Disrupted Homer scaffolds mediate abnormal mGluR5 function in a mouse model of fragile X syndrome

Jennifer A Ronesi1,4, Katie A Collins1,4, Seth A Hays1,4, Nien-Pei Tsai1, Weirui Guo1, Shari G Birnbaum2, Jia-Hua Hu3, Paul F Worley3, Jay R Gibson1 & Kimberly M Huber1

Enhanced metabotropic glutamate receptor subunit 5 (mGluR5) function is causally associated with the pathophysiology of fragile X syndrome, a leading inherited cause of intellectual disability and autism. Here we provide evidence that altered mGluR5-Homer scaffolds contribute to mGluR5 dysfunction and phenotypes in the fragile X syndrome mouse model, Fmr1 knockout (Fmr1−/−). In Fmr1−/− mice, mGluR5 was less associated with long Homer isoforms but more associated with the short Homer1a. Genetic deletion of Homer1a restored mGluR5–long Homer scaffolds and corrected several phenotypes in Fmr1−/− mice, including altered mGluR5 signaling, neocortical circuit dysfunction and behavior. Acute, peptide-mediated disruption of mGluR5-Homer scaffolds in wild-type mice mimicked many Fmr1−/− phenotypes. In contrast, Homer1a deletion did not rescue altered mGluR-dependent long-term synaptic depression or translational control of target mRNAs of fragile X mental retardation protein, the gene product of Fmr1. Our findings reveal new functions for mGluR5-Homer interactions in the brain and delineate distinct mechanisms of mGluR5 dysfunction in a mouse model of cognitive dysfunction and autism.

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability and a leading genetic cause of autism1,2. FXS is caused by transcriptional silencing of the FMR1 gene, which encodes fragile X mental retardation protein Homer10. The N-terminal EVH1 (Ena-VASP homology) domain of Homer proteins binds the intracellular C-terminal tail of group 1 mGluRs (mGluR5 and mGluR1a) and affects their trafficking, localization and function11. Long, constitutively expressed forms of Homer (Homer1b, 1c, 2 and 3) multimerize through their C-terminal coiled-coil domains and localize mGluRs to the PSD through interactions with Shank (SH3 and multiple ankyrin repeat domains protein), as well as link mGluRs to signaling pathways through Homer interactions with phosphoinositide-3 kinase enhancer (PIKE), elongation factor 2 kinase (EF2K) and the inositol-1,4,5-trisphosphate receptor11,12. Homer1a (H1a), a short, activity-inducible form of Homer, lacks the coiled-coil domain and cannot multimerize with other Homers. Consequently, H1a disrupts mGluR5–long Homer complexes, alters mGluR signaling and causes constitutive, agonist-independent activity of mGluR1 and mGluR5 (ref. 13).

In Fmr1−/− mice, mGluR5 is less associated with the long Homer isoforms and more associated with H1a (ref. 10). We hypothesized that the altered balance in mGluR5 interactions with Homer isoforms contributes to the mGluR5 dysfunction and pathophysiology of FXS. To test this hypothesis, we crossed Fmr1−/− mice with mice selectively lacking the H1a isoform of Homer1 (H1a−/−) and determined whether H1a deletion restored mGluR5 function and Homer interactions, as well as neurophysiological and behavioral phenotypes of Fmr1−/− mice. In addition, we determined whether acute peptide-mediated disruption of mGluR5-Homer scaffolds in wild-type (WT) mice

1Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas, USA. 2Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, Texas, USA. 3The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. 4These authors contributed equally to this work. Correspondence should be addressed to K.M.H. (kimberly.huber@utsouthwestern.edu).

Received 19 October 2011; accepted 14 December 2011; published online 22 January 2012; doi:10.1038/nn.3033
Supplementary Figure 7. The conditions as indicated. Right: group data for each protein (ratio of phosphorylated/total, normalized to basal, or untreated, slices from the same pretreated with the CT or MU peptide as indicated. Left: representative western blots of each phosphorylated and total protein, as well as (implicate different mechanisms of mGluR5 dysfunction in distinct and Homer (mice; (lent to mGluR5 that cannot interact with any Homer isoform 13–15.

ALTPPSPFR)14,16,17 binds the EVH1 domain of Homer, mGluR5CT (YGRKKRRQRRR-
the proline-rich motif (PPXXF) of the mGluR5 C-terminal tail that
To disrupt mGluR5-Homer, we incubated acute hippocampal slices

Figure 1 Peptide-mediated disruption of mGluR5-Homer scaffolds in WT mouse hippocampus bidirectionally regulates group 1 mGluR signaling to translation initiation and elongation. (a) Pretreatment of acute hippocampal slices from WT mice with mGluR5CT peptide (CT; 5 h; 5 µM) reduced mGluR5-Homer interactions as determined using coimmunoprecipitation (IP) with a pan-Homer antibody and immunoblotting (IB) for mGluR5.

A control peptide, mGluR5MU (MU; 5 h; 5 µM), had no effect on mGluR5-Homer co-IP in comparison to untreated (−) slices. One-half of the input for the co-IP was run on a separate blot (bottom). (b–e) Disruption of mGluR5-Homer interaction altered signaling to translation. Western blots of (b) phosphorylation (P-) of mTOR on Ser2448, (c) phosphorylation of S6K on Thr389, (d) phosphorylation of ERK on Thr202 and Tyr204 and (e) phosphorylation of EF2 on Thr56, in the basal (B) condition and DHPG (D) treated hippocampal slices (100 µM; 5 min) from WT mice. Slices were pretreated with the CT or MU peptide as indicated. Left: representative western blots of each phosphorylated and total protein, as well as β-tubulin, in the conditions as indicated. Right: group data for each protein (ratio of phosphorylated/total, normalized to basal, or untreated, slices from the same mouse). n = 4–15 slices per condition from 3–8 mice. * P < 0.05; ** P < 0.01; error bars, s.e.m. Full-length western blots for this figure are shown in Supplementary Figure 7.

RESULTS
Disruption of mGluR5-Homer regulates signaling to translation
To investigate whether the altered mGluR5-Homer scaffolds contribute to the mGluR5 dysfunction in Fmr1<sup>−/−</sup> mice, we determined whether disruption of mGluR5-Homer scaffolds with a peptide in WT mice mimics mGluR5 signaling alterations in the Fmr1<sup>−/−</sup> mice. The rationale for this approach is based on data that H1a-bound mGluR5, which is increased in the Fmr1<sup>−/−</sup>, is functionally equivalent to mGluR5 that cannot interact with any Homer isoform<sup>13–15</sup>. To disrupt mGluR5-Homer, we incubated acute hippocampal slices from WT mice in a cell-permeable (Tat-fused) peptide containing the proline-rich motif (PPXXF) of the mGluR5 C-terminal tail that binds the EVH1 domain of Homer, mGluR5CT (YGRKKRRQRRR-ALTPPSPFR)<sup>14,16,17</sup>, mGluR5CT reduced mGluR5-Homer interactions to 41 ± 6% of that observed in slices with no peptide treatment (n = 3 mice; P = 0.003) as determined by coimmunoprecipitation of mGluR5 and Homer (Fig. 1a). Notably, mGluR5CT peptide treatment roughly mimicked the 50% decrease in mGluR5–long Homer interaction observed in Fmr1<sup>−/−</sup> hippocampal lysates (described below). As a control, slices were incubated in a peptide with a mutated Homer binding motif, mGluR5MU (YGRKKRRQRRR-ALTPPLSPRR)<sup>14,16</sup>, mGluR5MU peptide had no effect on mGluR-Homer by comparison with slices with no peptide treatment (103 ± 8% of untreated; n = 3; Fig. 1a).

