LRRTM3 regulates activity-dependent synchronization of synapse properties in topographically connected hippocampal neural circuits

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Synaptic cell-adhesion molecules (CAMs) organize the architecture and properties of neural circuits. However, whether synaptic CAMs are involved in activity-dependent remodeling of specific neural circuits is incompletely understood. Leucine-rich repeat transmembrane protein 3 (LRRTM3) is required for the excitatory synapse development of hippocampal dentate gyrus (DG) granule neurons. Here, we report that Lrtm3-deficient mice exhibit selective reductions in excitatory synapse density and synaptic strength in projections involving the medial entorhinal cortex (MEC) and DG granule neurons, accompanied by increased neurotransmitter release and decreased excitability of granule neurons. LRRTM3 deletion significantly reduced excitatory synaptic innervation of hippocampal mossy fibers (MF) of DG granule neurons onto thorny excrescences in hippocampal CA3 neurons. Moreover, LRRTM3 loss in DG neurons significantly decreased mossy fiber long-term potentiation (MF-LTP). Remarkably, silencing MEC–DG circuits protected against the decrease in the excitatory synaptic inputs onto DG and CA3 neurons, excitability of DG granule neurons, and MF-LTP in Lrrtm3-deficient mice. These results suggest that LRRTM3 may be a critical factor in activity-dependent synchronization of the topography of MEC–DG–CA3 excitatory synaptic connections. Collectively, our data propose that LRRTM3 shapes the target-specific structural and functional properties of specific hippocampal circuits.

Significance

The present study utilized imaging, electrophysiology, and three-dimensional high-resolution electron microscopy analyses to address the neural circuit role of LRRTM3 in vivo, using both conventional and conditional Lrtm3-KO mice. We found that LRRTM3 is required for the specific assembly and function of the medial perforant path to dentate gyrus synapses. Moreover, LRRTM3 is required for proper activity-dependent synaptic connectivity and long-term synaptic plasticity at mossy fiber–CA3 synapses. Strikingly, presynaptic inactivation of medial perforant path–dentate gyrus (MPP–DG) circuit activities completely rescued the impaired excitatory synaptic inputs and long-term synaptic plasticity of Lrtm3-KO mice, demonstrating that LRRTM3 is involved in activity-dependent hippocampal excitatory synapse refinement/ stabilization, which is dictated and synchronized by glutamatergic neurotransmission.
(27). These results suggest that a subset of LRRTMs is expressed in presynaptic neurons of certain brain areas.

DG mossy fiber (MF)-CA3 synapses in the hippocampus have been extensively used as a model to investigate mechanisms of activity-dependent homeostatic synaptic plasticity and target-specific synaptic specificity (28, 29). Highly plastic presynaptic MF terminals with massive separate vesicle release sites of glutamatergic DG neurons connect to multiheaded postsynaptic spines of CA3 (called thorny excrescences [TEs]), and filopodia projecting from the MF boutons (MBs) connect with nearby GABAergic interneurons to provide feed-forward inhibition of CA3 neurons during a subset of cognitive tasks (30). In addition, the MF–CA3 synapses exhibit structural plasticity at both presynaptic MF terminals and postsynaptic TEs that are sensitive to changes in levels of activity within the hippocampus (31). Moreover, these structural alterations are involved in encoding CA3-dependent contextual memory formation. Several classes of synaptic proteins, including the cell-surface proteins SynCAM1 and Kirre3 and the transcription factor Npas4, for each Lrrtm mRNA are mainly expressed in granule neurons (Fig. S1). Parallel RNAscope ISH analyses using isotope-labeled gene-specific probes showed that Lrrtm3 mRNAs are not prominently expressed in other hippocampal subfields (26, 35) (SI Appendix, Fig. S1A).

LRRTM3 Loss Decreases Excitatory Synaptic Inputs Specifically from the Medial Entorhinal Cortex to the Dentate Gyrus. LRRTM3 was previously shown to be responsible for excitatory synapse development in hippocampal DG granule neurons, similar to other LRRTMs (13–17, 19–21, 26, 36). However, it remained unclear whether LRRTM3 is required for biasing entorhinal cortical projections that innervate specific dendritic compartments of DG granule neurons in vivo. Serial-section electron microscopy using LRRTM3 knockout (Lrrtm3-KO) mice revealed that the numbers of synapses formed on dendritic spines were significantly decreased in the middle (MML), but not the outer (OML), molecular layer in Lrrtm3-KO mice (Fig. 1 A–C), in line with our previous findings (26). The shaft synapse density of wild-type (WT) and Lrrtm3-KO mice, however, did not differ. In addition, Lrrtm3-KO mice had a significantly smaller projection density of MMEA than did WT mice (Fig. 1 A, D, and E), suggesting that LRRTM3 might have a layer- or input-specific role in excitatory synapse formation in the DG.

To further probe whether LRRTM3 deletion can functionally impact a specific perforant pathway (PP), we generated Lrrtm3 conditional KO (cKO) mice in which exons 1 and 2 were flanked by loxP sites (Lrrtm3flox/flox) to allow Cre recombinase–dependent LRRTM3 deletion (SI Appendix, Fig. S2 A and B). We then stereotactically injected adeno-associated viruses (AAVs) expressing either inactive (ΔCre) or active Cre recombinase into the hippocampal DG region of Lrrtm3flox/mice. LRRTM3 deletion in vivo was validated by qRT-PCR analyses (SI Appendix, Fig. S2C) and semiquantitative immunoblot analyses using hippocampal DG tissue lysates of AAV-injected mice (SI Appendix, Fig. S2 D and E).

LRRTM3 Loss Impairs Excitatory Synaptic Strength from the Medial Entorhinal Cortex to the Dentate Gyrus. To corroborate our anatomical results, we performed electrophysiological recording of DG granule neurons from both WT and Lrrtm3-cKO mice (Fig. 1 F–S and SI Appendix, Fig. S3). Notably, the frequency, but not the amplitude, of spontaneous excitatory postsynaptic currents (sEPSCs) was massively reduced in Lrrtm3-cKO mice (SI Appendix, Fig. S3 B–D). Moreover, the frequency, but not the amplitude, of miniature excitatory postsynaptic currents (mEPSCs) was also reduced in Lrrtm3-cKO mice (SI Appendix, Fig. S3 E–G). Furthermore, current-clamp recordings revealed that action potential firing rate of DG granule neurons induced by current injections was significantly lower in Lrrtm3-cKO slices than in control slices (SI Appendix, Fig. S3 H and I). In addition, we induced LTP of medial perforant path (MPP) to DG granule neurons through high-frequency stimulation to determine whether there are any alterations in MPP–DG LTP in Lrrtm3-cKO mice. These experiments revealed a significant reduction in PP-DG LTP (~34%) in Lrrtm3-cKO mice (SI Appendix, Fig. S3 J–M). These results reaffirm that conditional LRRTM3 deletion impairs excitatory synaptic transmission and the excitability of DG granule neurons, in addition to a reduction in MPP–DG LTP.

