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Eye opening and PSD95 are required for long-term potentiation in developing superior colliculus

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The major glutamate receptor membrane-associated guanylate kinase scaffolds expressed in the young superficial superior colliculus (SC) are synapse-associated protein 102 (SAP102) and postsynaptic density protein 95 (PSD95). In this, as in all visual brain regions examined, synaptic PSD95 increases rapidly following simultaneous eyelid opening (EO). We show that EO and PSD95 are necessary for SC NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) and this LTP is eliminated or reinstated by manipulating EO. PSD95 knockdown (KD) in vivo blocks this LTP, but not long-term depression, and reduces frequencies of miniature AMPA receptor and NMDAR currents with no change in presynaptic release. Furthermore, miniature NMDAR currents after PSD95 KD show an activity-dependent calcium sensitivity that is only normally found in the pre-EO period when SAP102 binds mixed GluN2B/GluN2D NMDARs. These data indicate that young SC LTP arises from PSD95 unsilencing of silent synapses, that unsilencing is labile in young brain, and that even though SAP102 and PSD95 can bind the same NMDARs, only PSD95 enables SC synaptic maturation.

It is significant that MAGUKs show different expression patterns at different ages because there is increasing evidence that each may bind different signaling molecules at glutamate synapses (15). For example, in rodent hippocampus, SAP102 is present in the early postnatal PSD, it binds NMDA receptors (NMDARs) via GluN2B, AMPARs via stargazin (16), and a complex containing SynGAP (17, 18). In visual cortex, SAP102 is replaced at the PSD by the PSD95 complex containing GluN2A and TrkB upon EO (9, 18). At this stage, SAP102 and GluN2B-rich NMDARs remain in extrasynaptic regions (4, 13) where they mediate mostly evoked currents, whereas the PSD95–GluN2A complex is responsible for minor NMDAR currents (mNMDARs). This scenario, first suggested in the hippocampus (19), was documented in the SC of the developing GluN2A KO mouse (20). Extrasynaptic NMDARs with functions that differ from PSD NMDARs have been identified in several brain regions (13, 21–24) and PSD95 selectivity for the GluN2A tail has now been verified in the developing hippocampus (25).

For NMDARs in forebrain and dorsal midbrain regions, the change from binding MAGUKs via GluN2B to binding via GluN2A has frequently been interpreted as a switch from GluN1/GluN2B diheteromeric receptor currents to short decay-time currents characteristic of GluN1/GluN2A diheteromeric receptors. However, in the SC of rats and mice when SAP102 is still the major PSD MAGUK but GluN2B subunits are increasing, NMDAR decay times decrease abruptly. This is mediated via a calcineurin (CaN)-dependent dephosphorylation of the GluN2A tail (26, 27). Therefore, NMDAR decay time cannot reveal when a subunit change at the PSD from GluN2B/GluN2D to all GluN2A occurs or whether SAP102 or PSD95 scaffolds the receptor.

Here, we used short hairpin RNA (shRNA) to KD PSD95 in single cells of the neonatal SC and studied their synaptic currents and NMDAR-dependent plasticity at intervals after EO. Our findings differ from those expected from PSD95 KD experiments in hippocampus CA1 (25, 28–31) in three fundamental respects: In SC, long-term potentiation (LTP) does not survive PSD95 KD; in SC, long-term depression (LTD) does survive PSD95 KD; in SC, reductions in AMPAR and NMDAR synaptic responses occur with PSD95 KD without any change in presynaptic release. In addition, in young SC, the ability to induce LTP is not stable; eyelid reclosure (ERC) for several days causes SC LTD to disappear and eye reopening (ERO) following ERC reintroduces LTD. Finally, the CaN-mediated decrease in NMDAR decay times, observed in normal SC neurons only as activity increases before EO (26), is present after EO in PSD95 KD neurons. This suggests that SAP102 binding triheteromeric GluN1/GluN2B/GluN2A NMDARs remains at the PSD in these cells.