Fmr1<sup>−/−</sup> mice show a deficit in stimulation of protein synthesis by mGluR1 and mGluR5 that may be a result of altered mGluR5-Homer interactions. Previously, we reported that mGluR5CT peptide-mediated disruption of mGluR5-Homer interactions in rat hippocampal slices inhibits group 1 mGluR activation of the phosphoinositide-3 kinase (PI3K)–mammalian target of rapamycin (mTOR) protein kinase pathway and of translation initiation. However, mGluR5CT does not inhibit mGluR activation of the mitogen–activated protein kinase 1 (ERK) pathway<sup>17</sup>. Here we observed similar effects in hippocampal slices from WT mice. mGluR5CT peptide incubation blocked activation of PI3K-mTOR pathway in response to the group 1 mGluR agonist (RS)-3,5-dihydroxypbenylglycine (DHPG; 100 µM; 5 min) as measured by phosphorylation of mTOR on Ser2448 and p70 ribosomal S6 kinase (S6K) on Thr389 (phospho-mTOR: mGluR5MU: 196 ± 27% of basal, n = 8; mGluR5CT: 94 ± 20% of basal, n = 8; P < 0.01; phospho-S6K: mGluR5MU: 300 ± 80% of basal, n = 16; mGluR5CT: 114 ± 13% of basal, n = 6; P < 0.05; Fig. 1b,c) and had no effect on activation of the ERK pathway as measured by phosphorylation of ERK on Thr202 and Tyr204 (mGluR5MU: 495 ± 117% of basal, n = 4; mGluR5CT: 477 ± 162% of basal, n = 4; not significant; Fig. 1d)<sup>17</sup>. The mGluR5CT peptide did not affect basal levels of phospho-mTOR, phospho-S6K or phospho-ERK (Supplementary Table 1)<sup>18</sup>.

Homer and mGluR5 each directly interact with another translational regulatory factor, EF2K<sup>12</sup>. Although phosphorylation of elongation factor 2 (EF2) by EF2K inhibits translation elongation globally, EF2K is required for translational activation of mRNAs...
such as Arc (activity-regulated cytoskeleton-associated protein) and Camk2a (calcium/calmodulin-dependent kinase IIα)\(^2\). A moderate inhibition of elongation globally by EF2K is thought to release translation factors that are then available for translational activation of poorly initiated transcripts. Unexpectedly, mGluR5CT-treated slices showed a robust increase in phosphorylation of EF2 on Thr56 in response to DHPG (590 ± 118% of basal; n = 15 slices, Fig. 1e) in comparison to mGluR5MU-treated slices (294 ± 63% of basal; n = 15; P < 0.05). Similar results were obtained in rat hippocampal slices (Supplementary Fig. 1). The mGluR5CT peptide did not affect basal levels of phospho-EF2 (Supplementary Table 1). Taken together, our data suggest that Homer interactions facilitate mGluR activation of the PI3K-mTOR pathway to translation initiation, but dampen mGluR-induced phosphorylation of phospho-EF2 and thus restrain inhibition of global elongation rates. Consequently, disruption of mGluR5-Homer would be expected to block mRNA translation by blocking activation of the PI3K-mTOR pathway and translation initiation and enhancing inhibition of elongation to a level that may block elongation of all transcripts, including Arc. Consistent with this model, disrupting mGluR5-Homer interactions in hippocampal slices with mGluR5CT peptide blocked DHPG-induced synthesis of Arc (mGluR5MU: 122 ± 5% of untreated, n = 7; mGluR5CT: 95 ± 7% of untreated, n = 7; Supplementary Fig. 1) and elongation factor 1α (EF1α)\(^1\).

**Deletion of Homer1a rescues mGluR signaling in Fmr1\(^{-/-}\)**
mGluR5–long Homer interactions are reduced in Fmr1\(^{-/-}\) mice, and we hypothesize that this contributes to altered mGluR5 function. If so, then mGluR5 signaling to translation in Fmr1\(^{-/-}\) slices may mimic what is observed with mGluR5CT peptide treatment of WT slices (Fig. 1). In support of our hypothesis, DHPG-induced activation of PI3K, mTOR and S6K is deficient in Fmr1\(^{-/-}\) hippocampal slices, and ERK activation is unaffected\(^18–20\) (Fig. 2b). Furthermore, DHPG-induced phosphorylation of EF2 was enhanced in Fmr1\(^{-/-}\) slices relative to that in slices from WT littermates (WT: 787 ± 135% of untreated, n = 7 mice; Fmr1\(^{-/-}\): 1,452 ± 176% of untreated, n = 6 mice; P < 0.001; error bars, s.e.m. Full-length western blots for this figure are shown in Supplementary Figure 8). Notably, these alterations in mGluR signaling parallel what was observed with mGluR5CT peptide treatment of WT slices (Fig. 1). Basal levels of phosphorylated or total EF2 or S6K were unchanged in Fmr1\(^{-/-}\) slices (Supplementary Table 2)\(^18\). These results indicate that mGluR5 function is not generally enhanced or decreased in Fmr1\(^{-/-}\) mice, but is changed in a complex way that is mimicked in WT mice by disruption of mGluR5-Homer interactions.

In Fmr1\(^{-/-}\) mice, mGluR5 is more associated with H1a (ref. 10; Supplementary Fig. 2a). Because long Homers compete with H1a for interactions with their effectors\(^14,15\), we hypothesized that genetic deletion of H1a in Fmr1\(^{-/-}\) mice may restore normal mGluR5–long Homer interactions and mGluR5 function. To test this idea, we bred Fmr1\(^{-/-}\) mice with mice with a genetic deletion of H1a to create H1a\(^{-/-}\)/Fmr1\(^{-/-}\) double knockout mice\(^21\). H1a\(^{-/-}\) mice have normal levels of long Homer isoforms 1, 2 and 3 (ref. 21). In agreement with previous results\(^10\), communoprecipitation of long Homer isoforms from Fmr1\(^{-/-}\) forebrain revealed a reduced association of mGluR5 to that in WT littermates (Fig. 2a), whereas communoprecipitation of H1a revealed an increased association with mGluR5 (Supplementary Fig. 2). Total levels of mGluR5 and long Homer proteins and of H1a protein and mRNA in Fmr1\(^{-/-}\) hippocampi were not different from those in WT (Fig. 2a and Supplementary Fig. 2). Genetic deletion of H1a restored normal mGluR5–Homer interactions in Fmr1\(^{-/-}\) mice (n = 4 mice per genotype; Fig. 2a) but did not affect levels of mGluR5, long Homers or their interactions on a WT background (Fig. 2a and Supplementary Fig. 2)\(^21\). To determine whether H1a deletion restored normal mGluR5 signaling in Fmr1\(^{-/-}\) mice, we examined mGluR5 signaling to mTOR and EF2K translational regulatory pathways. The deficit in mGluR5 signaling to mTOR in the Fmr1\(^{-/-}\), as measured by S6K Thr389 phosphorylation (WT: 242 ± 34% of basal, n = 21; Fmr1\(^{-/-}\): 135 ± 15%; n = 19; Fig. 2b), was restored in the H1a\(^{-/-}\)/Fmr1\(^{-/-}\) (220 ± 28% of untreated, n = 18). Similarly, enhanced mGluR activation of EF2K was rescued to WT levels by...
**H1a deletion (H1a\(^{-/-}\) Fmr1\(^{-/-}\), 826 ± 192% of basal, n = 7; Fig. 2c).** H1a\(^{-/-}\) mice showed normal DHPG-induced phosphorylation of EF2 (796 ± 159% of untreated, n = 6) and S6K (227 ± 34% of treated, n = 14). There was no effect of Fmr1 or H1a genotype on basal levels of phosphorylated or total EF2 or S6K (Supplementary Table 2). Furthermore, DHPG treatment did not alter levels of total EF2 or S6K in any genotype (Supplementary Table 2)\(^{17,19}\).

**H1a deletion rescues enhanced translation rates in Fmr1\(^{-/-}\).** Although DHPG-induced translation is absent in Fmr1\(^{-/-}\) mice, basal translation rates in brain are elevated\(^{2,8,20,22}\). Enhanced protein synthesis in hippocampal slices was reversed by pharmacological blockade of mGluR5 or of ERK activation, but not by an inhibitor of PI3K or of mTOR\(^{20}\) (Fig. 3 and Supplementary Fig. 3). Another consequence of mGluR5-H1a interactions is constitutive, or agonist-independent, mGluR5 activity\(^{13}\), which may drive translation rates through ERK activation, a pathway that remains intact in Fmr1\(^{-/-}\) mice and with Homer disruption (Fig. 1d)\(^{18,20}\). In support of this hypothesis, genetic deletion of H1a rescued enhanced translation rates as measured by incorporation of 35S-labeled methionine and cysteine in proteins in hippocampal slices (Fmr1\(^{-/-}\); 122 ± 4% of WT, n = 14 slices from 7 mice; H1a\(^{-/-}\): 102 ± 4% of WT, n = 8 slices from WT but not in Fmr1\(^{-/-}\) littermates despite an elevated level of eIF4F complex under basal (B) conditions. H1a deletion alone had no effect on eIF4F complex levels under basal or DHPG-stimulated conditions, whereas H1a deletion on the Fmr1\(^{-/-}\) background reversed the enhanced eIF4F complex levels and restored DHPG-induced eIF4F complex formation. Right: quantified group data for eIF4E coimmunoprecipitating with eIF4G (eIF4F complex) in DHPG-treated samples (normalized to the value in basal, or untreated, slices). n = 3 mice per genotype. Left: representative western blots of phospho- and total ERK and translation initiation factors that are regulated by ERK (phospho- and total eIF4E and 4E-BP) from cortical homogenates in each of four genotypes. Right: quantified group data reveal elevated phospho-P (4E-BP) and P-eIF4E in Fmr1\(^{-/-}\) brains as compared to WT, which is rescued by H1a deletion. P-ERK levels were not different across any genotype (n = 4–6 mice per genotype). Left: representative western blots from acute hippocampal slices prepared from WT and Fmr1\(^{-/-}\) mice treated with MPEP (10 μM) or vehicle (H₂O). Right: quantified group data for phosphoproteins in MPEP-treated slices expressed as a percentage of that in basal (untreated) slices reveal a genotypic difference in P-4EBP (Ser65) and P-eIF4E but not P-ERK (n = 2 slices per mouse; 4–8 mice per condition). *P < 0.05; **P < 0.01; ***P < 0.001; error bars, s.e.m. Full-length western blots for this figure are shown in Supplementary Fig. 9.

