Synapse maturation by activity-dependent ectodomain shedding of SIRPα

Anna B Toth1,4, Akiko Terauchi1,4, Lily Y Zhang1,4, Erin M Johnson-Venkatesh1,4, David J Larsen1, Michael A Sutton1,2 & Hisashi Umemori1,3

Formation of appropriate synaptic connections is critical for proper functioning of the brain. After initial synaptic differentiation, active synapses are stabilized by neural activity-dependent signals to establish functional synaptic connections. However, the molecular mechanisms underlying activity-dependent synapse maturation remain to be elucidated. Here we show that activity-dependent ectodomain shedding of signal regulatory protein-α (SIRPα) mediates presynaptic maturation. Two target-derived molecules, fibroblast growth factor 22 and SIRPα, sequentially organize the glutamatergic presynaptic terminals during the initial synaptic differentiation and synapse maturation stages, respectively, in the mouse hippocampus. SIRPα drives presynaptic maturation in an activity-dependent fashion. Remarkably, neural activity cleaves the extracellular domain of SIRPα, and the shed ectodomain in turn promotes the maturation of the presynaptic terminal. This process involves calcium/calmodulin-dependent protein kinase, matrix metalloproteinases and the presynaptic receptor CD47. Finally, SIRPα-dependent synapse maturation has an impact on synaptic function and plasticity. Thus, ectodomain shedding of SIRPα is an activity-dependent trans-synaptic mechanism for the maturation of functional synapses.

Synapses are the sites of information processing between neurons in the brain. Defects in synaptic circuitry in the hippocampus, a structure critical for long-term memory formation, emotional processing and social behavior, are associated with a variety of neurological and psychiatric disorders, including fragile X syndrome, autism, epilepsy and schizophrenia1–3. Thus, proper assembly of hippocampal synapses is essential for optimal functioning of the brain. To organize synapse formation, signals are exchanged between pre- and postsynaptic neurons. Two forms of signal are required for functional synapse formation during development: activity-independent and activity-dependent signals. Usually, initial synaptic differentiation is regarded as consisting of activity-independent steps, whereas a period of activity-dependent signaling, signals are exchanged between pre- and postsynaptic neurons. This period of activity-dependent synapse maturation shapes the ultimate structure of neural circuits4–7. During synapse maturation, activity-dependent signals either stabilize or eliminate axons and further maturate selected synapses to establish appropriate synaptic connections8–12. Thus, activity-dependent mechanisms are required for the structural refinement of neural circuits, to match pre- and postsynaptic function, and for the final arrangement of the appropriate synaptic map11–14. While synapse stabilization and destabilization and synapse maturation are clearly activity-dependent, little is known about molecular mechanisms underlying them. Defects in activity-dependent synapse maturation in the hippocampus have been implicated in various neurodevelopmental disorders, including schizophrenia and autism1–3. Therefore, an understanding of the molecules and manner by which hippocampal circuits are established by neural activity should yield new insights into both the etiology and treatment of these devastating disorders.

To understand the molecular mechanisms of synapse formation, we have performed an unbiased search for molecules that promote differentiation of axons into presynaptic nerve terminals. Using the ability to cluster synaptic vesicles in cultured motor neurons as a bioassay, we have purified from developing brains molecules that can promote differentiation of axons into presynaptic nerve terminals and identified two molecules, fibroblast growth factor 22 (FGF22)15 and SIRPα16, as such presynaptic organizers. We have shown that FGF22 and its close relative FGF7 are selectively involved in the initial organization of excitatory (glutamatergic) and inhibitory (GABAergic) synapses, respectively, in the hippocampus17. The other molecule, SIRPα, is a transmembrane immunoglobulin superfamily member that is involved in various hematopoietic cell functions18–20, but little is known about its roles in the brain. We therefore investigated the role, mechanism and impact of SIRPα-dependent synapse formation in the brain.

Here we show that (i) target-derived FGF22 and SIRPα sequentially organize presynaptic terminals; (ii) SIRPα is necessary for presynaptic maturation, but not for induction or maintenance, in the hippocampus in vivo; (iii) SIRPα drives presynaptic maturation in an activity-dependent manner; (iv) activity cleaves the ectodomain of SIRPα, and this cleavage is required for SIRPα’s presynaptic effects; (v) calcium, calcium/calmodulin-dependent protein kinase (CaMK) and matrix metalloproteinase (MMP) mediate SIRPα cleavage; (vi) CD47 is SIRPα’s presynaptic receptor; and (vii) SIRPα has an impact on synaptic function and plasticity. These results indicate that ectodomain shedding of SIRPα is an activity-dependent mechanism...
allowing pre- and postsynaptic terminals to communicate for the maturation of functional synapses.

RESULTS
Distinct expression of FGF and SIRPα during synaptogenesis
We first compared the expression patterns of SIRPα and FGFs in the hippocampus during synapse formation. In situ hybridization experiments with mouse brain sections showed little Sirpa mRNA expression in hippocampal neurons at postnatal day 8 (P8; Fig. 1a), an early stage of synapse formation, but substantially higher expression at P21, a late stage of synapse formation. Western blotting confirmed a robust increase in the amount of SIRPα protein from P8 to P21 (Fig. 1b). This expression pattern is in contrast to the patterns of Fgf22 and Fgf7 mRNA, which were found to be highly expressed at P8 (ref. 17) but not at P21 (Supplementary Fig. 1). These results suggest that FGFs and SIRPα are involved in the early and late stages of synapse formation, respectively, in the hippocampus.

We next examined the localization of SIRPα in hippocampal neurons. Biochemical fractionation experiments revealed that SIRPα was abundant in the synaptic membrane fraction, indicating that SIRPα is a synaptic molecule. Notably, it was most enriched in the extrajunctional fraction (Supplementary Fig. 2), which is similar to the distribution of some other synaptogenic molecules, including EphB2 (ref. 23). Immunostaining of cultured hippocampal neurons showed that SIRPα was preferentially localized at MAP2-positive dendrites relative to neurofilament-positive axons (Fig. 1c). In dendrites, SIRPα was concentrated at excitatory synapses: it was colocalized (~75%) with vesicular GABA transporter (VGAT), a marker for GABAergic presynaptic terminals, but showed little colocalization (~13%) with vesicular GABA transporter (VGAT), a marker for glutamatergic presynaptic terminals (Fig. 1d). These results suggest that SIRPα is localized in dendrites at glutamatergic synapses (that is, it is postsynaptic) and may serve as a target-derived glutamatergic presynaptic organizer in the hippocampus.

