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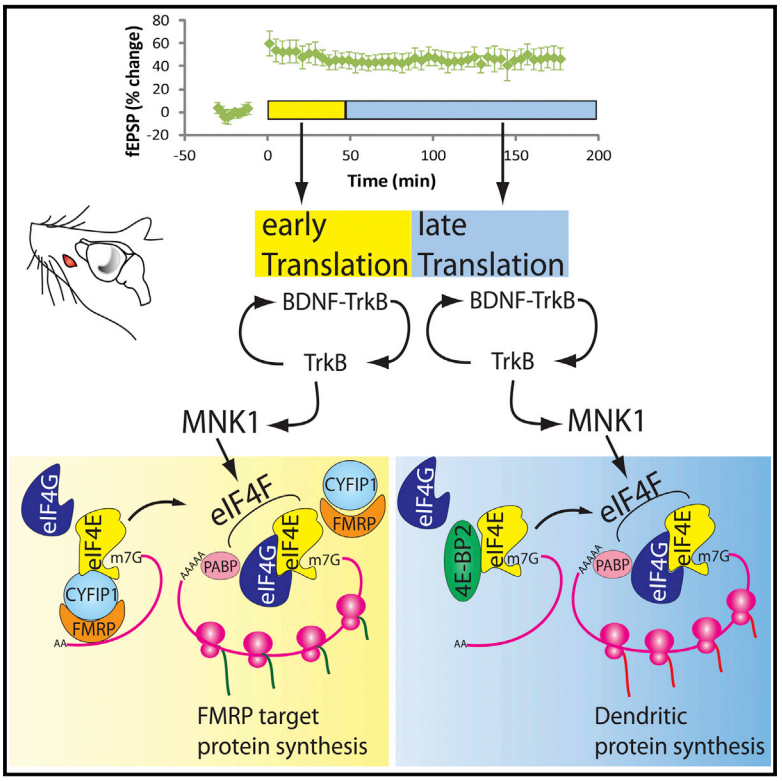
Two-Stage Translational Control of Dentate Gyrus LTP Consolidation Is Mediated by Sustained BDNF-TrkB Signaling to MNK

Graphical Abstract



Highlights

Sustained BDNF-TrkB signaling controls LTP consolidation in vivo

TrkB signaling to MNK mediates LTP consolidation

MNK regulates CYFIP1/FMRP translation repressor complex in early-stage LTP

MNK regulates 4E-BP2 and dendritic protein synthesis in late-stage LTP

Authors

Debabrata Panja, Justin W. Kenney, ..., Christopher G. Proud, Clive R. Bramham

Correspondence

clive.bramham@biomed.uib.no

In Brief

The logic of translational control in synaptic plasticity is not well understood. Panja et al. show that long-term potentiation in the dentate gyrus of live rodents is a two-stage process driven by brain-derived neurotrophic factor signaling to MAP-kinase-interacting kinase and activation of functionally and mechanistically distinct forms of translation.

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Two-Stage Translational Control of Dentate Gyrus LTP Consolidation Is Mediated by Sustained BDNF-TrkB Signaling to MNK

Debabrata Panja,[1,2] Justin W. Kenney,[3] Laura D'Andrea,[5] Francesca Zalfa,[6] Anni Vedeler,[1] Karin Wibrand,[1,2] Rikiro Fukunaga,[7] Claudia Bagni,[5,8,9] Christopher G. Proud,[3,4] and Clive R. Bramham[1,2,*]
[1]Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway
[2]KG Jebsen Centre for Research on Neuropsychiatric Disorders, University of Bergen, 5009 Bergen, Norway
[3]Centre for Biological Sciences, Life Sciences Building, Highfield Campus, University of Southampton, Southampton SO17 1BJ, UK
[4]South Australian Health and Medical Research Institute, and School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA 5000, Australia
[5]Department of Biomedicine and Prevention, University Tor Vergata, 00133 Rome, Italy
[6]CIR Department, Faculty of Medicine, University Campus Bio-Medico, 00128 Rome, Italy
[7]Department of Biochemistry, Osaka University of Pharmaceutical Sciences, Osaka 569-1094, Japan
[8]VIB Center for the Biology of Disease, 3000 Leuven, Belgium
[9]Center for Human Genetics and Leuven Institute for Neuroscience and Disease (LIND), KU Leuven, 3000 Leuven, Belgium
*Correspondence: clive.bramham@biomed.uib.no
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SUMMARY

BDNF signaling contributes to protein-synthesis-dependent synaptic plasticity, but the dynamics of TrkB signaling and mechanisms of translation have not been defined. Here, we show that long-term potentiation (LTP) consolidation in the dentate gyrus of live rodents requires sustained (hours) BDNF-TrkB signaling. Surprisingly, this sustained activation maintains an otherwise labile signaling pathway from TrkB to MAP-kinase-interacting kinase (MNK). MNK activity promotes eIF4F translation initiation complex formation and protein synthesis in mechanistically distinct early and late stages. In early-stage translation, MNK triggers release of the CYFIP1/FMRP repressor complex from the 5′-mRNA cap. In late-stage translation, MNK regulates the canonical translational repressor 4E-BP2 in a synapse-compartment-specific manner. This late stage is coupled to MNK-dependent enhanced dendritic mRNA translation. We conclude that LTP consolidation in the dentate gyrus is mediated by sustained BDNF signaling to MNK and MNK-dependent regulation of translation in two functionally and mechanistically distinct stages.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a key regulator of protein-synthesis-dependent synaptic plasticity and memory formation in the mammalian brain (Bekinschtein et al., 2014; Minichiello, 2009; Panja and Bramham, 2014; Park and Poo, 2013). BDNF is secreted at glutamatergic synapses in an activity-dependent manner (Aicardi et al., 2004; Edelmann et al., 2014; Hartmann et al., 2001; Matsuda et al., 2009). Endogenous activation of the BDNF receptor, tropomyosin-like kinase B (TrkB), promotes long-term potentiation (LTP) maintenance (Figurov et al., 1996; Kang et al., 1997; Korte et al., 1998; Minichiello et al., 2002; Rex et al., 2007), as well as protein-synthesis-dependent structural plasticity of dendritic spines (Tanaka et al., 2008). TrkB receptors couple to mRNA translation through multiple pathways, and exogenous BDNF is capable of inducing protein-synthesis-dependent LTP (Kang and Schuman, 1996; Leal et al., 2014; Panja and Bramham, 2014; Schratt et al., 2004; Takei et al., 2004). However, the dynamics of BDNF-TrkB signaling and the downstream mechanisms that mediate translation in LTP are little understood.

Translational control provides a means for regulating the time, place, and amount of cellular protein synthesis (Bramham and Wells, 2007; Jung et al., 2014; Kong and Lasko, 2012). Synaptic plasticity is thought to require changes in both general and mRNA-specific translation (Costa-Mattioli et al., 2009; Gal-Ben-Ari et al., 2012; Sossin and Lacaille, 2010). This suggests coordinated regulation of multiple forms of translation, but the logic of the process has not been described for any specific form of synaptic plasticity. Such information is essential for unraveling possible translational programs mediating plasticity in specific circuits.

Translation initiation, the multistep process by which the ribosome is recruited to the mRNA, is the most highly regulated step in eukaryotic protein synthesis (Kong and Lasko, 2012). A key event in translation initiation is the association of eukaryotic initiation factor 4E (eIF4E) to the mRNA 5′-m^{7}GpppN cap structure. eIF4E recruits the scaffolding protein, eIF4G, and the RNA helicase, eIF4A, to form the eIF4F complex. eIF4F formation is critical for recruitment of the 40S ribosome and scanning to the start codon. Under basal conditions, eIF4E-binding proteins (4E-BPs)

repress translation by blocking the recruitment of eIF4G to eIF4E. Phosphorylation of 4E-BP, catalyzed by the mammalian target of rapamycin complex 1 (mTORC1) kinase, triggers the release of 4E-BP and facilitates translation (Gingras et al., 2001; Proud, 2007). In addition, noncanonical 4E-BPs can regulate mRNA-specific translation through association with RNA-binding proteins (Kong and Lasko, 2012; Richter and Klann, 2009). One such protein in brain is CYFIP1, cytoplasmic fragile-X mental retardation protein (FMRP)-interacting protein (Napoli et al., 2008). Through dual binding of FMRP and eIF4E, CYFIP1 regulates translation of FMRP target mRNAs such as the mRNA encoding the activity-dependent cytoskeletal-associated protein, Arc (De Rubeis et al., 2013; Napoli et al., 2008; Zalfa et al., 2003).

Arc is an immediate early gene product required for several forms of long-term synaptic plasticity and memory formation (Bramham et al., 2010). *Arc* mRNA is rapidly induced and transported to dendritic processes for local storage and translation. In the dentate gyrus (DG), LTP consolidation requires a period of sustained Arc synthesis lasting from 2 to 4 hr after LTP induction (Messaoudi et al., 2007). Unlike hippocampal region CA1 and several other brain regions (Costa-Mattioli et al., 2009; Gal-Ben-Ari et al., 2012), LTP consolidation, eIF4F formation, and Arc synthesis in the DG are insensitive to mTORC1 inhibition by rapamycin (Panja et al., 2009). In DG LTP, ERK signaling to MAP-kinase-interacting kinases (MNKs) has been implicated in eIF4F formation (Panja et al., 2009). MNKs are known as eIF4E kinases (Banko et al., 2006; Gelinas et al., 2007; Proud, 2007), but the function of MNKs in the nervous systems is largely unknown.

