The role of SAP97 in synaptic glutamate receptor dynamics

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Contributed by Roger A. Nicoll, December 19, 2009 (sent for review November 30, 2009)

Proteins of the PSD-95–like membrane-associated guanylate kinase (PSD-MAGUK) family are vital for trafficking AMPA receptors (AMPARs) to synapses, a process necessary for both basal synaptic transmission and forms of synaptic plasticity. Synapse-associated protein 97 (SAP97) exhibits protein interactions, such as direct interaction with the GluA1 AMPAR subunit, and subcellular localization (synaptic, perisynaptic, and dendritic) unique within this protein family. Due in part to the lethality of the germline knockout of SAP97, this protein's role in synaptic transmission and plasticity is poorly understood. We found that overexpression of SAP97 during early development traffics AMPARs and NMDA receptors (NMDARs) to synapses, and that SAP97 rescues the deficits in AMPAR currents normally seen in PSD-93/-95 double-knockout neurons. Mature neurons that have experienced the overexpression of SAP97 throughout development exhibit enhanced AMPAR and NMDAR currents, as well as faster NMDAR current decay kinetics. In loss-of-function experiments using conditional SAP97 gene deletion, we recorded no deficits in glutamatergic transmission or long-term potentiation. These results support the hypothesis that SAP97 is part of the machinery that traffics glutamate receptors and compensates for other PSD-MAGUKs in knockout mouse models. However, due to functional redundancy, other PSD-MAGUKs can presumably compensate when SAP97 is conditionally deleted during development.

AMPA | hippocampus | membrane-associated guanylate kinase | NMDA | postsynaptic density | synaptic transmission | synaptic development

The excitatory postsynaptic density (PSD) stabilizes AMPA receptors (AMPARs) and NMDA receptors (NMDARs) opposed to presynaptic terminals, providing a foundation for synaptic transmission and bidirectional receptor trafficking during plasticity. A major PSD component is the PSD-95–like membrane-associated guanylate kinase (PSD-MAGUK) protein family, which includes PSD-95/SAP90, PSD-93/Chapsyn-110, synapse-associated protein (SAP) 102, and SAP97, the mammalian homolog of Drosophila tumor-suppressor discs large (Dlg1) (1, 2), All PSD-MAGUKs contain protein–protein interaction motifs, most notably three PSD95/Dlg/ZO-1 (PDZ) domains (3–5), which provide a scaffold for synaptic protein complexes. PSD-95 and PSD-93 play critical roles in AMPAR trafficking at mature synapses, whereas SAP102 is most important during synaptogenesis (6–13). PSD-MAGUKs also are important for localizing NMDA receptors, especially during synapse development (14–17).

Unlike other PSD-MAGUKs, SAP97β, the major isoform of the protein (also known as DLGH1; encoded by *Dlgh1*), is expressed at presynaptic and postsynaptic sites (2, 18) and perisynaptically in dendritic spines (19). SAP97β directly binds to AMPAR GluA1 subunits (20–23). Because SAP97 has been resistant to RNAi in our hands (but see 10) and germline KO causes lethal feeding deficits in neonatal mice (24), SAP97's synaptic function is uncertain and is based primarily on dissociated neuronal culture studies. Like all other single PSD-MAGUK KOs, dissociated embryonic neurons from SAP97 KO mice have normal glutamatergic transmission (25). Although SAP97 overexpression has no effect on AMPAR-mediated synaptic transmission (8, 11, 12), SAP97 rescues AMPAR transmission reduced by RNAi-mediated knockdown of PSD-95 (12). SAP97 overexpression during synapse formation increases presynaptic terminal size and activity in neuronal cultures via a transynaptic signal (26).

Here we report that SAP97 enhanced both AMPAR- and NMDAR-mediated synaptic transmission when overexpressed in immature neurons. Acute SAP97 overexpression did not alter glutamatergic synaptic transmission in mature neurons, but it did compensate for AMPAR trafficking deficits in mature PSD-93/-95 double-KO neurons. However, in vivo SAP97 overexpression throughout synaptogenesis enhanced AMPAR and NMDAR currents, sped up NMDAR kinetics, and decreased paired pulse facilitation in mature neurons. In loss-of-function experiments, using SAP97 conditional KO ($Dlgh1^{fl/fl}$) mice, glutamatergic synaptic transmission and long-term potentiation (LTP) were normal. Overall, our results indicate SAP97 involvement in glutamate receptor trafficking in vivo, particularly in early development, and a role in compensation for other PSD-MAGUKs. However, a functionally distinct role for SAP97 remains elusive.