**Figure 3** Altered mGluR5-Homer scaffolds in Fmr1\(^{-/-}\) mice mediate enhanced basal translation rates and initiation complex formation. (a) Acute hippocampal slices from Fmr1\(^{-/-}\) mice showed elevated protein synthesis rate in comparison to WT littermates as measured by incorporation of 35S-methionine and 35S-cysteine into total protein (n = 14 slices from 7 mice per genotype). Elevated protein synthesis rates in Fmr1\(^{-/-}\) slices were reversed by H1a deletion (n = 7 slices, 4 mice), whereas H1a deletion alone (n = 8 slices, 4 mice) had no effect. (b) Pretreatment of WT hippocampal slices with mGluR5CT (CT; 5 μM; 5 h; n = 16 slices, 4 mice) enhanced incorporation of 35S-methionine and 35S-cysteine in comparison to treatment with mGluR5M control peptide (MU; n = 16 slices, 4 mice). In contrast, pretreatment of Fmr1\(^{-/-}\) hippocampal slices (n = 15 slices, 4 mice) with CT peptide had no effect. (c) Preincubation of WT or Fmr1\(^{-/-}\) slices with U0126 (20 μM; 30 min) before 35S-methionine and 35S-cysteine incorporation (n = 12 slices, 6 mice per condition) (left) equalized protein synthesis rates. (d) Left: representative immunoblots (IB) of eIF4G coimmunoprecipitating (IP) with eIF4F from hippocampal slices. DHPG (D) induced an increase in eIF4F association with eIF4G, forming the eIF4F translation initiation complex, in WT but not in Fmr1\(^{-/-}\) littermates despite an elevated level of eIF4F complex under basal (B) conditions. H1a deletion alone had no effect on eIF4F complex levels under basal or DHPG-stimulated conditions, whereas H1a deletion on the Fmr1\(^{-/-}\) background reversed the enhanced eIF4F complex levels and restored DHPG-induced eIF4F complex formation. Right: quantified group data for eIF4E coimmunoprecipitating with eIF4G (eIF4F complex) in DHPG-treated samples (normalized to the value in basal, or untreated, slices). n = 3 mice per genotype. Left: representative western blots of phospho- and total ERK and translation initiation factors that are regulated by ERK (phospho- and total eIF4E and 4E-BP) from cortical homogenates in each of four genotypes. Right: quantified group data reveal elevated phospho-P (4E-BP) and P-eIF4E in Fmr1\(^{-/-}\) brains as compared to WT, which is rescued by H1a deletion. P-ERK levels were not different across any genotype (n = 4–6 mice per genotype). Left: representative western blots from acute hippocampal slices prepared from WT and Fmr1\(^{-/-}\) mice treated with MPEP (10 μM) or vehicle (H₂O). Right: quantified group data for phosphoproteins in MPEP-treated slices expressed as a percentage of that in basal (untreated) slices reveal a genotypic difference in P-4EBP (Ser65) and P-eIF4E but not P-ERK (n = 2 slices per mouse; 4–8 mice per condition). *P < 0.05; **P < 0.01; ***P < 0.001; error bars, s.e.m. Full-length western blots for this figure are shown in Supplementary Fig. 9.
Figure 4 Genetic deletion of Homer1a does not reverse the protein synthesis independence of mGluR-induced LTD or altered protein levels of FMRP target mRNAs. (a) Brief DHPG (100 μM; 5 min) induced long-term depression (LTD) of synaptic transmission in WT hippocampal slices that was reduced by the protein synthesis inhibitor cycloheximide (60 μM; P < 0.01). Plotted are group averages of field excitatory postsynaptic potential (FP) slope normalized to pre-DHPG baseline as a function of time. Inset, average of ten FPs taken during the baseline period (1) and 55–60 min after DHPG treatment (2). Scale bars: 0.5 mV, 5 ms. (b,c) In Fmr1−/− and H1a−/− Fmr1−/− mice, DHPG-induced LTD was unaffected by cycloheximide. (d) In H1a−/− mice, DHPG-induced LTD was normal and blocked by cycloheximide (P < 0.05). n, number of slices. (e) Map1b and CaMKIIα were elevated in Fmr1−/− mice and were unaffected by H1a deletion. Left: representative western blots of basal (B) and DHPG-stimulated slices of each genotype. Full-length western blots for this figure are shown in **Supplementary Figure 10**. Group averages reveal a deficit in DHPG-induced Arc synthesis slices taken from Fmr1−/− (n = 10 mice) and H1a−/− Fmr1−/− (n = 11 mice). DHPG induced Arc synthesis in both WT (n = 12) and H1a−/− (n = 14) littersmates. *P < 0.05; **P < 0.01; ***P < 0.001; error bars, s.e.m.

4 mice; double H1a−/− Fmr1−/−, 106 ± 6% of WT, n = 7 slices from 4 mice; Fig. 3a). Furthermore, mGluR5CT peptide-mediated disruption of mGluR5-Homer in WT slices was sufficient to mimic the enhanced protein synthesis rates observed in Fmr1−/− slices (mGluR5CT = 123 ± 6% of mGluR5MU treated, n = 16 slices per peptide from 10 mice; P = 0.002; Fig. 3b). In contrast, mGluR5CT had no effect on protein synthesis rates in Fmr1−/− slices (mGluR5CT = 106 ± 8% of mGluR5MU treated, n = 14 (mGluR5CT) or 15 (mGluR5MU) slices from 8 mice; P = 0.5; Fig. 3b).

Translation initiation is the rate-limiting step in translation. To determine whether enhanced translation rates in the Fmr1−/− stem from increased initiation, we measured eIF4F translation initiation complexes in hippocampal slices prepared from WT, Fmr1−/− H1a−/− and H1a−/− Fmr1−/− mice. The eIF4F complex is composed of the 5′ cap binding protein eIF4E, the scaffolding protein eIF4G and the RNA helicase eIF4A. eIF4F complex assembly can be measured by coimmunoprecipitation of eIF4G and eIF4E and is stimulated by DHPG in WT animals. Therefore, we also measured eIF4F complex in DHPG-stimulated slices from each genotype. Consistent with previous reports, eIF4F complex levels were enhanced basally in Fmr1−/− slices and no longer stimulated by DHPG (Fig. 3d). Like 35S-labeled methionine and cysteine incorporation, eIF4F complex levels in Fmr1−/− slices were restored to WT levels by H1a deletion (Fig. 3d; n = 3 mice per condition). Genetic deletion of H1a also rescued the deficit in DHPG-stimulated eIF4F complex assembly in the Fmr1−/− (Fig. 3d). These results suggest that elevated protein synthesis rates in Fmr1−/− mice are due to enhanced translation initiation that is driven by H1a-bound mGluR5. The deficit in mGluR-stimulated translation initiation in Fmr1−/− mice may be because eIF4F complex levels are saturated basally. Furthermore, mGluR-activation of mTOR is rescued in Fmr1−/− mice by H1a deletion (see Fig. 2), which may also contribute to restoration of DHPG-induced eIF4F complex assembly.

