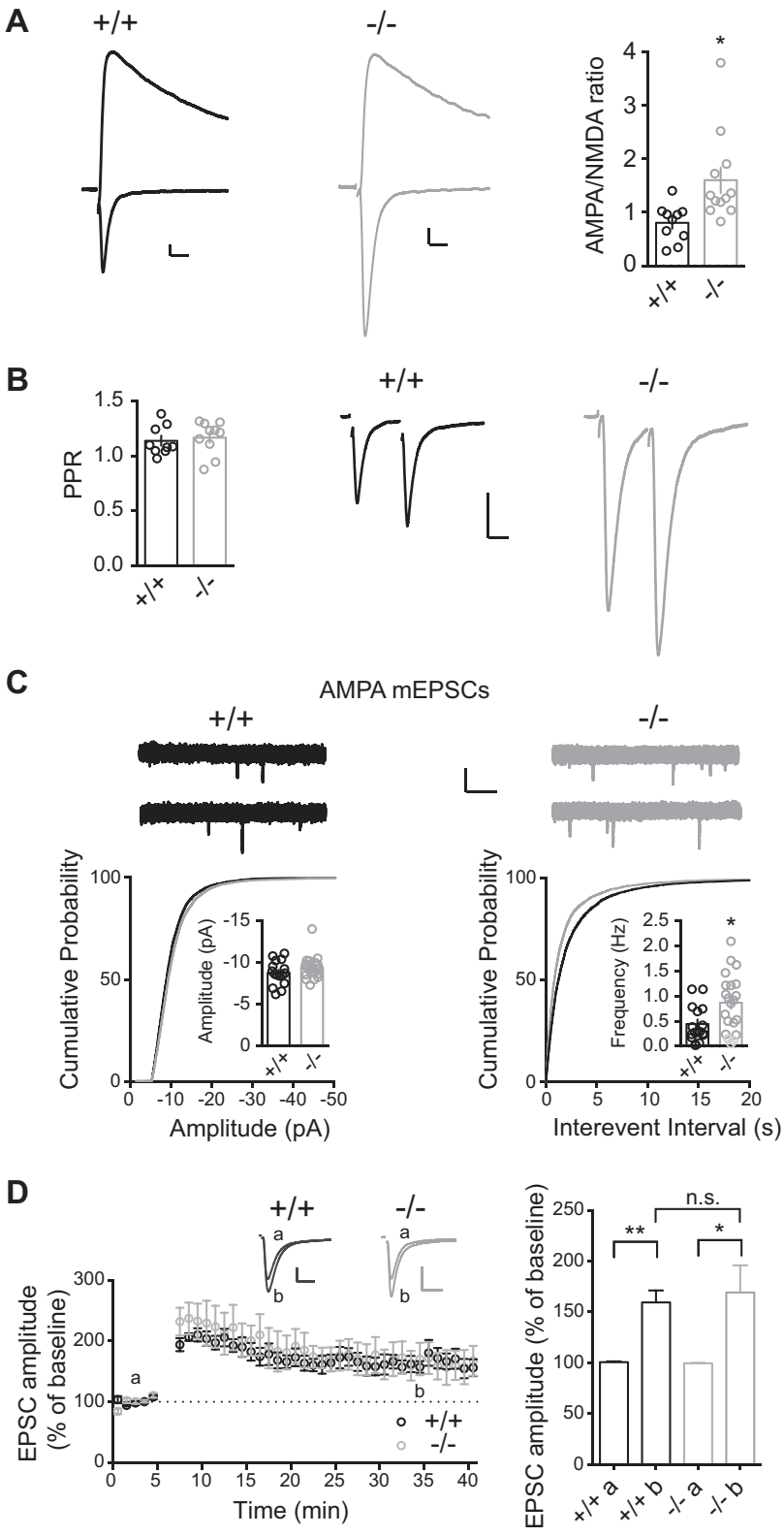

Fig. 2. GSG1L KO enhanced AMPA EPSCs in hippocampal dentate granule cells. *A*: ratio of AMPA EPSCs to NMDA EPSCs (AMPA EPSCs were recorded at −70 mV, and NMDA EPSCs were recorded at +40 mV in the presence of 100 μM picrotoxin) was significantly enhanced in KO rats (+/+: 0.8 ± 0.1; $n = 10$; −/−: 1.6 ± 0.2; $n = 11$; $^*P < 0.05$). Scale bar: 20 pA and 20 ms. *B*: there was no change of paired-pulse ratio of EPSCs recorded in granule neurons in GSG1L KO (+/+, 1.1 ± 0.04; $n = 9$; −/−, 1.2 ± 0.05; $n = 10$; $P = 0.66$). Scale bar: 50 pA and 20 ms. *C*: AMPAR-mediated mEPSC frequency (recorded at −70 mV) in granule neurons from P14–P19 KO rats was significantly increased (Amplitude: +/+, −8.8 ± 0.4; $n = 16$; −/−, 9.4 ± 0.3; $n = 21$; $P = 0.21$; Frequency: +/+, 0.4 ± 0.1; $n = 16$; −/−, 0.9 ± 0.1; $n = 21$; $^*P < 0.05$; Kolmogorov-Smirnov test was used for cumulative distributions, $P < 0.001$ for frequency and $P > 0.05$ for amplitude). Scale bar: 10 pA and 1 s. *D*: there was no change of LTP at dentate granule cells in GSG1L KOs (%increase: +/+, 58.1 ± 11.2%, $n = 9$, $^{**}P < 0.01$; −/−, 69.8 ± 27.2%, $n = 8$, $^{**}P < 0.01$; $n = 5$ animals for +/+ and $n = 4$ animals for −/−; $P > 0.05$ between +/+ and −/−; 1-way ANOVA test). a, Baseline EPSC; b, EPSC after 30 min of LTP induction; n.s., not significant. Scale bar: 50 pA and 20 ms. All data were presented as means ± SE.

decay kinetics of somatic outside-out patch currents evoked by 1- or 100-ms application of 1 mM glutamate, respectively.