Here, we report that sustained BDNF-TrkB signaling drives translation and mediates Arc synthesis-dependent LTP in the DG. Sustained TrkB receptor activation serves to maintain an otherwise short-lived signaling pathway from TrkB to MNK. In turn, MNK mediates eIF4F translation initiation complex formation in distinct early and late stages linked to CYFIP1 and 4E-BP2 regulation, respectively. Notably, the late stage is associated with pronounced MNK-dependent synaptic translation. Hence, LTP consolidation in the DG is mediated by BDNF signaling to MNK and MNK-dependent activation of translation in two functionally and mechanistically distinct stages.

RESULTS

Sustained BDNF-TrkB Activation Is Required for DG LTP Consolidation

The BDNF scavenger, TrkB-Fc, was used to probe the role of BDNF-TrkB activation in medial perforant-evoked DG LTP in adult anesthetized rats. TrkB-Fc or control immunoglobulin G (IgG)-Fc was acutely infused (100 μg, 1 μl, 12.5 min) 45 min before high-frequency stimulation (HFS), or at one of five time points after HFS. Infusions were made into deep stratum lacunosum-moleculare, immediately above the dorsal DG. As shown in Figure 1A, LTP of the field excitatory postsynaptic potential (fEPSP) slope was significantly reduced in rats receiving TrkB-Fc prior to HFS compared to the IgG-Fc-treated control. When HFS was omitted from the paradigm, TrkB-Fc infusion had no effect on fEPSP responses over a 4 hr period of baseline test stimulation (BTS). These findings concur with previous reports showing acute regulation of LTP induction by endogenous BDNF (Figurov et al., 1996; Gooney and Lynch, 2001; Kossel et al., 2001). During LTP maintenance, TrkB-Fc was infused at 10 min, 2 hr, 4 hr, 8 hr, or 10 hr after HFS (Figure 1; composite results in Figures 1G and 1H). Control IgG-Fc infusions had no effect on LTP maintenance. Strikingly, TrkB-Fc infusion at 10 min, 2 hr, or 4 hr after HFS resulted in rapid, complete, and permanent reversion of LTP (Figures 1B–1D). The decline in synaptic efficacy was significant within ~10 min of TrkB-Fc infusion onset and reached the pre-HFS baseline level by 30 min (Figure 1G). The effects of TrkB-Fc were strictly time sensitive; TrkB-Fc infusion only transiently diminished fEPSPs at 8 hr after HFS (Figure 1E) and had no significant effect at 10 hr after HFS (Figure 1F). The effects of TrkB-Fc on LTP maintenance were replicated by infusion of K252a (250 μM, 1 μl, 12.5 min), a Trk inhibitor that crosses cell membranes and directly inhibits Trk tyrosine kinase activity (Figure 1I; time-course plots shown in Figure S1A). The results indicate that LTP consolidation requires persistent activation of TrkB by BDNF. The period of TrkB dependency starts within 10 min of HFS and lasts between 4 and 8 hr.

Sustained BDNF-TrkB Signaling Maintains ERK Activation and Arc Expression

The rapid reversion of LTP observed at multiple time points suggests that TrkB couples to a labile signaling pathway. As BDNF-induced Arc expression requires ERK activation (Messaoudi et al., 2007; Ying et al., 2002), we examined TrkB-ERK signaling and Arc expression during LTP. To monitor signaling within the synaptic compartment, assays were performed in synaptoneurosomes, which are biochemical fractions highly enriched in pinched-off dendritic spines attached to axon terminals of excitatory synapses (De Rubeis et al., 2013; Håvik et al., 2003; Troca-Marín et al., 2011). The synaptic enrichment of the DG synaptoneurosome preparation was validated by immunoblotting for marker proteins (Figures 2A and 2B). PSD-95 and GluN1 postsynaptic components of glutamatergic synapses were enriched 14- and 17-fold, respectively, in DG synaptoneurosomes relative to lysates. The presynaptic markers synaptophysin and cysteine-string protein-α (CSPα) were also enriched in synaptoneurosomes, whereas the glial protein, GFAP, and the glycolytic enzyme, GAPDH, were depleted in synaptoneurosomes relative to lysates (Figures 2A and 2B). The nuclear membrane protein, laminin, was heavily stained in lysates but was not detected in synaptoneurosomes (Figures 2A and 2B).

HFS elicited increased $Tyr^{706/707}$ TrkB autophosphorylation and Thr^{202}/Tyr^{204} ERK phosphorylation and enhanced Arc expression in both whole-lysate samples and synaptoneurosome samples. Immunoblot analysis of tissue obtained at 10 min, 40 min, and 3 hr after HFS revealed sustained activation (Figures 2E–2J). Next, TrkB-Fc was infused at 10 min after HFS, and tissue was collected at 40 min after HFS, by which time fEPSP responses had declined to baseline levels (Figure 2C). At 40 min, phospho-TrkB, phospho-ERK, and Arc protein in whole lysate and synaptoneurosomes were significantly inhibited relative to time-matched IgG-Fc-infused controls (Figures 2E–2G). TrkB-Fc infusion at 2 hr similarly resulted in rapid

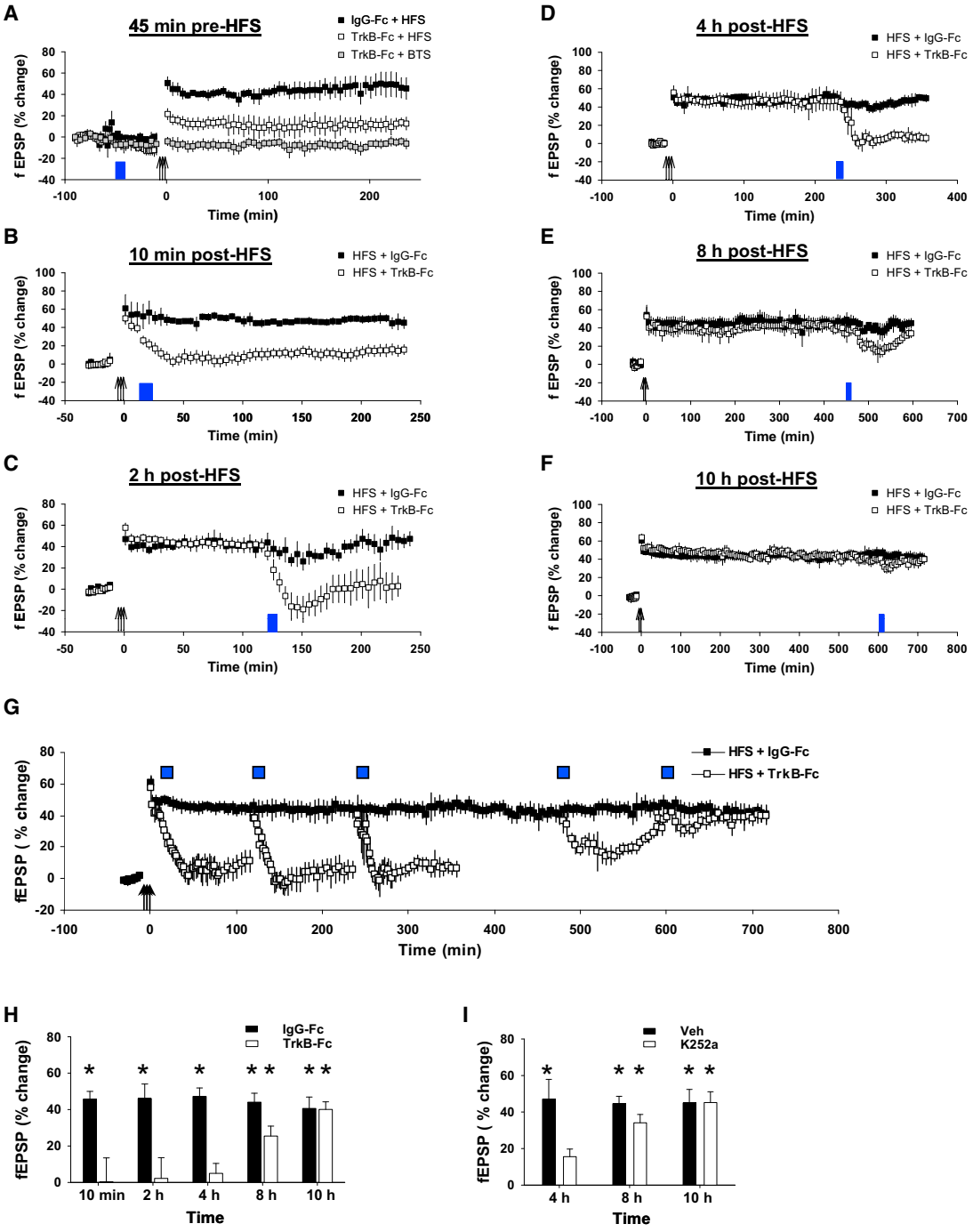


Figure 1. Sustained BDNF-TrkB Activation Is Required for DG LTP Consolidation In Vivo

Time-course plots of medial perforant path-dentate gyrus (DG) evoked fEPSPs recorded before and after high-frequency stimulation (HFS, indicated by arrows). Values are mean ± SEM of the maximum fEPSP slope expressed as percentage of baseline. Test pulses were applied at a 0.033 Hz. TrkB-Fc (1 μl, 12.5 min, 100 μg) or control IgG-Fc (1 μl, 12.5 min, 100 μg) was infused into the dorsal DG during the period indicated by the blue bar. In (A), a third treatment group received TrkB-Fc infusion and baseline test pulse stimulation (BTS) but not HFS. n = 7/group. Error bars represent SEM.

(A–F) fEPSP changes in rats receiving TrkB-Fc or IgG-Fc 45 min before HFS (A), or 10 min (B), 2 hr (C), 4 hr (D), 8 hr (E), or 10 hr (F) after HFS. LTP maintenance was permanently reverted by TrkB-Fc infusion at time points up to 4 hr after HFS, but not thereafter.