Figure 1 FGF22 and SIRPα promote the early or late stage of glutamatergic presynaptic differentiation, respectively. (a) In situ hybridization for Sirpa in the hippocampus (positive signals in black). Reproduced three times. (b) Western blotting for SIRPα protein (α-tubulin as control) in the hippocampus. Full-length blots are presented in Supplementary Figure 1. (c) SIRPα protein was abundant on MAP2+ dendrites but not on neurofilament+ (NF+) axons. (d) SIRPα was concentrated at VGLUT1+ glutamatergic synapses but not at VGAT+ GABAergic synapses. Reproduced five times. (e) HEK cells expressing SIRPα, neulroglin1 (NLGN1) or control HEK cells (labeled with GFP) were cocultured with hippocampal neurons for 2 d and stained for synapsin. The synapsin puncta formed on HEK cells expressing SIRPα were more intense and larger than those formed on control HEK cells and were comparable to the puncta of HEK cells expressing NLGN1. (f,g) SIRPα was applied to hippocampal cultures from DIV1–11. (f) SIRPα treatment significantly increased the number and size of VGLUT1 puncta as compared to PBS control (n = 57 fields from 5 cultures). (g) Representative traces and summary data of whole-cell recordings of mEPSCs from control and SIRPα-treated hippocampal neurons. n = 57 and 63 cells from 5 cultures. (h) Schematic timeline of the experiment shown in i,j. Cultured hippocampal cells were treated with FGF22 or SIRPα from DIV1–4 (beginning of synaptogenesis), DIV4–8, or DIV8–11 (ending of synaptogenesis). All cultures were fixed on DIV11. (i) Staining of hippocampal cultures for VGLUT1. (j) Numbers and sizes of VGLUT1 puncta. Data are shown as percentage of PBS control. n = 32, 43, 40, 34, 27 and 26 fields from 5 cultures. (k) SIRPα or FGF22 was applied to hippocampal cultures prepared from WT or Fgf22−/− mice, and the cultures were stained at DIV11. The number and size of VGLUT1 puncta on Py+ (CA3 pyramidal neuron) dendrites were quantified. n = 19, 23, 25, 25, 17 and 23 neurites from 3 cultures. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.0001 by Student’s t-test (f,g) or by ANOVA followed by Tukey test (e,j,k). Scale bars, 500 μm (a), 10 μm (e,k) and 5 μm (others).
SIRPα promotes glutamatergic presynaptic differentiation

To address whether SIRPα can promote presynaptic differentiation of hippocampal neurons, we examined the effect that SIRPα has on synaptic vesicle clustering using a coculture system\(^4\), in which neurons are cocultured with human embryonic kidney (HEK) cells. The number and size of synapsin puncta formed on HEK cells expressing SIRPα were significantly larger than those on control HEK cells (Fig. 1c). SIRPα’s presynaptic effects were comparable to those of neuroligin I, a well-characterized synaptogenic molecule, indicating that SIRPα is a synaptogenic molecule that can promote synaptic vesicle clustering in hippocampal neurons.

We then examined whether SIRPα can organize glutamatergic presynaptic differentiation. For this, we prepared the extracellular portion of SIRPα\(^{16}\) (soluble SIRPα; sSIRPα) and bath-applied it at 2 nM to cultured hippocampal neurons for 10 d. sSIRPα significantly increased the number and size of VGLUT1 puncta (Fig. 1f). Furthermore, SIRPα increased the number and size of baso- soon puncta, suggesting that SIRPα organizes active zones as well (Supplementary Fig. 3a). Electrophysiological recordings indicated that sSIRPα increased the frequency, but not the amplitude, of miniature excitatory postsynaptic currents (mEPSCs; Fig. 1g), consistent with an increase in synaptic contacts. sSIRPα did not noticeably affect dendrite and axon differentiation or the clustering of PSD95, a postsynaptic scaffolding protein at glutamatergic synapses (Supplementary Fig. 3b,c), but it did increase the colocalization between VGLUT1 and PSD95 (Supplementary Fig. 3c). These results indicate that SIRPα can specifically promote presynaptic differentiation of glutamatergic synapses in hippocampal neurons.

FGF22 and SIRPα promote distinct stages of synaptogenesis

On the basis of the developmentally different expression of Fgf22 and Sirpa mRNAs in the hippocampus, we hypothesized that FGF22 and SIRPα are preferentially involved in the early and late stages of glutamatergic synapse formation, respectively. To test this idea, we performed time course experiments using cultured hippocampal neurons. In our hippocampal cultures, glutamatergic synapse formation starts around day in vitro (DIV) 3 and slow around DIV12 (ref. 17). To determine the time during which FGF22 and SIRPα are most effective at promoting presynaptic differentiation, we cultured hippocampal neurons and applied recombinant FGF22 or sSIRPα during three different periods: DIV1–DIV4, DIV4–DIV8 or DIV8–DIV11, which correspond to the beginning, middle and ending of synapse formation, respectively (Fig. 1b). We then stained all cultures for VGLUT1 at DIV11. FGF22 treatment was most effective at increasing the number and size of VGLUT1 puncta when applied from DIV1–DIV4 (Fig. 1j) and Supplementary Fig. 4), consistent with an early role in synapse development. In contrast, sSIRPα treatment increased the number and size of VGLUT1 puncta most prominently when it was applied from DIV8–DIV11. These results suggest that FGF22 and SIRPα are presynaptic organizing molecules with temporally distinct roles during synapse formation, with FGF22 involved in early and SIRPα in late stages of synapse formation.

To further show that FGF22 and SIRPα have distinct roles in presynaptic differentiation, we cultured FGF22-deficient neurons\(^{17}\) and examined whether SIRPα could rescue their synaptic defects. There were fewer and smaller VGLUT1 puncta on CA3 neurons in FGF22-deficient cultures relative to wild-type cultures. These presynaptic defects were rescued by the application of FGF22, but not by sSIRPα, to cultures (Fig. 1k). These results suggest that, although both FGF22 and SIRPα can induce presynaptic differentiation, their specific roles in presynaptic differentiation are different.

SIRPα is required for presynaptic maturation in vivo

In Fgfg22\(^{−/−}\) mice, the differentiation of glutamatergic nerve terminals in the hippocampus is impaired early in synapse development, at P8 (ref. 17). To identify the developmental stages during which SIRPα is critical for synapse formation in vivo, we generated a conditional SIRPα knockout mouse (Supplementary Fig. 5a). To temporally control the expression of SIRPα, we mated mice with loxP-flanked Sirpa alleles to actin-Cre-ER (CAG-cre/Esr1\(^{+}\)) mice\(^{25}\) and injected their offspring with tamoxifen at different postnatal days to induce Cre-mediated excision of the Sirpa gene. Tamoxifen injections inactivated SIRPα effectively in the hippocampus of these mice as confirmed by immunostaining for SIRPα (Supplementary Fig. 5b).

