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mTORC2, but not mTORC1, is required for hippocampal mGluR-LTD and associated behaviors

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Abstract

The mechanistic target of rapamycin complex 1 (mTORC1) has been reported to be necessary for metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD). Here we found that mTORC1-deficient mice exhibit normal hippocampal mGluR-LTD and associated behaviors. Moreover, rapamycin blocks mGluR-LTD in mTORC1-deficient mice. Interestingly, both rapamycin and mGluR activation regulate mTOR complex 2 (mTORC2) activity, and mTORC2-deficient mice show impaired mGluR-LTD and associated behaviors. Thus, mTORC2 is a major regulator of mGluR-LTD.

Keywords

synaptic plasticity; memory; long-term depression; mTOR complexes; raptor; rictor

Activation of group I metabotropic glutamate receptors (mGluRs) at hippocampal CA1 synapses induces a form of LTD (mGluR-LTD) that depends on the synthesis of new proteins[1]. mGluR-LTD is altered in a variety of neurological disorders[1]. Thus, the understanding of the molecular mechanisms underlying mGluR-LTD is of crucial relevance because it could lead to the potential development of new treatments for mGluR-LTD-associated cognitive disorders[1].

The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) is a highly conserved signaling hub integrating a variety of synaptic inputs and a major regulator of protein synthesis rates in neurons[2,3]. The importance of mTORC1 signaling in brain processes is

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Authors Contribution

P. J. Z., C-J. C., J. M. and M.C-M. designed the experiments and wrote the manuscript, P. J. Z. conducted electrophysiology, behavioral and immunoblotting experiments and analyzed data. C-J. C performed behavioral, immunohistochemistry and immunoblotting experiments and analyzed data. J. M. conducted immunoblotting experiments and analyzed data. L. S. performed immunoblotting experiments and analyzed data.

Competing interests

The authors declare no competing financial interests.

underscored by its postulated function in long-lasting forms of synaptic plasticity and many neurological disorders in which mTORC1 activity is perturbed[2,3]. Indeed, mTORC1 has been reported to be necessary for hippocampal mGluR-LTD[1,4]. However, most of the evidence supporting the role of mTORC1 in mGluR-LTD is based on its pharmacological inhibition with the drug rapamycin, but recent results have challenged these findings[5].

To further investigate the role of mTORC1 in mGluR-LTD, we used molecular genetics and conditionally delete *Raptor* (Regulatory associated protein of mTOR), a defining component of mTORC1, Fig. 1a)[6,7], in the murine forebrain by crossing floxed *Raptor* mice with CamKIIα-Cre mice, thus generating mTORC1-deficient mice (see methods). As expected, in the hippocampus and cortex of *Raptor* forebrain knockout (*Raptor* fb-KO) mice, raptor levels and mTORC1 activity—as determined by the phosphorylation its downstream target ribosomal S6—, were significantly reduced compared to control littermates (Fig. 1b–c). Accordingly, immunohistochemistry shows that mTORC1 activity is undetected at CA1 neurons and stratum radiatum from *Raptor* fb-KO mice (Supplementary Fig. 1). However, in the cerebellum, where Cre is expressed only modestly, mTORC1 activity and raptor levels remained unaltered (Fig. 1b–c). Thus, genetic deletion of *Raptor* selectively reduces mTORC1 activity in the forebrain.

To investigate the role of mTORC1 in mGluR-LTD, hippocampal slices from control and *Raptor* fb-KO were treated with the selective mGluR1/5 agonist DHPG (R,S-dihydroxyphenylglycine; 100 μM, 10 min), which is known to reliably induce mGluR-LTD at CA1 synapses[8]. Surprisingly, we found that DPHG resulted in normal depression of field excitatory postsynaptic potentials (fEPSPs) in *Raptor* fb-KO slices, with a magnitude and time course similar to control littermates (Fig. 1d). Accordingly, paired pulse stimulation at low frequency (PP-LFS), elicited a similar mGluR-LTD of synaptic transmission in both control and *Raptor* fb-KO slices (Fig. 1e). It is noteworthy that basal synaptic transmission is normal in hippocampal slices from *Raptor* fb-KO and control littermates, as determined by the analysis of paired-pulse facilitation and input-output relationships (Supplementary Fig. 2). Thus, irrespective of the mGluR-LTD inducing protocol, conditional deletion of mTORC1 in CA1 neurons had no effect on mGluR-induced LTD.