We found that overexpression of GSG1L significantly sped up both deactivation and desensitization kinetics of AMPARs measured in somatic outside-out patches pulled from dentate granule neurons in cultured organotypic hippocampal slices (Fig. 3, *A* and *C*). These results are consistent with the data obtained in hippocampal CA1 pyramidal neurons overexpressing GSG1L (Gu et al. 2016), suggesting that GSG1L is capable of speeding AMPAR kinetics in both neuronal types. We also noticed that the peak amplitudes of AMPAR-mediated outside-out patch currents evoked by 1- or 100-ms application of 1 mM

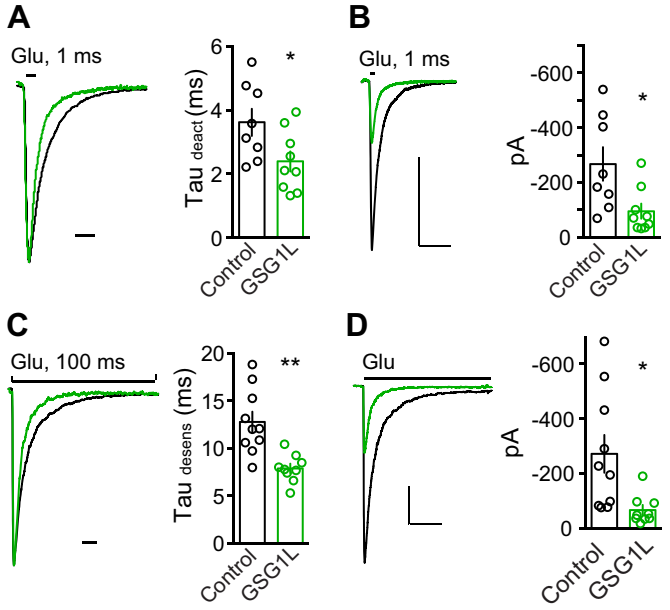

Fig. 3. Overexpression of GSG1L accelerated AMPAR deactivation and desensitization kinetics in hippocampal dentate granule cells. *A* and *B*: overexpression of GSG1L in cultured organotypic hippocampal slices sped up AMPAR deactivation [*A*, weighted deactivation time constant (Tau $_{deact}$): control, 3.6 ± 0.4 ms; $n = 8$; GSG1L, 2.4 ± 0.3 ms; $n = 9$; $^{*}P < 0.05$; scale bar, 2 ms; *B*, peak amplitude: control, −267.6 ± 61.3 pA, $n = 8$; GSG1L, −95.1 ± 27.2 pA, $n = 9$; $^{*}P < 0.05$; *t*-test; scale bar: 10 ms and 100 pA] in dentate granule neurons. *C* and *D*: overexpression of GSG1L in cultured organotypic hippocampal slices sped up AMPAR desensitization [*C*, weighted desensitization time constant (Tau $_{desens}$): control, 12.8 ± 1.1 ms; $n = 8$; GSG1L, 7.9 ± 0.5 ms; $n = 9$; $^{**}P < 0.01$; *D*, peak amplitude: control, −271.7 ± 68.6 pA, $n = 10$; GSG1L, −67.4 ± 17.7 pA, $n = 9$; $^{*}P < 0.05$; *t*-test; scale bar: 10 ms and 100 pA] in dentate granule neurons. All data were presented as means ± SE.