In the rodent hippocampus, synapse formation starts in the first postnatal week\(^{21,22}\). After their initial formation, synapses are then refined in an activity-dependent manner: effective synapses are stabilized and mature, while inactive contacts are destabilized and eliminated\(^{4,10}\). We have previously shown that activity-dependent synapse refinement in the hippocampus occurs between P15 and P25 (ref. 26). Thus, we chose three time periods corresponding to three different stages of synapse development to inactivate SIRPα: P0–P14 (initial synapse differentiation), P15–P29 (synapse maturation) and P30–P44 (synapse maintenance). When we injected SIRPα conditional knockout mice with tamoxifen to generate Sirpa\(^{−/−}\) mice at P0 and stained their hippocampi for VGLUT1 at P14, the intensity of VGLUT1 staining was not significantly different (P = 0.514, stratum lucidum; 0.828, stratum radiatum) in the Sirpa\(^{−/−}\) mice as compared to the wild-type littermate control (Fig. 2a), indicating that, unlike FGF22 (ref. 17), SIRPα is not critical for initial synapse development. In contrast, when we injected tamoxifen at P15 and analyzed at P29, the intensity of VGLUT1 staining was significantly reduced in the knockout hippocampus as compared to controls (Fig. 2b). Further analyses revealed that the size and intensity of each VGLUT1 punc- tum were decreased in the Sirpa\(^{−/−}\) mice. In addition to VGLUT1, the intensity of bassoon staining, a marker for active zones, was also decreased in the Sirpa\(^{−/−}\) mice relative to that in control mice (Fig. 2c). These results suggest that SIRPα inactivation affects the maturation of presynaptic terminals. Finally, when we injected tamoxifen at P30 and analyzed at P44, the intensity of VGLUT1 staining was not significantly different in the knockout mice as compared to control (P = 0.556, stratum lucidum; 0.469, stratum radiatum; Fig. 2d). These results demonstrate that SIRPα is critical for presynaptic maturation (P15–P29) but is not necessary for initial synapse development or synapse maintenance in vivo.

We also performed a series of experiments to examine whether the inactivation of SIRPα primarily affects presynaptic maturation. In the Sirpa\(^{−/−}\) mice with tamoxifen injected at P15, the hippocampus looked anatomically normal, and the fate of the cells in the hippocampus appeared to be unchanged (Supplementary Fig. 6a–d). In addition, the clustering of PSD95 was not significantly decreased in Sirpa\(^{−/−}\) as compared to control mice (P = 0.095, stratum lucidum; 0.134, stratum radiatum; Supplementary Fig. 6e). Thus, SIRPα appears to be primarily involved in presynaptic maturation in the hippocampus in vivo. Consistent with the Sirpa expression pattern (Fig. 1a and data not shown), presynaptic maturation in Sirpa\(^{−/−}\) mice was impaired throughout the hippocampus (Supplementary Fig. 6f), as well as in the cerebellum. The presynaptic defects in Sirpa\(^{−/−}\) mice were still present at P130 (Supplementary Fig. 6g), suggesting that SIRPα inac- tivation prevents presynaptic maturation rather than just delaying it.

To further confirm the role of SIRPα in presynaptic maturation, we examined the ultrastructure of excitatory (asymmetric) synapses in the hippocampus in P29 Sirpa\(^{−/−}\) mice injected with.
tamoxifen at P15 (Fig. 2e). We found significantly fewer synaptic vesicles and fewer docked synaptic vesicles in the asymmetric synapse in Sirpa−/− mice relative to the number in controls. In addition, the shape of synaptic vesicles in Sirpa−/− mice looked irregular compared to those in controls.

**Diminished transmitter release probability in Sirpa−/− mice**

To directly address the functional state of synapses in Sirpa−/− mice, we recorded evoked field excitatory postsynaptic potentials (fEPSPs) at CA3–CA1 synapses in acute hippocampal slices (tamoxifen injections at P15, analyses at ~P29). Input-output curves of fEPSP slope were strongly diminished in Sirpa−/− mice relative to those in control littersmates (Fig. 2f), whereas fiber volley amplitude (reflecting the number of axons firing to each stimulation) was unaffected, indicating that synaptic transmission is impaired in the absence of Sirpα.

Moreover, paired-pulse facilitation was dramatically increased in Sirpa−/− mice relative to controls (Fig. 2g), suggesting that neurotransmitter release probability is diminished in the knockout mice. Sirpa−/− neurons therefore have substantial defects in excitatory presynaptic function. Taken together, the histological and electrophysiological results from Sirpa−/− mice demonstrate that Sirpα is necessary for the maturation, but not induction or maintenance, of excitatory presynaptic terminals in the hippocampus in vivo.

**Presynaptic maturation by Sirpα requires neural activity**

During the maturation stage of synapse formation, activity-dependent signals either stabilize or destabilize the synapses to establish efficient synaptic connections. Therefore, we hypothesized that Sirpα contributes to mechanisms that stabilize and promote maturation of presynaptic terminals in response to neural activity.

To test this idea, we examined whether the presynaptic effects of Sirpα require neural activity. When Sirpα was transfected into cultured hippocampal neurons, the VGLUT1 puncta were significantly larger on the dendrites of Sirpα−/− mice and control littersmates (tamoxifen injections at P15, analyses at P29). Sample traces of fEPSP recordings are shown. In input-output curves (right), fEPSP slope, but not fiber volley amplitude, was significantly lower in Sirpα−/− mice than in control littersmates (P < 0.001 by two-way ANOVA; n = 9 and 13 cells from 4 mice). (g) Paired-pulse facilitation across a range of inter-stimulus intervals for evoked EPSCs from Sirpα−/− mice and control littersmates. Paired-pulse facilitation was significantly enhanced in Sirpα−/− mice (P < 0.001 by two-way ANOVA; n = 11 and 15 cells from 4 mice). Data are mean ± s.e.m.
SIRPα protein we identified from the brain extract was the extracellular portion of SIRPα. In fact, from cultured neurons, we were able to collect secreted SIRPα in the media, and its molecular weight was smaller than full-length SIRPα expressed in neurons (Fig. 3c). In addition, we detected a short fragment of SIRPα containing its C-terminal domain (~16 kDa, which corresponds to the intracellular domain) in the synaptic membrane fraction (Supplementary Fig. 2). Therefore, we hypothesized that the extracellular domain of SIRPα is cleaved by neural activity and that this cleavage is required for its presynaptic effects (see Supplementary Fig. 7a).