Given the conflicting results regarding the effects of rapamycin on mGluR-LTD[4,5], we treated hippocampal slices from wild-type control mice with different concentrations of rapamycin. Interestingly, mGluR-LTD was insensitive to treatment with low concentrations of rapamycin (20 nM and 200 nM, Supplementary Fig. 3a–b). By contrast, a high concentration of rapamycin (1 μM) prevented mGluR-LTD in control slices (Supplementary Fig. 3c), but had no effect on basal synaptic properties (Supplementary Fig. 4). All concentrations of rapamycin reduced mTORC1 activity (Supplementary Fig. 3d–e), suggesting that the high concentration of rapamycin (1 μM) may block mGluR-LTD in an mTORC1-independent manner. To directly test this possibility, we examined the effect of a high concentration of rapamycin (1 μM) on mGluR-LTD in hippocampal slices from mTORC1-deficient mice. Since rapamycin is reported to be highly specific for mTORC1[9], it is expected to have no effect on mGluR-LTD in *Raptor* fb-KO mice. However, as in control slices (Supplementary Fig. 3c), rapamycin (1 μM) inhibited mGluR-LTD in *Raptor* fb-KO

slices (Fig. 1f). Hence, these data support the notion that the effects of rapamycin on mGluR-LTD at CA1 synapses are independent of mTORC1.

In addition to mTORC1, another structurally and functionally distinct mTOR-containing complex named mTORC2 has been identified more recently[6,7]. While little is known regarding its up-stream regulation and downstream effectors, mTORC2 contains *Rictor* (Rapamycin-insensitive companion of mTOR; Fig. 2a) as an essential component that is largely insensitive to acute rapamycin treatment[6,7]. However, in cancer cells, prolonged rapamycin treatment[10] or higher concentrations of rapamycin[11] suppress mTORC2 activity. Could mTORC2, but not mTORC1, be the major regulator of mGluR-LTD in the mammalian brain? We began addressing this question by examining whether mGluR activation engages mTORC2 function. We found that treatment with DHPG (100 μM, 10 min) increased the activity of mTORC2, as determined by the phosphorylation of its downstream target Akt at Ser-473, a reliable readout of mTORC2 activity[6,7] (Supplementary Fig. 5a–b).

We next asked whether the high concentration of rapamycin (1 μM) sufficient to suppress mGluR-LTD would also block mTORC2 activity in control slices. Indeed, we found that high (1 μM), but not low (20 nM and 200 nM), concentrations of rapamycin reduced mTORC2 activity (Supplementary Fig. 5c–d). To investigate whether mTORC2 is required for mGluR-LTD at CA1 synapses, we studied mTORC2-deficient mice, in which *Rictor* (mTORC2's defining component) was conditionally deleted in the murine forebrain postnatally (*Rictor* fb-KO mice)[12]. As we have previously shown, mTORC2 activity is selectively reduced in the hippocampus from *Rictor* fb-KO mice (Fig. 2b–c) and basal synaptic transmission is not altered in these mice[12]. As expected, DHPG induced a typical LTD of fEPSPs in control slices (Fig. 2d). However, in *Rictor* fb-KO slices, the same stimulation protocol failed to elicit mGluR-LTD (Fig. 2d). In agreement with these observations, synaptic induction of mGluR-LTD with PP-LFS was also impaired in *Rictor* fb-KO slices (Fig. 2e). Moreover, a high concentration of rapamycin (1 μM) did not further reduce mGluR-LTD in *Rictor* fb-KO slices (Fig. 2f). Taken together, our results indicate that mTORC2, but not mTORC1, is required for mGluR-LTD.

mGluR-LTD contributes to different types of hippocampal learning and memory processes. Specifically, spatial recognition of objects has been reported to trigger a long-lasting hippocampal LTD at Schaffer collateral CA1 synapses in freely-moving animals (during training, see Fig. 3a)[13,14]. Re-exposure to the same objects on the following day is associated with reduced exploration time and absence of LTD *in vivo*[13,14]. Inhibition of mGluR receptors immediately before exposure to novel objects (training) blocks LTD and the concomitant reduction in re-exploration during re-exposure[14], indicating that this *in vivo* LTD depends, at least in part, on mGluR receptors. Because mGluR-LTD is impaired in slices from mTORC2-deficient mice, we next examined whether spatial recognition is also deficient in these mice. Indeed, *Rictor* fb-KO mice spent more time exploring the same objects compared to control littermates (Fig. 3b), indicating that mTORC2 is required for learning mediated by hippocampal mGluR-LTD. Accordingly, mTORC2-deficient mice were also impaired in novel object recognition (Fig. 3e), another hippocampal LTD-inducing task[14] (Fig. 3d). The impaired hippocampal mGluR-LTD-mediated behavior in mTORC2-

deficient mice is not caused by non-specific exploratory responses because distance travelled and exploratory behavior were similar between control and *Rictor* fb-KO mice (Supplementary Fig. 6a–b).