We next asked whether postsynaptic deletion of LRRTM3 could impair the excitatory synaptic strength at specific PPs, as predicted by the decreased synapse number seen in the MML region of Lrrtm3-KO mice (Fig. 1 A, B, and D). To this end, we electrically stimulated the axonal fibers of the MPP or lateral PP (LPP), which target the medial or outer molecular layers of...
Fig. 1. Reduced excitatory synaptic density and strength at MPP–DG synapses of LRRTM3-deficient mice. (A) Representative EM images of synapses on dendritic shafts or spines in the MML and OML of the DG in WT and Lrrtm3-KO mice. Areas marked in light sky blue and yellow indicate presynaptic and postsynaptic sites, respectively (Scale bar, 1 μm). (B and C) Mean number of synapses per unit volume of MML (B) and OML (C) in WT (gray bar) and Lrrtm3-KO (orange or blue bar) mice. Data represent means ± SEMs (n = 12 dissector volumes from two mice per genotype per layer; Student’s t test). (D and E) Cumulative distribution plots for the longest PSD lengths of individual spines in the MML (D) and OML (E) in WT (gray line) and Lrrtm3-KO (orange or blue line) mice. Data represent means ± SEMs (WT [MML], n = 485; KO [MML], n = 385 spines; WT [OML], n = 527; KO [OML], n = 466 spines; Kolmogorov–Smirnov test). (F–H) Measurements of excitatory synaptic strength via I-O curves of control and L3 cKO mice, showing representative AMPAR-EPSC traces (F), summary plotting of the EPSC amplitudes as a function of MPP stimulation current (G), and summary graphs of fitted linear I-O slopes (H). Data represent means ± SEMs (n denotes the number of recorded neurons; control, n = 21; L3 cKO, n = 23; Mann–Whitney U test). (I and J) Measurements of NMDAR/AMPAR-EPSC ratios at MPP–DG synapses of control and L3 cKO mice. AMPAR-EPSCs were recorded in the same neurons as in F–H as a function of LPP stimulation. Data represent means ± SEMs (n denotes the number of recorded neurons; control, n = 19; L3 cKO, n = 13; Mann–Whitney U test). (K and L) Same as F–H, except that AMPAR-EPSCs were recorded in the same neurons as in F–H as a function of MPP stimulation. Data represent means ± SEMs (n denotes the number of recorded neurons; control, n = 21; L3 cKO, n = 23; Mann–Whitney U test). (M–O) Measurements of NMDAR/AMPAR-EPSC ratios at MPP–DG synapses of control and L3 cKO mice. AMPAR-EPSCs were recorded in the same neurons as in F–H as a function of LPP stimulation. Data represent means ± SEMs (n denotes the number of recorded neurons; control, n = 19; L3 cKO, n = 13; Mann–Whitney U test). (P and Q) Representative traces of PPRs of EPSCs at MPP–DG synapses (P) or LPP–DG synapses (Q) at two different interstimulus intervals (50 and 100 ms). Data represent means ± SEMs (n denotes the number of recorded neurons; control [MPP], n = 12; L3 cKO [MPP], n = 14; control [LPP], n = 11; L3 cKO [LPP], n = 10; Mann–Whitney U test). NS, not significant.
the DG, respectively (37–39), and then recorded the evoked excitatory postsynaptic currents (eEPSCs) of DG granule neurons using input–output (I–O) curves to control for the variability in stimulus strength (Fig. 1 F–H and K–M). The postsynaptic deletion of LRRTM3 decreased MPP-EPSCs by ~52.6%, but had no significant effect on LPP-EPSCs (Fig. 1 F–H and K–M). In these measurements, the EPSCs almost exclusively reflected α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR)–mediated responses. However, parallel experiments assessing N-methyl-D-aspartate receptor (NMDAR)–mediated responses also showed a decrease in synaptic strength, such that the NMDAR/AMPA ratio was comparable between control and Lrrtm3–KO neurons (Fig. 1 I, J, N, and O). Moreover, the reduced excitatory synaptic strength in the MPP was strikingly accompanied by an increase in the release probability, as reflected by a decreased paired-pulse ratio (PPR) under Lrrtm3 deletion (Fig. 1 P and Q). There were no changes in PPRs of the LPP–DG pathway in LRRTM3-deficient neurons (Fig. 1 R and S). Therefore, our results suggest that LRRTM3 is specifically required to maintain excitatory synaptic strength within MPP–DG projections and might be involved in the up-regulation of presynaptic function.

LRRTM3 Loss Reduces Excitatory Synapse Maintenance at Mossy Fiber–CA3 Excitatory Synapses. We previously reported that the excitatory synaptic transmission and excitability of DG granule neurons were reduced in constitutive Lrrtm3–KO mice (26). To confirm that conditional LRRTM3 deletion in DG neurons recapitulates the similar histological phenotypes of constitutive Lrrtm3–KO mice, we stereotactically injected AAVs expressing ΔCre or Cre into the hippocampal DG region of Lrrtm3Δ mice (SI Appendix, Fig. S4 A and B), and analyzed the density of excitatory and inhibitory synaptic puncta, which were labeled with antibodies to vesicular glutamate transporter 1 (VGLUT1) and vesicular GABA transporter (VGAT), respectively, in sections from the hippocampal DG and CA3 (SI Appendix, Fig. S4 C–F). Immunohistochemical analyses revealed that the VGLUT1 puncta density was decreased in the DG molecular layers and the hilus (SI Appendix, Fig. S4 C and D). Strikingly, the conditional loss of LRRTM3 from the DG area also reduced the density and area of VGLUT1 puncta in the hippocampal CA3 stratum oriens (SO) and stratum radiatum (SR) layers (SI Appendix, Fig. S4 C and D), findings similar to those in conventional Lrrtm3–KO mice (SI Appendix, Fig. S5). The density and area of VGAT puncta were comparable in control and Lrrtm3–cKO mice (SI Appendix, Fig. S4 E and F). Taken together, these results show that postsynaptic LRRTM3 is required for innervation of excitatory synaptic inputs at MPP–DG synapses and for excitatory synapse maintenance in a subset of hippocampal CA3 layers.

To assess whether altered synapse properties in DG granule neurons influence the structural connectivity with output neurons, we next performed quantitative immunofluorescence analyses using VGLUT1 or the MB marker, synaptoporin (SPO), in control and Lrrtm3–cKO mice (SI Appendix, Fig. S6).