To examine whether the extracellular domain of SIRPα is cleaved and released from hippocampal neurons in response to neural activity, we cultured hippocampal cells with either KCl (50 mM) to depolarize neurons, bicuculline (50 μM) to enhance endogenous network activity or TTX (1 μM) to suppress network activity. We then collected media and assessed the amount of cleaved and released SIRPα by immunoprecipitation followed by western blot. KCl and bicuculline treatments significantly increased the amount of released SIRPα in media as compared to that in untreated control (Fig. 3d–f), while TTX treatment significantly decreased the amount of cleaved SIRPα in the media, indicating that the SIRPα ectodomain is released by neural activation. These effects were not due to altered cell numbers, as the amount of α-tubulin in the cell lysate was not altered by any treatment condition. In addition, the amount of full-length SIRPα remaining on the cell was decreased in KCl-treated cultures (medium) and increased in TTX-treated cultures (medium), consistent with an increase or a decrease in SIRPα cleavage by KCl or TTX treatment, respectively.
Shedding of SIRPα is necessary for presynaptic maturation

We then investigated whether the cleavage of the extracellular domain of SIRPα is required for presynaptic maturation mediated by SIRPα. For this, we prepared a mutant form of SIRPα (MT-SIRPα) that is resistant to ectodomain shedding (Fig. 3h). In the HEK cell–hippocampal neuron coculture system (see Fig. 1e), the number and size of synapsin puncta formed on HEK cells expressing MT-SIRPα were similar to those on control HEK cells (Fig. 3i), indicating that MT-SIRPα cannot promote synaptic vesicle clustering in hippocampal neurons.

We next transfected cultured hippocampal neurons with wild-type SIRPα (WT-SIRPα) or MT-SIRPα. The localization of MT-SIRPα was similar to that of WT-SIRPα (Supplementary Fig. 7b,c). Overexpression of WT-SIRPα led to an increase in the size of VGLUT1 puncta on the transfected neurons; however, overexpression of MT-SIRPα failed to do so (Fig. 3j). These results indicate that shedding-resistant SIRPα cannot promote presynaptic maturation both in coculture and neuronal culture, suggesting that the cleavage and secretion of the SIRPα ectodomain are necessary for its presynaptic effects.

Neural activity is responsible for SIRPα cleavage

If neural activity is responsible for cleaving SIRPα, suppressing neural activity should inhibit the presynaptic effect of full-length SIRPα (Fig. 3a,b) but not that of soluble SIRPα (sSIRPα). To test this idea, we cultured hippocampal neurons with sSIRPα with or without TTX. Application of sSIRPα increased the size of VGLUT1 puncta, and, unlike what was observed with full-length SIRPα, this effect was
completely resistant to TTX (Fig. 3k). Thus, after cleavage, presynaptic maturation by SIRP\(\alpha\) no longer depends on neural activity.

To exclude the possibility that SIRP\(\alpha\) is subjected to constitutive cleavage in neurons followed by activity-dependent secretion of its cleaved product, we performed an experiment with a secretable form of SIRP\(\alpha\) that contains only the extracellular domain of SIRP\(\alpha\) (Ext-SIRP\(\alpha\); the construct used to prepare sSIRP\(\alpha\)). When transfected into cultured neurons, Ext-SIRP\(\alpha\) efficiently induced maturation of glutamatergic presynaptic terminals on the Ext-SIRP\(\alpha\)-expressing neurons (Fig. 3i). This presynaptic effect was not inhibited by TTX application, indicating that neural activity is not important for the secretion of Ext-SIRP\(\alpha\). These results are consistent with the notion that neural activity is responsible for cleaving, and not secretion, of the extracellular domain of SIRP\(\alpha\).

**CaMK and MMP mediate activity-dependent SIRP\(\alpha\) cleavage**

We further investigated the signaling pathway that is involved in activity-dependent SIRP\(\alpha\) cleavage. CaMK is a major signaling molecule at synapses\(^{27}\), prompting us to explore the possibility that CaMK contributes to SIRP\(\alpha\) cleavage. Consistent with this hypothesis, treatment of hippocampal cultures with CaMK inhibitors, KN62 or KN93 (5 \(\mu\)M), significantly decreased the amount of cleaved SIRP\(\alpha\) in the media (Fig. 4a). We next examined the effects of a CaMK inhibitor (KN62) and a calcium channel blocker (nifedipine; 10 \(\mu\)M) on activity-dependent cleavage of SIRP\(\alpha\). Both inhibitors suppressed KCl-induced SIRP\(\alpha\) cleavage (Fig. 4b), suggesting that neural activity-dependent calcium entry followed by CaMK activation are important for the ectodomain shedding of SIRP\(\alpha\).

We then characterized the proteases that cleave the extracellular domain of SIRP\(\alpha\). MMPs are zinc-dependent endopeptidases that cleave extracellular molecules and are implicated in synaptic function\(^{28}\). We found that incubation of hippocampal neurons with MMP inhibitors, GM6001 (10 \(\mu\)M) or tissue inhibitors of metalloproteinases (TIMPs) (0.5 \(\mu\)g \(\text{ml}^{-1}\)), markedly inhibited SIRP\(\alpha\) shedding, including the augmented cleavage induced by KCl (Fig. 4c,d). These results suggest that calcium, CaMK and MMP are involved in the activity-dependent shedding of SIRP\(\alpha\) from hippocampal neurons, although it is known that any inhibitors have off-target effects, and we cannot completely rule out an influence of such effects at this time.

**CD47 is the presynaptic receptor for SIRP\(\alpha\)**

Because our data indicated that the shed SIRP\(\alpha\) ectodomain promotes presynaptic maturation, we next examined the identity of its presynaptic receptor. We asked whether CD47, a receptor for SIRP\(\alpha\) in hematopoietic cells\(^{18-20}\), mediates the presynaptic effects of SIRP\(\alpha\). Immunostaining experiments showed that CD47 puncta were abundant in neurofilament-positive axons and not in MAP2-positive dendrites (Fig. 5a) and that CD47 colocalized with SIRP\(\alpha\) (Fig. 5b), consistent with the idea that CD47 serves as a presynaptic receptor for SIRP\(\alpha\). We then used \(\text{Cd47}^{-/-}\) neurons\(^{29}\) to determine whether CD47 mediates SIRP\(\alpha\)’s effects. \(\text{Cd47}^{-/-}\) neurons extended axons and dendrites normally (Supplementary Fig. 8) but did not increase the number and size of VGluT1 puncta in response to sSIRP\(\alpha\) application (Fig. 5c). The following two experiments suggest that CD47 acts as a presynaptic receptor for SIRP\(\alpha\): (i) HEK cells expressing SIRP\(\alpha\), which can induce presynaptic differentiation in cocultured WT hippocampal neurons, failed to do so in cocultured \(\text{Cd47}^{+/+}\) neurons, while neuroligin1 was able to induce presynaptic differentiation in both WT and \(\text{Cd47}^{-/-}\) neurons (Fig. 5d), and (ii) \(\text{Cd47}^{-/-}\) neurons did not respond to sSIRP\(\alpha\) application to induce presynaptic differentiation as assessed by synaptophysin-YFP clustering, but the responsiveness was restored by presynaptic expression of CD47 (Fig. 5e).