glutamate from granule cells overexpressing GSG1L were strongly reduced (Fig. 3, *B* and *D*). These results were consistent with data presented in Fig. 1*B* recorded in the presence of cyclothiazide, demonstrating that increased expression of GSG1L strongly suppresses AMPAR currents recorded at somatic extrasynaptic membranes. Taken together, these data show that increased expression of GSG1L in dentate granule cells can speed deactivation and desensitization kinetics of AMPARs and reduces AMPAR density on neuronal somatic membrane.

Surprisingly, kinetic analysis of glutamate-evoked somatic outside-out patch currents in granule neurons in acute hippocampal slices prepared from GSG1L KOs showed that genetic deletion of GSG1L did not change either deactivation or desensitization kinetics (Fig. 4, *A* and *C*). There was also no difference for glutamate-evoked somatic outside-out patch currents in GSG1L KO dentate granule cells (Fig. 4, *B* and *D*). In addition, there was no change of decay kinetics of mEPSCs (Fig. 4*E*). These data indicate that although GSG1L is capable of modulating AMPAR kinetics in dentate granule cells, endogenous GSG1L in these cells does not play an important role in the receptor deactivation and desensitization properties. Thus it appears that in dentate granule neurons, endogenous GSG1L is specifically involved in the regulation of AMPA EPSCs but played a negligible role in the modulation of AMPAR deactivation and desensitization.

DISCUSSION

In this study, we examined the role of GSG1L, a newly identified AMPAR auxiliary subunit (Gu et al. 2016; McGee et al. 2015; Schwenk et al. 2012; Shanks et al. 2012), in the regulation of AMPARs in hippocampal dentate granule cells. We found that overexpression of GSG1L strongly reduced AMPAR-mediated synaptic transmission and glutamate-evoked AMPAR-mediated somatic outside-out patch currents in dentate granule cells. In addition, GSG1L overexpression sped deactivation and desensitization kinetics of AMPARs on granule cell somatic membranes. Furthermore, AMPAR-mediated synaptic transmission was enhanced in GSG1L KO granule cells. In contrast, LTP and somatic AMPAR deactivation and desensitization kinetics remain unchanged in GSG1L KO granule cells. These data suggest that although GSG1L is important for the regulation of the strength of basal AMPAR-mediated synaptic transmission, it is not critical for the modulation of LTP and AMPAR deactivation and desensitization kinetics in hippocampal dentate granule neurons.