The density and area of VGLUT1 puncta were significantly reduced in the SR and SO layers, but not the stratum lucidum (SL) layer, of the hippocampal CA3 area (SI Appendix, Fig. S4). Mf axons are composed of two distinct bundles targeting CA3 pyramidal neurons: large suprapyramidal bundles (SPBs) traveling adjacent to and above the pyramidal layer and small infrapyramidal bundles (IPBs) traveling below the pyramidal layer, both of which target CA3 pyramidal neurons (40). Thus, we separately quantified the density and area of SPO puncta in each bundle of Mf axons in WT and Lrrtm3–cKO mice. We found that the density of SPO puncta tended to be lower in SPB axons of Lrrtm3–cKO than of WT mice (SI Appendix, Fig. S6 B and F). Moreover, SPO puncta sizes in IPB axons were greater in Lrrtm3–cKO than in WT mice (SI Appendix, Fig. S6 B and F). However, the length of SPBs was specifically increased while the length of IPBs was significantly decreased in Lrrtm3–cKO mice, resulting in a slight decrease of the IPB/SPB ratio (SI Appendix, Fig. S6 A and C–E), an indicator of MB maturation (41). Overall, these analyses suggest that LRRTM3 might contribute to the balanced projection of Mf bundles and be involved in the normal maturation of presynaptic axonal terminals projected to the CA3 area.

LRRTM3 Loss Remodels Apposed Thorny Excrescence Structures In Vivo. Mf presynaptic complexes connect DG granule neurons not only to glutamatergic CA3 pyramidal neurons but also to GABAergic interneurons (42). Because light microscopy is unable to precisely determine the involvement of LRRTM3 in connections between DG granule neuron axons and specific postsynaptic partner neurons (i.e., CA3 excitatory pyramidal neurons and GABAergic interneurons), serial block-face scanning electron microscopy (SBF-SEM) was performed to collect large datasets in the SL layer of the hippocampal CA3 subfield. This method was employed to analyze the Mf–CA3 circuitry at nanoscale resolution in transgenic mice (32, 42, 43). The volume for each image stack was 60 × 60 × 20 μm in the x, y, and z dimensions, and was composed of at least 400 serial images obtained at 50-nm thickness, resulting in a typical volume of 72,000 μm³ (Movie S1). Eight proximal dendritic segments of CA3 pyramidal neurons were manually reconstructed. There were four segments per genotype, with mean linear dendritic lengths of 38.58 ± 3.37 μm per segment for WT and 40.45 ± 4.72 μm per segment for Lrrtm3–KO, running throughout their entire volumes including their TEs and corresponding MBs. Note that conventional Lrrtm3–KO mice were used for SBF-SEM analyses, and major morphological issues were further addressed in Lrrtm3–cKO mice using transmission electron microscopy (TEM).

The density of MBs along the proximal dendrites of CA3 neurons was also significantly lower in Lrrtm3–KO than in WT mice (Fig. 2 A and C). Subsequent morphological analyses of individual MBs showed that the population means of MB volume were significantly higher in Lrrtm3–KO mice (Fig. 2 D). Representative MBs taken every 10th percentile (in order of MB volume) showed differences between the two genotypes in MB size (Fig. 2 B and D). Notably, there were no differences in the number of synaptic contacts per MB or the number of TEs per MB between Lrrtm3–KO and WT mice (Fig. 2 E and F). To determine whether LRRTM3 deletions specifically affect Mf–CA3 TE synapses or the general integrity of other CA3 synapses, we analyzed SBF-SEM datasets of the SR layer of the CA3 region, just adjacent and distal to the SL layer. Dendritic segments of CA3 neurons in this layer mainly receive recurrent inputs from other CA3 neurons formed by association and commissural connections. Three-dimensional (3D) reconstruction of dendritic segments revealed no obvious between-group differences in mean dendritic spine density and PSD area (SI Appendix, Fig. S7 A–C).

TE density in hippocampal CA3 neurons was also lower in Lrrtm3–KO than in WT mice (Fig. 2 G and J). Individual TEs along the apical proximal dendritic segments of the hippocampal CA3 neurons were color-coded and separated from the dendritic shafts as discrete objects to facilitate subsequent morphological analyses (Fig. 2H). The population means of TE size showed no between-group differences (Fig. 2I). While the number of PDSs per TE volume was lower in Lrrtm3–KO (Fig. 2K), the number of MBs per TE did not differ between genotypes (Fig. 2L), suggesting that the synaptic ratio between MBs and CA3 TEs was similar in Lrrtm3–KO and WT mice. Importantly, the reduced density of Mf–CA3 synapses in Lrrtm3–KO mice was also confirmed in Lrrtm3–cKO mice (Fig. 2 M and N),
LRRTM3 Loss Has No Effect on Mf Filopodial Synapse Structures. Because Kirrel3 was previously reported to participate in the formation of Mf filopodia to GABAergic neuron synapses (42) and IgSF8 was more recently reported to be involved in maintaining MF boutons and filopodial density (44), we assessed whether loss of LRRTM3 also affects connections at these particular synapses. Close examination of the presynaptic filopodia from the reconstructed MfBs showed that each individual MfB in adult WT mice possesses approximately two presynaptic filopodia, as previously reported (43). Further analyses showed that LRRTM3 deficiency significantly increased the mean number of Mf filopodia or branched filopodia per MfB, with no change in the length of individual Mf filopodia (SI Appendix, Fig. S8 A–E).

Mf filopodial synapses can be classified into three categories: 1) a synapse-free type, containing no presynaptic vesicle clusters or PSDs; 2) a partial synapse type, with clusters of synaptic vesicles but no visible PSDs; and 3) a complete synapse type, containing vesicle clusters adjacent to corresponding PSDs (42). Of these three categories of Mf filopodia, the partial synapse type constituted the highest percentage in both WT (81.3%) and Lrrtm3-KO (86.0%) mice (SI Appendix, Fig. S8F). We then determined the postsynaptic targets of Mf filopodial synapses. As expected, most complete synapse-type Mf filopodia were connected to nearby GABAergic neurons as postsynaptic targets (SI Appendix, Fig. S8 A, B, and F). Moreover, a few Mf filopodia formed synapses onto the dendritic shaft and TE of CA3 neurons (SI Appendix, Fig. S8 C, D, and F), which can be readily distinguished from GABAergic neurons by the presence of TEs in the dendrites of CA3 neurons. Although Lrrtm3-KO mice displayed more filopodia per MfB (SI Appendix, Fig. S8E), there were no differences between genotypes in the proportion of Mf filopodia in the three synaptic categories and in the percentage of their postsynaptic target cell types (SI Appendix, Fig. S8F), indicating that loss of LRRTM3 had little influence on Mf filopodial synapses.