Results

SAP97 Overexpression Enhances Glutamatergic Synaptic Transmission in Immature Neurons. Previous studies have shown that SAP97β overexpression does not increase synaptic AMPAR currents in mature neurons as does overexpression of other PSD-MAGUKs, such as PSD-95 (11, 12). We tested the effects of SAP97β in very immature neurons from hippocampal slice cultures made on postnatal day 2 (P2). P2 slices were cultured in vitro for 5–7 days, after which individual CA1 pyramidal neurons were infected with Semliki Forest viral particles encoding SAP97β-EGFP. Dual whole-cell paired recordings were made 18–24 h after infection. SAP97β was previously studied in slice cultures made on P8 or later, when synapses are more mature. We made recordings from pairs of neighboring SAP97β–overexpressing neurons and WT neurons and stimulated glutamatergic afferents. SAP97β overexpression resulted in significant increases in evoked AMPAR excitatory postsynaptic currents (EPSCs) (Fig. 1*A*) (control, −8.6 ± 1.1; SAP97β overexpression, −53.6 ± 11.8 pA; $n = 12$ pairs; $P < 0.005$). SAP97β also significantly enhanced the amplitude of NMDAR currents, recorded at a holding potential of +40 mV (control, 6.6 ± 1.3 pA; SAP97β overexpression, 20.7 ± 5.8 pA; $n = 11$ pairs; $P < 0.05$). To clarify the locus of this EPSC enhancement, we used a paired-pulse stimulation paradigm to measure short-term plasticity. We evoked two EPSCs with an interstimulus interval of 40 ms and calculated the paired pulse ratio (PPR) as the quotient of the second event (EPSC2) divided by the first event (EPSC1). SAP97β did not alter PPR (Fig. 1*B*),

Author contributions: M.A.H., G.M.E., L.A.B.E., W.S., and R.A.N. designed research; M.A.H., G.M.E., and L.A.B.E. performed research; M.A.H., G.M.E., L.A.B.E., W.S., and R.A.N. analyzed data; and M.A.H., G.M.E., L.A.B.E., W.S., and R.A.N. wrote the paper.

The authors declare no conflict of interest.

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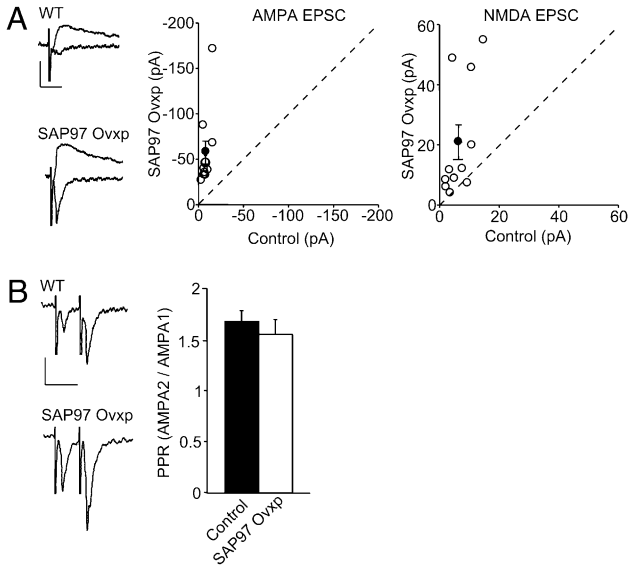

Fig. 1. SAP97β overexpression enhances AMPAR and NMDAR currents in immature neurons. Hippocampal slice cultures were made at P2. Slices were cultured for 5 days and then infected with Semliki Forest viruses to overexpress SAP97β for 18–24 h. (*A*) EPSCs are larger in a SAP97β-overexpressing (Ovxp) neuron (*Lower*) compared with a paired control neuron (*Upper*). (Scale bars: 20 pA and 50 ms.) Open symbols in the scatterplots indicate EPSC amplitudes for single pairs of control and SAP97β-expressing neurons; filled circles indicate mean values. SAP97β overexpression results in significant increases in AMPA currents (*Left*; $P < 0.01$; $n = 12$) and NMDA currents (*Right*; $P < 0.05$; $n = 11$). Error bars indicate SEM. (*B*) Paired pulse ratio is equivalent between control and SAP97β-expressing neurons. (Scale bars: 20 pA and 50 ms.)