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The authors declare no conflict of interest.

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Fibrin Glutamate receptor membrane-associated guanylate kinase scaffolds expressed in the young superficial superior colliculus (SC) are synapse-associated protein 102 (SAP102) and postsynaptic density protein 95 (PSD95). In this, as in all visual brain regions examined, synaptic PSD95 increases rapidly following simultaneous eyelid opening (EO). We show that EO and PSD95 are necessary for SC NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) and this LTP is eliminated or reinstated by manipulating EO. PSD95 knockdown (KD) in vivo blocks this LTP, but not long-term depression, and reduces frequencies of miniature AMPA receptor and NMDAR currents with no change in presynaptic release. Furthermore, miniature NMDAR currents after PSD95 KD show an activity-dependent calcium sensitivity that is only normally found in the pre-EO period when SAP102 binds mixed GluN2B/GluN2D NMDARs. These data indicate that young SC LTP arises from PSD95 unsilencing of silent synapses, that unsilencing is labile in young brain, and that even though SAP102 and PSD95 can bind the same NMDARs, only PSD95 enables SC synaptic maturation.

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Results

LTP Is Labile in the Young SC. In SC, acute slices from postnatal day 15 (P15) to P17 rat pups with EO at P13 or P14, stimulating the stratum opticum at 20 Hz for 20 s produces an NMDAR- and L-type Ca$^{2+}$-channel-dependent LTP in a major excitatory SC neuron population, narrow field vertical (NFS) neurons: a group we have focused LTP studies on because they are visually driven, abundant, and of relatively uniform size (32). This same stimulation applied to slices from pups of the same age with eye closure (EC) showed no LTP in NFS neurons. However, if similarly deprived pups received 4–5 h of visual experience after simultaneous EO, LTP could be induced (Fig. 1A). The same EO-dependent LTP was induced in slices from pups killed 2–4 d after EO, but not in pups with EC during the same period (Fig. 1B). Also when only one eye remained shut and the other was opened at P13 with patterned visual experience for 3–4 h before killing, LTP was induced only in neurons that had received input from the contralateral open eye (Fig. 1C). In a final paradigm, glued eyelids were opened at P13, and then reclosed on P16. At P20, one-half of the animals had their eyelids reopened for 4–5 h before killing and recording. SC LTP was obtained in slices from these eye-reopened animals. However, LTP was not present in the reclosed animals whose eyes were not reopened before P20 recording (Fig. 1D). Therefore, young SC LTP had a pronounced dependence on pattern vision as well as a pronounced lability to loss of pattern vision. Importantly, these EC and EO regimes were identical to ones used in the initial study of EC- and EO-associated changes in synaptic levels of PSD95 in visual cortex and SC synapses (9). This tight correlation suggested that PSD95, or molecules in a complex with PSD95 at the synapse were necessary for induction of SC LTP.

SAP102 and PSD95 Are the Only MAGUKs in Young SC. We documented the MAGUKs present in the SC with quantitative Western blotting of PSD95, PSD93, and SAP102. SAP97 immunoreactivity is not present in the SC (33). Homogenates from the SC at P15 and hippocampus at P19, two roughly corresponding stages of synapse maturity (4, 9, 25), were analyzed. Identical protein concentrations from SC and hippocampus were run in adjacent lanes (Fig. 2A), blotted, and probed with antibodies for PSD95, SAP102, and PSD93. SAP102 and PSD95 bands were present in both lanes, with the expected, higher levels in the hippocampus. However, PSD93 was present as an intense band only in hippocampal lanes and barely detectable control lanes (Fig. 2B).