![Fig. 2](https://doi.org/10.1073/pnas.2110196119)

Although the average MfB area in Lrrtm3-cKO mice was not different from that of control mice (Fig. 2O). Again, excitatory synaptic density and PSD length in the SR layer of the CA3 region were comparable in Lrrtm3-cKO and control mice (SI Appendix, Fig. S7 D–F). Collectively, these results indicate that LRRTM3 deletions selectively influence synaptic organization at DG–CA3 TE circuits, but not at CA3–CA3 recurrent connections.

### Methodologies

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LRRTM3 Is Required for Stabilization of Long-Term Potentiation at MF–CA3 Synapses. We next performed patch-clamp recordings to examine whether the altered TEs and MIBs in Lrrtm3-cKO mice influenced the electrophysiological properties of CA3 pyramidal neurons. We first performed experiments in current-clamp mode using 500-ms current injection steps (from −100 to 250 pA in 25-pA increments), determining that firing rates of CA3 pyramidal neurons were comparable between control and Lrrtm3-cKO mice (SI Appendix, Fig. S9 A and B). To examine NMDA-EPSCs and AMPA-EPSCs from CA3 pyramidal neurons, we next performed whole-cell patch-clamp recordings using stimulating DG granule neurons. We found that NMDAR/AMPAR ratios in CA3 pyramidal neurons were similar in control and Lrrtm3-cKO mice (SI Appendix, Fig. S9 C and D). PPRs of MF–CA3 synapses were also measured by stimulating DG granule neurons, but no detectable differences were found between control and Lrrtm3-cKO mice (SI Appendix, Fig. S9 E and F). These results indicate that conditional deletion of LRRTM3 in DG granule neurons does not affect the electrophysiological properties of CA3 pyramidal neurons under basal conditions.

We next examined whether LTP was altered when LRRTM3 was absent from MF–CA3 synapses. MF-LTP was induced by high-frequency tetanic stimulation (100 Hz for 1 s given three trains with a 10-s interval between trains) in the presence of the NMDAR antagonist, D-amino-5-phosphonopentanoic acid; this trains with a 10-s interval between trains) in the presence of the high-frequency tetanic stimulation (100 Hz for 1 s given three trains) in the presence of the NMDAR antagonist, D-amino-5-phosphonopentanoic acid (Fig. 3 A). NMDAR antagonist, D-amino-5-phosphonopentanoic acid; this was absent from Mf–CA3 synapses. Mf-LTP was induced by (100 Hz, 1 s, three trains with a 10-s interval between trains) is given at the time indicated by the arrow. Data represent means ± SEMs (Mann–Whitney U test). (D) Quantification of fEPSP amplitudes in the MF-LTP recording experiments. Data represent means ± SEMs (n denotes the number of analyzed slices; control, n = 10; L3 cKO, n = 12; Mann–Whitney U test).

LRRTM3 Coordinates Medial Entorhinal Cortical Inputs to the DG to Regulate MF–CA3 Excitatory Synaptic Inputs and LTP. The above-described results collectively suggest that LRRTM3 might shape specific topographic architectures of MEC—DG and DG—CA3 circuits. However, it is unclear whether the activities of these two distinct neural circuits involving DG granule neurons are synchronized. To address this question, we attempted to specifically block neurotransmitter release at MPP–DG synapses and performed quantitative immunohistochemical analyses to monitor alteration of excitatory synapse structures in the DG and CA3 areas. To this end, we injected control and Lrrtm3-cKO mice with an AAV encoding an Flpo recombinase–mediated double-inverted open reading frame (fDIO), inverted enhanced green fluorescent protein (EGFP), and tetanus toxin (TeNT) gene (fDIO-TeNT-F2A-EGFP), or inverted EGFP alone (fDIO-F2A-EGFP) into the MEC, and a trans-neuronally transported version of Flpo fused to wheat germ agglutinin (WGA-Flpo) into the DG (Fig. 4 A). This scheme allowed EGFP and TeNT to be expressed only after inversion of the double-flxed expression cassette in the MEC neurons by injecting AAVs coexpressing mCherry WGA-Flpo recombinases (Fig. 4 A). We first validated whether expression of WGA-Flpo in the DG activated EGFP and TeNT expression in the MEC. We found that injection of WGA-Flpo AAV into the DG specifically induced EGFP expression in the MEC neurons and selectively reduced excitatory synaptic strength and neurotransmitter release at MPP–DG synapses (Fig. 4 B–E), validating the specific targeting of MEC circuits (Fig. 4 C). This scheme allowed EGFP and TeNT to be expressed only after inversion of the double-flxed expression cassette in the MEC neurons by injecting AAVs coexpressing mCherry WGA-Flpo recombinases (Fig. 4 A). We first validated whether expression of WGA-Flpo in the DG activated EGFP expression in the MEC neurons and selectively reduced excitatory synaptic strength and neurotransmitter release at MPP–DG synapses (Fig. 4 B–E), validating the specific targeting of MEC projections. Neither the number of VGLUT1 puncta in the DG ML region nor the number of VGLUT1 puncta in SO and SR layers of CA3 was affected by inactivation of the MEC with TeNT in control mice.

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and mCherry [hM3Dq], or a control (AAV-fDIO-mCherry) into modified human M3 muscarinic receptor (AAV-fDIO-hM3Dq-dependent AAVs expressing the designer receptor; Gq-coupled deficient neurons (synapse maintenance in DG and CA3 layers of LRRTM3-Exclusively Activated by Designer Drugs injections did not water–based administration of the designer ligand, clozapine-

Finally, we examined whether the activity of MPP–DG synapses was involved in the maintenance of Mf-LTP (Fig. 4 H–J). TeNT expression in MPP–DG projections did not affect Mf-LTP in control mice but completely rescued the Mf-LTP impairment in Lrrtm3-cKO mice (Fig. 4 H–J). Strikingly, silencing MPP–DG circuit activities in Mpp3-cKO mice rescued the reduced excitability of DG granule neurons (Fig. 5 A–C). In contrast, CNO-mediated hM3Dq excitation of LRRTM3-deficient DG granule neurons failed to normalize the increase in neurotransmitter release (Fig. 5 D–F). These results suggest that increased neurotransmitter release in hippocampal DG granule neurons of Lrrtm3-cKO mice is likely coupled with reduced excitatory synaptic inputs and long-term plasticity. Collectively, our data demonstrate that LRRTM3 activity in the MEC projections onto DG granule neurons is involved in an activity-dependent excitatory synapse–remodeling process that is crucial for certain functions of DG–CA3 circuits.

Discussion

The best understood form of homeostatic plasticity arguably involves an up-regulation of presynaptic efficacy to counterbalance a reduction in postsynaptic neuron excitability (48). The current study showed that LRRTM3 is differentially involved at two interdependent hippocampal neural circuits, namely MPP–DG and DG–CA3 synapses, providing insights into a neural circuit–organizing role of LRRTM3 that involves a coordinated homeostatic presynaptic response.