Finally, we confirmed that the source of SIRP\(\alpha\) is postsynaptic: we found that presynaptic defects in \(\text{Sirpa}^{-/-}\) neurons were rescued by postsynaptic expression of SIRP\(\alpha\) (Fig. 5f). Altogether, these results strongly suggest that postsynaptically derived SIRP\(\alpha\) interacts with presynaptic CD47 to organize presynaptic maturation.

**LTP is impaired in \(\text{Sirpa}^{-/-}\) mice**

What are the functional consequences of defects in SIRP\(\alpha\)-dependent presynaptic maturation? To explore this question, we examined the impact of SIRP\(\alpha\)-deficiency on activity-dependent synaptic plasticity in the hippocampus. Long-term potentiation (LTP) was impaired at CA3–CA1 synapses in the hippocampus of \(\text{Sirpa}^{-/-}\) mice (Fig. 6); of relevance, \(\text{Cd47}^{-/-}\) mice also show impaired LTP\(^{30}\). This is consistent with the altered presynaptic function in \(\text{Sirpa}^{-/-}\) mice (Fig. 2 and Supplementary Fig. 9) and demonstrates that SIRP\(\alpha\)-dependent synapse maturation has an enduring impact on long-lasting forms of plasticity in hippocampal circuits.

**DISCUSSION**

Activity-dependent synapse maturation is a critical step for the refinement of neural circuits and the establishment of an appropriate and efficient synaptic map in the brain. However, little is known about the molecular mechanisms that control this important aspect of synapse development. Here we have uncovered a new process by which neural activity contributes to synapse maturation. From our results, we propose that, after initial synaptic differentiation by molecules such as FGF22 (ref. 17), synaptic activity regulates extracellular domain cleavage of postsynaptic SIRP\(\alpha\) through CaMK and MMP, and the released SIRP\(\alpha\) ectodomain, in turn, promotes the maturation of the presynaptic terminal through CD47 (Supplementary Fig. 7a). SIRP\(\alpha\)-dependent synapse maturation affects synaptic function and plasticity, as demonstrated by impaired basal transmission, diminished neurotransmitter release probability and impaired LTP in \(\text{Sirpa}^{-/-}\) mice.
Many molecules have been implicated in presynaptic development, including neuroligins, SynCAMs, ephrins and Eph receptors, leucine-rich repeat transmembrane neuronal proteins (LRRTMs), netrin-G ligands (NGLs), FGFs, Wnts, neurotrophins, cerebellin and thrombospondins. Why are there multiple presynaptic organizers? We hypothesized that different presynaptic organizers exist for the organization of different types of synapses (spatial specificity) and for the regulation of different stages of synapse formation (temporal specificity). Concerning spatial specificity, we have previously shown that two FGFs, FGF22 and FGF7, are involved in the differentiation of two distinct types of synapses in vivo: FGF22 in excitatory and FGF7 in inhibitory neurons. Several presynaptic organizers, such as neuroligin1, SynCAMs, Eph receptors, LRRTMs and NGLs, seem to be relatively specific to excitatory synapses, whereas others, such as neuroligin2 and BDNF, may be preferentially involved in inhibitory synapses. Thus, distinct presynaptic organizers indeed appear to contribute to the organization of different synapses in the brain. As for temporal specificity, we have here shown that, in the hippocampus, FGF22 and SIRPα are important for two sequential stages of synapse formation, with FGF22 influencing initial synaptic differentiation and SIRPα influencing synapse maturation. Together, we propose that multiple spatially and temporally defined presynaptic organizers cooperate to organize specific and functional synaptic networks in the brain.

The role of SIRPα has been mainly studied in the immune system. Little is known about its function in the nervous system, but possible roles for SIRPα’s intracellular domain have been suggested: it promotes neurite outgrowth and enhances the effect of BDNF in culture, and mice expressing mutant SIRPα that lacks the intracellular domain show prolonged immobility in the forced swim test. We focused on the role of SIRPα’s extracellular domain: using hippocampal cultures and conditional SIRPα knockout mice, we found that the extracellular domain of SIRPα serves as a target-derived presynaptic organizer in the hippocampus and is critical in the maturation stage of synapse formation in vitro and in vivo. SIRPα’s extracellular domain is cleaved in response to neural activity, acting as an activity-dependent, target-derived presynaptic organizer. Why does SIRPα need to be cleaved for presynaptic maturation? Cleavage may be necessary for the extracellular domain of SIRPα to bind to its presynaptic receptor, CD47. Crystal structure models of SIRPα and CD47 suggest that the extracellular region of the SIRPα–CD47 complex is ~14 nm in length. However, the cleft of excitatory synapses is ~25 nm across, which may require the release of SIRPα ectodomain to bind to CD47. It will be also interesting to address the fate and roles of the SIRPα intracellular domain after cleavage.

Ectodomain shedding is important in various processes, including sperm-egg interaction, cell migration and adhesion, cell fate determination, wound healing, axon guidance and immune responses. Here we have identified a new role for ectodomain shedding: activity-dependent ectodomain shedding of SIRPα is involved in synapse maturation. Notably, while we were preparing our paper, two groups showed that activity-dependent cleavage of neuroligin1 is involved in synapse disassembly and negatively regulates synaptic function in a homeostatic manner. In contrast, our results demonstrate that activity-dependent cleavage of SIRPα is a critical positive regulator of synapse maturation during synapse development for the establishment of functional circuits. It is also noteworthy that the cleavages of both SIRPα and neuroligin1 involve common pathways, CaMK and MMP, yet they have opposite effects at synapses. Thus, our results, together with the neuroligin results, expand the role of activity-dependent shedding in controlling synapse maturation and function. How activity-dependent shedding of SIRPα and neuroligin1 cooperate or antagonize to regulate synapses will be an interesting question to address next. Finally, since defects in activity-dependent synapse maturation in the hippocampus have been implicated in various neurological and psychiatric disorders, such as schizophrenia and autism, our results may help design strategies to prevent and treat such disorders.