To determine how increased H1a-mGluR5 interactions lead to enhanced signaling to translation downstream of ERK, we examined phosphorylation of initiation factors known to be regulated by ERK in WT and Fmr1−/− cortical homogenates and the effects of H1a deletion. ERK phosphorylates and activates MAPK-interacting kinase (Mnk), which in turn phosphorylates the cap-binding protein eIF4E on Ser209 (ref. 23). ERK also phosphorylates eIF4E binding protein (4EBP) at Ser65, a site distinct from mTOR-regulated sites (Thr37 and Thr46). ERK-dependent phosphorylation of eIF4E and 4EBP (Ser65) is associated with increased translation rates in neurons and other cell types. Consistent with a role for ERK in phosphorylation of these initiation factors in hippocampal slices, phospho-(Ser209)-eIF4E and phospho-(Ser65)-4EBP were strongly reduced or abolished by treatment with U0126, an inhibitor of the upstream kinase that...
activates ERK (MAP/ERK kinase; MEK) (Supplementary Fig. 4). Phospho-4EBP and phospho-eIF4E levels were enhanced in cortical homogenates from Fmr1−/− mice, an effect that was rescued by H1a deletion (Fig. 3e). As reported in hippocampal slices (Fig. 1d)12,20, phospho-ERK levels were unchanged in Fmr1−/−lysates (Fig. 3e).

To determine whether mGluR5 activity abnormally drives phosphorylation of ERK, eIF4E and 4EBP (Ser65) in Fmr1−/− mice, we treated hippocampal slices from both WT and Fmr1−/−mice with the mGluR5 inverse agonist 2-methyl-6-(phenylethynyl)pyridine (MPEP; 10 μM). MPEP treatment did not affect phospho-4EBP or phospho-eIF4E in WT slices. However, in Fmr1−/−slices, MPEP reduced phospho-4EBP and phospho-eIF4E by ~50% (Fig. 3f). Unexpectedly, MPEP had no effect on phospho-ERK levels in either WT or Fmr1−/−slices. These results support our hypothesis that H1a-mediated mGluR5 activity drives translation initiation through ERK phosphorylation of initiation factors. Because phospho-ERK levels are not affected by Fmr1 knockout or with MPEP, this suggests that mGluR5 may regulate accessibility or localization of eIF4E or 4EBP with ERK, as opposed to ERK activity per se.

**Altered LTD is not rescued by deletion of H1a**

In WT animals, mGluR-dependent LTD in the CA1 region of the hippocampus requires dendritic protein synthesis of FMRP-interacting mRNAs such as Arc and Map1b (also known as Map1b)5,12,27. Although mGluR activation induces robust LTD in Fmr1−/−mice, mGluR-induced synthesis of Arc and Map1b is deficient and LTD is independent of new protein synthesis12. From this result, it has been suggested that loss of FMRP-mediated translational suppression leads to enhanced steady-state levels of ‘LTD proteins’ that allow mGluR-LTD to persist without new protein synthesis5,12. Consistent with this hypothesis, elevated Map1b and Arc have been reported in Fmr1−/−neurons5,28. Alternatively, H1a-bound and constitutively active mGluR5, which drives translation initiation through ERK phosphorylation of initiation factors, could elevate LTD protein levels and lead to protein synthesis-independent LTD.

To distinguish between these possibilities, we determined whether genetic deletion of H1a reverses the protein synthesis independence of mGluR-LTD and enhances levels of specific FMRP target mRNAs. To test the protein synthesis dependence of mGluR-LTD, we preincubated slices in the translation inhibitor cycloheximide (60 μM). Although mGluR-LTD was reliably induced with DHPG in H1a+/−Fmr1−/−mice (81 ± 3% of baseline 60–70 min after DHPG application, n = 11 slices), LTD was not sensitive to cycloheximide (78 ± 1% of baseline, n = 9), and was similar to that in Fmr1−/−mice (control: 74 ± 4% of baseline, n = 7; cycloheximide: 79 ± 3% of baseline, n = 9; Fig. 4). LTD was inhibited by cycloheximide treatment in both WT (control: 76 ± 1% of baseline, n = 7; cycloheximide: 92 ± 4% of baseline, n = 8; P = 0.002) and H1a−/−mice (control: 65 ± 5% of baseline, n = 6; cycloheximide: 88 ± 5% of baseline, n = 6; P = 0.01; Fig. 4a). These results suggest that the altered mGluR5-Homer scaffolds in Fmr1−/−mice do not mediate the protein synthesis independence of mGluR-LTD.

To determine whether H1a deletion in Fmr1−/−mice rescues elevated steady state levels of LTD-promoting proteins or other FMRP target mRNAs, we performed western blots of Map1b, Arc and CaMKII in hippocampal homogenates of WT, Fmr1−/−, H1a−/− and H1a+/−Fmr1−/−mice (Fig. 4e). In our experiments, levels of Map1b and CaMKII in hippocampal homogenates of WT, Fmr1−/−, H1a−/− and H1a+/−Fmr1−/−mice (Fig. 4e). In both Fmr1−/−mice and H1a+/−Fmr1−/−mice in comparison to WT and H1a−/−mice (Fig. 4e), indicating that H1a deletion does not restore normal levels of Map1b and CaMKII in Fmr1−/−mice. Although we did not detect elevated steady-state protein levels of Arc in hippocampal homogenates from Fmr1−/−mice, we observed a deficit in DHPG-induced synthesis of Arc in hippocampal slices from Fmr1−/−mice in comparison to WT (WT: 140 ± 17% of basal or untreated, n = 12; Fmr1−/−: 98 ± 12% of untreated, n = 12; Fig. 4f). However, H1a deletion did not restore mGluR-induced synthesis of Arc in Fmr1−/−mice12 (H1a+/−Fmr1−/−: 91 ± 12% of untreated, n = 11). DHPG-induced synthesis of Arc was normal in H1a−/−mice (139 ± 11% of untreated, n = 11). These results show that altered mGluR5-Homer scaffolds in the Fmr1−/−mice do not mediate abnormal mGluR-LTD or altered translational control of specific FMRP target mRNAs. Instead, these results support an essential role for interaction of FMRP with its target mRNAs in mGluR-LTD and translational control of these mRNAs2,29.

**mGluR5-Homer and hypereexcitable neocortical circuits**

Humans with FXS and Fmr1−/− mice show sensory hypersensitivity, epilepsy and/or audiogenic seizures suggestive of an underlying sensory circuit hypereexcitability3,28. We recently discovered synaptic and circuit alterations indicative of hypereexcitability in the somatosensory
barrel neocortex of Fmr1−/− mice. Neocortical slices of Fmr1−/− mice have decreased excitatory drive onto layer 4 fast-spiking interneurons and prolonged thalamically evoked and spontaneously occurring persistent activity, or UP, states.30,31 UP states represent a normal physiological rhythm generated by the recurrent neocortical synaptic connections and are observed in alert and slow-wave sleep states in vivo, as well as in neocortical slice preparations.25,33 Of note, genetic or pharmacological reduction of mGluR5 in Fmr1−/− mice rescues the prolonged UP states in acute slices and in vivo.31 To determine whether altered mGluR5–Homer interactions contribute to altered neocortical circuit function and hyperexcitability in Fmr1−/− mice, we measured spontaneously occurring UP states in acute slices from somatosensory barrel cortex using extracellular multiunit recordings.32 As previously reported,25,31 UP states were longer in slices from Fmr1−/− mice than in those from WT littermates (WT: 797.4 ± 31.5 ms, n = 22 slices; Fmr1−/−: 1,212 ± 87.9 ms, n = 13; Fig. 5a,b). In support for a role altered Homer interactions, UP state duration was shortened to WT levels by H1a deletion (H1a−/−Fmr1−/−: 872.2 ± 42.1 ms, n = 44; Fig. 5a,b). Loss of H1a alone did not affect UP states, ruling out general alterations in excitability (H1a−/−: 767.7 ± 35.8 ms, n = 18).

We next determined whether mGluR5CT peptide-mediated disruption of mGluR5–Homer was sufficient to prolong UP states in WT slices. Preincubation of WT neocortical slices in mGluR5CT peptide increased the duration of UP states in comparison to treatment with mGluR5SMU control peptide (WT mGluR5SMU: 909.8 ± 103.7 ms, n = 13 slices; WT mGluR5CT: 1,408.8 ± 156.4 ms, n = 15; Fig. 5c,d). Therefore, acute disruption of mGluR5–Homer complexes is sufficient to mimic the circuit hyperexcitability in Fmr1−/− mice. In contrast, mGluR5CT had no effect on the duration of UP states in Fmr1−/− slices (Fmr1−/− mGluR5SMU: 2,014.9 ± 117.9 ms, n = 15; Fmr1−/− mGluR5CT: 1,819.8 ± 163.8 ms, n = 12; Fig. 5c,d), likely because Homer complexes are already disrupted in these mice.