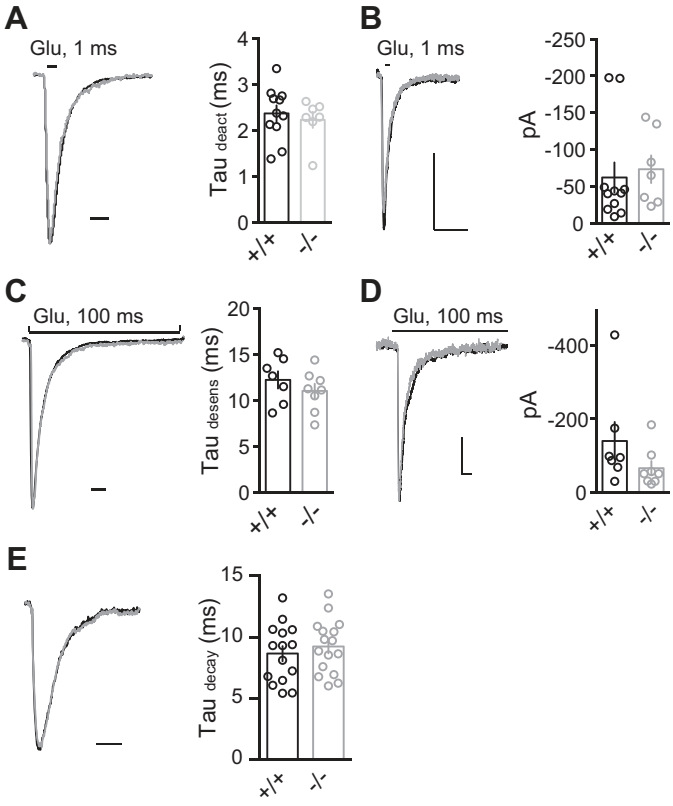

Fig. 4. GSG1L KO did not change AMPAR deactivation and desensitization kinetics in hippocampal dentate granule cells. *A* and *B*: in dentate granule neurons from GSG1L KO rats, there was no change of AMPAR deactivation (*A*, weighted deactivation time constant: +/+, 2.4 ± 0.2 ms; $n = 11$; −/−, 2.2 ± 0.2 ms; $n = 7$; $P = 0.57$; scale bar, 2 ms; *B*, peak amplitude: +/+, −62.2 ± 20.6 pA, $n = 11$; −/−, −73.4 ± 18.9 pA, $n = 7$; $P = 0.69$; *t*-test; scale bar: 10 ms and 100 pA). *C* and *D*: GSG1L KO did not change AMPAR desensitization in dentate granule neurons (*C*, weighted desensitization time constant: +/+: 12.2 ± 0.9; $n = 7$; −/−: 11.0 ± 0.8; $n = 8$; $P = 0.34$; scale bar, 2 ms; *D*, peak amplitude: +/+, −140.6 ± 50.8 pA, $n = 7$; −/−, −66.1 ± 19.0 pA, $n = 8$; $P = 0.21$; *t*-test; scale bar: 10 ms and 100 pA). *E*: AMPAR mEPSC decay time constant was not significantly changed in GSG1L KO dentate granule neurons (+/+, 8.7 ± 0.6 ms, $n = 15$; −/−, 9.2 ± 0.6 ms, $n = 16$; $P = 0.48$; *t*-test; scale bar, 20 ms). Peak-normalized sample traces are shown to the *left*. All data were presented as means ± SE.

Previously, we have shown that GSG1L regulates both the receptor trafficking and functional properties in hippocampal CA1 pyramidal neurons (Gu et al. 2016). Indeed, overexpression of GSG1L sped AMPAR kinetics and dramatically reduced AMPAR-mediated synaptic transmission and extrasynaptic AMPARs in CA1 pyramidal neurons (Gu et al. 2016). In addition, genetic KO of GSG1L enhanced AMPAR-mediated synaptic transmission and also slowed AMPAR deactivation and desensitization kinetics in CA1 pyramidal neurons (Gu et al. 2016). Thus GSG1L negatively regulates AMPAR-mediated excitatory synaptic transmission but differentially modulates AMPAR kinetics in these two types of hippocampal neurons. In granule cells, increased expression of GSG1L appears to be sufficient to speed up AMPAR deactivation and desensitization rates, suggesting that AMPARs in granule cells are accessible for the GSG1L regulation. However, the lack of effect of GSG1L KO on AMPAR deactivation and desensitization kinetics in dentate granule cells suggests that GSG1L is not important for the regulation of AMPAR kinetics in this type of neurons. Alternatively, the function of GSG1L in this type of neurons could be substituted by other AMPAR auxiliary subunits with similar functions. Currently, there are no other characterized AMPAR auxiliary subunits that have been reported to play similar roles to GSG1L in the regulation of AMPAR trafficking and function. For example, TARPs, CNIHs, CKAMP44, and porcupine have been shown to regulate positively AMPAR forward trafficking to the neuronal surface and synapses (Bats et al. 2007, 2013; Chen et al. 2000; Erlenhardt et al. 2016; Gill et al. 2011; Herring et al. 2013; Kato et al. 2010a,b; Kessels et al. 2009; Khodosevich et al. 2014; Rouach et al. 2005; Schwenk et al. 2009; Studniarczyk et al. 2013; Sumioka et al. 2011; Tomita et al. 2005; von Engelhardt et al. 2010). In contrast, GSG1L inhibits AMPAR-mediated synaptic transmission. In addition, although TARPs, CNIHs, and Shisa6 increase time constants of AMPAR deactivation and desensitization (Boudkkazi et al. 2014; Cais et al. 2014; Cho et al. 2007; Coombs et al. 2012; Herring et al. 2013; Jackson and Nicoll 2011; Kato et al. 2010a; Klaassen et al. 2016; Milstein et al. 2007; Priel et al. 2005; Schwenk et al. 2009; Shi et al. 2009, 2010), GSG1L speeds these kinetic properties in CA1 neurons. CKAMP44 also slows AMPAR deactivation kinetics but speeds up desensitization (Khodosevich et al. 2014; von Engelhardt et al. 2010). Furthermore, GSG1L and TARPs differ in the regulation of calcium-permeable AMPAR channel properties (McGee et al. 2015). A recent study has also shown that ABHD6 can negatively regulate AMPAR-mediated synaptic transmission, although it enhances AMPAR mEPSC decay kinetics (Wei et al. 2016; but also see Erlenhardt et al. 2016). Interestingly, several recent proteomic studies have uncovered over a dozen membrane proteins or secreted proteins that are present in neuronal AMPAR complexes, and many of them have not been examined for their function (Chen et al. 2014; Schwenk et al. 2009; Shanks et al. 2012). Thus it is possible that these uncharacterized AMPAR auxiliary subunit(s) with similar function to GSG1L may be expressed in hippocampal dentate granule cells. Finally, it is also possible that there are some unknown compensatory mechanisms in GSG1L KOs that masked the effect of loss of GSG1L on AMPAR kinetic properties in dentate granule cells.