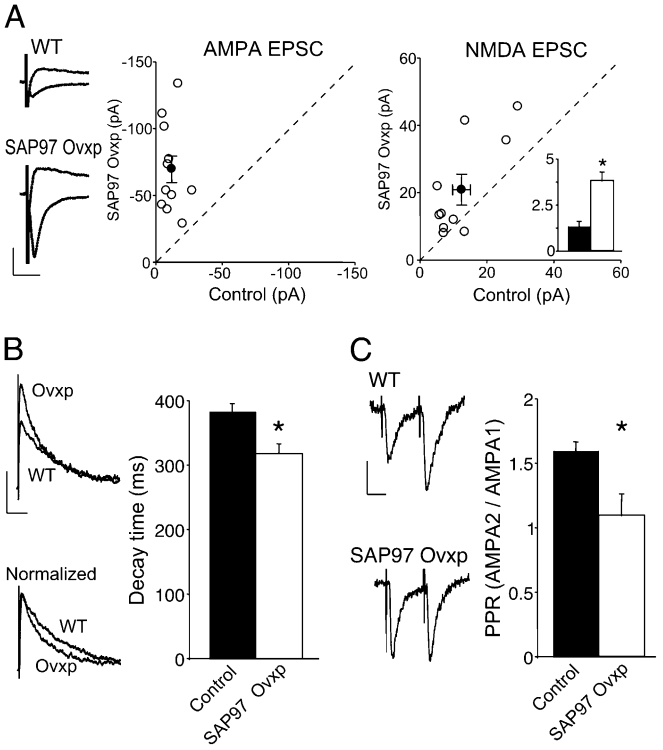

Fig. 2. Long-term in vivo SAP97β overexpression enhances synaptic transmission. SAP97β was overexpressed in utero at E16, and acute hippocampal slices were made at P8–9. (*A*) AMPAR and NMDAR EPSCs recorded from a control (*Top*) and SAP97β (*Bottom*) pair of neurons illustrate larger currents due to SAP97β Ovxp (Scale bars: 20 pA and 50 ms). Analysis of group data indicates significant enhancement of both AMPAR (*Middle*; $P < 0.001$; $n = 11$) and NMDAR currents (*Right*; $P < 0.05$; $n = 10$) induced by SAP97β overexpression. The larger relative increase in AMPAR currents resulted in a significant increase in AMPA/NMDA (*Inset*; $P < 0.05$, *t* test). (*B*) Isolated NMDAR currents (20 μM NBQX) in SAP97β-overexpressing neurons were larger in amplitude and when normalized exhibited significantly faster decay kinetics ($P < 0.05$, *t* test). (Scale bars: 40 pA and 200 ms.) (C) Paired stimuli were used to evoke EPSCs in control and SAP97β neurons. PPR was significantly reduced in SAP97β neurons ($P < 0.05$, *t* test), indicating an increased presynaptic probability of release. (Scale bars: 10 pA and 20 ms.)

indicating that the increases in glutamate receptor currents are most likely due to postsynaptic changes. Isolated NMDAR current kinetics were not recorded in these neurons. These data suggest that SAP97β is highly effective at trafficking both AMPARs and NMDARs to synapses early in development, at a time when receptor density at synapses is low.

In Vivo SAP97 Overexpression Throughout Development Enhances Synaptic Transmission. We wished to test whether SAP97 could traffic glutamate receptors in vivo during development. To address this question, we electroporated E16 embryos in utero with FUGW plasmids encoding SAP97β-EGFP. At P8, we prepared acute hippocampal slices from these pups and made paired recordings from SAP97β-expressing neurons and neighboring control neurons. As in our slice culture experiments, AMPAR currents were significantly enhanced in SAP97β −overexpressing neurons (Fig. 2*A*) (control, −12.3 ± 2.1 pA; SAP97β overexpression, −68.7 ± 10.0 pA; $n = 11$ pairs; $P < 0.001$). NMDAR currents also were significantly enhanced in the experimental group (control, 12.7 ± 2.7 pA; SAP97β overexpression, 20.9 ± 4.6 pA; $n = 10$ pairs; $P < 0.05$). The enhancement of AMPAR currents was much greater than that of NMDAR currents. This is reflected by the significant change in the AMPA/NMDA ratio (i.e., the quotient of the peak AMPA current amplitude at a holding potential of −70 mV and the NMDAR current amplitude 100 ms after the stimulus at a holding potential of +40 mV). After pharmacologically isolating NMDAR currents by adding of 20 μM NBQX to artificial cerebrospinal fluid (ACSF), we measured the weighted decay times for NMDAR currents and found significantly faster kinetics in SAP97β neurons (Fig. 2*B*) (control, 383 ± 14 ms, $n = 15$; SAP97β overexpression, 318 ± 15 ms, $n = 12$; $P < 0.005$). This suggests that SAP97, like PSD-95 but unlike SAP102, may have the ability to influence the relative contribution of GluN2A- and GluN2B-containing NMDARs present at synapses. In contrast to the immature slice culture experiments described above, we recorded a significant decrease in PPR in SAP97β neurons relative to control (Fig. 2*C*) (control, 1.6 ± 0.17, $n = 10$; SAP97β overexpression, 1.1 ± 0.06, $n = 9$; $P < 0.05$). This indicates a change in presynaptic release probability and supports hypotheses of SAP97 involvement in trans-synaptic signaling posited in previous studies (26). Given that a PPR change of this magnitude is associated with a doubling in synaptic transmission (27), the change in the size of NMDA EPSC can be fully accounted for by the enhanced release. Overall, these experiments indicate that SAP97, when overexpressed in a chronic fashion in vivo throughout development rather than acutely in vitro, can greatly enhance synaptic glutamate receptor trafficking.

Deletion of SAP97 Does Not Alter Synaptic Transmission in Immature Neurons. Based on our SAP97β overexpression data, we hypothesized that removing SAP97 via conditional genetic deletion would decrease synaptic AMPARs and NMDARs in immature neurons. To ensure complete loss of SAP97 in immature neurons, we used *Dlgh1*$^{fl/fl}$ mice expressing Cre driven by the nestin promoter, in which recombination occurs throughout the brain by E16. Previous experiments in our laboratory have shown that elimination of

PSD-MAGUK protein following knockdown may take longer than a week (7). To verify that SAP97 protein was absent in our neurons of interest following this conditional gene deletion, we used Western blot analysis to examine protein levels in hippocampi of WT and *Dlgh1*$^{fl/fl}$ mice (Fig. 3*A*). Whereas SAP97 expression was robust in WT at both P9 and P21, SAP97 was not measurable in the hippocampi of *Dlgh1*$^{fl/fl}$ mice.