Consistent with previous ISH analyses (21, 26, 35), we confirmed that the expressions of LRRTM3 are restricted to the DG granule neurons within the hippocampus, in contrast to the widespread expression of LRRTM1 and LRRTM2 in all hippocampal subfields. In an extension of our previous analyses on hippocampal DG synapses of Lrrtm3-KO mice, we first asked whether LRRTM3 is specifically involved in a particular PP. Our data showed that LRRTM5 is selectively required for assembly and/or maintenance of MEC projections to the DG pathway. We confirmed a subset of anatomical and electrophysiological phenotypes reported from constitutive Lrrtm3-KO mice (26) as also being observed after conditional postsynaptic KO of LRRTM3, with consistently reduced PP–DG LTP, excitability, and excitatory synaptic transmission in DG granule neurons, together with reduced excitatory synapse density in both DG granule neurons and CA3 pyramidal neurons. However, there were marked differences in sEPSCs and mEPSCs between constitutive and conditional Lrrtm3-KO mice. At present, the mechanistic basis of these differences is obscure, but developmental compensation, likely by reflecting differences in the timing of Lrrtm3 deletion, is a possibility. Our hypothesis is that LRRTM3 expressed in proximal dendrites of DG granule neurons likely mediates specific recognition processes by interacting with an extracellular ligand(s) expressed in axons of MEC neurons. It remains to be determined, however, whether and how an LRRTM3 ligand(s) operates in a specific projection, which might be irrelevant of their specific localization/expression in the EC region. Regardless of LRRTM3’s mechanism of action, our data establish that LRRTM3 acts as a critical factor in conferring the properties of a defined specific neural circuit.

Upon deletion of LRRTM3 at Mf–CA3 synapses, we made a series of observations that were initially unexpected and surprising. Importantly, high-resolution ultrastructural analyses showed that loss of LRRTM3 specifically reduced the density of Mf–CA3 synapses in proximal dendrites of both Lrrtm3-deficient mouse lines (Fig. 4) and conditional Lrrtm3-deficient mice in vivo. Paradoxically, the sizes of the MBs were increased in Lrrtm3-KO, but not in Lrrtm3-cKO, mice. This discrepancy may result from differences in genetic deletion strategies or the potential absence of developmental compensation in Lrrtm3-cKO mice. The presence of these structural alterations in Lrrtm3-deficient mice suggests that LRRTM3 loss influences inputs onto CA3 neurons (i.e., by presynaptic deletion effects) or exerts homeostatic adjustment in a downstream region of the connected hippocampal circuits. To discriminate between these possibilities, the exact localization of LRRTM3 should be determined with high-resolution approaches. Because our in-house and currently available commercial LRRTM3-specific antibodies were not suitable for immunohistochemical analyses to determine the precise localization of this protein at synapses in vivo, it remains unclear if a fraction of LRRTM3 might be expressed in the terminals of DG granular axons in vivo. Notably, LRRTM4 is presynaptically localized to retinal bipolar cells (27, 49). Nevertheless, based on various prior results related to LRRTM family members at hippocampal synapses (11, 12), we favor the latter possibility that LRRTM3 likely regulates the homeostatic synaptic plasticity that occurs predominantly at proximal synapses between DG and CA3 neurons (31, 50).

The morphological alterations of DG MI and CA3 TEIs are likely due to homeostatic adaptations, driven by reduced inputs from MPPs and functioning to balance excitatory and inhibitory network activity in the hippocampus. There was no difference in density or PSD area of distal dendrites of CA3 pyramidal neurons derived from Lrrtm3-KO and WT mice.

LRRTM3 is dispensable for development of Mf filopodia–CA3 pyramidal neuron synapses, although the proportion of these synapses was much lower (~0.9%) in juvenile postnatal day (P)42 WT and Lrrtm3-KO mice than in P14 WT mice (~5.2%) (42). These data suggest that different sets of cell-surface proteins are involved in organizing the structural projections of DG MI to distinct postsynaptic targets (i.e., LRRTM3 targeting CA3 pyramidal neurons, Kirrel3 targeting GABAergic interneurons, and IgSF8 targeting DG, CA3, and GABAergic interneurons) (33, 44). The number of Mf filopodia was significantly higher in Lrrtm3-KO mice than in WT mice, although there was no difference in the length of their Mf filopodia. In contrast, Lrrtm3-KO mice exhibited a comparable number of complete synapses with GABAergic

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LRRTM3 regulates activity-dependent synchronization of synapse properties in topographically connected hippocampal neural circuits
Fig. 4. Impaired excitatory synapse refinement and MF-LTP in LRRTM3-deficient mice are rescued by blocking synaptic transmission at MPP–DG synapses. (A) Design of AAVs used to inactivate neurotransmitter release from MEC neurons to DG granule neurons. Double-flxed inverted TeNT-AAV (2xFlx-TeNT-AAV) encodes a bicistronic expression encoding EGFP (for visualizing infected MEC neurons) and TeNT (to block synaptic transmission). The coding region of the double-flxed inverted TeNT-AAV is not translated until Flpo recombinase flips the inverted coding region into the correct orientation. WGA-Flpo AAV mediates bicistronic expression of mCherry and WGA-Flpo. When this AAV infects DG neurons, WGA-Flpo is trans-neuronally transferred to connected MEC neurons, whereas mCherry is only expressed in the infected DG neurons. (Scale bar, 100 μm.) (B and C) Measurements of excitatory synaptic strength via I-O curves of control- and TeNT-expressing WT mice, presenting representative AMPAR-EPSC traces (B), summary plotting of the EPSC amplitudes as a function of MPP stimulation current (C, Left), and summary graph of fitted linear I-O slopes (C, Right). Data represent means ± SEMs (n denotes the number of recorded neurons; control, n = 10; TeNT, n = 11; Mann–Whitney U test). (D and E) Same as B and C, except that AMPAR-EPSCs were recorded in the same neurons as in B and C as a function of LPP stimulation. Data represent means ± SEMs (n denotes the number of recorded neurons; control, n = 8; TeNT, n = 9; Mann–Whitney U test). (F and G) Representative immunofluorescence images (F) and quantification (G) of VGLUT1 puncta density. Data shown are means ± SEMs (n denotes the number of analyzed mice; ΔCre [Ctrl], n = 4; Cre [Ctrl], n = 4; ΔCre [TeNT], n = 4; Cre [TeNT], n = 4 mice; ANOVA followed by Shapiro–Wilk normality test). (Scale bar, 20 μm.) IHC, immunohistochemistry. (H) Representative average traces of fEPSPs, evoked in WT and Lrrtm3-cKO mice injected with either control or TeNT by stimulation of Mf before LTP induction by HFS (black) and 50 to 60 min after LTP induction (light gray, control [Ctrl]; red, L3 cKO [Ctrl]; blue, control [TeNT]; green, L3 cKO [TeNT]). (I) fEPSP amplitudes plotted against MF-LTP experiments as a function of recording time. An HFS (100 Hz, three 1-s trains at 10-s intervals) is given at the time indicated by the arrow. Data represent means ± SEMs. (J) Quantification of fEPSP amplitudes in the MF-LTP recording experiments. Data represent means ± SEMs (n denotes the number of analyzed slices; WT [Ctrl], n = 6; WT [TeNT], n = 8; L3 cKO [Ctrl], n = 11; L3 cKO [TeNT], n = 7; nonparametric ANOVA with post hoc Tukey’s multiple-comparison test).
interneurons. The cause for the increased number of Mf filopodia upon LRRTM3 deletion is currently unclear, although LRRTM3 loss in the Mf filopodia and/or GABAergic interneurons may concertedly drive this structural rearrangement.