(G) Composite time-course plots of TrkB-Fc effects.

(H) Mean changes ± SEM in fEPSP slope 1 hr after the start of IgG-Fc or TrkB-Fc infusion. *$p < 0.05$, significantly different from baseline, Student's t test for independent samples. n = 5–6/group.

(I) Effect of K252a infusion on LTP maintenance. Vehicle is 0.1% DMSO in 1 × PBS. Time-course plots shown in Figure S1.

inhibition of enhanced TrkB-ERK activity and Arc expression at 3 hr after HFS (Figures 2D and 2H–2J). We conclude that BDNF persistently activates a short-lived signaling pathway from TrkB to ERK activation and Arc protein expression.

Early Translation: TrkB Regulates CYFIP1/FMRP and eIF4F Formation

There are three mammalian 4E-BP paralogs (4E-BP1, -2, and -3) and 4E-BP2 is the major form expressed in brain (Banko et al., 2005). mTORC1-dependent release of 4E-BP2 and the resulting enhancement in eIF4F formation (eIF4E-eIF4G interaction) are critical to multiple forms of translation-dependent synaptic plasticity (Richter and Klann, 2009). However, DG LTP is mTORC1-independent and eIF4F formation occurs in the absence of 4E-BP2 release from eIF4E, as measured in cap pull-down analyses in lysates samples (Panja et al., 2009).

We therefore asked whether CYFIP1, a noncanonical 4E-BP, functions in DG LTP. TrkB-Fc or IgG-Fc was infused 10 min after HFS, and DG lysates were collected at 40 min after HFS (same electrophysiological procedure as shown in Figure 2C). m^7GTP-Sepharose (cap analog) pull-downs were performed, and changes in the amount of eIF4G, 4E-BP2, CYFIP1, and FMRP normalized to levels of cap-bound eIF4E were determined by immunoblotting (Figures 3A, top, and 3B). In HFS-treated DG of IgG-Fc-infused rats, recovery of CYFIP1 and FMRP was significantly reduced, whereas loading of eIF4G was enhanced 2-fold relative to the contralateral DG. Infusion of TrkB-Fc abolished these changes in CYFIP1/FMRP and eIF4G recovery on m^7GTP beads (Figures 3A and 3B). Given evidence of sustained eIF4F formation in LTP, we predicted sustained regulation of CYFIP1/FMRP. TrkB-Fc was infused at 2 hr and whole DG tissue was collected at 3 hr after HFS (same electrophysiology procedure as shown in Figure 2D). Surprisingly, although TrkB-Fc prevented the enhanced eIF4G-eIF4E interaction, there was no difference between HFS-treated and control DG lysate in CYFIP1/FMRP recovery (Figures 3A, bottom panel, and 3B). The results support a model in which TrkB signaling regulates CYFIP1/FMRP association with eIF4E at the early (40 min) but not late (3 hr) time points in LTP maintenance.

4E-BP2 undergoes brain-specific deamidation postnatally, resulting in three primary bands detected by western blot (Bidinosti et al., 2010). Two upper bands at ~18–20 kDa correspond to deamidated 4E-BP2, whereas the unmodified form migrates at ~16 kDa (see blots in Figure 3B). Confirming our previous findings (Panja et al., 2009), the amount of deamidated (both bands combined) and unmodified 4E-BP2 recovered with eIF4E in cap pull-downs did not differ between treated and control DG at 40 min or 3 hr after HFS (Figures 3A and 3B).

Late Translation: TrkB Regulates 4E-BP2 and eIF4F Formation in the Synaptic Compartment

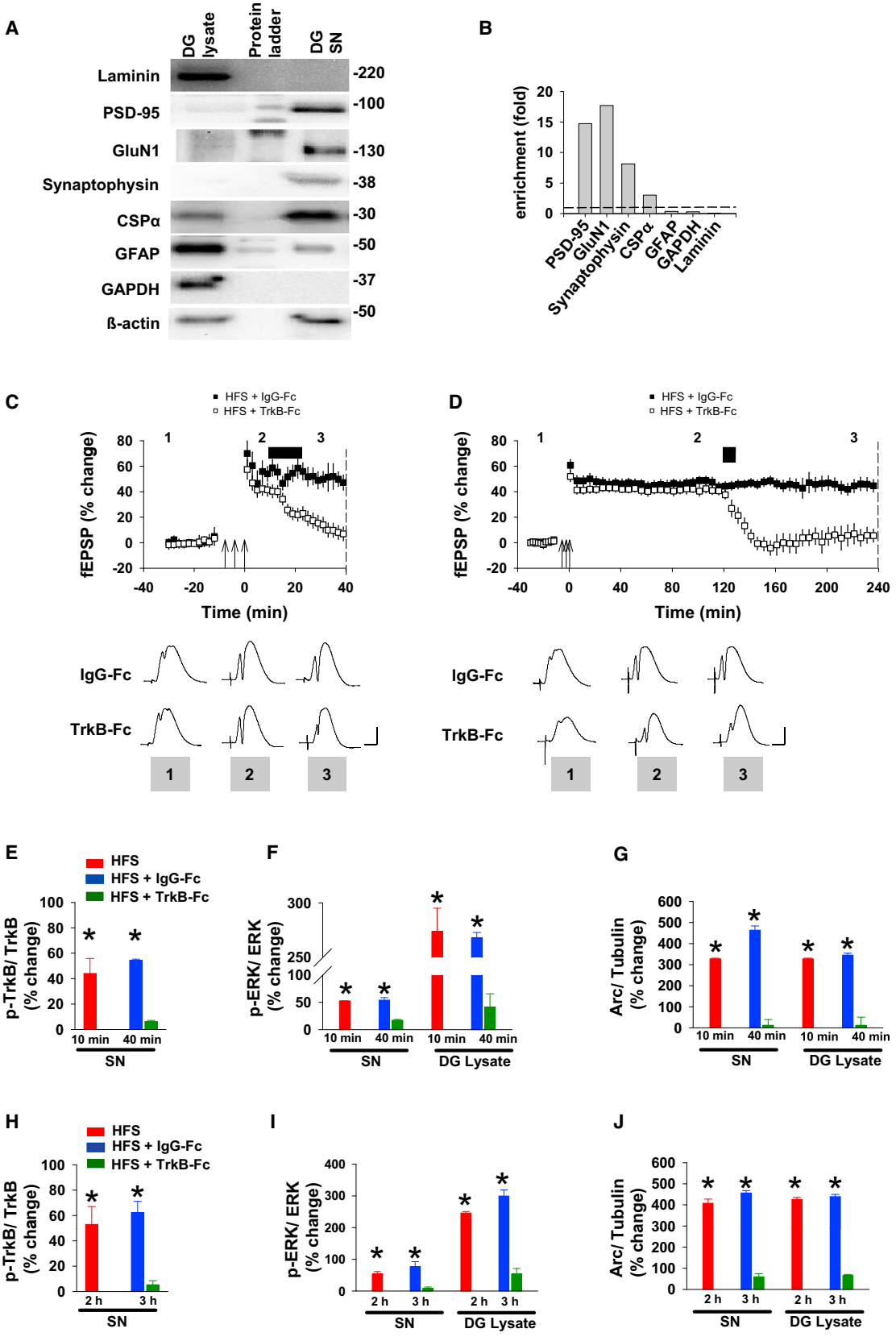
Local protein synthesis in dendrites and dendritic spines is important for synaptic regulation and plasticity (Bramham and Wells, 2007; Martin and Ephrussi, 2009). We therefore performed cap pull-downs in DG synaptoneurosomes. eIF4F formation in synaptoneurosomes was enhanced at both 40 min and 3 hr after HFS (Figures 3C and 3D). Reduction of CYFIP1 and FMRP binding to the m^7GTP beads was also detected in synaptoneurosomes, but, as in lysates, this effect was confined to the early time point (Figure 3C, upper panel). Surprisingly, immunoblot analysis of 4E-BP2 in cap pull-downs revealed a regulation that was specific to the synaptoneurosome compartment and the late time point (Figures 3C, bottom panel, and 3D). At 3 hr after HFS, levels of 4E-BP2 normalized to eIF4E were reduced by 50.6% ± 1.8% and 33.4% ± 0.8% for deamidated and unmodified 4E-BP2, respectively. Finally, TrkB-Fc blocked both the early regulation of CYFIP1 and the late regulation of 4E-BP2, while preventing the enhanced eIF4G-eIF4E interaction at both time points in the synaptic compartment (Figures 3C and 3D). Thus, TrkB activity mediates sequential regulation of distinct translational repressors and persistently enhances eIF4F formation in DG LTP.

TrkB-MNK Signaling Regulates CYFIP1 and 4E-BP2

Next, we examined TrkB signaling to MNK as a potential mechanism for DG LTP maintenance. Infusion of the MNK inhibitor, CGP57380 (2 mM, 1 μl, 12.5 min; Tschopp et al., 2000), at 10 min or 2 hr after HFS induced a rapid and stable reversion of LTP (Figures 4A and 4B), whereas CGP57380 infusion at 10 hr no longer affected LTP maintenance (composite time course in Figure 4F). The MNK inhibitor did not alter basal synaptic transmission in response to test-pulse stimulation (Figure 4B). Levels of active, $Thr^{197/202}$ phosphorylated MNK1 were inhibited by infusion of CGP57380 (Figure 4C) and TrkB-Fc at both early and late time points (Figures 4D and 4E). CGP57380 infusion at 10 min or 2 hr after HFS also led to a rapid decline in Arc protein levels relative to vehicle-infused control (Figure 4C). Hence, LTP maintenance across a defined time window depends on sustained TrkB signaling to MNK.