It is well-established that trafficking and function of AMPARs in the brain are profoundly regulated by a number of AMPAR auxiliary subunits (Erlenhardt et al. 2016; Gu et al. 2016; Haering et al. 2014; Jackson and Nicoll 2011; McGee et al. 2015; Straub and Tomita 2012). Interestingly, AMPAR auxiliary subunits often play cell type-specific roles. Indeed, CNIH2 is important for AMPAR trafficking to synapses at CA1 pyramidal neurons but not in hippocampal hilar mossy cells (Boudkkazi et al. 2014; Herring et al. 2013). On the other hand, decreased expression of CNIH2 similarly led to acceleration of AMPAR EPSC decay in both types of neurons (Boudkkazi et al. 2014; Herring et al. 2013). Similarly, another AMPAR auxiliary subunit, CKAMP44, plays overlapping but also distinguishable physiological roles in hippocampal CA1 pyramidal neurons and dentate granule cells (Khodosevich et al. 2014; von Engelhardt et al. 2010). Our data now show that GSG1L functions similarly in the regulation of the strength of AMPAR-mediated synaptic transmission, but differentially in the modulation of AMPAR kinetics, in hippocampal CA1 pyramidal neurons and dentate granule cells. In addition, whereas LTP is enhanced in CA1 pyramidal neurons (Gu et al. 2016), it is not changed in dentate granule cells in GSG1L KOs. Currently, it remains unclear what accounts for the differential role of GSG1L in these two cell types. It has been reported that dentate granule cells express more flop relative to flip species of AMPARs and also express less amount of GluA1 and GluA2/3 compared with CA1 pyramidal neurons (Coultrap et al. 2005; Sommer et al. 1990). In addition, AMPARs in dentate granule cells are more susceptible to desensitization in the presence of low concentration of glutamate than in CA1 pyramidal neurons, and the time course of AMPAR recovery from desensitization is slower in dentate granule cells than in CA1 pyramidal neurons (Colquhoun et al. 1992). AMPAR-TARP stoichiometries also differ in these two cells types (Shi et al. 2009). Furthermore, CKAMP44 expression in dentate granule cells is higher than in CA1 pyramidal neurons (Khodosevich et al. 2014; von Engelhardt et al. 2010). These data indicate that AMPAR subunits, auxiliary subunit abundance, and the receptor and auxiliary subunit stoichiometry differ in dentate granule cells and in CA1 pyramidal neurons. Thus it is possible that in dentate granule cells, GSG1L interaction with AMPARs regulates the receptor trafficking to synapses, but unique AMPAR subunit and/or auxiliary subunit composition and abundance render the receptor kinetics insensitive to GSG1L modulation. Future work to explore these possibilities further will provide important understanding of how auxiliary subunits control AMPAR-mediated synaptic transmission. Interestingly, a recent study also shows that GSG1L does not regulate AMPAR trafficking in cerebellar granule cells and stellate cells (McGee et al. 2015). Furthermore, in chronic TTX-treated dissociated hippocampal neuron cultures to induce expression of calcium-permeable AMPARs, GSG1L knockdown led to an acceleration of mEPSC decay (McGee et al. 2015). Such cell type-specific or activity-dependent regulation of AMPAR trafficking and function by AMPAR auxiliary subunits may contribute to functional diversity of AMPARs across the central nervous

system and to a large variety of the molecular mechanisms for the regulation of excitatory synaptic transmission that have been reported in different types of neurons in the brain.

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The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

X.M., X.G., and W.L. designed and performed whole cell electrophysiological experiments; X.M. performed AMPAR kinetics experiments; W.L. wrote the manuscript; all authors read and commented on the manuscript.

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