Notably, presynaptic neurotransmitter release was increased at MPP–DG synapses of LRRTM3-deficient neurons, but not at Mf–CA3 synapses. Presynaptic silencing of MEC neurons at MPP–DG synapses blunted the effects of LRRTM3.

Fig. 5. Action of LRRTM3 in regulating excitatory synaptic transmission at MPP–DG synapses is a prerequisite for synchronized activity-dependent circuit properties. (A) Design of AAVs used to inactivate neurotransmitter release from MEC neurons to DG granule neurons. Double-floxed inverted TeNT-AAV (2xFlx-TeNT-AAV) encodes a bicistronic expression construct encoding EGFP (for visualizing infected MEC neurons) and TeNT (to block synaptic transmission). The coding region of the double-floxed inverted TeNT-AAV is not translated until the Flpo recombinase flips the inverted coding region into the correct orientation. WGA-Flpo AAV mediates bicistronic expression of mCherry and WGA-Flpo. Upon infection of DG neurons with this AAV, WGA-Flpo is trans-neuronally transferred to connected MEC neurons, whereas mCherry is only expressed in the infected DG neurons. (B and C) Representative traces (B), summary graphs (C, Top), and average (C, Bottom) of intrinsic excitability measured as firing rates in response to step depolarizing currents (duration, 500 ms) in DG granule neurons. Individual points represent means ± SEMs (n denotes the number of recorded neurons; control, n = 16; control [+] TeNT, n = 16; L3 cKO, n = 26; L3 cKO [+] TeNT, n = 16; ANOVA followed by Tukey’s post hoc test after Shapiro–Wilk normality test). (D) Schematic of an electrophysiological recording configuration showing stimulating and recording sites in hippocampal CA3. (E) Representative traces of PPRs of EPSCs at MPP–DG synapses at two different interstimulus intervals (50 and 100 ms). (F) Average of EPSC-PPRs at MPP–DG synapses at four different interstimulus intervals (25, 50, 100, and 200 ms). Data represent means ± SEMs (n denotes the number of recorded neurons; control, n = 20; L3 cKO [+] hM3Dq [vehicle], n = 16; L3 cKO [+] hM3Dq [CNO], n = 12; nonparametric ANOVA with Kruskal–Wallis test followed by post hoc Dunn’s multiple-comparison test).
deletion on DG granule neuron excitability or excitatory synaptic inputs and LTP at MF–CA3 synapses. Our interpretation of these results is that the elimination of excitatory synapses at MPP–DG synapses triggered by LRRTM3 loss activates a homeostatic presynaptic response, as recently shown in an autaptic circuit (51), that further drives activation of the molecular machinery involved in excitatory synapse refinement. Nlgn1 was shown to modulate presynaptic release probability in hippocampal CA1 synapses, likely through Nrns (52). Given that these Nlgn1 and LRRTM3 loss-of-function phenotypes and the analyzed neural circuits are distinct, a complete understanding of trans-synaptic effects on presynaptic functions will require further investigation into what dictates how LRRTM3 deletion induces the homeostatic presynaptic response. Loss of LRRTM3 also reduced the excitability of DG granule neurons, but not one of the CA3 neurons; instead, it significantly reduced excitatory synapse development in a subset of CA3 layers. Intriguingly, chemogenetic activation of DG granule neurons rescued the deficits in excitatory synapse maintenance in DG and CA3 areas, but did not rescue the impaired maintenance of MF-LTP and failed to normalize the increase in neurotransmitter release at MPP–DG synapses (Fig. 5 and SI Appendix, Fig. S11). Thus, our observations suggest that LRRTM3 deletions induce an alteration or dysregulation of homeostatic plasticity and excitatory synaptic properties that are distinctively manifested at two different hippocampal circuits, and that LRRTM3 acts primarily at a postsynaptic locus in the MPP–DG circuit, with its actions likely propagating to the DG–CA3 circuit to regulate specific excitatory synapse properties. More sophisticated experiments will be required to discern the temporal order of various biological phenomena documented in the present study.

Although no published study has reported behavioral analyses of LRRTM3-deficient mice, it is plausible to speculate that LRRTM3 might be involved in a subset of cognitive tasks that are mediated by neural circuits involving the DG (53, 54), and other DG-linked regions (55, 56). Structural plasticity in MF neural circuits was previously reported to be associated with long-lasting memory (57, 58), with LTP induced in the MF granular and remodeling (59, 60). Moreover, molecular profiles in MF terminals are dynamically regulated when induced by spatial learning (61, 62). Future studies using various transgenic animals with similar structural and functional features should elucidate how the information flow in hippocampal circuits is carried to downstream structures involved in governing cognitive tasks.

In summary, the present study delineated the in vivo role of LRRTM3 in organizing the specific topography of hippocampal synaptic connections and the activity-dependent refinement of excitatory synapses for discrete granule cell activity.

Materials and Methods

Animals. All mice were maintained and handled in accordance with protocols (DGIST-IACUC-19052109-00) approved by the Institutional Animal Care and Use Committee of DGIST under standard, temperature-controlled laboratory conditions. Mice were kept on a 12:12-light/dark cycle (lights on at 7:00 AM), and received water and food ad libitum. Constitutive Lrrtm3-KO mice were described previously (26). LRRTM3-cKO mice were generated at Biotechnology. Floxed LRRTM3 (Lrrtm3fl/fl) mice were generated by flanking exons 1 and 2 with loxP sites (SI Appendix, Fig. S2).

In detail, Lrrtm3 floxed mice were generated using an embryonic stem cell (ESC)-based gene-targeting method. The CCD53925 transcript of the Lrrtm3 gene was used in designing a targeting vector into which a loxP site was inserted ~2 kb upstream of the 5′ untranslated region of the Lrrtm3 gene. The FRT site, neomycin resistance gene, and second loxP site were inserted in introns 2 and 3 of the Lrrtm3 gene. This arrangement positions loxP sites to direct Cre-mediated deletion of the Lrrtm3 allele. A diapherine toxin minigene was inserted for negative selection.