To examine the consequence of deleting SAP97, we obtained acute hippocampal slices from *Dlgh1*$^{fl/fl}$ mice. Overall, hippocampal glutamatergic synaptic transmission was unchanged in these mice compared with WT littermates. AMPAR currents, NMDAR currents, and the AMPA/NMDA ratio were all equivalent in *Dlgh1*$^{fl/fl}$ neurons and WT neurons (Fig. 3*B*). PPR also remained unchanged (Fig. 3*C*). We also recorded spontaneous mEPSCs after the addition of 0.1 μM TTX and 100 mM sucrose to the ACSF (Fig. 3*D*). There was no significant difference in mEPSC amplitude or frequency between WT and *Dlgh1*$^{fl/fl}$ neurons. Based on these results, it appears that SAP97 is not absolutely necessary for glutamate receptor trafficking early in development. However, whether compensatory mechanisms are sufficient to rescue the normal phenotype in the absence of SAP97, or whether SAP97 plays only a minimal role in normal AMPAR trafficking at this developmental stage, remains unclear. This pattern reflects that of PSD-95 in mature neurons, in that some reports show that PSD-95 KO neurons exhibit normal synaptic transmission, and deficits in AMPAR trafficking are revealed only when PSD-95 is acutely knocked down by RNAi (7, 28, but see 9, 29). To trigger compensatory mechanisms, either more time or knockout of the protein throughout development is required.

Unfortunately, endogenous neuronal SAP97 has proven resistant to RNAi in our hands, and we are not confident that we can distinguish between negative results and insufficient protein reductions after RNAi in immature neurons. SAP97 may possibly play a role at an earlier developmental stage, before P9; however, because synaptic transmission in cultured neurons from late-stage SAP97 KO embryos is normal (25), the role of SAP97 is either compensated for or minimal during embryonic development. We cannot entirely eliminate the possibility that SAP97 plays a more important role in synaptic transmission between P0 and P9, because whether the protein has been entirely eliminated in the conditional knockout mice during this phase is unclear.

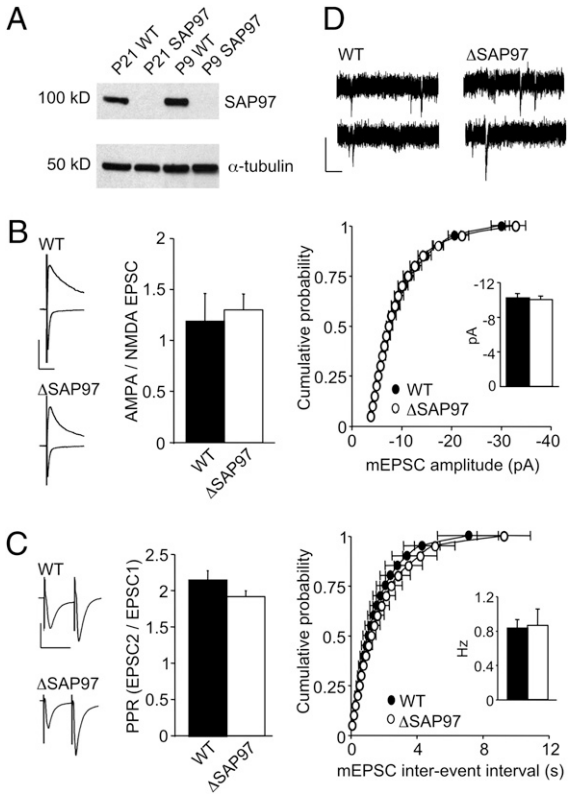

Fig. 3. Deleting SAP97 does not alter basal synaptic transmission in immature neurons. (*A*) Western blot analysis shows abundant SAP97 protein in isolated hippocampi from WT mouse at age P9 and P21, but no discernible signal from *Dlgh1*$^{fl/fl}$ (ΔSAP97) hippocampi at those ages. α-Tubulin was used as a loading control. Acute hippocampal slices were made from WT and *Dlgh1*$^{fl/fl}$ mice at P9. (*B*) AMPAR and NMDAR EPSCs from representative WT neurons (*Left*) and *Dlgh1*$^{fl/fl}$ neurons (*Right*). (Scale bars: 50 pA and 100 ms.) AMPA/NMDA is equivalent between these two genotypes. [$P = 0.74$; $n = 35$ (KO), 12 (WT)] (*C*) Neurons of both genotypes exhibit paired pulse facilitation and an equivalent paired pulse ratio. (Scale bars: 50 pA and 40 ms.) (*D*) Example traces of spontaneous mEPSCs recorded from WT and *Dlgh1*$^{fl/fl}$ neurons (in the presence of TTX and picrotoxin) show similar activity. (Scale bars: 10 pA and 250 ms.) Cumulative probability curves for mEPSC amplitude [*Middle*; mean amplitude inset; P = 0.69; n = 12 (KO), 19 (WT)] and interevent interval [*Bottom*; mean frequency inset; $P = 0.92$; $n = 12$ (KO), 19 (WT)] show that mEPSC activity is unchanged by loss of SAP97.