Because mGluR-LTD is not altered in mTORC1-deficient mice, we predicted that mGluR-LTD-related behaviors should be normal in these mice. Consistent with this prediction, we found that both spatial recognition (Fig. 3c) and object recognition (Fig. 3f) are normal in *Raptor* fb-KO mice, indicating that hippocampal mGluR-LTD and correlated behaviors do not depend on mTORC1. Finally, chronic rapamycin treatment, inhibit both mTORC1 and mTORC2[15], blocks spatial and object recognition (Supplementary Fig. 7). Thus, rapamycin-treated mice resemble *Rictor* fb-KO mice, but not *Raptor* fb-KO mice with respect to their requirement for mGluR-LTD associated behaviors. Taken together, these data indicate that mTORC2, but not mTORC1, is required for hippocampal mGluR-LTD and associated behaviors.

mGluR-LTD is dependent on new protein synthesis[16]. While mTORC1 is a major regulator of protein synthesis in the brain[2,3], our results indicate that the translational program underlying mGluR-LTD at CA1 synapses is independent of mTORC1. Regulation of protein synthesis at the levels of i) initiation, by the translation initiation factor eIF2α[17], or ii) elongation[18], may better explain this protein synthesis-dependent form of synaptic plasticity. While not required for hippocampal mGluR-LTD, mTORC1 is necessary for hippocampal long-term potentiation (LTP), another major form of synaptic plasticity in the mammalian brain, and related behaviors[19] (but also see[20]). Thus, we propose that mTORC1 at CA1 synapses is selectively required for protein synthesis-dependent increases (LTP), but not decreases (LTD), in synaptic efficacy.

Finally, mGluR-LTD is altered in a variety of neurological disorders including autism spectrum disorders, intellectual disability, Alzheimer's disease, epilepsy and drug addiction[1]. In the last few years, the study of the molecular mechanisms implicated in mGluR-LTD has led to the development of "mechanism-based treatments" for some of these disorders. Unexpectedly, our results support the notion that mTORC2, but not mTORC1, is the major mTOR complex driving mGluR-LTD in the adult mammalian brain. Thus, modulation of mTORC2 may emerge as promising new avenues for the treatment of mGluR-LTD-related disorders.

Online Methods

Mouse husbandry

All experiments were conducted on 3–6 month old male and female mice from the C57Bl/6 background. *Raptor*$^{loxP/loxP}$ mice were purchased from Jackson laboratory (Stock# 013188) and crossed with mice expressing *Cre* recombinase under the control of the α subunit of calcium/calmodulin-dependent protein kinase II (*Camk2α*) promoter (*Camk2α-Cre*)[21,22], thus generating *Raptor* fb-KO mice. Mice were weaned at the third postnatal week and genotyped by PCR. Raptor mutant and wild-type alleles were detected by PCR assay in which primer F11117 (5′-CTCAGTAGTGGTATGTGCTCA-3′) and primer R11118 (5′-GGGTACAGTATGTCAGCACAG-3′) amplify a 141 base pair fragment (wild type) and a

180 base pair fragment (exon 6 of the *Raptor* conditional allele). Cre expression was detected by PCR with primers CreF3 (5′-GGCCCAGCTTTCTCATATTTG-3′) and CreR3 (5′-'TCAGCTACACCAGAGACG -3′), which amplify a 488 base pair fragment. *Rictor* fb-KO mice were previously described[12]. Mice were kept on a 12h/12h light/dark cycle (lights on at 7:00 am) and had access to food and water *ad libitum*. Animal care and experimental procedures were approved by the institutional animal care and use committee (IACUC) at Baylor College of Medicine, according to NIH Guidelines.