PSD95 KD Decreases mAMPARc and mNMDARc Frequency. We designed two KD shRNAs (KD I and II) against PSD95, and two scrambled shRNAs (Scr I and II) as negative controls. These shRNAs were inserted into the lentiviral plasmid carrying GFP, and lentiviruses were produced. Efficacy and specificity of these shRNAs were documented in HEK cells and cultured occipital cortical neurons (Fig. 2B and C). The lentiviruses were injected into the SC of neonates (P1–P3). The eyes of these pups were opened at P14 and they were killed between P15 and P17 for slice physiology with the investigator blind to the shRNA lentiviruses injected. In acute SC slices, infected neurons were identified by their GFP fluorescence followed by labeled differential interference contrast microscopy (Figs. 2A and F–H). Rise and decay times and amplitudes of evoked mAMPARcs were isolated with appropriate agonists. All neurons expressing the PSD95 KD shRNAs showed significantly lower mAMPARc frequencies than the corresponding Scr controls (Fig. 3), and neither KD I nor KD II had an effect on mAMPARc amplitude (Fig. S1). Frequencies and amplitudes in uninfected NFV neurons in the same slices were not significantly different from those recorded in neurons from the same animal infected with the Scr control lentiviruses (Fig. S2 A–C and F–H). Rise and decay time of mAMPARcs and mNMDARcs were also unchanged between PSD95 KD, Scr, and uninfected neurons (Fig. S2 D and E, and I and J). Decreases in mAMPARc frequency have been documented in virtually all PSD95 KD or KO studies in CA1 pyramids where they were examined (28–30); however, none of these studies reported changes in mNMDARcs. In the relatively small NFV neurons, it was possible to record mNMDARcs, and similar to the mAMPARcs, their frequencies (Fig. 4 A–D) but not their amplitudes (Fig. S3) were significantly reduced in all PSD95 KD cells. To assay for an effect of PSD95 KD shRNA on pre-synaptic release (34) that might cause a change in both mAMPARc and mNMDARc frequencies, we examined paired-pulse ratios (PPRs). In all cases, PPRs were identical for PSD95 KD and Scr neurons (Fig. 4 E and F). Also consistent with decreases in both mAMPARc and mNMDARc frequencies, we found no differences in evoked AMPAR current (eAMPARc)/evoked NMDAR current (eNMDARc) ratios between neurons expressing KD I or II and their corresponding Scr controls (Fig. 4 G and H).

In Vivo PSD95 KD Eliminates NMDAR-Dependent LTP. Characterization of SC LTP in whole-cell patch-clamped NFV neurons expressing the PSD95 KD or the corresponding Scr shRNA revealed that LTP was absent in all PSD95 KD neurons but present in all Scr-expressing neurons (Fig. 5 A and B). In addition, because the mAMPARc and mNMDARc recordings indicated reduced numbers of AMPAR- and NMDAR-containing...
synapses in PSD95 KD cells, we compared the amplitude and frequency of responses to inducing stimuli that were determined for each neuron as the intensity producing a response of half-maximal size. We found no differences in the amplitudes of the evoked responses or in their ability to follow each stimulating pulse between neurons expressing the PSD95 KD or corresponding Scr shRNAs (Fig. S4).

**LTD Is Normal in SC PSD95 KD Neurons.** Colledge et al. (35) proposed that PSD95 removal could be causative in NMDAR-dependent LTD. Also, LTD is absent in the hippocampus of PSD95 KO mice (14, 15, 28) and impaired in PSD95 KD neurons in cultured hippocampal slices (30, 31). However, there is evidence that only extrasynaptic NMDARs are coupled to LTD generation (36, 37), and in the SC, normal NMDAR-dependent LTD is present in young GluN2A KO mice (38). These mice have no mNMDARcs and spontaneous NMDAR currents (sNMDARcs) probably because once PSD95 is at the PSD, it requires the GluN2A tail to bind NMDARs (13, 20). Moreover, in both WT and GluN2A KO mice, SC LTD can be eliminated by blockade of either GluN2B receptors or L-type Ca\(^{2+}\) channels (38). Consequently, we hypothesized that NMDAR-dependent LTD in the PSD95 KD SC neurons would not show disrupted LTD. Indeed, we found that NMDAR-dependent LTD remained in these neurons and was identical in amplitude to the LTD induced in neurons expressing the corresponding Scr shRNA (Fig. C and D).