Acute SAP97β Overexpression Compensates for Other PSD-MAGUKs but Does Not Alter WT Synaptic Transmission in Mature Neurons. Previous work has indicated that in mature neurons, SAP97β overexpression alone has no effect on glutamatergic synaptic transmission, but it can compensate for deficits resulting from PSD-95 RNAi (12). We examined whether SAP97β also could compensate for the large decrements in AMPAR trafficking that result from PSD-93/-95 double KO. We prepared hippocampal slice cultures from WT and double-mutant mice at age P8, overexpressed SAP97β EGFP by infection with Semliki Forest virus and then made dual whole-cell recordings 18–24 h later. As in previous studies, SAP97β overexpression did not enhance AMPAR or NMDAR currents over WT levels in these more mature neurons (Fig. 4*A*).

As reported previously, neurons in acute slices made from PSD-93/-95 double-KO mice exhibited significantly reduced AMPAR currents but normal NMDAR amplitudes. Paired recordings of SAP97β-expressing neurons and control double-KO neurons show that SAP97β enhanced AMPAR currents in the context of the reduced AMPAR-mediated transmission of the multiple PSD-MAGUK mutant background (Fig. 4*B*) (control, 36.8 ± 10.2 pA; SAP97β overexpression, 109.3 ± 25.2 pA; $n = 5$ pairs; $P < 0.05$). NMDAR currents, which are normal in the double-KO mice, were unchanged. This resulted in a significant change in the AMPA/NMDA ratio (Fig. 4*B Inset*) ($P < 0.01$). Although a direct quantitative comparison between the AMPA/NMDA ratio of this experiment with the WT ratio is not possible, it is interesting to note that the ratio is very similar to that of WT neurons. This suggests a virtually complete compensation by SAP97β for the mutant phenotype caused by the elimination of two key PSD-MAGUK proteins.

Deletion of SAP97 Does Not Alter Synaptic Transmission and LTP in Mature Neurons. To assess the effect of SAP97 elimination on synaptic transmission in mature neurons, we recorded EPSCs from acute slices made from P19-21 *Dlgh1*$^{fl/fl}$ mice. Overall, we recorded no change in basal glutamatergic synaptic transmission, as was the case for slices from immature *Dlgh1*$^{fl/fl}$ mice. In *Dlgh1*$^{fl/fl}$ neurons AMPAR and NMDAR EPSCs and the AMPA/NMDA ratio (Fig. 5*A*), PPR (Fig. 5*B*), NMDAR kinetics (Fig. 5*C*), and mEPSC amplitude and frequency (Fig. 5*D*) were all equivalent to control measures from WT littermates.

Given the distributed nature of SAP97 expression (i.e., synaptic, perisynaptic, and extrasynaptic), we hypothesized that this protein might play a role in the active redistribution of AMPARs

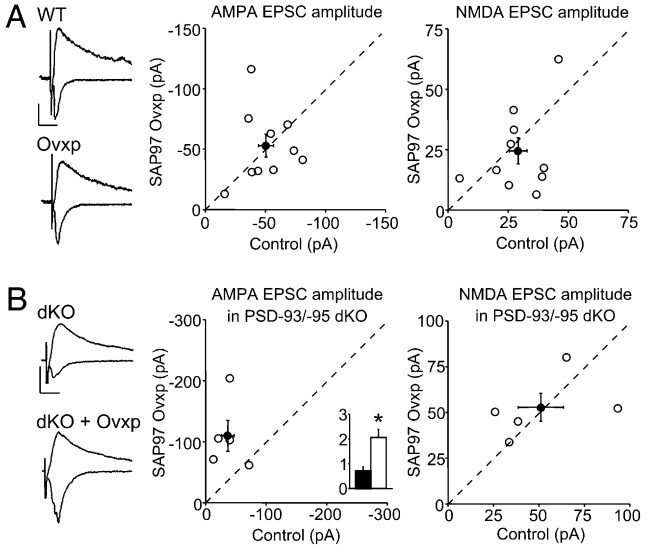

Fig. 4. SAP97β overexpression does not enhance synaptic transmission in mature neurons but compensates for PSD-93/-95 double KO. Hippocampal slice cultures were made at P8–9. Slices were cultured for 6–8 days in vitro, and SAP97β was overexpressed using Semliki Forest viruses. Recordings were made 18–24 h after infection. (*A*) Traces of AMPAR and NMDAR EPSCs recorded from a pair of control (*Upper*) and SAP97β (*Lower*) mature cultured neurons. Scatterplots indicate that AMPA (*Left*) and NMDA (*Right*) currents were unchanged by SAP97β. (Scale bars: 50 pA and 25 ms.) (*B*) Traces of EPSCs recorded from a pair of PSD-93/-95 double-KO neurons (*Upper*, control; *Lower*, SAP97β) shows that AMPAR currents are enhanced by SAP97β. Scatterplots of pairs of neurons indicate a significant increase in AMPAR currents (*Left*; $P < 0.05$; $n = 5$) but no change in NMDAR currents (*Right*) due to SAP97β expression. This change results in a significant increase in the AMPA/NMDA ratio (*Inset*; WT: black bar, dKO: white bar) in double-KO neurons overexpressing SAP97β. (Scale bars: 50 pA and 25 ms.)