**H1a deletion reverses Fmr1−/− behavioral phenotypes**

To determine whether H1a deletion rescues any in vivo or behavioral phenotypes in Fmr1−/− mice, we measured the incidence of audiogenic seizures and anxiety as measured using the open field activity test across the four genotypes. We chose these phenotypes because they are robust in the C57BL6 strain of Fmr1−/− mice and sensitive to mGluR5 antagonists.8 Consistent with previous reports, Fmr1−/− mice showed increased seizure incidence and severity upon exposure to a loud sound relative to WT and H1a−/− mice, who exhibited little or no incidence of seizure (seizure score 0–3; 3 being most severe; see Online Methods): WT, 0.12 ± 0.12; n = 16 mice; H1a−/−: 0.04 ± 0.04; n = 24; Fmr1−/−: 1.6 ± 0.2; n = 39; P < 0.001, Fmr1−/− versus WT or H1a−/−; Fig. 6a and Supplementary Table 3). H1a−/−Fmr1−/− mice responded with a reduced incidence and severity of seizure in comparison to Fmr1−/− littermates (H1a−/−Fmr1−/− mice, seizure score 1.1 ± 0.2; n = 37; P < 0.05, H1a−/−Fmr1−/− versus Fmr1−/−). However, H1a−/−Fmr1−/− mice showed increased seizures in comparison to WT or H1a−/− mice (P < 0.001; Fig. 6a). Such a partial rescue of the audiogenic seizures by H1a deletion is similar to what is observed with genetic reduction of mGluR5 in Fmr1−/− mice.7

As previously reported, Fmr1−/− mice spent more time in the center of a lit open field than WT littermates, which has been interpreted as reduced generalized anxiety in the mice (Fig. 6b; WT, 85 ± 8 s; n = 18 mice; Fmr1−/−, 138 ± 12 s; n = 24; P < 0.01). H1a−/− mice did not differ from WT mice in this behavior (H1a−/−, 75 ± 10 s; n = 17). We found that H1a−/−Fmr1−/− mice behaved like WT mice (H1a−/−Fmr1−/−, 91 ± 10 s; n = 17), spending significantly less time than Fmr1−/− mice (P < 0.01) in the center of an open arena. There were no differences in locomotor activity between any of the genotypes (Supplementary Fig. 5). Therefore, H1a deletion completely rescued the open field activity phenotype, suggesting that altered mGluR5–Homer interactions contribute to altered behavior in Fmr1−/− mice and may be relevant for altered behaviors in people with FXS.}

**DISCUSSION**

**Two mechanisms for mGluR dysfunction in FXS**

Here we demonstrate a causative role for reduced Homer scaffolds in mGluR5 dysfunction in a model of human neurological disease. MGlur5 dysfunction in animal models of FXS is well established, and genetic or pharmacological reduction of mGluR5 activity reduces or rescues many disease phenotypes in animal models and most recently in patients. However, the molecular basis for mGluR5 dysfunction in FXS was essentially unknown. It has been suggested that loss of an FMRP-mediated translational ‘brake’ downstream of mGluR5 leads to enhanced mGluR5 function, but this mechanism cannot account for the deficits in mGluR5 signaling or translation-independent dysfunction of mGluR5 associated with FXS.8,31

Our results reveal two mechanisms for mGluR5 dysfunction in Fmr1−/− mice. First, an imbalance of mGluR5 interactions from long to short Homer1a isoforms leads to altered mGluR5 signaling, enhanced basal translation rates, neocortical hyperexcitability, audiogenic seizures and open field activity (Supplementary Fig. 6a). Because H1a-bound mGluR5 is constitutively active or agonist independent, our results strongly suggest that the therapeutic action of mGluR5 inverse agonists, such as MPEP11, in FXS phenotypes are due, in part, to inhibition of H1a-bound, constitutively active mGluR5. Second, our results reveal that disrupted Homer scaffolds in Fmr1−/− mice cannot account for altered mGluR-LTD or abnormal translational control of FMRP target mRNAs (Supplementary Fig. 6b) and implicate an essential role for FMRP binding to and translational regulation of specific mRNAs in mGluR-LTD. The discovery that altered Homer scaffolds account for much of the complex dysfunction of mGluR5 in FXS will help to develop alternative, targeted therapies for the disease and provide mechanistic links to other genetic causes of autism.

**Homer scaffolds coordinate mGluR regulation of translation**

Our data demonstrate new functions of Homer scaffolds in coordination of mGluR-stimulated translation by facilitating activation.
of the PI3K-mTOR pathway and translation initiation, as well as limiting activation of EF2K and subsequent inhibition of elongation. Homer links to PIKE, a small GTPase that binds and activates PI3K in response to mGluR activation. The PI3K pathway stimulates mTOR to phosphorylate eIF4E binding protein (4EBP), which in turn releases eIF4E to interact with eIF4G and form the eIF4F translation initiation complex. Furthermore, mTOR phosphorylates S6K to stimulate translation of 5′ terminal oligopyrimidine tract (5′TOP) mRNAs that encode ribosomal proteins and translation factors, thus increasing the translational capacity of the cell. mGluR activation of the PI3K-mTOR-S6K pathway was blocked by mGluR5CT, and the deficits in mGluR activation of mTOR and initiation complex (eIF4F) formation in Fmr1−/− mice were restored by H1a deletion, indicating that Homer scaffolds are key for mGluR-stimulated translation initiation.

Although somewhat counterintuitive given the fact that mGluRs stimulate translation initiation, mGluRs also stimulate phosphorylation of EF2, which inhibits elongation. EF2K and moderate inhibition of elongation are necessary for mGluR-induced synthesis of Arc, as well as mGluR-LTD. Submaximal inhibition of global elongation may make available rate-limiting factors to translate mRNAs that are poorly initiated and cannot compete effectively for these factors. Long Homer interactions limit EF2K activation by mGluRs and thus would be expected to temper mGluR-mediated inhibition of general elongation and, in turn, promote translation of poorly initiated mRNAs. Consequently, disruption of mGluR5-Homer enhances EF2K activity and would be expected to strongly inhibit elongation and block translational activation. Although we did not rescue abnormal mGluR-LTD in Fmr1−/− neurons by deletion of H1a, in WT slices mGluR5CT peptide treatment blocked mGluR-induced synthesis of Arc and mGluR-LTD. These results indicate that in WT neurons, where mGluR-LTD requires de novo protein synthesis, mGluR5-Homer interactions are necessary to properly stimulate translation and induce LTD.

In Fmr1−/− mice there was a deficit in mGluR stimulation of PI3K-mTOR and eIF4F initiation complex formation, whereas EF2K activation was markedly enhanced. These changes would be expected to block mGluR-induced translational initiation and strongly inhibit elongation. mGluR-induced rapid synthesis of many proteins (for example, PSD-95, EF1α, amyloid precursor protein, Arc, CaMKIIα and Map1b) is absent in Fmr1−/− mice, which may be mediated, in part, by disrupted mGluR-Homer scaffolds and altered signaling to the translation machinery. Alternatively or in addition, because FMRP interacts with these mRNAs, it is likely required for mGluR-triggered translational activation of specific target mRNAs. The fact that H1a deletion rescues mGluR-mediated translation initiation complex formation but not synthesis of Arc suggests a requirement for both mGluR5-Homer scaffolds and FMRP in mGluR-triggered Arc translation.