ES culture and selection for homologous recombination were performed using a C57BL/6 mouse ESC line. The targeting vector was introduced into C57BL/6 mouse ESCs by electroporation. Positive ESC clones, identified by screening for successful recombination of the target DNA, were injected into C57BL/6 mouse blastocysts. Blastocysts were then implanted into host mice, resulting in chimeras. Chimeras were bred with strains of an Fip mouse strain to remove the neomycin resistance cassette and produce mice with germline transmission of the target gene. Mice were maintained in the C57BL/JN background. All experimental procedures were performed on male mice, using a littermate control without Cre expression. Mouse genotyping for constitutive Lrrtm3-KO mice was previously described (26).

Antibodies. The following primary antibodies were generated in the laboratory of Jaewon Ko: guinea pig polyclonal anti–pan-LRRTM3 (JK132; RRID: AB_2810943). Synthesized peptides of mouse LRRTM3 (amino acids 564 to 582, DLSFTISAGRTSDHPKQLA) were conjugated with keyhole limpet hemocyanin, and injected three times into guinea pigs. Antisera were collected and affinity-purified using Sephadex columns (Pierce), onto which the same conjugated peptide had been immobilized. Commercially purchased antibodies included rabbit polyclonal anti–SPO (Synaptic Systems; RRID: AB_887841), guinea pig polyclonal anti–VGLUT1 (Sigma-Aldrich; RRID: AB_2301751), rabbit polyclonal anti–VGLUT3 (Synaptic Systems; RRID: AB_887869), mouse monoclonal anti–LRRTM3 (clone 2N259822; Neomab; RRID: AB_10674105), and mouse monoclonal anti–β-actin (clone C4; Santa Cruz Biotechnology; RRID: AB_826632).

Plasmids. The AAV vectors were generated by standard molecular biology procedures as follows: AAV-EF1α–FDO–EGFP–F2A–TeNT was constructed by PCR amplification of the FDO–EGFP–F2A–TeNT segment using the pAAV-2Flox–TeNT (63) plasmid as a template; the product was digested with Ascl and Nhel, and subcloned into the pAAV-EF1α–FDO–EYFP vector (Addgene 55641). AAV-EF1α–FDO–EGFP–F2A was constructed by PCR amplification of the EGFP–F2A segment, which was performed similar to the above-described generation of AAV-EF1α–FDO–EGFP–F2A–TeNT. AAV-EF1α–FDO–EGFP–F2A–HM3Dq was constructed by PCR amplification of the HM3Dq sequence from the pAAV-H5sync–DIO–GFP–IRES–Ia (Addgene; 50454); the product was digested with Ascl and subcloned into the pAAV-EF1α–FDO–EYFP–F2A vector. AAV-phSyn–WGA–IRES–mCherry–Fipio–BghPA was constructed by PCR amplification of the WGA–IRES–mCherry segment from pAAV–WGA–Cre–IRES–mCherry (64); the product was digested with KpnI and HindIII, and subcloned into the AAV–phSyn–Fipio–BghPA vector (Addgene; 51669). AAV–hSyn–HM3Dq–mCherry was purchased (Addgene; 50474). The following plasmids were previously described: AAV–WGA–Cre–IRES–tdTomato and AAV–WGA–Cre–IRES–tdTomato (64), and AAV–F2–TeNTox (63).

Also see SI Appendix, Materials and Methods for details for fluorescence in situ hybridization (RNAseq assay), immunocytochemistry, confocal microscopy imaging, preparation of AAVs and titration, stereotactic surgery, electrophysiology, tissue processing for SBF-SEM and TEM, acquisition of SBF-SEM datasets and 3D reconstruction, sterology, physical dissector counting, and chemogenetics experiments.

Experimental Design and Statistical Analysis. All statistical analyses were performed using GraphPad Prism 7 software (RRID: SCR_002798). The normality of distributed data was determined using the Shapiro–Wilk normality test. Normally distributed data were compared using Student’s t test or the ANOVA test, and nonnormally distributed data were compared by the Mann–Whitney U test, nonparametric ANOVA with Kruskal–Wallis test followed by post hoc Dunn’s multiple-comparison test, or nonparametric ANOVA with post hoc Tukey’s multiple-comparison test. If a single value made the data distribution nonnormal and was found to be a significant outlier (P < 0.05) by Grubb’s test, it was regarded as an outlier. Dataset S1 presents detailed statistics.

Data Availability. All study data are included in the article and supporting information.