Slice electrophysiology

Electrophysiological recordings were performed, as previously described[12,23]. The investigators were blind to the mouse genotypes. Briefly, horizontal hippocampal slices (320 μm thick) were cut with a vibratome (Leica VT 1000S, Leica Microsystems, Buffalo Grove, IL) at 4°C in artificial cerebrospinal fluid solution (ACSF; 95% O_2 and 5% CO_2) containing in mM: 124 NaCl, 2.0 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 10 glucose (2–3 ml/min). Slices were incubated for at least 60 min prior to recording in an interface chamber and continuously perfused with artificial cerebrospinal fluid (ACSF) at 30°C and a flow rate of 2–3 ml/min. The recording electrodes were placed in the stratum radiatum. Field excitatory postsynaptic potentials (fEPSPs) were recorded with ACSF-filled micropipettes, and were elicited by bipolar stimulating electrodes placed in the CA1 stratum radiatum to excite Schaffer collateral and commissural fibers. The intensity of the 0.1-ms pulses was adjusted to evoke 40–50% of maximal response. A stable baseline of responses at 0.033 Hz was established for at least 30 min. mGluR-LTD was induced by bath-application of DHPG (100 μM) for 10 min or by pairing stimuli (interstimulus interval, 50 ms) delivered at 1 Hz for 15 min (900 pulses; PP-LFS), as previously described[24]. For the experiments with rapamycin, hippocampal slices were pre-incubated in the recording chamber with rapamycin (20 nM, 200 nM or 1μM) for 30 min before DHPG application and rapamycin was kept throughout the recording. All data are presented as means ± sem and “n” refers to both the number of slices and the number of mice. All drugs were obtained from Tocris (Ellisville, MO).

Western Blotting

The hippocampus, cortex and cerebellum from control and *Raptor* fb-KO mice were isolated, homogenized in cold homogenizing buffer [200 mM HEPES, 50 mM NaCl, 10% Glycerol, 1% Triton X-100, 1 mM EDTA, 50 mM NaF, 2 mM Na_3VO_4, 25 mM β-glycerophosphate, and EDTA-free complete ULTRA tablets (Roche, Indianapolis, IN)] and centrifuged at 13,000 × *g* for 10 min. The supernatants (of 30 μg of protein/sample) were resolved on SDS–PAGE (10%) and transferred onto nitrocellulose membranes (Pall, Port Washington, NY). Treatment with DHPG followed by biochemical analysis, was performed as previously described[4]. Briefly, after treatments, slices were snap-frozen on dry ice, then suspended in lysis buffer and analyzed by western blotting, which was performed as we previously described[12,19]. Antibodies against p-S6 (1:1000, Ser240/244 #5364), p-Akt (1:1000, Ser473 #9271), total S6 (1:1000 #2217), total Akt (1:1000 #9272), raptor (1:1000 #2280), and Rictor (1:1000 #2114) were purchased from Cell Signaling and Technology Laboratories (Danvers, MA) and β-actin (1:5000 #1501) from Millipore (Temecula, CA).

Spatial and Object Recognition

The investigators performing and scoring the behavior were blind to the genotype and treatment. Spatial and object recognition were performed as previously described[13,14,17], with only slight modifications. For all behavioral experiments, we included similar numbers of male and female mice for each genotype. No differences were between males and females were found (data not shown). Mice were handled for 5–10 min and habituated ("Habituation") to a black Plexiglas rectangular chamber (31 × 24 cm, height 27 cm) for 10 min under dim ambient light for 3 days. Exploration of the objects was defined as sniffing of the objects (with nose contact or head directed to the object) within at 2 cm radius of the objects. Sitting or standing on the objects was not scored as exploration. Behavior was recorded from cameras positioned above the training chamber. Data are expressed as a percentage of re-exploration ("Re-exposure) relative to the initial exploration time (during "Training").

For novel object recognition training, two identical objects were presented to mice to explore for 5 min, after which, mice were returned to the home cage. Twenty-four hours later, one object was replaced by one novel object and the mouse was again placed in the chamber 5 min. The novel object has the same height and volume but different shape and appearance. Discrimination Index (DI) was computed as, DI = (Novel Object Exploration Time – Familiar Object Exploration Time/Total Exploration Time) × 100. To control for odor cues, the open field arena and the objects were thoroughly cleaned with ethanol, dried, and ventilated between mice.