**FK506 Increases sNMDAR Decay Time in PSD95 KD Neurons.** After PSD95 KD, the only other significant MAGUK in the young SC, SAP102, was expected to be the remaining scaffold at the PSD. However, in all of the PSD95 KD cells we recorded sNMDARcs with the short decay-time characteristic of the diheteromeric GluN1/GluN2A NMDARs usually bound by PSD95 after EO (9) in both the PSD95 KD and Scr neurons (Fig. A, C, E, and G, upper three traces). In normal SC neurons, the effect of the CaN blockade disappears before EO as the level of PSD95 and GluN2A increases (9, 12). However, the GluN2A subunit protein, first detectable in the SC at ~P7, is significantly increased in the P15–P17 EO pups studied here (12). It is also likely that these increases are independent of PSD95. Consequently, we tested the hypothesis that the pre-EO decrease in NMDARc decay times that were observed with GluN1/GluN2A/GluN2B triheteromeric NMDARs bound by SAP102 had reappeared in PSD95 KD neurons by applying the membrane-permeable CaN antagonist FK506. In the study by Shi et al. (26), both FK506 and the CaN inhibitory peptide effectively eliminated the decay-time decrease. We bath-applied FK506 and within 12 min PSD95 KD neurons developed long sNMDARc decay times (Fig. C and G, lower three traces, and D and F) typical of GluN2A/GluN2B-rich NMDARcs in younger animals with SAP102 as the PSD MAGUK. FK506 had no effect on neurons carrying the corresponding Scr shRNA (Fig. A and E, lower three traces, and B and F). The results indicate that SAP102 binding NMDARcs by the GluN2B C-terminal (16) supported GluN2B/GluN2A NMDARs at the PSD despite EO, and that the generally higher levels of activity after EO when it impinges on the PSD95 KD neurons triggered the same activity-dependent CaN response documented by Shi et al. (26). Thus, the normal NMDAR activity-dependent CaN shortening of NMDARc decay times found before EO could remain active on triheteromeric GluN1/GluN2A/GluN2B NMDARcs at older SC synapses because activity was sufficiently high and because SAP102 binding NMDARcs by the GluN2B C-terminal remained at the PSD.

**Discussion**

Despite many reports describing the functions of PSD95 at glutamate synapses, identification of specific roles for synaptic MAGUKs has been difficult because the KD and KO work has been focused on the hippocampus where four MAGUK family members...
members can scaffold glutamate receptors and several can compensate for each other (15, 29). The hippocampus is one of the most highly evolved structures in the mammalian brain necessary for many forms of learning and memory and therefore equipped with many compensating mechanisms to maintain its critical functions with the specialized mammalian neocortex. By contrast, the SC continues to subserve most of the functions of localization in space, persistent activity, and initiation of single or multisensory motor output that has been crucial to the survival of the vertebrate line throughout evolution (39, 40). However, the SC and its nonmammalian homolog the optic tectum, like most other central nervous system regions, maintain activity-dependent interactions critical to adaptive circuitry during development (41, 42). In this report, we document effects of SC PSD95 KD during development that are unexpected from the MAGUK manipulations performed in hippocampal CA1. We show here that PSD95 KD in the developing SC eliminates NMDAR-dependent LTP, and in CA1 hippocampus it does not. PSD95 KD in SC does not eliminate NMDAR-dependent LTD, and in the hippocampus it does. PSD95 KD in SC also causes reductions in both mAMPArc and mNMDARc frequency with no change in presynaptic release. In addition, we show that young SC LTP in vivo is critically linked to pattern vision. This LTP is not stable at least within the week after EO when ERC for several days causes the LTP to disappear and ERO reintroduces LTP. Lability of LTP on the order of minutes to hours has been previously noted in the optic tectum and hippocampus upon changes in activity (43), but to date there have been no attempts to abnormally reduce activity for days in other young brain regions where PSD95 has recently appeared.