METHODS
Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
H.U. designed experiments and prepared the manuscript. A.T., A.T., L.Y.Z., E.M.J.-V. and D.J.L. performed experiments. M.A.S. and H.U. supervised the project. All authors analyzed data and commented on the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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1. Lipska, B.K., Halim, N.D., Segal, P.N. & Weinberger, D.R. Effects of reversible inactivation of the neonatal ventral hippocampus on behavior in the adult rat. J. Neurosci. 22, 2835–2842 (2002).
2. Pfeiffer, B.E. et al. Fragile X mental retardation protein is required for synapse elimination by the activity-dependent transcription factor MEF2. Neuron 66, 191–197 (2010).
3. Kasar, H., Fukuda, M., Watanabe, S., Hayashi-Takagi, A. & Noguchi, J. Structural dynamics of dendritic spines in memory and cognition. Trends Neurosci. 33, 121–129 (2010).
4. Sanes, J.R. & Lichtman, J.W. Development of the vertebrate neuromuscular junction. Annu. Rev. Neurosci. 22, 389–442 (1999).
5. Walres, C.L., Craig, A.M. & Garner, C.C. Mechanisms of vertebrate synaptogenesis. Annu. Rev. Neurosci. 28, 251–274 (2005).
6. Fox, M.A. & Umemori, H. Seeking long-term relationship: axon and target communicate to organize presynaptic differentiation. J. Neurochem. 97, 1215–1231 (2006).
7. Dalva, M.B., McClelland, A.C. & Kayser, M.S. Cell adhesion molecules: signaling functions at the synapse. Nat. Rev. Neurosci. 8, 206–220 (2007).
8. Goda, Y. & Davis, G.W. Mechanisms of synapse assembly and disassembly. Neuron 40, 243–264 (2003).
9. Tessier-Lavigne, M. & Biedie, C.R. Activity-dependent modulation of neural circuit synaptic connectivity. Funt. Mol. Neurosci. 2, 8 (2009).
10. Kano, M. & Hashimoto, K. Synapse elimination in the central nervous system. Curr. Opin. Neurobiol. 19, 154–161 (2009).
11. Zhang, L.I. & Poo, M. Electrical activity and development of neural circuits. Nat. Neurosci. 4, 1207–1214 (2001).
12. Bleckert, A. & Wong, R.O. Identifying roles for neurotransmission in circuit assembly: insights gained from multiple model systems and experimental approaches. Bioessays 33, 61–72 (2011).
13. Kay, L., Humphreys, L., Eckholt, B.J. & Burrone, J. Neuronal activity drives matching of pre- and postsynaptic function during synapse maturation. Nat. Neurosci. 14, 688–690 (2011).
14. Flavell, S.W. & Greenberg, M.E. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu. Rev. Neurosci. 31, 563–590 (2008).
15. Umemori, H., Linhoff, M.W., Onitz, D.M. & Sanes, J.R. FGF22 and its close relatives are presynaptic organizing molecules in the mammalian brain. Cell 118, 257–270 (2004).
16. Umemori, H. & Sanes, J.R. Signal regulatory proteins (SIRPs) are secreted presynaptic organizing molecules. J. Biol. Chem. 283, 34053–34061 (2008).
17. Terauchi, A. et al. Distinct FGFs promote differentiation of excitatory and inhibitory synapses. Nature 465, 783–787 (2010).
18. van Beek, E.M., Cochrane, F., Barclay, A.N. & van den Berg, T.K. Signal regulatory proteins in the immune system. J. Immunol. 175, 7781–7787 (2005).
19. Barclay, A.N. & Brown, M.H. The SIRP family of receptors and immune regulation. Nat. Rev. Immunol. 6, 457–464 (2006).
20. Matozaki, T., Murata, Y., Okazawa, H. & Ohnishi, H. Functions and molecular mechanisms of the CD47-SIRPα signaling pathway. Trends Cell Biol. 19, 72–80 (2009).
21. Danglot, L., Triller, A. & Marty, S. The development of hippocampal interneurons in rodents. Hippocampus 16, 1032–1060 (2006).
22. Steward, O. & Falk, P.M. Selective localization of polyribosomes beneath developing synapses: a quantitative analysis of the relationships between polyribosomes and developing synapses in the hippocampus and dentate gyrus. J. Comp. Neurol. 314, 545–557 (1991).
23. Bouvier, D. et al. Pre-synaptic and post-synaptic localization of EphA4 and EphB2 in adult mouse forebrain. J. Neurochem. 106, 682–695 (2008).
24. Biederer, T. & Scheiffele, P. Mixed-culture assays for analyzing neuronal synapse formation. Nat. Protoc. 2, 670–676 (2007).
25. Guo, C., Yang, W. & Lobe, C.G. A Cre recombinase transgene with mosaic, widespread tamoxifen-inducible action. Genesis 32, 8–18 (2002).
26. Yasuda, M. et al. Multiple forms of activity-dependent competition refine hippocampal circuits in vivo. Neuron 70, 1128–1142 (2011).
27. Wayman, G.A., Lee, Y.S., Tokumitsu, H., Silva, A.J. & Soderling, T.R. Calmodulin-kinases: modulators of neuronal development and plasticity. Neuron 59, 914–931 (2008).
28. Ethell, I.M. & Ethell, D.W. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J. Neurosci. Res. 85, 2813–2823 (2007).
29. Lindberg, F.P. et al. Decreased resistance to bacterial infection and granulocyte defects in IAP-deficient mice. Science 274, 795–798 (1996).
30. Chang, H.P., Lindberg, F.P., Wang, H.L., Huang, A.M. & Lee, E.H. Impaired memory retention and decreased long-term potentiation in integrin-associated protein-deficient mice. Learn. Mem. 6, 448–457 (1999).
31. Shen, K. & Cowan, C.W. Guidance molecules in synapse formation and plasticity. Cold Spring Harb. Perspect. Biol. 2, a001842 (2010).
32. Williams, M.E., de Wit, J. & Ghosh, A. Molecular mechanisms of synaptic specificity in developing neural circuits. Neuron 68, 9–18 (2010).
33. Siddiqui, T.J. & Craig, A.M. Synaptic organizing complexes. Curr. Opin. Neurobiol. 21, 132–143 (2011).
34. Wang, X.X. & Pfenninger, K.H. Functional analysis of SIRPα in the growth cone. J. Cell Sci. 119, 172–183 (2006).
35. Ohnishi, H. et al. Stress-evoked tyrosine phosphorylation of signal regulatory protein α regulates behavioral immobility in the forced swim test. J. Neurosci. 30, 10472–10483 (2010).
36. Hatherley, D. et al. Paired receptor specificity explained by structures of signal regulatory proteins alone and complexed with CD47. Mol. Cell 31, 266–277 (2008).
37. Hatherley, D., Graham, S.C., Hartos, K., Stuart, D.I. & Barclay, A.N. Structure of signal-regulatory protein α: a link to antigen receptor evolution. J. Biol. Chem. 284, 26613–26619 (2009).
38. Edwards, D.R., Handsley, M.M. & Pennington, C.J. The ADAM metalloproteinases. Mol. Aspects Med. 29, 258–289 (2008).
39. Reiss, K. & Saftig, P. The “a disintegrin and metalloprotease” (ADAM) family of sheddases: physiological and cellular functions. Semin. Cell Dev. Biol. 20, 126–137 (2009).
40. Bai, G. & Pfaff, S.L. Protease regulation: the yin and yang of neural development and disease. Neuron 72, 9–21 (2011).
41. Peixoto, R.T. et al. Transsynaptic signaling by activity-dependent cleavage of neurologin-1. Neuron 76, 396–409 (2012).
42. Suzuki, K. et al. Activity-dependent proteolytic cleavage of neurologin-1. Neuron 76, 410–422 (2012).
ONLINE METHODS

In situ hybridization. In situ hybridization was performed as described42 using digoxigenin-labeled riboprobes (Roche). The probes were generated by PCR from the 3′ untranslated regions15–17.