Immunofluorescence

Mice were anesthetized by isoflurane and perfused transcardially with cold 0.9% phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Brain samples were then post-fixed in 4% paraformaldehyde at 4°C overnight and cyroprotected in 30% sucrose in BPS for 3 days. Free-floating frozen sagittal sections (25 μm) were incubated in blocking solution (10% normal goat serum, 0.3% Triton X-100, 0.01% Sodium-Azide in PBS) for 1 hour at RT and then transfer into diluted primary antibodies (mouse anti-NeuN, Abcam #104224,1:500; rabbit anti-pS6-Ser$^{240/244}$, Cell Signaling Technology #5364, 1:300) for incubation overnight. Primary antibodies were visualized using florescence-conjugated antibodies (1:1000, goat anti-rabbit Alexa Fluor 488, ThermoFisher Scientific, #A-11034; goat anti-mouse Alexa Fluor 594, ThermoFisher Scientific, #A-11032). Image acquisition and processing was performed as we previously described[25].

Rapamycin administration

Mice received intraperitoneal (i.p) injections of rapamycin (10 mg/kg, LC Laboratories, Woburn MA) or vehicle (4% ethanol, 4% Tween-80, and 4% PEG-400) daily for six days before re-exposure or novel object exposure sessions of behaviors tests.

Statistical analyses

No statistical methods were used to pre-determine sample sizes, but our sample sizes are selected base on previous studies published in the field (see Life Science Reporting Summary for references). Animals in the same litter were randomly assigned to different

treatment groups in various experiments. No animals or data points were excluded from the analysis. Normality test and F-test of equality of variances were performed before choosing statistical test. Statistics were based on two-sided Student's *t*-test or Mann-Whitney Rank Sum test for two-group comparisons (for data sets that were not normally distributed). One-way ANOVA followed by Bonferroni *post hoc* analysis was performed for multiple comparisons, unless otherwise indicated. $P < 0.05$ was considered significant ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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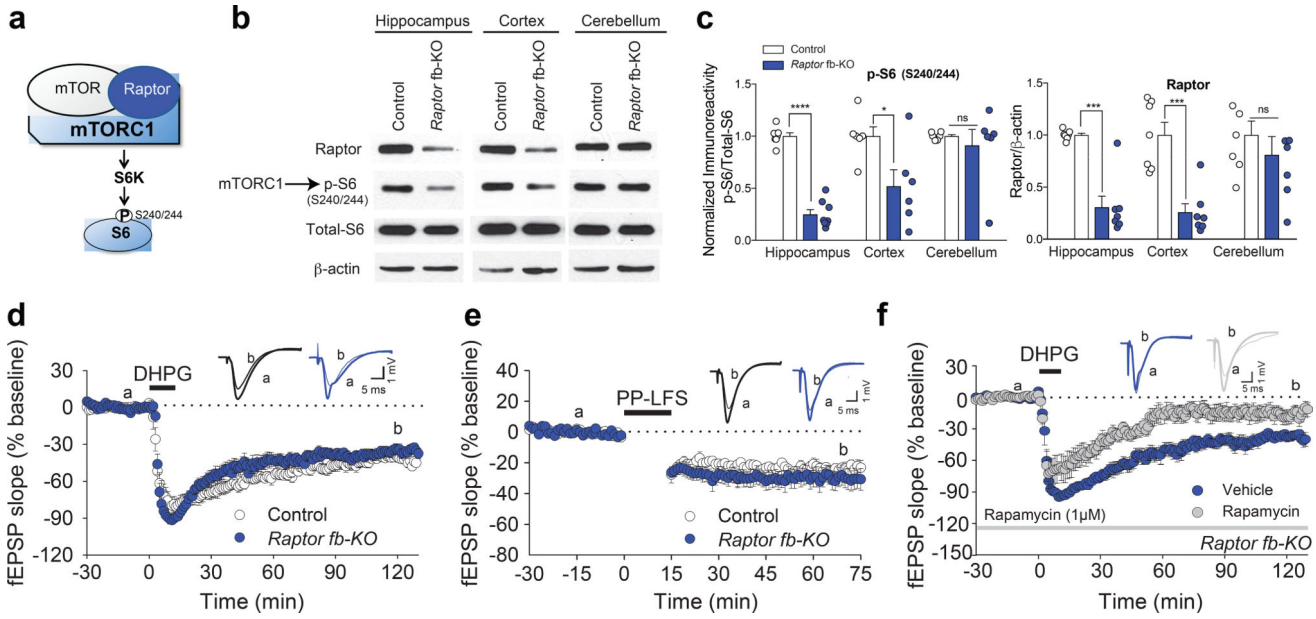