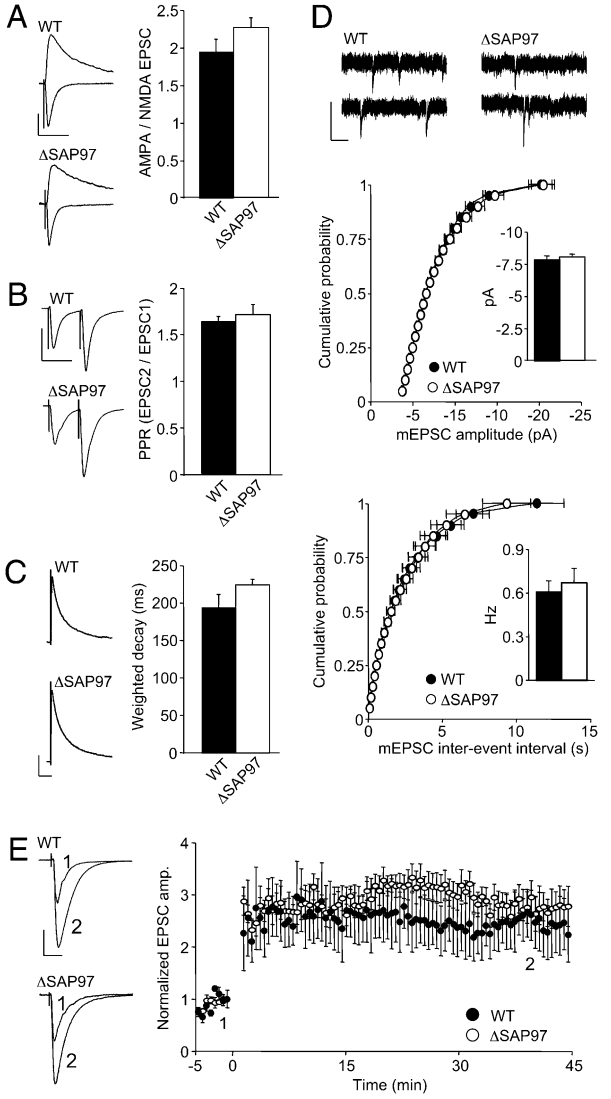

Fig. 5. Deleting SAP97 does not alter basal synaptic transmission or LTP. Acute hippocampal slices were made from WT and SAP97 conditional KO mice at P18–21. (*A*) AMPAR and NMDAR EPSCs from representative WT neurons (*Upper*) and *Dlgh1*$^{fl/fl}$ neurons (*Lower*) are qualitatively similar. AMPA/NMDA is unchanged between genotypes [P = 0.11; n = 17 (KO), 9 (WT)]. (Scale bars: 80 pA and 50 ms.) (*B*) Paired pulse facilitation in individual neurons and mean paired pulse ratio also are unaffected in *Dlgh1*$^{fl/fl}$ neurons. (Scale bars: 40 pA and 40 ms.) (*C*) Decay kinetics of isolated NMDAR EPSCs are unaffected in *Dlgh1*$^{fl/fl}$ neurons. (Scale bars: 20 pA and 100 ms.) (*D*) Example traces of mEPSC recordings show similar activity in both genotypes. Cumulative probability curves for mEPSC amplitude [*Middle*; mean amplitude inset; P = 0.67; n = 18 (KO), 22 (WT)] and interevent interval [*Bottom*; mean frequency inset; P = 0.63; n = 18 (KO), 22 (WT)] show no changes in mEPSCs in *Dlgh1*$^{fl/fl}$ neurons compared with WT. (Scale bars: 10 pA and 250 ms.) (*E*) (*Upper*) EPSCs recorded from a representative WT (*Top*) and *Dlgh1*$^{fl/fl}$ neuron (*Bottom*) before (1) and 40 min after (2) LTP induction exhibit similar potentiation. LTP was induced by holding neurons at 0 mV and stimulating at 2 Hz for 90 s. (Scale bars: 50 pA and 25 ms.) (*Lower*) Mean EPSC amplitudes, normalized to baseline, show equivalent potentiation in WT neurons (filled circles) and *Dlgh1*$^{fl/fl}$ neurons (open circles; n = 9 KO and WT).

during induction of LTP. Thus, we recorded EPSCs in WT and *Dlgh1*$^{fl/fl}$ hippocampal slices before and after induction of LTP with a pairing induction protocol (2 Hz, 90 s duration, holding potential 0 mV). Compared with baseline EPSC amplitude, EPSCs were equally potentiated in WT and *Dlgh1*$^{fl/fl}$ neurons (Fig. 5*E*). This indicates that SAP97 is not necessary for the expression of LTP at this synapse in mature neurons.

Discussion

PSD-MAGUK proteins are essential for normal localization of both AMPARs and NMDARs at synapses (5). When synaptic AMPAR content is low (e.g., early in development, in PSD-93/-95 double KO), our findings demonstrate that the PSD-MAGUK SAP97 has the ability to traffic both of these subtypes of glutamate receptors to synapses. In addition, when the expression of other PSD-MAGUKs is disrupted, overexpression of SAP97 compensates for the missing proteins and traffics a normal complement of receptors to synapses in mature neurons. We have shown that removal of SAP97, accomplished by conditional knockout, results in no deficits in glutamatergic transmission. These results indicate that other PSD-MAGUKs can compensate for SAP97 in immature and mature neurons, or that SAP97 plays a minimal role in basal glutamatergic transmission in CA1 pyramidal neurons.