Primary neuronal cultures and transfection. Hippocampal cultures were prepared as described17. For immunostaining, hippocampal cells (1.5 × 10^4 to 4 × 10^5) were plated on glass coverslips (diameter 12 mm) coated with poly-lysine. Transfection was performed using the CalPhos Mammalian transfection kit (Clontech). For immunoprecipitation, hippocampal cells (3 × 10^5 to 5 × 10^5) were plated on poly-b-lysine–coated tissue culture dishes (diameter 35 mm). For coculture experiments, HEK cells were transfected using Lipofectamine 2000 (Invitrogen), and 24 h after transfection they were dissociated and added onto cultured hippocampal neurons (DIV 8). Cocultures were maintained for 48 h before fixation.

Knockout and transgenic mice. Sirpα knockout mice: A Sirpα genomic clone containing exons 1 (BAC clone 394B7; Invitrogen) was used to construct a targeting vector. A gene cassette composed of floxed full-length mouse Sirpα cDNA with SV40 intron–poly(A), EGFP–poly(A) and FRT6d Tin5 neo was introduced into the first exon, deleting 71 nucleotides containing the start codon (Supplementary Fig. 5). The deletion disrupts the expression of the endogenous Sirpα gene but allows the expression of the inserted gene. Floxed Sirpα mice were generated by embryonic stem cell–based homologous recombination.

Actin-Cre–ER mice were mated with floxed Sirpα mice. Tamarixen (100 µg per 1 mg) was injected at P0, P15 or P30 to induce the Cre recombinase–mediated excision of the Sirpα gene.

CD47 knockout mice were from W. Frazier (Washington University).

Mice used were C57/B6J background. Both male and female mice were used in our experiments. We did not detect any significant differences between males and females. All animal care and use was in accordance with the institutional guidelines and approved by the University of Michigan Committee on Use and Care of Animals.

Immunochemistry. Cultures were fixed with methanol for 3 min at −20 °C or with 4–1% paraformaldehyde (PFA) for 10 min at 37 °C and stained as described17. Mouse brains were fixed for 24 h with 4% PFA in PBS. Sagittal sections of 20 µm thickness were cut in a cryostat and stained. For immunostaining for PSD95, mouse brains were fresh-frozen and sectioned. Sections were then fixed with methanol for 5 min at −20 °C and stained. Dilutions and sources of antibodies were as follows: anti-VGLUT1 (1:5,000; Millipore; AB5905), anti-PSD95 (1:250; NeuroMab; 75-028), anti-VGAT (1:1,500; Synaptic Systems; 131003), anti-β-actin (1:1,000; Sigma; Aldrich; M4400), anti-neurofilament (1:1,000; Covance; SMI-312), anti-basoon (1:500; Enzo Life Sciences; ADI-VAM-P5003), anti-GFP (1:1,000; Millipore; AB16901), anti-GAP-43 (1:1,500; Synaptic Systems; 1730002), anti-NeuN (1:500; Millipore; MAB377), anti-calbindin (1:500; Sigma; C9848), anti-synapsin (1:2,000; a kind gift from P. Greengard and A. Nairn, Rockefeller University)15,16, antibody Py (1:50; a kind gift from W. Webb and P.L. Woodhams)17,45, anti-CD47 (1:200; BD; miap301), polyclonal anti-SIRPα (against the SIRPα extracellular domain; αC-terminal domain; clone p84; 1:200; BD)16,46. Clone p84, which recognizes the extracellular domain of Sirpα, was produced by transiently transfecting Sirpα extracellular–domain plasmids (Ex-Sirpα) into HEK cells and purifying secreted SIRP proteins from culture media as described16. Recombinant FGF2 was from R&D Systems.

Immunochemistry. Hippocampal neurons were cultured for 10–12 d, after which the medium was replaced by new medium (control) or medium containing 50 mM KCl, 50 µM bicuculline, 1 mM TTX, 5 µM KN62, 5 µM KN93, 10 µM nifedipine, 10 µM GM6001 and/or TIMPs (0.5 µg/ml each of TIMP1 and TIMP2). After incubation for 1–3 d with these treatments, media and cells were collected. The media were preloaded with Immobilized Protein-L (Pierce) and incubated with 1 µg in 2 µl of anti-SIRPα extracellular domain antibody (p84)16,35,47 for 4 h at 4 °C. The immune complexes were precipitated with Protein-L and the immunoprecipitates were subjected to western blotting as described below to assess the amount of secreted Sirpα.

Western blotting. Cells, collected as described above, were lysed on ice for 1 h in 200 µl of lysis buffer (1% Nonidet P-40, 50 mM Tris buffer, pH 8.0) with a protease inhibitor cocktail tablet (Roche). Dissected hippocampi were lysed by homogenization in 10 volumes of lysis buffer per gram of tissue. Lysis buffer was 1% Triton, 50 mM Tris buffer (pH 7.4) and 150 mM NaCl with a protease inhibitor cocktail tablet. The immunoprecipitates and lysates were subjected to SDS-PAGE. Equal amounts of lysate from each group were applied to the gel, as confirmed by assaying for α-tubulin. Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane and probed using anti-SIRPα extracellular domain antibody (p84; 1:200; BD)16,35,47 and anti-α-tubulin antibody (1:5,000; Sigma; T6074)16,42. The proteins were visualized by chemiluminescence (GE Healthcare) and the band intensities were quantified with ImageJ software.