Figure 1. Hippocampal mGluR-LTD is normal in mTORC1-deficient mice, but is sensitive to rapamycin

(**a**) Schematic of mTOR complex 1 (mTORC1). (**b–c**) Representative western blots (**b**) and quantification (**c**) show reduced raptor levels and mTORC1 activity (p-S6) in hippocampus and cortex, but not cerebellum, of *Raptor* fb-KO mice (p-S6-Ser$^{240–244}$: hippocampus control n = 7, *Raptor* fb-KO n = 8, $t_{13} = 13.53$, $P < 0.0001$; cortex control n = 6, *Raptor* fb-KO n = 6, $t_{10} = 2.69$, $P < 0.0244$, cerebellum control n = 6, *Raptor* fb-KO n = 6, $t_{10} = 0.58$, $P = 0.57$; Raptor: hippocampus control n = 7, *Raptor* fb-KO n = 7, $t_{12} = 6.72$, $P < 0.001$, cortex control n = 7, *Raptor* fb-KO n = 7, $t_{12} = 5.03$, $P < 0.001$, cerebellum control n = 6, *Raptor* fb-KO n = 6, $t_{10} = 0.58$, $P = 0.59$).

(**d–e**) LTD induced either with DHPG (**d**; 100 μM, 10 min; control $n = 12$, *Raptor* fb-KO mice $n = 8$, LTD magnitude during last 10 min: control = −43.1 ± 2.4; *Raptor* fb-KO mice = −37.9 ± 3.7, $t_{18} = 1.354$, $P = 0.193$) or paired pulses of low frequency stimulation (**e**, PP-LFS, pairs of pulses, 50 ms interval, delivered at 1Hz, 900 pulses; control $n = 7$, *Raptor* fb-KO mice $n = 12$, LTD magnitude during last 10 min: control = −25.1 ± 4.5; *Raptor* fb-KO mice = −32.1 ± 3.4, $t_{17} = 1.354$, $P = 0.583$) is intact in *Raptor* fb-KO mice.

(**f**) DHPG-induced LTD in *Raptor* fb-KO is sensitive to rapamycin (1 μM; vehicle $n = 8$; rapamycin $n = 7$; LTD magnitude during last 10 min: vehicle = −35.1 ± 3.8; rapamycin = −17.9 ± 5.9, Mann-Whitney Rank Sum test, $U = 5.0$, $P < 0.01$). Horizontal bars indicate period of drug application or synaptic stimulation. (Inset) Superimposed traces obtained before (a) and after (b) stimulation. All data are presented as mean ± SEM. Statistics were based on two-sided Student's *t*-test unless otherwise specified. ns is not significant. Images of western blots were cropped to show only representative figures. Full-length blots can be found in Supplementary Fig. 8