**Functional Effects of EO and PSD95 on SC LTP and Synapse Stabilization.** In acute SC slices, robust LTP requires NMDAR and L-type Ca²⁺ channel activity (32) as well as GluN2A subunits at the PSD (20, 38), and, as shown here (Fig. 1), SC LTP in the superficial visual layers only occurs after EO. This potentiation results almost entirely from unsilencing of silent synapses (32). Fig. 5A and B demonstrates that PSD95 KD eliminates SC LTP; therefore, both high levels of synaptic PSD95 and its unique ability to bind NMDARs having two GluN2A subunits at the center of SC synapses are necessary for NMDAR-dependent LTP in the developing SC. Furthermore, the finding that mixed trihemispheric NMDARs reappear at the PSD of PSD95 KD cells supports our previous proposal that the insertion of PSD95 actively displaces SAP102 bound receptors from the center to the extrasynaptic region of synapse (13). The current evidence for a crucial function of PSD95 in SC synaptic increases is fully consistent with the study by Phillips et al. (7) where another type of SC neurons, dorsally oriented vertical neurons, in the PSD95 KO mouse (14) fail to show the significant increase in synapse number found in WT SC neurons upon EO and the onset of pattern vision. These new synapses are from the cortico-collicular projection, which develops later than retinal inputs, and, without EO, this set of converging inputs is not only functionally but also structurally withdrawn (7). The data of Phillips et al. therefore reinforce the present results by showing that new SC synapses resulting from activity increases cannot be stabilized unless PSD95 is present. This report shows that SC LTP disappears with several days of ERC after EO, and reappears with ERO. This is completely consistent with the data of Yoshi et al. (9) showing corresponding decreases and increases of PSD95 levels in visual synapses using the same EO, EC paradigms used.

![Fig. 4. PSD95 KD reduces mNMDARc frequency, but not PPR or evoked AMPARc/NMDARc ratio. (A and C) Sample traces recorded at −70 mV of mNMDARcs from shRNA Scr I and KD I-expressing neurons and from shRNA Scr II and KD II-expressing neurons. (Scale bars: 10 pA, 200 ms.) (B and D) Cumulative distributions of mNMDARc interevent intervals (IEI) from the all sets of neurons. (Insets) The bar graphs showing significant differences in mNMDARc frequency [(B) Scr I: 0.17 ± 0.03 Hz, n = 9/3, vs. KD I: 0.1 ± 0.009 Hz, n = 9/3, P = 0.01; (D) Scr II: 0.23 ± 0.04 Hz, n = 9/3, vs. KD II: 0.12 ± 0.009 Hz, n = 8/3, P = 0.02]. (E and F) (Upper traces) Samples of average paired-pulse evoked AMPARc from Scr I and KD I neurons, and from Scr II and KD II neurons. (Scale bars: 20 pA, 50 ms.) (Lower) Bar graphs showing no significant differences in PPR (E) Scr I: 1.08 ± 0.13, n = 14/3, vs. KD I: 1.3 ± 0.18, n = 11/3, P = 0.35; (F) Scr II: 1.13 ± 0.26, n = 7/2, vs. KD II: 0.93 ± 0.13, n = 11/3, P = 0.42). (G and H) (Upper traces) Samples of averaged eAMPARc and eNMDARc from Scr I and KD I neurons and from Scr II and KD II neurons. (Scale bars: 20 pA, 100 ms.) (Lower) Bar graphs showing no significant differences in eAMPARc/eNMDARc ratio [(G) Scr I: 1.91 ± 0.18, n = 11/4, vs. KD I: 2.29 ± 0.13, n = 9/3, P = 0.21; (H) Scr II: 1.83 ± 0.18, n = 10/3, vs. KD II: 1.73 ± 0.12, n = 12/4, P = 0.56].