PSD-MAGUK Trafficking of AMPARs. PSD-MAGUKs generally interact with AMPARs through AMPAR auxiliary subunits known as transmembrane AMPAR regulatory proteins (TARPs), which have PDZ-binding motifs (29). SAP97 is alone among the PSD-MAGUKs in interacting directly with AMPAR subunits, namely GluA1 (20, 23). Some reports show that germline knockout of any single PSD-MAGUK family member, PSD-93, PSD-95, or SAP 102, causes no major disturbance in glutamatergic synaptic transmission (7, 15, 30, but see 9, 28). Whereas mice harboring a truncation of SAP97 do not survive as neonates, because craniofacial deformities prevent feeding (24), cultured dissociated neurons from these embryos exhibit normal synaptic transmission (25). However, acute removal of PSD-MAGUKs (by, e.g., RNAi) reveals their functional significance to excitatory neurotransmission (7, 8, 12). PSD-93 and

PSD-95 are each responsible for trafficking a large portion of AMPARs to synapses in mature neurons. Interestingly, these two proteins traffic AMPARs to nonoverlapping subsets of synapses. The mechanism for this segregation of PSD-MAGUKs to different synapses within a neuron remains unknown. When both PSD-93 and PSD-95 are genetically deleted, AMPAR currents are radically reduced in mature neurons. SAP102 expression is up-regulated in these double-KO neurons, whereas SAP97 expression remains unchanged (7). The remaining deficit in synaptic transmission indicates that SAP102 and SAP97 together are unable to naturally rescue the phenotype exhibited by PSD-93/-95 double KO. These data imply that these proteins have functionally distinct roles in AMPAR trafficking. We have shown that overexpression of SAP97 rescues normal levels of synaptic AMPARs in double-KO neurons. Previous studies also have described rescue of normal phenotypes by SAP97 following acute reduction of other PSD-MAGUKs (12). These data beg the following question: If expression of one PSD-MAGUK is sufficient to provide full compensation for a deficiency, then why does this compensation occur under the circumstances of a single gene KO but not following the double KO?

The relative roles of PSD-MAGUKs differ in early stages of development. Acute knockdown of SAP102 results in dramatic decreases in synaptic AMPAR currents in neonatal brains. Neither acute knockdown of PSD-95 nor PSD-93/-95 germline deletion (both of which result in dramatic AMPAR deficits in mature neurons) disrupts synaptic transmission at this stage of development; however, even in this early time period, PSD-95 overexpression increases the number of synaptic AMPARs (16). We have shown that SAP97 overexpression in immature synapses also increases AMPAR transmission. Taken together, our findings and results of previous studies highlight important questions about compensation and redundancy among PSD-MAGUKs in the task of AMPAR trafficking. Whereas overexpression of exogenous proteins is sufficient to rescue mutant phenotypes, this compensatory machinery is not necessarily activated in vivo. This again points to functionally distinct roles for PSD-MAGUKs and possibly to different mechanisms through which PSD-MAGUK expression is regulated, processes that are still not well understood.

PSD-MAGUK Trafficking of NMDARs. PSD-MAGUKs interact directly via their PDZ domains with NMDAR PDZ-binding motifs (31). Whereas it was initially hypothesized that PSD-MAGUKs would thus have a major influence on synaptic NMDAR trafficking, most studies of mature neurons have reported that neither overexpression nor acute knockdown of individual PSD-MAGUKs results in major changes in synaptic NMDAR number. Double KO of PSD-93/-95 did not decrease synaptic NMDAR currents, but further reduction of PSD-MAGUK levels using RNAi to decrease SAP102 in PSD-93/-95 double-KO neurons did result in a small decrease in NMDAR currents in mature neurons (7). Acute double knockdown of PSD-93 and -95 by RNAi did decrease synaptic NMDAR currents, although not to the extent of synaptic AMPAR currents (7). In line with these results, we report no changes to synaptic NMDAR currents following either SAP97 overexpression or conditional knockout in mature neurons.

As is the case with PSD-MAGUK–AMPAR interactions, NMDAR trafficking by PSD-MAGUKs is different in immature and mature neurons. In the neonatal brain, SAP102 expression directly correlates with synaptic NMDAR currents; SAP102 overexpression increases and acute SAP102 knockdown decreases NMDAR EPSC amplitudes. Interestingly, this pattern can persist into maturity. NMDAR currents are enhanced in mature neurons when SAP102 expression is induced in utero and continues throughout development (16). We found dramatic increases in synaptic NMDAR currents following SAP97 overexpression in immature neurons; however, we recorded no phenotype in immature SAP97 conditional knockout neurons. Again, this may be due to compensation by SAP102 for the loss of SAP97 protein.