Altered mGluR5-Homer scaffolds increase translation rates

Although there is a deficit in mGluR agonist–stimulated translation in Fmr1−/− mice, steady-state translation rates and levels of specific proteins are elevated, thus reflecting the complexity of translational control. Because one function of FMRP is to suppress translation of its mRNA targets, an obvious possibility was that the elevated protein synthesis rates and protein levels directly result from loss of FMRP-mediated suppression of mRNA targets. In support of this hypothesis, H1a deletion does not reverse enhanced protein levels of Map1b and CaMKIIα. However, elevated total protein synthesis rates and translation initiation (eIF4F) complexes were rescued by H1a deletion and mimicked in WT mice by mGluR5CT peptide treatment. Thus, increased steady-state translation rates in Fmr1−/− tissue are a result of altered mGluR5-Homer scaffolds that are a secondary consequence of FMRP loss. Elevated protein synthesis rates that are downstream of mGluR5 (Grm5+) slices are reversed by the genetic reduction of mGluR5 (Grm5−/−), the mGluR5 inverse agonist MPEP and inhibitors of ERK.2,8,20. In Fmr1−/− cortical lysates, we observed enhanced phosphorylation of translation initiation factors that are downstream of ERK (eIF4A and 4EBP) that was reversed by H1a deletion. Furthermore, blocking mGluR5 activity with MPEP strongly reduced phospho-eIF4A and phospho–4EBP in Fmr1−/−, but not WT, hippocampal slices. Together these results suggest that H1a-bound and constitutively active mGluR5 in Fmr1−/− neurons drives ERK-dependent phosphorylation of eIF4E and 4EBP, which enhances eIF4F initiation complex formation and translation rates. Because we do not observe an effect of Fmr1−/− or MPEP on phospho-ERK levels as detected by western blot, this suggests that mGluR5 either drives ERK activity that is not detectable by phosphorylation at Thr202 and Tyr204 and/or regulates accessibility of eIF4E and 4EBP to ERK.

In contrast to hippocampus, recent results from neocortical synaptoneurosomes of Fmr1−/− mice demonstrate a role for PI3K activity in enhanced protein synthesis rates.22 We observed that the PI3K inhibitor wortmannin equalized translation rates between WT and Fmr1−/− hippocampal slices, but this was because wortmannin actually increased translation rates in WT, but not Fmr1−/−, slices (Supplementary Fig. 3). Furthermore, elevated basal phosphorylation of PI3K, mTOR and 4EBP (at the mTOR sites) was recently reported in fresh hippocampal lysates of Fmr1−/− mice, perhaps as a result of increased PIKE.2,20. Although we are unable to detect elevated basal activation of the PI3K-mTOR pathway in our slice preparation, persistent activation of downstream effectors of PI3K and mTOR together with constitutive mGluR5-driven ERK may elevate translation rates in Fmr1−/− mice. Phospho-EF2 levels are unchanged in Fmr1−/− slices, suggesting that basal or constitutive mGluR5 activity is not sufficient to activate EF2K. The detailed mechanisms by which ERK, PI3K and mTOR, and EF2K contribute to elevated basal protein synthesis rates in Fmr1−/− mice requires further study and may differ depending on the brain region, subcellular compartment or preparation.

H1a deletion and restoration of mGluR5-Homer scaffolds in Fmr1−/− mice did not rescue the protein synthesis independence of mGluR-LTD nor elevated steady-state levels of Map1b and CaMKIIα proteins produced from FMRP target mRNAs. This supports the hypothesis that the protein synthesis independence of mGluR-LTD in Fmr1−/− mice is a result of loss of FMRP-mediated translational suppression of LTD-promoting proteins, such as MAP1b.26. Because H1a deletion rescued the elevated incorporation of 35S-labeled methionine and cysteine, but not altered mGluR-LTD and enhanced MAP1b and CaMKIIα levels, this suggests that altered LTD and elevated MAP1b and CaMKIIα are not a result of elevated total protein synthesis rates. Furthermore, the fact that mGluR-triggered Arc synthesis was not rescued by H1a deletion supports an essential role for FMRP in mGluR-triggered translational activation of Arc. Recent work has implicated mGluR-triggered dephosphorylation of FMRP in translational activation of FMRP target mRNAs, such as Dlgap3 (SAPAP3) and Dlg4 (PSD95) (refs. 2,29).

Altered mGluR5-Homer and neocortical network dysfunction

Altered neocortical circuit function and hyperexcitability have been predicted to contribute to cognitive disorders and autism.29,38. The epilepsy and electroencephalographic abnormalities observed in people with FXS are indicative of brain hyperexcitability. Furthermore, these individuals are hypersensitive to sensory stimuli, and Fmr1−/−
mice have audiogenic seizures reflecting hyperexcitability of sensory circuits\textsuperscript{3,5}. Although UP states are a normal physiological rhythm and are not epileptiform activity, they provide an effective readout of the state of circuit function and excitability\textsuperscript{32}. Furthermore, UP states underlie the slow oscillations that occur during slow wave sleep and are implicated in memory consolidation, as well as sensory processing in waking states\textsuperscript{33}. Therefore, altered neocortical UP states in the \textit{Fmr1}\textsuperscript{−/−} mouse\textsuperscript{30,31} may be relevant to the sensory processing and cognitive abnormalities in humans with FXS.

Our findings indicate that the longer UP states in \textit{Fmr1}\textsuperscript{−/−} neocortex are mediated by enhanced, likely constitutive, activity of H1a-bound mGluR5 (ref. 31). In support of this conclusion, prolonged UP states in the \textit{Fmr1}\textsuperscript{−/−} mouse are reversed by genetic or acute, pharmacological blockade of mGluR5 (ref. 31) and genetic deletion of H1a. Peptide-mediated disruption of mGluR5-Homer interactions prolonged UP states in WT, but not \textit{Fmr1}\textsuperscript{−/−}, slices, which suggests that regulation of Homer scaffolds, by H1a or other means, may regulate neocortical slow oscillations in the normal brain. Of note, H1a is induced in neocortex with sleep deprivation and contributes to the homeostatic increase in slow wave sleep that occurs in response to sleep deprivation\textsuperscript{39}. Consequently, altered UP states, Homer interactions and the increased open field activity in \textit{Fmr1}\textsuperscript{−/−} mice have been associated with FXS pathologies\textsuperscript{3,8}. Although UP states are a normal physiological rhythm and are not epileptiform activity, they provide an effective readout of the state of circuit function and excitability\textsuperscript{32}. Furthermore, UP states underlie the slow oscillations that occur during slow wave sleep and are implicated in memory consolidation, as well as sensory processing in waking states\textsuperscript{33}. Therefore, altered neocortical UP states in the \textit{Fmr1}\textsuperscript{−/−} mouse\textsuperscript{30,31} may be relevant to the sensory processing and cognitive abnormalities in humans with FXS.

Mechanism of disrupted Homer scaffolds in \textit{Fmr1}\textsuperscript{−/−}

Mechanism of disrupted Homer scaffolds in \textit{Fmr1}\textsuperscript{−/−}

How does loss of FMRP lead to altered mGluR5-Homer scaffolds? Protein levels of long Homers and H1a are unchanged in total homogenates of \textit{Fmr1}\textsuperscript{−/−} hippocampi\textsuperscript{10}, and FMRP is not reported to interact with mRNA for any Homer isoforms\textsuperscript{43}. Previous work reported a decrease in tyrosine phosphorylation of long Homer in \textit{Fmr1}\textsuperscript{−/−} forebrain\textsuperscript{10}, but it is unknown whether or how this affects interactions with mGluR5. Phosphorylation of Homer3 regulates interactions with other Homer effectors\textsuperscript{44,45}. Similarly, phosphorylation of mGluR5 at the C-terminal Homer interaction domain reduces the affinity of mGluR5 for Homer\textsuperscript{46}. Therefore, post-translational modification of mGluR5 and/or Homer in \textit{Fmr1}\textsuperscript{−/−} mice may underlie the decreased interactions.

Disrupted or destabilized synaptic scaffolds that affect Homer and/or mGluR5 may also contribute more generally to cognitive disorders and autistic behaviors. Mutations in the Homer binding domain of SHANK3 and the Homer binding protein oligophrenin 1 are implicated in autism and intellectual disability, respectively\textsuperscript{47,48}. Expression of a truncated SHANK3 without the Homer binding domain in mice results in degrada- tion of SHANK3 and autistic behaviors in mice. Notably, reduction of long Homers or induction of H1a recapitulates the degradation of synaptic SHANK3 (ref. 47). One possibility is that mGluR5 dysfunction of the kind we describe here may occur in individuals with mutations in SHANK3 or other genes that destabilize Homer scaffolds.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We would like to thank L. Ormazabal and N. Cabalo for technical assistance. This research was supported by the grants from the US National Institutes of Health NS045711, HD052731 (K.M.H.), HD056370 (J.R.G.), GM008203 (S.A.H.), Autism Speaks (K.M.H.), FRAXA Research Foundation and The Hartwell Foundation (J.A.R.).