![Fig. 5. In vivo PSD95 KD affects LTP, but not LTD. (A and B) LTP was normal in shRNA Scr I and Scr II-expressing neurons, but absent in shRNA KD I and KD II-expressing neurons [(A) Scr I: 1.21 ± 0.04, n = 6/2, P < 0.01; KD I: 0.99 ± 0.04, n = 7/2, P = 0.73; (B) Scr II: 1.24 ± 0.06, n = 7/2, P < 0.01; KD II: 0.99 ± 0.04, n = 7/2, P = 0.94). (C and D) LTD was induced in both Scr I and KD I and Scr II and KD II neurons [(C) Scr I: 0.78 ± 0.07, n = 7/3, P < 0.03; KD I: 0.74 ± 0.03, n = 11/3, P < 0.01; Scr I vs. KD I, P = 0.59; (D) Scr II: 0.76 ± 0.04, n = 11/4, P < 0.01; KD II: 0.81 ± 0.05, n = 13/5, P < 0.01; Scr II vs. KD II, P = 0.43). The black bars indicate application of LTD induction stimulation.](image-url)
before application of the CaN inhibitor FK506. (Scale bars: 10 pA, 200 ms.) (B, F) Left) Superposition of average scaled sNMDARcs obtained before and 12–15 min after FK506 application from the same PSD95 KD I and KD II neurons. (Right) Pooled data showing no significant differences in the decay times of average sNMDARcs before and after FK506 application in Scr I or Scr II neurons (8/3 Scr I, n = 7/2, P = 0.06, 8/2 Scr II, n = 6/2, P = 0.64). (D and H) Left) Superposition of average scaled sNMDARcs obtained from the same PSD95 KD I and KD II neurons before and 12–15 min after FK506 application. (Right) Pooled data showing significant lengthening of sNMDARc decay times 12–15 min after FK506 application (KD I, n = 8/2, P < 0.01; KD II, n = 8/3, P < 0.01). Paired t tests were used, the open diamonds represent individual experiments, and the filled diamonds are means of all of the experiments in groups (B, D, F, and H).

CaN-Mediated Decrease of sNMDARc Decay Time. A final finding in this study is that the CaN-mediated decrease in sNMDARc decay times (26) observed in normal SC neurons can be retained after EO in SC PSD95 KD neurons. This CaN effect involves a dephosphorylation of at least one protein kinase A (PKA) site, serine 900, on the GluN2A cytoplasmic tail (27). Similar CaN activity was described by Lieberman and Moody (46) as a CaN-dependent change in NMDAR channel open time, in analyses of single channel currents. Krupp et al. (47) found that the same decrease in NMDARc decay time resulted from CaN dephosphorylation of two PKA sites on the GluN2A cytoplasmic tail. The present data show that this NMDARc decay-time shortening can reappear when PSD95 KD causes the GluN2B subunit composition of NMDARs at PSDs to be unusually high and when input activity is increased due to pattern vision. The finding is potentially significant for understanding differences between the subunit makeup of NMDARs throughout the brain and the activity-dependent control of the receptor’s currents. For example, Flint et al. (48) documented short NMDARc decay times characteristic of GluN1/GluN2A diheteromeric NMDARs in young somatostatin neurons when measured levels of GluN2A were still extremely low. They concluded that just one GluN2A subunit in a triheteromeric receptor with GluN2B was sufficient to shorten the decay times of the NMDARcs. It is likely that the CaN-mediated dephosphorylation of GluN2A is responsible for the short NMDARc decay times reported by Flint et al. (48). The same CaN mechanism may also explain why recent biochemical analyses of synaptic NMDAR composition report a prevalence of GluN1/GluN2A/GluN2B triheteromers at mature glutamate synapses even though sNMDARc decay times are short in the mature brain (49, 50). Finally, the reappearance of this CaN effect in older SC neurons when levels of triheteromeric NMDARs are abnormally present and when converging glutamatergic activity is high suggests that it may represent yet one more mechanism for homeostatically regulating cytoplasmic Ca2+ concentrations in the brain (51).