MNKs binds to eIF4G and phosphorylate eIF4E (Pyronnet et al., 1999; Scheper et al., 2002; Shveygert et al., 2010). In cap pull-down assays performed in DG lysates, TrkB-Fc and CGP57380 inhibited HFS-evoked Ser^{209} eIF4E phosphorylation at both early and late time points (Figures 3 and 5A–5C). At the early time point, the MNK inhibitor prevented changes in CYFIP1/FMRP and eIF4G association with eIF4E. At the late time point in synaptoneurosomes, CGP57380 inhibited changes in 4E-BP2 and eIF4G association with cap-bound eIF4E (Figures 5D and 5E).

The effect of CGP57380 and rapamycin on Arc protein expression during the period of sustained Arc synthesis was visualized by immunohistochemical staining (Figure S4). In rats infused with vehicle at 2 hr after HFS, Arc immunostaining was uniformly enhanced in the DG granule cell body and molecular (dendritic) layer. In CGP57380-infused rats, Arc immunostaining was strongly reduced in the molecular layer of both the upper and lower blades of the DG (Figure S4A). Clear Arc immunostaining remained in the granule cell body layer, consistent with residual expression of Arc in the immunoblot analysis (Figure 4C). In contrast, infusion of rapamycin at high concentrations (100 μM) did not impair LTP maintenance (Figure S4A) or affect Arc protein immunostaining (Figure S4B), yet it inhibited Ser^{2448} mTOR phosphorylation (not shown) and downstream enhancement of ribosomal protein S6 phosphorylation in the granule cell body layer (Figure S4B).



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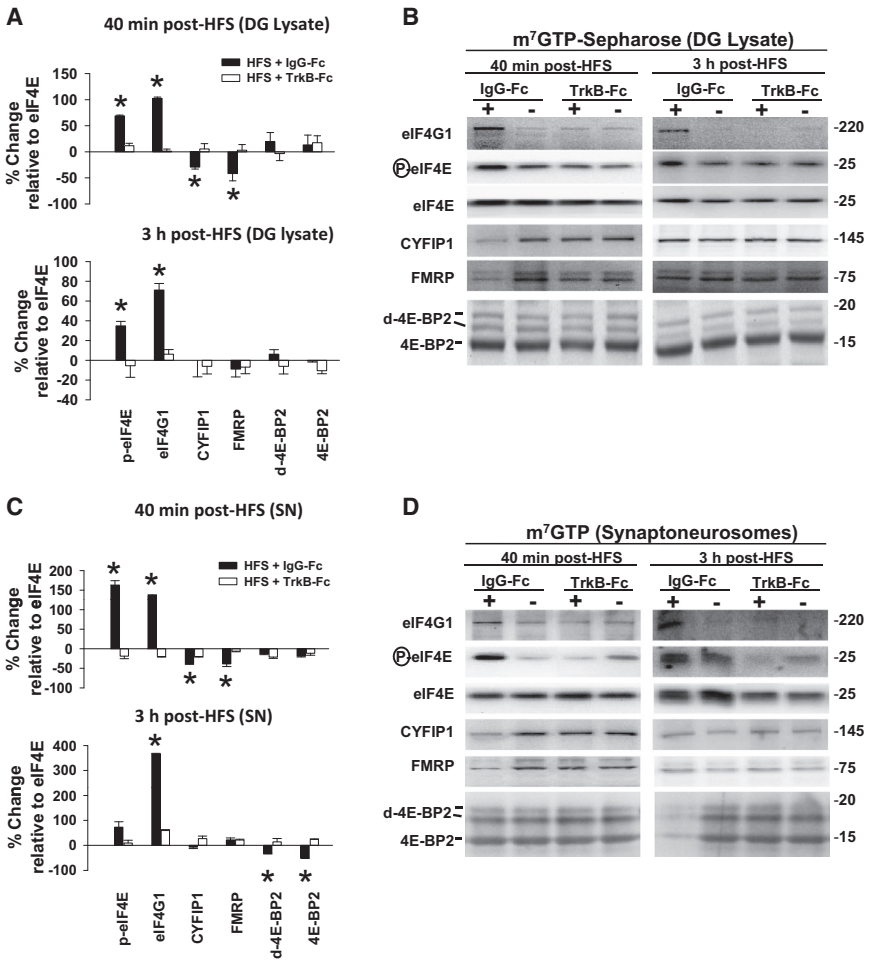


Figure 3. Sustained TrkB-Dependent eIF4F Formation and Sequential Regulation of CYFIP1 and 4E-BP2 Translational Repressors

(A and B) m^{7}GTP pull-down analysis in dentate gyrus (DG) total lysates. (A) Upper panel: TrkB-Fc or IgG-Fc was infused at 10 min after HFS and tissue was collected at 40 min after HFS. Lower panel: infusion at 2 hr, tissue collection at 3 hr after HFS. Quantification of immunoblots expressed as percentage change (mean ± SEM) of for p-eIF4E, eIF4G, CYFIP1, FMRP, deamidated (d), and non-modified 4E-BP2, normalized to total precipitated eIF4E in the corresponding treated DG (+) relative to the contralateral control DG (−). Error bars represent SEM. Student's t test in Table S1. (B) Representative immunoblot for (A).

(C and D) m^{7}GTP pull-down analysis in DG synaptoneurosomes (SN). (C) Quantification of immunoblots for indicated protein. n = 6/group *p < 0.05. (D) Representative immunoblot for (C). No bands were detected in control pull-downs using Sepharose 4B beads alone (Figure S2A). Results from immunoblot analysis of the input samples are shown in Figure S3.

4E-BP2 binding to eIF4E is known be regulated by mTORC1 catalyzed phosphorylation of 4E-BP2 on Thr$^{37/46}$. It was therefore of interest to examine Thr$^{37/46}$ 4E-BP2 phosphorylation state in synaptoneurosomes at 3 hr after HFS. No phospho-4E-BP2 signal was detected in synaptoneurosome cap pull-down samples from HFS-treated or control DG (Figure 5E). In synaptoneurosome inputs, strong phospho-4E-BP immunoreactivity specific to the two deamidated forms of 4E-BP2 was found (Figure 5E). Phospho specificity was confirmed by elimination of these bands in phosphatase-treated samples (Figure S2B). However, there was no change in phosphorylation state after HFS (Figure 5E), ruling out a role for HFS-induced Thr$^{37/46}$ 4E-BP2 phosphorylation in 4E-BP2 release.

Taken together, the results suggest that MNK regulates cap-dependent translation through control of CYFIP1 and 4E-BP2 interactions with eIF4E. To assess the role of cap-dependent translation in LTP, we infused 4EGI-1, a competitive blocker of eIF4G binding to eIF4E (Hoeffer et al., 2011; Moerke et al., 2007). Infusion of 4EGI-1 (1 mM, 1.5 μl) at 10 min or 2 hr after HFS resulted in rapid reversion of ongoing LTP (Figures S5A and S5B), mimicking the effects of TrkB-Fc and CGP57380. New protein synthesis was demonstrated by puromycin labeling in a method known as surface sensing of translation (SUnSET) (Hoeffer et al., 2013; Schmidt et al., 2009). Puromycin is a structural analog of tyrosyl-tRNA that becomes incorporated into nascent polypeptides, causing their release from ribosomes, and the puromycin-tagged

Figure 2. Sustained BDNF-TrkB Signaling Maintains ERK Activation and Arc Expression during LTP Consolidation

(A and B) Immunoblot characterization of dentate gyrus (DG) synaptoneurosomes.

DG lysate and synaptoneurosomes (SN) samples were immunoblotted with indicated antibodies. (A) Representative immunoblots. Molecular mass (in kDa at right) corresponds to the protein standard ladder in the middle lane. (B) Quantified immunoblots from (A); n = 6/ group. Measurement of direct chemiluminescence was normalized to β-actin protein. Normalized chemiluminescence for respective protein in total lysate was set at 1 (arbitrary units).

(C and D) Upper panels: fEPSP changes in rats receiving TrkB-Fc or IgG-Fc (black bar) at 10 min (C) or 2 hr (D) after HFS. Lower panels: sample field potential traces (mean of ten consecutive responses) from baseline (1), after HFS (2), and after TrkB-Fc (3). Scale bars, 2 ms and 5 mV.

(E–G) Immunoblot analysis of DG total lysates and SN obtained at 10 min after HFS (corresponding to start of infusion) or 40 min after HFS (stippled line in C). Quantification of immunoblots for p-TrkB (Tyr$^{706/707}$) (E), p-ERK(Thr^{202}/Tyr^{204}) (F), and Arc (G), n = 6–7/ group. Mean ± SEM and Student's t test in Table S1. Error bars represent SEM.

(H–J) Immunoblot analysis at 2 hr (start of infusion) and 3 hr after HFS (stippled line in D). Immunoblot data in this and all subsequent figures are calculated as percentage change in normalized densitometric values between treated and control DG on the same immunoblot. *p < 0.05, n = 5–6/group. Student's t test in Table S1. Representative immunoblots are shown in Figure S1B.