AUTHOR CONTRIBUTIONS

J.A.R. designed, performed and analyzed experiments included in Figures 2–4 and 6, Supplementary Figures 1 and 2 and Supplementary Tables 1 and 2 and wrote a first draft of the manuscript. K.A.C. performed and analyzed experiments in Figures 1 and 3, Supplementary Figures 2–4 and Supplementary Table 1. S.A.H. performed and analyzed experiments in Figures 5 and 6. N.-P.T. performed and analyzed commonprecipitation experiments for Figures 2 and 3 and Supplementary Figure 2. W.G. performed and analyzed experiments for Figures 1–4. S.G.B. provided intellectual input on the behavioral experiments and designed and performed experiments in Figure 6 and Supplementary Figure 5. J.-H.H. and P.F.W. provided intellectual input and generated and provided the H1a\textsuperscript{−/−} mice. J.R.G. contributed intellectually to the overall project and in particular to the UP state experiments (Fig. 5). J.R.G. trained and supervised S.A.H., designed experiments for Figure 5, contributed funding and edited the manuscript. K.M.H. supervised the overall project, designed experiments, contributed funding, edited figures and wrote the final version of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/natureneuroscience/. Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.
1. Abrahams, B.S. & Geschwind, D.H. Advances in autism genetics: on the threshold of a new neurobiology. Nat. Rev. Genet. 9, 341–355 (2008).
2. Bassell, G.J. & Warren, S.T. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron 60, 201–214 (2008).
3. Berry-Kravis, E. Epilepsy in fragile X syndrome. Dev. Med. Child Neurol. 44, 1–8 (2002).
4. Hagerman, R. The physical and behavioral phenotype. In Fragile X Syndrome: Diagnosis, Treatment, and Research (eds. Hagerman, R. & Hagerman, P.) 3–109 (Johns Hopkins Univ. Press, Baltimore, 2002).
5. Gross, C. & Huber, K.M. Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron 65, 445–459 (2010).
6. Bear, M.F., Huber, K.M. & Warren, S.T. The mGluR theory of fragile X mental retardation. Trends Neurosci. 27, 370–377 (2004).
7. Dölen, G. et al. Correction of fragile X syndrome in mice. Neuron 56, 955–962 (2007).
8. Dölen, G., Carpenter, R.L., Ocain, T.D. & Bear, M.F. Mechanism-based approaches to treating fragile X. Pharmacol. Ther. 127, 78–93 (2010).
9. Jacquesmont, S. et al. Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. Sci. Transl. Med. 3, 6ra461 (2011).
10. Giuffrida, R. et al. A reduced number of metabotropic glutamate subtype 5 receptors are associated with constitutive homer proteins in a mouse model of fragile X syndrome. J. Neurosci. 25, 8908–8916 (2005).
11. Shiraishi-Yamaguchi, Y. & Furuichi, T. The Homer family proteins. Genome Biol. 8, 206 (2007).
12. Parrish, S.S. & Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arg/Arg3.1 essential for mGluR-LTD. Neuron 59, 70–83 (2008).
13. Ango, F. et al. Agonist-independent activation of metabotropic glutamate receptors by the intracellular protein Homer. Nature 411, 962–965 (2001).
14. Tu, J.C. et al. Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. Neuron 21, 717–726 (1998).
15. Xiao, B. et al. Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. Neuron 21, 707–716 (1998).
16. Mao, L. et al. The scaffold protein Homer1c links metabotropic glutamate receptor 5 to extracellular signal-regulated protein kinase cascades in neurons. J. Neurosci. 25, 2741–2752 (2005).
17. Ronesi, J.A. & Huber, K.M. Metabotropic glutamate receptor and fragile X mental retardation protein: partners in translational regulation at the synapse. Sci. Signal. 1, peb (2008).
18. Ronesi, J.A. & Huber, K.M. Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. J. Neurosci. 28, 543–547 (2008).
19. Sharma, A. et al. Dysregulation of mTOR signaling in fragile X syndrome. J. Neurosci. 30, 15661–15667 (2010).
20. Osterwei, E.K., Krueger, D.D., Reinhold, K. & Bear, M.F. Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. J. Neurosci. 30, 15616–15627 (2010).
21. Hu, J.H. et al. Homeostatic scaling requires group 1 mGluR activation mediated by Homer1a. Neuron 68, 1128–1142 (2012).
22. Gross, C. et al. Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. J. Neurosci. 30, 10624–10638 (2010).
23. Prou, G. Signalling to translation: how signal transduction pathways control the protein synthetic machinery. Biochem. J. 403, 217–234 (2007).
24. Banko, J.L., Hou, L., Poulin, F., Sonenberg, N. & Klann, E. Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. J. Neurosci. 26, 2167–2173 (2006).
25. Herbert, T.P., Tee, A.R. & Proud, C.G. The extracellular signal-regulated kinase pathway regulates the phosphorylation of 4E-BP1 at multiple sites. J. Biol. Chem. 277, 11591–11596 (2002).
26. Kelleher, R.J., III, Govindarajan, A., Jung, H.Y., Kang, H. & Tonegawa, S. Translational control by MAPK signaling in long-term synaptic plasticity and memory. Cell 116, 724–736 (2004).
27. Waung, M.W., Pfeffer, B.E., Nosyreva, E.D., Ronesi, J.A. & Huber, K.M. Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron 59, 84–97 (2008).
28. Zalta, F. et al. The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. Cell 112, 317–327 (2003).
29. Muddashetty, R.S. et al. Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. Mol. Cell 42, 673–688 (2011).
30. Gibson, J.R., Barton, A.F., Hays, S.A. & Huber, K.M. Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. J. Neurophysiol. 100, 2615–2626 (2008).
31. Hays, S.A., Huber, K.M. & Gibson, J.R. Altered neocortical rhythm activity states in Fmr1 KO mice are due to enhanced mGluR5 signaling and involve changes in excitatory circuitry. J. Neurosci. 31, 14223–14234 (2011).
32. Sanchez-Vives, M.V. & McCormick, D.A. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat. Neurosci. 3, 1027–1034 (2000).
33. Haider, B. & McCormick, D.A. Rapid neocortical dynamics: cellular and network mechanisms. Neuron 62, 171–189 (2009).
34. Liu, Z.H. & Smith, C.B. Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. Neurosci. Lett. 454, 62–66 (2009).
35. Scheetz, A.J., Nairn, A.C. & Constantine-Paton, M. NMDA receptor-mediated control of protein synthesis during synaptic development. Nat. Neurosci. 3, 211–216 (2000).
36. Waung, M.W. & Huber, K.M. Protein translation in synaptic plasticity: mGluR-LTD, Fragile X. Curr. Opin. Neurobiol. 19, 319–326 (2009).
37. Rubenstein, J.L. & Merzenich, M.M. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2, 255–267 (2003).
38. Ulhaia, P.J. & Singer, W. Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. Neurosci. 52, 155–168 (2006).
39. Mackie, M., Paigen, B., Naito, N. & Pack, A.I. Analysis of the QTL for sleep homeostasis in mice: Homer1a is a likely candidate. Physiol. Genomics 33, 91–99 (2008).
40. Brown, M.R. et al. Fragile X mental retardation protein controls gating of the ionic-activated potassium channel Slack. Nat. Neurosci. 13, 819–821 (2010).
41. Thomas, A.M. et al. Genetic reduction of group 1 metabotropic glutamate receptors alters select behaviors in a mouse model for fragile X syndrome. Behav. Brain Res. 186, 230–232 (2011).
42. Thomas, A.M., Bui, N., Perkins, J.R., Yuva-Paylor, L.A. & Paylor, R. Group I metabotropic glutamate receptor antagonists alter select behaviors in a mouse model for fragile X syndrome. Psychopharmacology (Berl.) 219, 47–58 (2012).
43. Darnell, J.C. et al. FMRP stalls ribosomal translation on mRNAs linked to synaptic function and autism. Cell 146, 247–261 (2011).
44. Mizutani, A., Kuroda, Y., Futatsugi, A., Furuchi, T. & Mikoshiba, K. Phosphorylation of Homer3 by calcium/calmodulin-dependent kinase II regulates a coupling state of its target molecules in Purkinje cells. J. Neurosci. 28, 5369–5382 (2008).
45. Huang, G.N. et al. NFAT binding and regulation of T cell activation by the cytoplasmic scaffolding Homer proteins. Science 319, 476–481 (2008).
46. Orlando, L.R. et al. Phosphorylation of the homer-binding domain of group I metabotropic glutamate receptors by cyclin-dependent kinase 5. J. Neurochem. 110, 557–569 (2009).
47. Bangash, M.A. et al. Enhanced polyubiquitination of Shank3 and NMDA receptor in a mouse model of autism. Cell 145, 758–772 (2011).
48. Billuart, P. et al. Oligophenin-1 encodes a rhGAP protein involved in X-linked mental retardation. Nature 392, 923–926 (1998).
ONLINE METHODS

Animals. Congenic Fmr1<sup>y</sup> mice<sup>49</sup> were bred on the C57/B6J background. Homer1a-specific knockout mice were generated as described<sup>23</sup> and backcrossed at least five generations onto the C57/B6J mice from the University of Texas Southwestern mouse breeding core facility. All experiments were performed on littermate controls and blind to mouse genotype. Long Evans Hooded rats were obtained from Charles River Laboratories. Approximately 750 mice and 15 rats were used for experiments. Only male mice were used. The animal use protocols used in this manuscript were approved by the UT Southwestern Institutional Animal Care and Use Committee.