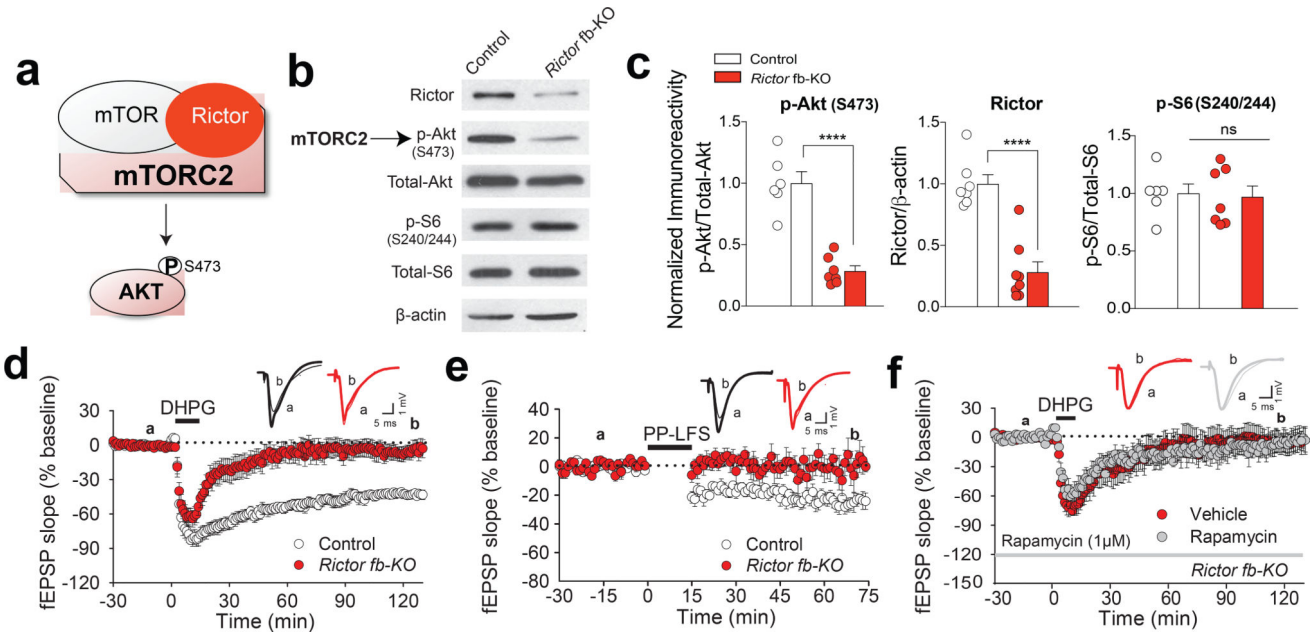

Figure 2. Hippocampal mGluR-LTD is impaired in mTORC2-deficient mice

(**a**) Schematic of mTOR complex 2 (mTORC2). (**b–c**) Representative western blots (**b**) and quantification (**c**) show reduced Rictor levels and mTORC2 activity (p-Akt-Ser^{473}), but not mTORC1 activity (p-S6-Ser$^{240–244}$), in the hippocampus of *Rictor* fb-KO mice (p-Akt-Ser473: control n = 6, *Rictor* fb-KO n = 7, $t_{11} = 7.27$, $P < 0.0001$; *Rictor*: control n = 7, *Rictor* fb-KO n = 8, $t_{13} = 6.31$, $P < 0.0001$; p-S6-Ser$^{240–244}$: control n = 6, *Rictor* fb-KO n = 7: $t_{11} = 0.23$, $P = 0.82$). (**d–e**) LTD induced either with DHPG (**d**; 100 μM, 10 min; control n = 10; *Rictor* fb-KO mice n = 12, LTD magnitude during last 10 min: control = −44.3 ± 2.5; *Rictor* fb-KO mice = −9.1 ± 5.9, $t_{20} = 6.113$, $P < 0.001$) or paired pulses of low frequency stimulation (**e**, PP-LFS, pairs of pulses, 50 ms interval, delivered at 1Hz, 900 pulses; control n = 9; *Rictor* fb-KO mice n = 8, LTD magnitude during last 10 min: control = −25.7 ± 4.3; *Rictor* fb-KO mice = 2.5 ± 8.7, $t_{15} = 2.989$, $P = 0.009$) is impaired in *Rictor* fb-KO mice. (**f**) DHPG-induced LTD in *Rictor* fb-KO mice is not further reduced by rapamycin (1 μM; vehicle n = 7, rapamycin, n = 6, LTD magnitude during last 10 min: vehicle = −7.2 ± 2.7; rapamycin = −5.9 ± 8.1, $t_{11} = 0.128$, $P = 0.91$). (Inset) Superimposed traces obtained before (a) and after (b) stimulation. All data are presented as mean ± SEM. The statistics were based on two-sided Student's t-test. ns is not significant. Images of western blots were cropped to show only representative figures. Full-length blots can be found in Supplementary Fig. 8