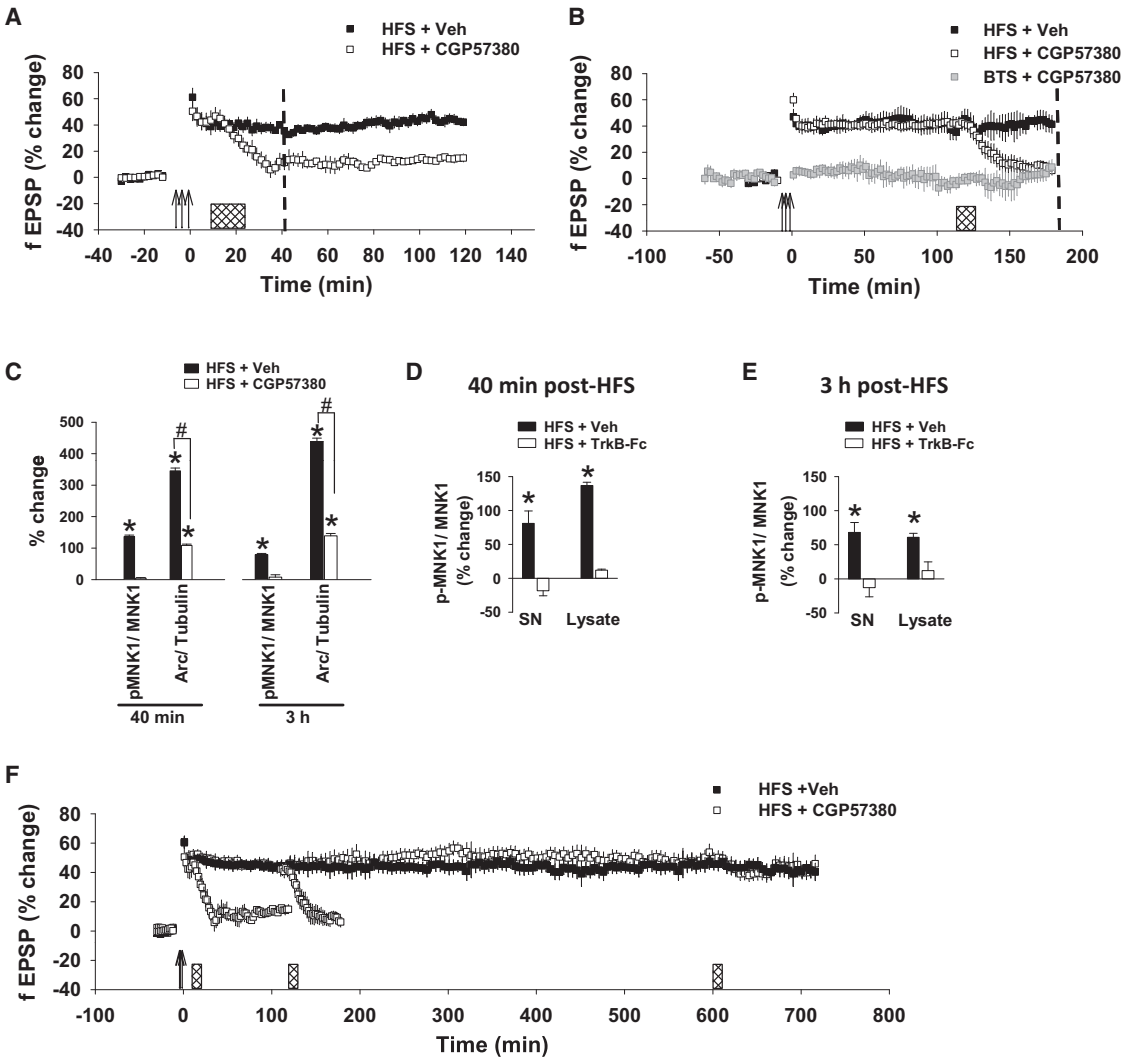


Figure 4. TrkB Signaling to MNK Is Required for LTP Consolidation

(A and B) Time-course plots of fEPSP changes in medial perforant path granule-cell-evoked fEPSPs. (A) CGP57380 (1 μl, 12.5 min, 2 μM, n = 10) or vehicle control (1 μl, 12.5 min, 0.1% DMSO-PBS) was infused at 10 min (meshed bar). (B) Infusion CGP57380 and vehicle at 2 hr. A third group received only baseline test stimulation (BTS) and CGP57380.

(C) Quantification of immunoblots (mean ± SEM) in DG lysates obtained at 40 min after HFS (stippled line in A) and 3 hr after HFS (stippled line in B). n = 6/group, *p < 0.05.

(D and E) Percentage changes (mean ± SEM) in phospho-MNK1 in DG lysates and SN obtained 40 min and 3 hr after HFS. n = 6/group, *p < 0.05.

(F) Composite time-course plots of the CGP57380 infusion at 10 min, 2 hr, and 10 hr after HFS. Error bars represent SEM. Statistics information and source data in Table S1.

polypeptides can be detected by immunoblotting with anti-puromycin. Puromycin labeling of proteins was significantly increased in DG tissue obtained 3 hr after HFS (Figures S5C and S5D). Moreover, increased puromycin labeling and enhanced Arc expression was inhibited by infusion of TrkB-Fc, CGP57380, and 4EGI-1 at 2 hr after HFS (Figures S5C and S5D).

Total levels of the eIF4E-associating proteins were also quantified for all cap pull-down experiments. Levels of eIF4G are known to be elevated after LTP induction (Panja et al., 2009). Here, we find enhanced TrkB-dependent and MNK-dependent regulation at both 40 min and 3 hr after HFS (Figure S3). Levels of 4E-BP2 in input samples did not change during LTP (Figure S3). In contrast, CYFIP1 and FMRP expression was reduced at 40 min, but not 3 hr, after HFS (Figure S3). The results suggest a model in which CYFIP1 and FMRP are degraded and resynthesized following their MNK-dependent release from eIF4E.

Impaired LTP Maintenance and Arc Synthesis in *Mnk1* Knockout Mice In Vivo

Acute infusion of CGP57380 into the hippocampus or cerebral cortex inhibits eIF4E phosphorylation without affecting activation of ERK, CaMKII, mTORC1, and several other signaling

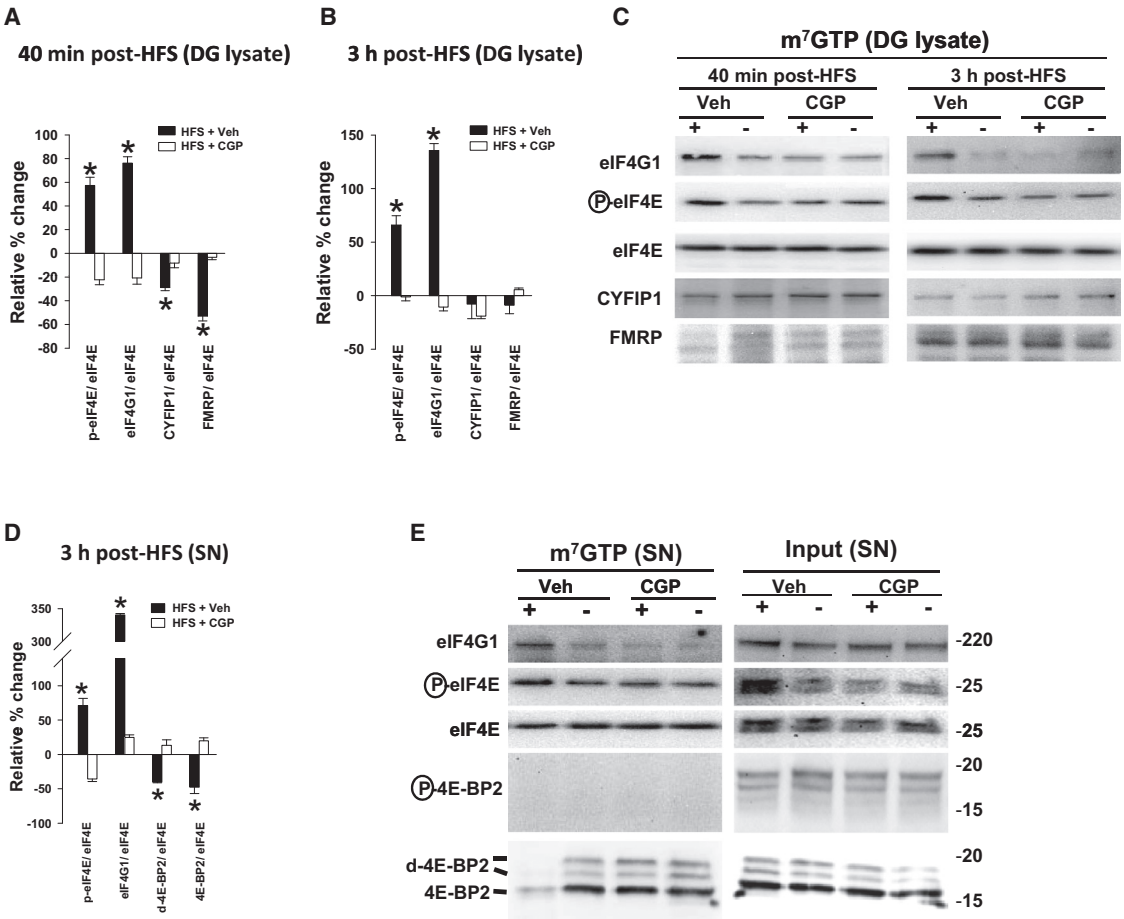


Figure 5. MNK Triggers Sequential Regulation of CYFIP1 and 4E-BP2 and Sustained eIF4F Formation

(A and B) m^{7}GTP pull-down assays on dentate gyrus total lysates (DG lysates) with vehicle (DMSO-PBS) or CGP57380 infused at 10 min (A) or 2 hr (B) after HFS and DG tissue collected as previously indicated in Figure 4. Quantification of immunoblots (mean ± SEM) in treated DG (+) relative to the contralateral control DG (–). n = 6/group, *p < 0.05.