Reagents. Drugs were prepared as stocks, stored at −20 °C and used within 2 weeks. The Tat-fused peptides mGluR5CT (YGRKKRRQRRR-ALTPPSPFR) and mGluR5MU (YGRKKRRQRRR-ALTPLSPRR)<sup>18</sup> were synthesized at the University of Texas Southwestern Protein Chemistry Technology Center. Peptide was dissolved in H<sub>2</sub>O at 5 mM, aliquoted and stored at −20 °C, and used at 5 µM. Frozen aliquots were used within 10 d. The mixed group I mGluR agonist DHPG, U0126 and wortmannin were obtained from Tocris Bioscience and described<sup>20,22</sup>. Cycloheximide was purchased from Sigma and freshly prepared daily by dissolving directly in artificial cerebrospinal fluid (ACSF).

Hippocampal slice preparation and LTD recordings. Acute hippocampal brain slices were prepared from rats 3–6 weeks of age or WT, Fmr1<sup>y</sup>, H1α<sup>y</sup> or H1α<sup>y</sup>-/Fmr1<sup>y</sup> mouse littersmates as described<sup>18,19</sup>. LTD recordings were performed and analyzed as described<sup>18</sup>.mGluR signaling in slices and western blotting. Western blotting on slices was performed as described<sup>18</sup>. Hippocampal slices were preincubated in a static incubation chamber in ACSF containing 5 µM mGluR5CT or mGluR5MU for 4 h before DHPG treatment (Fig. 1) or 30 min in MPEP (10 µM; Fig. 3f). Blotting membranes were incubated with the following antibodies according to the manufacturer's instructions: phospho-(Thr56)-EF2, total EF2, phospho-(Thr389)-SEK, phospho-(Ser2448)-mTOR, total mTOR, phospho-(Thr202 and Tyr204)-ERK1/2, total ERK1/2, phospho-(Ser209)-eIF4E, phospho-(Ser65)-4EBP, 4EBP, eIF4G, eIF4E (all from Cell Signaling Technology), Homer (Sc-8921; Santa Cruz), mGluR5 (Millipore), β3 tubulin (Abcam), Arc (Synaptic Systems), Map1b (gift from I. Fischer, Drexel University), CaMKIIα (Santa Cruz, sc-5391), actin (Millipore, MAB1501). For comparison of phosphoprotein levels across conditions or genotypes, immunoreactive phosphoprotein bands were normalized to total protein from the same slice homogenates (for example, phospho-mTOR/mTOR immunoreactivity ratio), each of which was first normalized to loading control (either tubulin, actin or total ERK as indicated).

Coimmunoprecipitation. Hippocampi were lysed in coimmunoprecipitation buffer (50 mM Tris, pH 7.4, 120 mM NaCl, 0.5% Nonidet P-40), and protein was tumbled overnight at 4 °C with 1 µg of antibody (either Homer (Santa Cruz, D-3), Homer1a (Santa Cruz, M-13) or eIF4G (Cell Signaling)). Protein A/G agarose bead slurry (Thermo Scientific) was added for 1 h more, and the beads were then washed with coimmunoprecipitation buffer. Western blotting was performed with antibodies to Homer (Santa Cruz, E-18 sc-8921), Homer1a (ref. 21), mGluR5 (Millipore), eIF4E (Cell Signaling) or eIF4G (Cell Signaling). Full-length blots are shown in Supplementary Figures 7–10.

Real-time RT-PCR. Hippocampi were homogenized in TRIzol reagent (Invitrogen) followed by RNA extraction according to manufacturer's protocol. RNA (2 µg from each sample) was subjected to first-strand cDNA synthesis by SuperScript III First-Strand synthesis system (Invitrogen) with two independent primers targeting the 3′ UTR of the mRNAs (Homer1a: 5′-GTT GGA AAG CTT TCT TCT ATG AGA G-3′ and 5′-GGG ACC TCT GTG GGC CTT TGG-3′, Gapdh: 5′-GTT CAA GAG AGT AGG GAG-3′ and 5′-GGG TGC AGC AGA CTT TAT TG-3′). PCR was performed by GoTag Green DNA polymerase (Promega) with specific primers against Homer1a (5′-TGA TCG CTG AAT TGA ATG TGT TGC ACC-3′ and 5′-GAA GTC GCA GGA GAT G-3′)<sup>50</sup> and Gapdh (5′-AGG TCG GTG TGC GAT GAT TTG-3′ and 5′-TGT AGA CCA TCT GGT TGA GGT CA-3′).

Metabolic labeling of hippocampal slices. Hippocampal slices were prepared as described<sup>18,20</sup>. For these experiments (Fig. 3), the most ventral slices (two per hippocampus) were used because basal protein synthesis rates differ between dorsal and ventral hippocampal slices<sup>20</sup>. Slices recovered for 3.5 h in ACSF at 32 °C and then were incubated in actinomycin D (25 µM) for 30 min. Where indicated, 20 µM U0126 or 100 nM wortmannin was added at this step. For experiments with mGluR5 peptides, slices were preincubated in ACSF containing 5 µM mGluR5CT or mGluR5MU for 4 h before actinomycin incubation.

Neocortical slice preparation and UP state recordings. UP state experiments in neocortical slices were performed and analyzed as described<sup>11</sup>. To allow time for the mGluR5 peptides to permeate slices, the peptide-containing ACSF was perfused onto the slices in the interface chamber for 4 h before recording and was supplemented with 10 µM HEPES buffer, pH 7.4, 0.05% BSA and 5 µM of the appropriate peptide. The ACSF containing peptide and BSA was not oxygenated directly, but slices were oxygenated in the interface recording chamber.

Audigenic seizures. To evaluate audiogenic seizures, mice were placed in a plastic chamber (30 × 19 × 12 cm) containing a 120-dB siren (GE 50246 personal security alarm), which was covered with a Styrofoam lid. The 120-dB siren was presented to mice for 5 min. Mice were videotaped and scored for behavioral phenotype as described<sup>27</sup>: 0 = no response, 1 = wild running, 2 = tonic-clonic seizures, 3 = status epilepticus or death.

Behavioral measurements. Open field activity. Mice were placed individually into the periphery of a novel open field environment (44 cm × 44 cm, walls 30 cm high) in a dimly lit room and allowed to explore for 5 min. They were monitored from above by a video camera connected to a computer running video tracking software (Ethovision 3.0, Noldus) to determine the time, distance moved and number of entries into two areas: the periphery (5 cm from the walls) and the center (all areas excluding the periphery). The open field arenas were wiped and allowed to dry between mice. Time in the center was used as a measure of anxiety.

Locomotor activity. Mice were placed individually into a new, plastic mouse cage (18 cm × 28 cm) that was located inside a dark Plexiglas box. Movement was monitored by five photobeams in one dimension (Photobeam Activity System, San Diego Instruments) for 2 h, with the number of beam breaks recorded every 5 min. Data were analyzed with an analysis of variance (ANOVA) with genotype and time as the dependent variables.

Statistics. Data plotted in the figures represent the mean ± s.e.m. Significant differences were determined using independent or paired t-tests (for determining effects of mGluR5CT peptide). For comparisons between WT, Fmr1<sup>y</sup>, H1α<sup>y</sup> and H1α<sup>y</sup>-/Fmr1<sup>y</sup>, a two-way ANOVA and Bonferroni post-tests were used. For statistics on nominal data, such as seizure severity and incidence (Fig. 6a and Supplementary Table 3), a chi-squared (Fisher's exact) test was used. Group data are presented in the figures as mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001.