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22. J. S. Ko, 18. M. W. Linhoff, 20. T. J. Siddiqui, R. Pancaroglu, Y. Kang, A. Rooyakkers, A. M. Craig, LRRTMs and neurotopographically connected hippocampal neural circuits. *Neuron* 100, 276–293 (2018).
23. T. J. Sudhof, Towards an understanding of synapse formation. *Neuron* 100, 276–293 (2018).
24. T. Biederer, P. S. Kaeser, T. A. Blanpied, Transcellular nanoalignment of synaptic functions. *Neuron* 98, 680–696 (2017).
25. T. J. Sudhof, Synaptic neurexin complexes: A molecular code for the logic of neural circuits. *Cell* 171, 745–769 (2017).
26. D. S. Berns, L. A. DeNardo, D. T. Pederick, L. Luo, Teneurin-3 controls topographic circuit assembly in the hippocampus. *Nature* 554, 328–333 (2018).
27. G. R. Anderson et al., Postsynaptic adhesion GPCR lstrophillin-2 mediates target recognition in entorhinal hippocampal synapse assembly. *J. Cell Biol.* 216, 3881–3846 (2017).
28. M. E. Williams, J. de Wit, A. Ghosh, Molecular mechanisms of synaptic specificity in developing neural circuits. *Neuron* 68, 9–18 (2010).
29. L. Y. Chen, M. Jiang, B. Zhang, O. Gokce, T. C. Sudhof, Conditional deletion of all neurexins defines diversity of essential synaptic organizer functions for neurexins. *Neuron* 94, 611–6254 (2017).
30. L. M. Richter, J. Gjorgieva, Understanding neural circuit development through theory and models. *Curr. Opin. Neurobiol.* 46, 39–47 (2017).
31. J. Ko, The leucine-rich repeat superfamily of synaptic adhesion molecules: LRRTMs and Stricks. *Mol. Cells* 34, 335–340 (2012).
32. R. T. Rollipop et al., Role of LRRTMs in synapse development and plasticity. *Neurosci. Res.* 116, 18–28 (2017).
33. J. Ko, M. V. Fuccillo, R. C. Malenka, T. C. Sudhof, LRRTM2 functions as a neurexin ligand in promoting excitatory synapse formation. *Neuron* 64, 791–798 (2009).
34. J. Ko, G. J. Soler-Llavin, M. V. Fuccillo, R. C. Malenka, T. C. Sudhof, Neuroligin-1 LRRTM1 prevents activity- and Ca2+-dependent synapse elimination in cultured neurons. *J. Cell Biol.* 194, 332–333 (2011).
35. G. J. Soler-Llavin, M. V. Fuccillo, J. Ko, T. C. Sudhof, R. C. Malenka, The neurexin ligands, neuroligins and leucine-rich repeat transmembrane proteins, perform convergent and divergent synaptic functions in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 108, 16502–16509 (2011).
36. G. J. Soler-Llavin et al., Leucine-rich repeat transmembrane proteins are essential for maintenance of long-term potentiation. *Neuron* 79, 439–446 (2013).
37. M. Bhouri et al., Deletion of LRRTM1 and LRRTM2 in adult mice impairs basal AMPA receptor transmission and LTP in hippocampal CA1 pyramidal neurons. *Proc. Natl. Acad. Sci. U.S.A.* 115, E5382–E5389 (2018).
38. M. W. Linhoff et al., An unbiased expression screen for synaptogenic proteins identifies HSRRG as a hippocampal mossy fiber synapse. *Neurosci. J.* 30, 507–522 (2013).
39. N. Apóstolos et al., Synapse type-specific proteinic dissection identifies Isg58 as a hippocampal CA3 microcircuit organizer. *Nat. Commun.* 11, 5171 (2020).
40. A. S. Wilke et al., Deconstructing complexity: Serial block-face electron microscopic analysis of the hippocampal mossy fiber synapse. *Neurosci. J.* 33, 507–522 (2013).
41. S. Chierzi, T. J. Stachiask, E. Trudel, C. W. Bourque, K. M. Murai, Activity maintains structural plasticity of mossy fiber terminals in the hippocampus. *Mol. Cell. Neurosci.* 50, 260–271 (2012).
42. C. D. Velasco, A. Llobet, Synapse elimination activates a coordinated homeostatic synaptic response in an autaptic circuit. *Commun. Biol.* 3, 260 (2020).
43. K. Futai et al., Retrograde modulation of presynaptic release probability through signaling mediated by PSD-95-neuroulin. *Nat. Neurosci.* 10, 186–195 (2007).
44. T. J. McGlach et al., Dentate gyms NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 317, 94–99 (2007).
45. B. E. Bernard et al., Dentate gyms contribute to retrieval as well as encoding: Evidence from context fear conditioning, recall, and extinction. *J. Neurosci.* 37, 6359–6371 (2017).
46. R. R. Rozeske et al., Prefrontal-periaqueductal gray projecting neurons mediate context fear discrimination. *Neuron* 97, 898–910 (2018).
47. A. Grosso, G. S. Santoni, E. Mansaros, A. Renna, B. Sacchetti, A neuronal basis for fear discrimination in the lateral amygdala. *Nat. Commun.* 9, 1214 (2018).
48. D. A. Henze, N. N. Urban, G. Barrionuevo, The multifarious hippocampal mossy fiber pathway: A review. *Neuroscience* 98, 407–427 (2000).
49. I. Galimberti et al., Long-term rearrangements of hippocampal mossy fiber terminal connectivity in the adult regulated by experience. *Neuron* 50, 749–763 (2006).
50. V. Ramirez-Amaya, I. Balderas, J. Sandoval, M. L. Escobar, F. Bermudez-Rattoni, Spatial long-term memory is related to mossy fiber synapticaptogenesis. *J. Neurosci.* 21, 7340–7348 (2001).
51. A. Routtenberg, Adult learning and remodeling of hippocampal mossy fibers: Unheralded participant in circuitry for long-lasting spatial memory. *Hippocampus* 20, 44–45 (2010).
52. M. Agost, T. G. Wensel, LRRTM4 is a member of the transsynaptic complex between rod photoreceptors and bipolar cells. *J. Comp. Neurol.* 529, 221–233 (2021).
53. S. C. Chierzi, T. J. Stachiask, E. Trudel, C. W. Bourque, K. M. Murai, Activity maintains structural plasticity of mossy fiber terminals in the hippocampus. *Mol. Cell. Neurosci.* 50, 260–271 (2012).
54. C. D. Velasco, A. Llobet, Synapse elimination activates a coordinated homeostatic synaptic response in an autaptic circuit. *Commun. Biol.* 3, 260 (2020).
55. K. Futai et al., Retrograde modulation of presynaptic release probability through signaling mediated by PSD-95-neuroulin. *Nat. Neurosci.* 10, 186–195 (2007).
56. T. J. McGlach et al., Dentate gyms NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 317, 94–99 (2007).
57. B. E. Bernard et al., Dentate gyms contribute to retrieval as well as encoding: Evidence from context fear conditioning, recall, and extinction. *J. Neurosci.* 37, 6359–6371 (2017).
58. R. R. Rozeske et al., Prefrontal-periaqueductal gray projecting neurons mediate context fear discrimination. *Neuron* 97, 898–910 (2018).
59. A. Grosso, G. S. Santoni, E. Mansaros, A. Renna, B. Sacchetti, A neuronal basis for fear discrimination in the lateral amygdala. *Nat. Commun.* 9, 1214 (2018).
60. D. A. Henze, N. N. Urban, G. Barrionuevo, The multifarious hippocampal mossy fiber pathway: A review. *Neuroscience* 98, 407–427 (2000).
61. I. Galimberti et al., Long-term rearrangements of hippocampal mossy fiber terminal connectivity in the adult regulated by experience. *Neuron* 50, 749–763 (2006).
62. V. Ramirez-Amaya, I. Balderas, J. Sandoval, M. L. Escobar, F. Bermudez-Rattoni, Spatial long-term memory is related to mossy fiber synapticaptogenesis. *J. Neurosci.* 21, 7340–7348 (2001).
63. A. Routtenberg, Adult learning and remodeling of hippocampal mossy fibers: Unheralded participant in circuitry for long-lasting spatial memory. *Hippocampus* 20, 44–45 (2010).
64. M. R. Holahan, J. L. Rekart, J. Sandoval, A. Routtenberg, Spatial learning induces synaptic structural remodeling in the hippocampal mossy fiber system of two rat strains. *Hippocampus* 16, 560–570 (2006).
65. R. McGrigor, N. Tabatabadz, A. Routtenberg, Selective presynaptic terminal remodeling induced by spatial, but not cued, learning: A quantitative confocal study. *Hippocampus* 22, 1242–1252 (2012).
66. W. Xu, T. C. Sudhof, A neural circuit for memory specificity and generalization. *Sci. 433, 1290–1295 (2013).
67. D. C. Martinelli et al., Expression of CreI3 in discrete neuronal populations controls efferent synapse numbers and diverse behaviors. *Neuron* 91, 1034–1051 (2016).