(C) Representative immunoblots for (A) and (B). Quantification of input samples and representative immunoblots in Figures S3E and S3F.

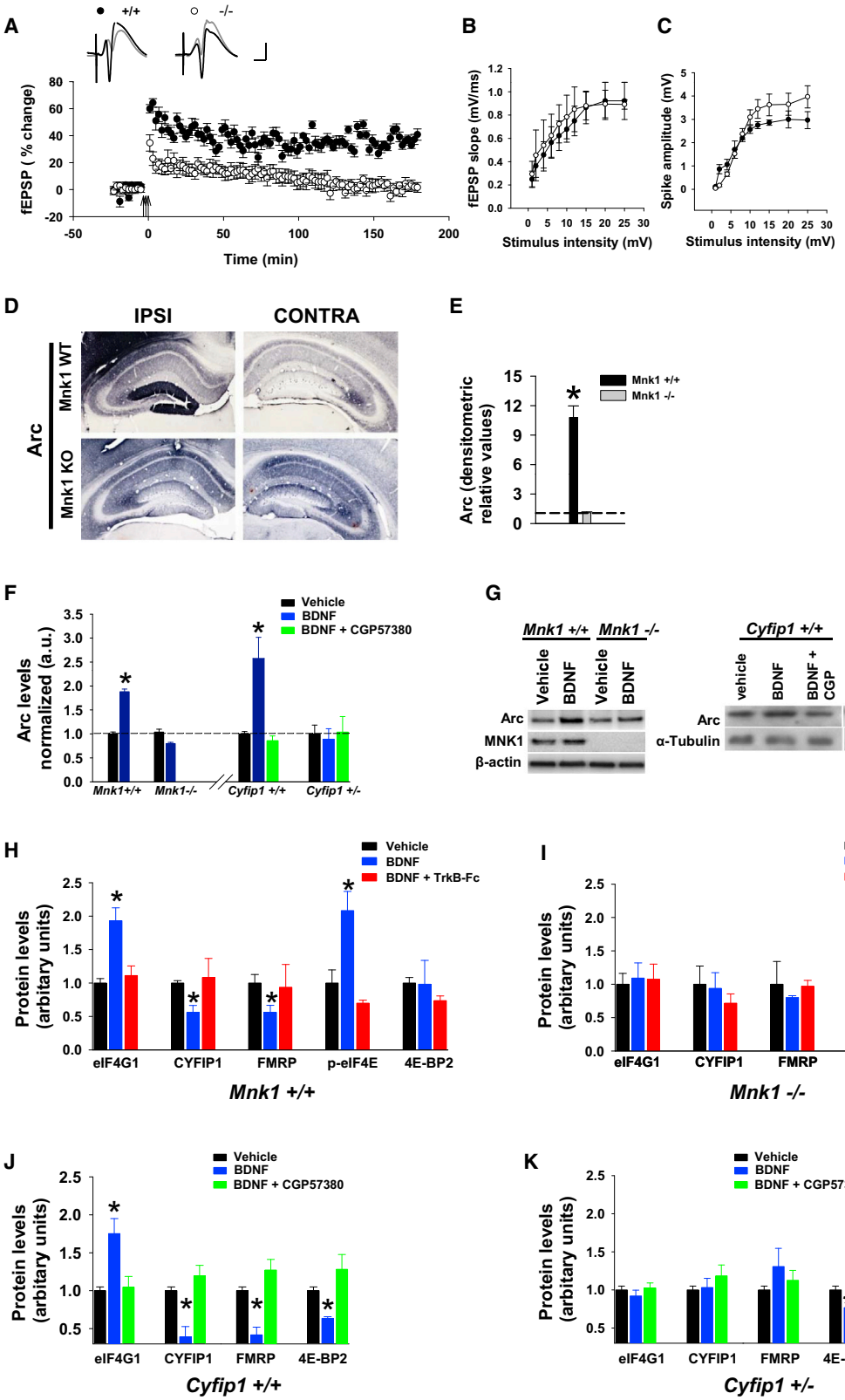
(D) m^{7}GTP pull-down assays on DG synaptoneurosomes (SN) samples. *p < 0.05, n = 6/group.

(E) Representative immunoblots from DG SN input and m^{7}GTP pull-down in (D). Error bars represent SEM. Statistics and source data are in Table S1.

(Dumoulin et al., 2013; Panja et al., 2009; Figures S6A and S6B). However, in vitro kinase assays show that CGP57380 inhibits other kinases (MKK1, CK1, BRSK2) in addition to MNK1 and MNK2 (Bain et al., 2007). As a complementary approach to pharmacological inhibition, we examined DG LTP in homozygous *Mnk1* knockout mice (Ueda et al., 2004). In urethane-anesthetized wild-type mice, 200 Hz HFS of the perforant path induced stable LTP of the fEPSP slope measured over a 3 hr period. In *Mnk1*$^{-/-}$ mice the magnitude of LTP was significantly reduced (mean 19% increase at 30 min, compared to 40% in wild-type) and decayed to baseline level by 2 hr after HFS (Figure 6A). Baseline evoked fEPSPs and population spikes did not differ between wild-type and *Mnk1* knockout mice over a range of stimulus intensities (input-output curves in Figures 6B and 6C). In brains of wild-type mice, Arc immunostaining was robustly increased in the granule cell layer and molecular layer of the ipsilateral DG at 3 hr after HFS (Figures 6D and 6E). In *Mnk1*$^{-/-}$ mice, no change in Arc staining was observed after HFS (Figures 6D and 6E) further showing that Arc synthesis is regulated by MNK1.

Selective Loss of CYFIP1/FMRP Regulation in DG Synaptoneurosomes from *Mnk1* Knockout Mice

To study regulation of the CYFIP1 and 4E-BP2 repressor complexes specifically within the synaptic compartment, we performed in vitro BDNF stimulation of isolated DG synaptoneurosomes from wild-type and *Mnk1* knockout mice. Arc expression was enhanced in BDNF-treated DG synaptoneurosomes in wild-type, but not *Mnk1*$^{-/-}$, mice (Figures 6F and 6G). In synaptoneurosomes from wild-type mice, but not *Mnk1* mutant mice, BDNF treatment for 30 min reduced CYFIP1/FMRP recovery while increasing eIF4G recovery and eIF4E phosphorylation (Figures 6H and 6I; sample blots in Figures S7A and S7B). Notably, 4E-BP2 binding to eIF4E was not affected by BDNF treatment in wild-type or *Mnk1* mutant mice (Figures 6H and 6I). Hence, BDNF treatment of synaptoneurosomes mimics the



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early translation stage of LTP associated with selective CYFIP1/FMRP regulation. Furthermore, levels of CYFIP1 and FMRP in input samples were unchanged, showing that BDNF first triggers release of CYFIP1/FMRP from eIF4E in the absence of protein degradation (Figures S7C and S7D). Finally, basal formation of the translation repressor complexes did not differ between wild-type and *Mnk1* knockout mice (Figure S7A).

MNK and CYFIP1-Dependent Regulation of eIF4F in BDNF-Treated Cortical Synaptoneurosomes

Previous work in cortical and hippocampal synaptoneurosomes showed that BDNF treatment induces release of CYFIP1/FMRP from eIF4E and enhanced translation of multiple FMRP-target mRNAs (De Rubeis et al., 2013; Napoli et al., 2008). In agreement with these data, we find that BDNF stimulation of cortical synaptoneurosomes decreases the association of CYFIP1/FMRP and 4E-BP2 with m^7GTP-bound eIF4E, while enhancing eIF4G-eIF4E association (Figures 6J and 6K). Treatment with CGP57380 inhibited these effects, indicating that BDNF-induced regulation of CYFIP1 and 4E-BP2 at cortical synapses is MNK dependent (Figures 6J and 6K).

To assess a causal role for CYFIP1, we used *Cyfip1* ± mice (Napoli et al., 2008; De Rubeis et al., 2013), which exhibited significantly reduced expression of CYFIP1 of 45% in lysates and 65% in synaptoneurosomes (Figures S7E and S7F). BDNF-induced Arc expression was impaired in *Cyfip1*$^{+/-}$ mice and inhibited by CGP57380 treatment in wild-type mice (Figures 6F and 6G). BDNF failed to alter the association of CYFIP1/FMRP and eIF4G with eIF4E (Figures 6J and 6K; sample blots in Figure S7B), indicating that a critical level of CYFIP1 is required for BDNF-induced eIF4F formation. Interestingly, BDNF stimulated the release of 4E-BP2 from eIF4E in both wild-type and CYFIP$^{+/-}$ mice, and this regulation was inhibited by CGP57380 (Figures 6J and 6K). These results provide further support for independent regulation of CYFIP1 and 4E-BP2 by MNK.

A Switch to Synapse-Specific Translation during Late-Stage LTP In Vivo

Polysome analysis was used to directly measure changes in translational activity following LTP induction. Samples from rat DG lysates and synaptoneurosomes were loaded onto a 10%–50% linear sucrose gradient and subjected to velocity sedimentation. Total polysomal formation was determined by averaging the spectrophotometric absorption at 260 nm (area under the curve) across gradient fractions 2–7 for the treated and contralateral control DG. Total polysome formation was then expressed as the percentage of the total RNA signal across all fractions in order to correct for changes in RNA abundance in the free ribonucleoprotein fraction (fractions 8–12). Immunoblot characterization of the gradient fractions confirmed cosedimentation of rpS6, eEF2, and poly(A) binding protein in polysomal fractions, and eIF4E, CYFIP1, and a fraction of FMRP and rpS6 in the nonpolysomal fraction (Figure S8A).