A specific aspect of NMDAR trafficking in which PSD-MAGUKs have been implicated is in the developmental switch in receptor subunits. In general, immature neurons express receptors containing two obligatory GluN1 subunits and two GluN2 subunits. Over the course of development, and in response to increased levels of activity, GluN2B-containing receptors are switched for GluN2A-containing receptors. The physiological hallmark of this switch is a change in receptor kinetics from slow to fast (32). Data from knockout, knockdown, and overexpression studies indicate that this switch is influenced largely by the developmental change from SAP102 to PSD-93/-95 as the dominant PSD-MAGUKs. In general, more PSD-95 results in faster NMDAR kinetics. This is true even at early developmental stages if PSD-95 is overexpressed. Similarly, acute knockdown of PSD-95 results in slower NMDAR kinetics in mature neurons (16). In contrast, although overexpression of SAP102 or knockdown of SAP102 affects the amplitude of NMDARs EPSC, it does not change the NMDAR kinetics relative to control. Thus, it has been proposed that SAP102, unlike PSD-95, is promiscuous with respect to the trafficking of NMDARs with either GluN2A or GluN2B subunits; SAP102 appears to traffic both equally well (16). We report that in utero SAP97 overexpression also can drive the NMDAR subunit switch, resulting in both larger and faster NMDAR currents than normal neurons at the same developmental stage, indicating a potential role for SAP97 in controlling both the number and the specific subunit composition of synaptic NMDARs. Whereas the mechanisms underlying these effects are unclear, and the loss of SAP97 can still be compensated for, these experiments demonstrate that SAP97 behaves in a distinctly different manner than other PSD-MAGUKs. In immature neurons SAP97 favors GluN2A over GluN2B, unlike SAP102, and SAP97 overexpression can enhance synaptic NMDAR number, unlike PSD-95 overexpression.

Conclusion. SAP97 shows marked differences from other PSD-MAGUKs in both protein–protein interactions and subcellular distribution (5). SAP97 has been implicated in trafficking many types of proteins important for cellular function, including ion transporters, voltage-gated ion channels, and ligand-gated receptors. SAP97 is involved in patterning the dendritic arbor (22) and setting presynaptic activity via trans-synaptic signaling (26). Like other PSD-MAGUKs, SAP97 is sufficient, but not individually necessary, for AMPAR trafficking to synapses. Furthermore, SAP97 can traffic NMDARs in a subunit-specific manner. Our results also suggest functional distinctions between SAP97 and other PSD-MAGUKs, particularly in terms of trafficking AMPARs and subunit-specific trafficking of NMDARs during early development. Although our data suggest that SAP97 does not play a functionally distinct role in trafficking of glutamate receptors in CA1 pyramidal neurons of the hippocampus, our overexpression studies indicate that SAP97 could play an important role in other brain regions in which this protein is enriched.

Methods

Generation of SAP97 Conditional KO Mice. In *Dlgh1*$^{fl/fl}$ mice, exon 4 of the *Dlgh1* gene is flanked upstream and downstream by loxP sites (33). Recombination that these sites produces a truncated transcript that is not translated into a functional protein (34). Mice homozygous for this genotype were bred with nestin-Cre transgenic mice. Genotypes were confirmed by PCR as described previously (33), and depletion of SAP97 protein level was measured by Western blot analysis.

Transfection and Slice Preparation. In utero electroporation of plasmids was carried out as described previously in timed-pregnant (E16) dams (16, 35). Cultured hippocampal slices were prepared from 2- to 9-day-old mice, as described previously (36). After 5–10 days in vitro, slice cultures were injected with Semliki Forest virus to overexpress SAP97β, as described previously (7). Electrophysiological recordings were made 18–24 h after infection. For acute recordings, 300-μm transverse hippocampal slices were made at P8–21. All electrophysiological recordings were done at room temperature in ACSF con-

taining 119 mM NaCl, 2.5 mM KCl, 1 mM $NaHPO_4$, 26.2 mM $NaHCO_3$, 11 mM glucose, 4 mM $MgSO_4$, 4 mM $CaCl_2$, and 0.1 mM picrotoxin. Patch pipettes (3–5 MΩ) were filled with a solution containing 135 mM $CsMeSO_4$, 8 mM NaCl, 5 mM QX-314, 10 mM Hepes, 4 mM ATP-Mg, 0.3 mM GTP-Na, 0.3 mM EGTA, and 0.1 mM spermine.

Statistical comparisons between groups were made using two-tailed paired *t* tests. Cumulative probability curves for mEPSC amplitude and interevent interval were analyzed using the Kolmogorov-Smirnov test. The threshold for statistical significance was $P < .05$.

ACKNOWLEDGMENTS. We wish to specifically acknowledge the assistance of Eric Schnell, Kirsten Bjorgan, Pierre Apostolides, Manuel Cerpas, without whose expertise this study would not have been possible. We thank all the members of the Nicoll laboratory for support and assistance, with special acknowledgment of Jonathan Levy and John Gray for their help with this manuscript. We also thank members of Arnold Kriegstein's laboratory for assistance with in utero electroporation. R.A.N. is funded by grants from the National Institute of Mental Health. W.S. is funded by grants from the National Institutes of Health (P30-AR048335 and RO1-AI061077). G.M.E. was supported by the University of California at San Francisco (UCSF) Chancellor Graduate Award and the UCSF President Dissertation-Year Award.

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