Synaptic protein fractionation. The fractionation protocol was adapted from a previous report49. Cortex (300–350 mg) from P21 mice was homogenized in 1.5 ml of homogenization buffer (0.32 M sucrose, 1 mM NaHCO3, 1 mM MgCl2, 0.5 mM CaCl2, protease inhibitor). Homogenate was then adjusted to 1.25 M sucrose and 0.1 mM CaCl2 in a total of 5 ml. Homogenate was overlaid on 5 ml of 1 M sucrose and spun at 100,000g (SW41-Ti rotor, Beckman) for 3 h at 4 °C. The supernatant was collected and designated as synaptic membrane fraction (SPM). SPM (500 µl) was then added to 2 ml of 0.1 mM CaCl2 and 2.5 ml of 40 mM Tris pH, 6 with, 2% Triton X-100, and placed on rocking platform for 20 min at 4 °C. The sample was then spun at 35,000g (SS-34 rotor, Sorvall) for 20 min.
at 4 °C and supernatant was collected as the extrajunctional fraction. The pel-
et was air-dried and resuspended in 1 ml of 0.1 mM CaCl$_2$ and 1 ml of 40 mM
Tris, pH 8, with 2% Triton X-100, and placed on rocking platform for 60 min.
Resuspended pellets were then spun at 140,000 g (SW41-Ti rotor, Beckman) for
30 min at 4 °C, and supernatant was collected as presynaptic fraction. The
insoluble fraction was resuspended in 1 ml 20 mM Tris pH 7.4 with 1% SDS and
designated as the postsynaptic fraction. Extrajunctional and presynaptic fractions
were acetone-precipitated and resuspended in 1 ml of 20 mM Tris, pH 7.4, with
1% SDS. Snyptic membrane fraction and equivalent volumes of extrajunctional,
presynaptic and postsynaptic membrane fractions were then transferred to PVDF
membrane and probed with anti-PSD95 antibody (1:500; NeuroMab; 75-028)\cite{17},
anti-synaptotagmin antibody (1:100; Hybridoma Bank; mab48)\cite{49} and polyclonal
anti-SIRPα antibody (1:500; Upstate; 06-729)\cite{16,34}.

Whole-cell patch-clamp recordings in cultures. Neurons were bathed in
HEPES-buffered saline, consisting of 119 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 2 mM MgCl$_2$, 30 mM glucose and 10 mM HEPES (pH 7.4), supplemented with
1 µM TTX and 50 µM picrotoxin to isolate mEPSCs. Whole-cell internal solu-
tion consisted of 100 mM gluconic acid, 0.2 mM EGTA, 5 mM MgCl$_2$, 2 mM
ATP, 0.3 mM GTP and 40 mM HEPES (pH 7.2). Recording pipettes had a resist-
cance of 4–6 MΩ. Recordings were made with an Axopatch 200B amplifier and
collected with Clampex 8.0 (Molecular Devices). mEPSCs were analyzed using
MiniAnalysis 6.0 (Synaptosoft).

Acute hippocampal slice preparation and field electrophysiology. Mice were
decapitated and the hippocampi were isolated. Transverse slices (400 µm) were cut
using a tissue chopper (Stoelting) and incubated at 25 °C in a humidified cham-
ber for at least 2 h before recording. Slices were then transferred to a recording
chamber, maintained at 27–28 °C and continuously perfused at rate of 1.5 ml/min
with oxygenated artificial cerebral spinal fluid (aCSF). aCSF consisted of 119 mM
NaCl, 2.5 mM KCl, 1 mM Na$_2$HPO$_4$, 26.3 mM NaHCO$_3$, 11 mM glucose, 1.3 mM
MgSO$_4$ and 2.5 mM CaCl$_2$. In most experiments (except those in Fig. 6), aCSF
also contained 50 µM picrotoxin during recording. Picrotoxin was perfused for
at least 15 min before data were collected. Recording electrodes were pulled from
borosilicate capillary glass (1.7 mm o.d.; VWR International), filled with 3 M
NaCl and placed in the stratum radiatum of CA1. mEPSCs were stimulated using
cluster electrodes (FHC) also placed in the stratum radiatum of CA1. Current
was delivered with an ISO-flex stimulus isolation unit (AMPI). Recordings were
made with a MultiClamp 700B amplifier, collected and analyzed using Clampfit
10.2 (Molecular Devices). An input-output curve was obtained for each slice by
increasing the stimulus intensity from 0.02 to 0.25 mA. For paired-pulse experi-
ments, the intensity was set at 0.2 mA, which was the maximum response size.
To obtain the paired-pulse ratio, two pulses were delivered with an inter-pulse
interval from 25 to 200 ms. For LTP experiments, the stimulus intensity was set
so that the response size was 50% of maximum, and test stimuli were delivered
every 30 s. After 20 min of stable baseline EEPSPs, LTP was induced by delivering
two trains (each 1 s in duration) of 100 Hz separated by 30 s each.

Statistical analysis. The statistical tests performed were two-tailed Student’s
t-test or one- or two-way ANOVA, as indicated in the figure legend. In the case of a
two-way ANOVA, post hoc analysis was done with Tukey’s test. All data are expressed
as mean ± s.e.m. No statistical methods were used to pre-determine sample sizes, but our sample sizes were similar to those reported in previous
publications in the field\cite{15–17,41,42}. Data distribution was assumed to be normal.
From the experiment presented in Figure 6, 3 out of 13 (control) and 2 out of
22 (Sirpa$^{-/-}$) data points were excluded because the fiber volley amplitude has
changed by more than 10%. No data points were excluded from any other experi-
ments. All steps of the experiments were randomized to minimize the effects of
confounding variables. This includes how mice were chosen for injections,
order of cell culture treatments, etc. Electrophysiology experiments were done
blind. Imaging was done in similar fashions among conditions: fields from brain
sections were chosen randomly from the region of interest, and images of cell
cultures were taken randomly from all areas of the culture.

43. Schaeren-Wiemers, N. & Gerfin-Moser, A. A single protocol to detect transcripts of
various types and expression levels in neural tissue and cultured cells: in situ
hybridization using digoxigenin-labelled cRNA probes. Histochemistry 100,
431–440 (1993).
44. Uesaka, T. et al. Conditional ablation of GFRα1 in postmitogatory enteric neurons
triggers unconventional neuronal death in the colon and causes a Hirschsprung’s
disease phenotype. Development 134, 2171–2181 (2007).
45. Woodhams, P.L., Webb, M., Atkinson, D.J. & Seeley, P.J. A monoclonal antibody,
Py, distinguishes different classes of hippocampal neurons. J. Neurosci. 9,
2170–2181 (1989).
46. Oldenborg, P.A. et al. Role of CD47 as a marker of self on red blood cells. Science
288, 2051–2054 (2000).
47. Ohnishi, H. et al. Ectodomain shedding of SHPS-1 and its role in regulation of cell
migration. J. Biol. Chem. 279, 27878–27887 (2004).
48. Hahn, C.G. et al. The post-synaptic density of human postmortem brain tissues:
an experimental study paradigm for neuropsychiatric illnesses. PLoS ONE 4,
e5251 (2009).
49. Fox, M.A. & Sanes, J.R. Synaptotagmin I and II are present in distinct subsets of
central synapses. J. Comp. Neurol. 503, 280–296 (2007).