In lysates, total polysome formation was significantly increased at 40 min, but not 3 hr, in the HFS-treated DG relative to contralateral control (Figures 7A–7C). In synaptoneurosomes, HFS induced a shift from light polysomes (fractions 5–7) into heavy polysomes (fractions 2–4) at 40 min (Figure 7G), with no net change in the total polysome formation (Figure 7H). At 3 hr after HFS, total polysome formation in synaptoneurosomes was significantly increased (184.6% ± 13.0%) in the HFS-treated DG relative to the contralateral control (Figures 7E and 7H), and this increase was abolished by CGP57380 infusion at 2 hr after HFS (Figures 7F and 7H). The results demonstrate ongoing translation in DG LTP in vivo and reveal a shift toward MNK-dependent synaptic translation at the late time point.

The shift from CYFIP1/FMRP to 4E-BP2 regulation in DG LTP implies a functional shift in mRNA translation. To begin to investigate patterns of individual mRNA translation, quantitative PCR analysis was used to measure changes in the translational efficiency of five dendritically expressed FMRP targets (*Arc*, *APP*, *αCaMKII*, *NR2A/GluN2A*, *Lingo*) (Pasciuto and Bagni, 2014). mRNA levels in polysomes (sum of sucrose gradient fractions 2–7) were normalized to the summed levels in monosomal and mRNP fractions, and values in the HFS-treated DG were expressed as fold change relative to the contralateral control (Figures 7I and 7J). In lysate samples, all FMRP targets exhibited enhanced polysomal association at 40 min, but not 3 hr, after HFS. In synaptoneurosomes, polysomal abundance of *Arc*, *APP*, and *αCaMKII* was upregulated more than 300-fold at

Figure 6. LTP Maintenance, eIF4F Formation, and Arc Expression Are Impaired in *Mnk1* Knockout Mice

(A) Time course of perforant path-DG evoked fEPSPs. The magnitude of LTP was significantly reduced in the DG of *Mnk1*$^{-/-}$ mice relative to wild-type *Mnk1*$^{+/+}$ mice at 170–180 min (knockout [KO]: 5.3% ± 5.39%, n = 12; wild-type: 34.09% ± 6.17%, n = 10) Student's t test $t_{(12)} = -3.284$, $p < 0.001$). Inset: sample field potentials (mean of five consecutive sweeps); calibration, 5 ms, 3 mV.

(B and C) Input-output curves of fEPSP slope (B) and population spike (C) responses show no difference in basal synaptic transmission between wild-type and *Mnk1* KO mice. Repeated-measures ANOVA: $F_{1,9} = 1.0561$, $p = 0.3082$ for fEPSP and $F_{1,9} = 0.8431$, $p = 0.362$, for population spike).

(D) Representative Arc protein immunohistochemistry staining of coronal section obtained at 3 hr after HFS in wild-type and *Mnk1* KO.

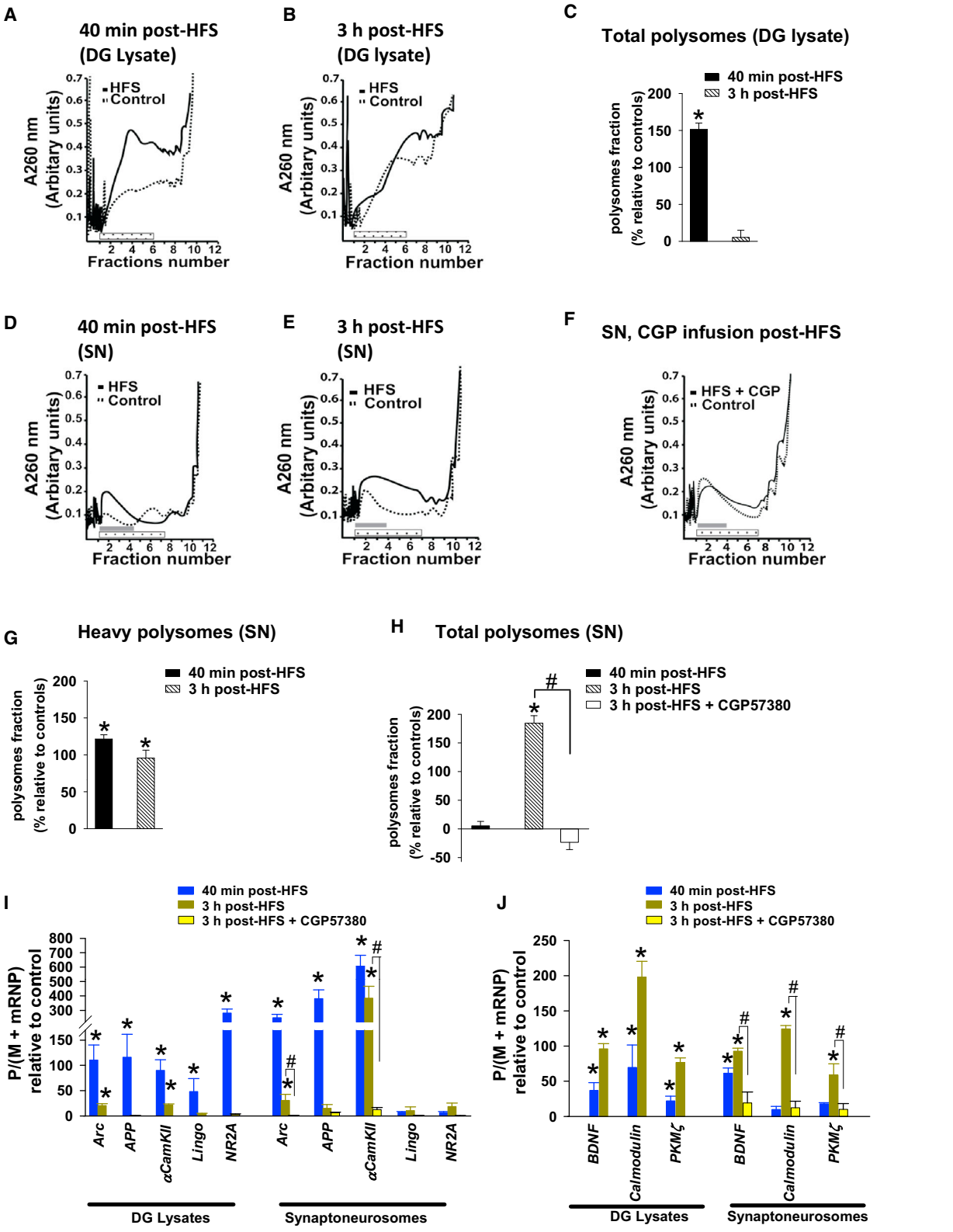
(E) Quantification of Arc protein expression based on mean densitometric intensity in ipsilateral (IPSI) DG relative to contralateral (CONTRA) control DG set at 1 (arbitrary units) in wild-type (10.77% ± 1.2%; $p < 0.001$) and *Mnk1* KO mice (1.09% ± 0.1%; $p = 0.87$). *$p < 0.05$.

(F) Quantification of Arc protein expression in BDNF-stimulated DG SN from wild-type and *Mnk1* KO mice and BDNF-treated cortical SNs from *Cyfip1*$^{+/-}$ mice, in the presence or absence of the MNK inhibitor CGP57380. The BDNF-treated SN were normalized to β-actin or tubulin as indicated and expressed relative to vehicle-treated SN (stippled line) set at 1 (arbitrary unit). n = 5. *$p < 0.05$.

(G) Representative immunoblots for results in (F).

(H and I) m^7GTP pull-down assays of DG SN from wild-type and *Mnk1* KO mice. Quantification show mean changes in protein levels normalized to eIF4E and expressed relative to vehicle treatment set at 1 (arbitrary units) in wild-type and *Mnk1* KO mice. n = 6, *$p < 0.05$. Representative immunoblots in Figure S7A.

(J and K) m7GTP pull-down assays of cortical SN from wild-type and Cyfip1$^{+/-}$ mice. Mean changes in protein levels normalized to eIF4E and expressed relative to vehicle treatment set at 1 (arbitrary units) in wild-type and *Cyfip1*$^{+/-}$ mice. Representative immunoblots in Figure S7B. Error bars represent SEM. Statistics information and source data are in Table S1.



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40 min after HFS; *αCaMKII* translation remained strongly elevated at 3 hr, whereas *Arc* and *APP* declined, though Arc translation remained significantly elevated ~30-fold above control. We also examined polysomal abundance of three dendritic, non-FMRP target mRNAs (*PKM-ζ*, *BDNF*, *calmodulin*) that are implicated in synaptic plasticity. These mRNAs exhibited a pattern of regulation almost inverse to that of the FMRP targets. Translation of *PKM-ζ*, *BDNF*, and *calmodulin* was increased at both time points but was significantly higher at 3 hr after HFS (Figure 7J). In synaptoneurosomes, enhanced translation of *PKM-ζ* and *calmodulin* occurred only at the late time point (Figure 7J). Finally, CGP57380 infusion at 2 hr after HFS inhibited the increased synaptoneurosomal translation of *Arc*, *αCaMKII*, *PKM-ζ*, *calmodulin*, and *BDNF* (Figures 7I, 7J, and S8B), thus supporting a key role for MNK in synaptic translation underlying DG LTP in vivo.

DISCUSSION

DG LTP Consolidation Requires Sustained BDNF-TrkB Activation

The present work shows that LTP consolidation in the DG of live rats is a highly dynamic, active process driven by persistent BDNF-TrkB signaling. TrkB receptors activate a transient signaling pathway to MNK, and this pathway is persistently activated by BDNF to mediate LTP consolidation. In turn, MNK activity regulates translation initiation complex formation and protein synthesis in mechanistically distinct early and late stages. The dynamics of LTP consolidation were revealed by time-sensitive reversion of LTP in response to acute infusion of TrkB-Fc, CGP57380, and 4EGI-1. The rapid reversion of LTP and Arc synthesis in the present study mimics the effects obtained upon acute inhibition of Arc synthesis with antisense oligodeoxynucleotides (Messaoudi et al., 2007). The work thus identifies sustained TrkB signaling to MNK as a key mechanism in Arc-dependent LTP consolidation.