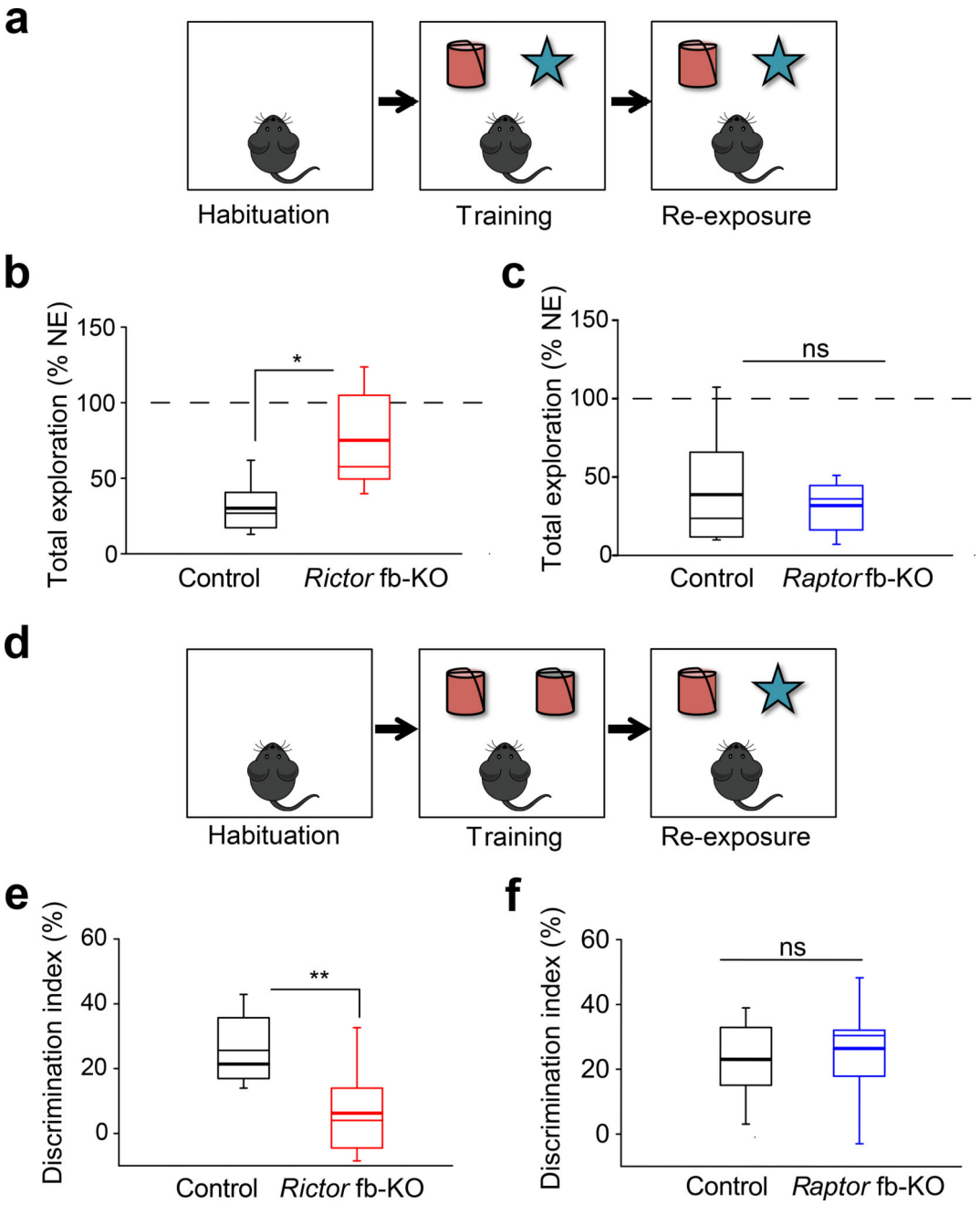

Figure 3. mTORC2, but not mTORC1, is required for hippocampal-mediated mGluR-LTD related behavior

(**a**) Experimental paradigm of the spatial recognition task. After habituating in an empty box, mice are exposed to two novel objects on the following day (training), and then re-exposed to the same objects 24 hours later (re-exposure). (**b**) During re-exposure, *Rictor* fb-KO mice ($n = 10$) spent more time exploring the objects than control littermates ($n = 13$; Mann-Whitney Rank Sum test, $U = 13.0$, $P < 0.001$). (**c**) Hippocampal-dependent spatial recognition is intact in *Raptor* fb-KO mice ($n = 8$) as they spend similar times as control littermates ($n = 10$) exploring the objects during re-exposure (Mann-Whitney Rank Sum test,

$U = 61$, $P = 0.828$). (**d**) Experimental design for the object recognition task. (**e**) *Rictor* fb-KO mice ($n = 11$) show significantly less preference for novel objects than control littermates ($n = 12$; Mann-Whitney Rank Sum test, $U = 18$, $P < 0.001$). (**f**) Object recognition is intact in *Raptor* fb-KO mice ($n = 11$) as they spend similar times as control littermates ($n = 12$) exploring the novel objects during re-exposure (two-sided Student's t-test, $t_{21} = 0.613$, $P = 0.547$). Box plots show the minimum, maximum, median, 25th, and 75th percentile of the groups. The mean values are indicated in thick lines.