Conclusion
We document a requirement for PSD95 for the maintenance of both mAMPARc and mNMDARc frequency, normal synaptic input, and for the appearance of NMDAR-dependent LTD in the SC where only SAP102 and PSD95, but not PSD94, MAGUKs are normally prominent. Unlike normal SC neurons where diheteromeric GluN1/GluN2A NMDARs are at the PSD, in PSD95 KD SC neurons triheteromeric NMDARs bound by SAP102 are at the PSD. Nevertheless, in the P15–P17 rats studied, the SAP102 MAGUK cannot replace the PSD95 function of binding GluN2A diheteromeric receptors or facilitating NMDAR-dependent LTD. Most significantly, this study suggests that much of the intensive and sophisticated work on MAGUK function in hippocampal CA1, may not generalize to synaptogenesis and developmental plasticity in many other regions of the vertebrate central nervous system.

Methods

Animals. Sprague Dawley rat pups were treated with synchronized EC, EO, ERC, and ERO procedures as described previously (9). All experiments were performed using Sprague Dawley rats. Pups were housed in a controlled environment with 12-h light/dark cycles and ad libitum access to food and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of the National Institute of Mental Health. 

Here, it remains to be seen whether this highly labile form of synapse potentiation and stabilization is also present in older brains and other visual centers after prolonged pattern vision deprivation.

LTD Survives PSD95 KD in SC. This finding also differs from those in the hippocampus where the KD or KO of PSD95 eliminates NMDAR-dependent LTD. In the hippocampus where PSD95 remains to compensate for PSD95 KO or KD (14, 15, 30, 31), LTD but not LTP disappears. Carlisle et al. (15) suggest that PSD95 may bind the signaling complex necessary for LTD while PSD95 is involved in LTD and normally mitigates the potentiating effects of LTD resulting in the enhanced LTD seen in the hippocampus of genetic PSD95 KOs (14, 28). However, the lack of PSD93 in the young SC is not consistent either with the loss of LTD or the maintenance of LTD in this structure when PSD95 is depleted. Xu et al. (31) also provided evidence for PSD95 involvement in LTD. With a series of deletion constructs and point mutations in the PSD95 C terminus following WT PSD95 depletion by shRNA, they showed that the C-terminal domain of PSD95 normally scaffolds the signaling complex necessary for CA1 LTD. This explanation is also not consistent with our finding that NMDAR-dependent LTD is maintained in SC neurons after PSD95 KD. However, CaN activity is believed to be critical to NMDAR-dependent LTD at least in the hippocampus (44, 45), and, as noted above, both GluN2B and L-type Ca2+ channels are necessary for SC LTD. In addition, this study demonstrates CaN involvement in decreasing sNMDARc decay time when SAP102 is the only remaining major MAGUK in SC neurons. These findings suggest that the early appearing MAGUK SAP102 that is still highly expressed in the young SC after EO can also scaffold the complex for inducing LTD.

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performed in accord with the guidelines of the Massachusetts Institute of Technology Institutional Animal Care and Use Committee.

Construction of Lentiviral Vectors. Two shRNAs (shRNA KD I and II) against mRNAs from mouse and also two similar but shRNAs (shRNA Sc I and II) as negative controls were designed. These were inserted into the lentiviral plasmid carrying GFP, and lentiviruses were produced in HEK cells (see SI Methods for shRNA sequences and further details).

Expression Analysis of MAGUKs in Developing SC and Hippocampus. Homogenates from rat SC and hippocampus at P15 and P19, respectively, were analyzed by Western blotting for evaluation of PSD95, SAP102, and PSD93 protein levels (SI Methods).

Injection of Lentivirus into the SC. P1–P3 rat pups were cold-anesthetized, and 0.1 µL was injected into the SC (SI Methods).

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