TrkB-MNK signaling to translation within the synaptic compartment is shown by ex vivo and in vitro analysis of synaptoneurosomes. Persistent BDNF-TrkB activity could reflect (1) repeated activation of a stationary pool of TrkB, (2) continuous membrane insertion and activation of new TrkB, or (3) ligand-mediated endocytosis and recycling of TrkB to the membrane. Repeated activation of stationary TrkB is highly unlikely, given evidence that TrkB is rapidly endocytosed upon ligand binding. Recent work in hippocampal neurons and HEK293 cells shows that endocytosed full-length TrkB rapidly recycles to the membrane to promote sustained ERK signaling (Chen et al., 2005; Huang et al., 2009, 2013; Nagappan and Lu, 2005). TrkB activation is known to stimulate BDNF release, and endocytosed BDNF can be recycled for neuronal activity-dependent secretion (Canossa et al., 1997; Santi et al., 2006). In neuronal development, self-amplifying autocrine actions of BDNF-TrkB ensure axonal differentiation and growth (Cheng et al., 2011). Based on these studies, it is tempting to speculate that sustained TrkB activation in the DG LTP involves regenerative secretion of BDNF coupled with TrkB endocytosis and recycling to the membrane. In cultured embryonic cortical neurons grown in microfluidic chambers, BDNF application to the dendrite can generate a TrkB signaling endosome that travels to the soma where it activates ERK and induces Arc transcription (Cohen et al., 2011). Signaling from TrkB endosomes should be resistant to extracellular TrkB-Fc. In DG LTP, ERK phosphorylation was blocked by extracellular TrkB-Fc (Figures 2F and 2I), arguing against a primary role for signaling endosomes. Further studies are needed to explore roles for TrkB recycling endosomes and signaling endosomes in synaptic plasticity.

Two-Step Translational Control of LTP Mediated by MNK-Dependent Regulation of CYFIP1 and 4E-BP2

Based on combined pharmacological and genetic approaches in vivo and in vitro, we show that sustained TrkB signaling to MNK drives persistent enhancement of eIF4F formation (eIF4G-eIF4E interaction) underlying protein-synthesis-dependent LTP. Moreover, we show that MNK facilitates translation in discrete early and late stages of LTP consolidation associated with regulation of CYFIP1 and 4E-BP2, respectively.

Napoli and colleagues (2008) showed that exogenous BDNF stimulates CYFIP1 release and translation of FMRP-target mRNAs in neuronal cultures and cortical synaptoneurosomes in vitro. Here, we found that MNK regulates CYFIP1/FMRP downstream of endogenous BDNF signaling early in LTP consolidation. In live *Mnk1* knockout mice, DG LTP maintenance is impaired and Arc synthesis is blocked. In DG synaptoneurosomes from *Mnk1* knockout mice, BDNF regulation of CYFIP1/FMRP, eIF4F, and Arc synthesis is inhibited. In cortical

Figure 7. Enhanced Polysome Formation in DG LTP In Vivo: A Shift to Late MNK-Dependent Synaptic Translation

Samples from DG total lysate and synaptoneurosome (SN) samples obtained at 40 min and 3 hr after HFS were loaded onto a 10%–50% linear sucrose gradient and subjected to velocity sedimentation. Gradients were collected in 12 fractions starting with the heavy fraction. HFS-treated and contralateral DG tissues were processed in tandem for each time point. Total polysomal formation was determined calculating the area under the curve across gradient fractions 2–7. To correct for differences in RNA abundance within the free ribonucleoprotein fraction (fractions 8–12), total polysome formation was expressed in percentage of the RNA signal across all fractions.

(A and B) RNA absorbance profiles for DG lysate samples obtained at 40 min (A) and 3 hr (B) after HFS.

(C) Percentage change in absorbance in polysome fractions (indicated by dotted bar in A and B) as percentage of the total area under the absorbance curve, normalized to the unstimulated contralateral DG lysate control.

(D–F) RNA absorbance profiles for DG SN samples obtained at 40 min (left panel) and 3 hr (right panel) after HFS. (F) RNA absorbance profiles for DG SN samples obtained from experiments where CGP57380 was infused at 2 hr and tissue collected at 3 hr after HFS.

(G) Percentage change in absorbance for heavy polysomes in SNs collected at 40 min and 3 hr after HFS in the ipsilateral DG relative to contralateral DG.

(H) Percentage of change in absorbance for total polysomes in SNs (dotted bar) for 40 min and 3 hr and CGP57380-infused 3 hr samples. n = 4, *$p < 0.05$.

(I and J) Quantification by qRT-PCR analysis of mRNA abundance loaded onto polysomes relative to mRNA loaded onto monosomes and mRNPs at 40 min (blue), 3 hr (green), and 3 hr and CGP infusion (yellow) in total lysates and SN for the tested mRNAs. Error bars represent SEM. Statistics information and source data in Table S1.

synaptoneurosomes from *Cyfip1*$^{+/-}$ mice, MNK-dependent regulation of eIF4F is inhibited. In contrast, the late stage of LTP consolidation is associated with MNK-dependent regulation of 4E-BP2 and enhanced total polysome formation specifically within the synaptoneurosome compartment.

Stable LTP formation involves expansion of the postsynaptic density, enlargement of pre-existing dendritic spines, and de novo synapse formation (Bourne and Harris, 2008; Lisman and Raghavachari, 2006). These processes likely require coordinated regulation of mRNA-specific and general translation (Costa-Mattioli et al., 2009; Gal-Ben-Ari et al., 2012; Sossin and Lacaille, 2010). The present study provides evidence for spatial and temporal regulation of distinct forms of translational control in LTP and further suggests the existence of a specific translational program: early translation of CYFIP1-controlled FMRP targets followed by 4E-BP2-mediated translation in dendrites. Quantitative PCR (qPCR) analysis of sucrose-density gradient fractions from DG lysate and synaptoneurosomes served to validate the plausibility of the model and quality of the polysome preparation. Accordingly, enhanced early translation was observed for five dendritically expressed FMRP target mRNAs and enhanced late translation for three non-FMRP target mRNAs. Global profiling techniques are needed to define the MNK-dependent translatome, and many forms of translation control may be involved (Niere et al., 2012; Sossin and Lacaille, 2010; Udagawa et al., 2012).

Studies in nonneuronal cells show that 4E-BP, the main regulator of eIF4E availability, is particularly critical for translation of mRNAs with structured 5′ UTRs encoding proteins that regulate cellular growth (De Benedetti and Graff, 2004; Hay and Sonenberg, 2004). Here, MNK activation was coupled to synapse-specific regulation of 4E-BP2, enhanced polysome formation, and translation of dendritic mRNAs (*Arc*, *αCaMKII*, *PKM-ζ*, *calmodulin*, *BDNF*). Translocation of polyribosomes and specific mRNAs into spines or synaptic fractions has been shown to occur after LTP induction (Bourne et al., 2007; Håvik et al., 2003). In the DG, TrkB signaling to MNK could drive protein synthesis needed for synaptic reorganization and growth. Interestingly, the critical period of TrkB-MNK signaling outlasts the critical period of Arc synthesis, as defined by Arc antisense oligo infusion (Messaoudi et al., 2007), and this could reflect the continued MNK-dependent synthesis of other proteins needed for LTP consolidation.

Polysome analysis shows that CGP57380 infusion rapidly blocks LTP-associated Arc translation in LTP. Notably, CGP57380 inhibits but not does abolish Arc protein expression as assayed by immunoblotting. Immunohistochemical staining also shows residual Arc protein expression across the DG granule cell body layer. The same pattern of complete inhibition of LTP maintenance, but partial inhibition of Arc expression, was observed with Arc antisense oligos (Messaoudi et al., 2007). The CGP57380-resistant Arc may reflect a pool of newly synthesized Arc with slow turnover. This stable Arc would not be affected by the application of translation inhibitors during LTP maintenance but could conceivably function in homeostatic plasticity or LTD (Korb et al., 2013; Okuno et al., 2012).

MNKs bind to eIF4G and phosphorylate eIF4E, resulting in decreased affinity of eIF4E for the 5′-mRNA cap structure (Scheper et al., 2002; Wang et al., 1998). MNKs may phosphorylate eIF4G (Pyronnet et al., 1999), and MNK activity has been reported to regulate binding of MNK to eIF4G (Scheper et al., 2002; Shveygert et al., 2010). There is currently no evidence that eIF4E phosphorylation regulates eIF4F formation (Scheper et al., 2002). Here, MNK-dependent eIF4E phosphorylation and eIF4F formation were consistently linked. However, the fact that CYFIP1 and 4E-BP2 are independently regulated indicates that factors other than eIF4E phosphorylation are involved.

Previous analysis of whole-brain and hippocampal extracts demonstrated brain-specific postnatal deamidation of 4E-BP2 (Bidinosti et al., 2010). Deamidated 4E-BP2 is sequestered from eIF4E through high-affinity association with raptor in the mTORC1 complex. In DG LTP, both deamidated and unmodified 4E-BP2 are released from eIF4E in an MNK-dependent manner. In mTORC1-mediated translation, Thr$^{37/46}$ phosphorylation of 4E-BP2 is expected. Here, we found no change in 4E-BP2 phosphorylation state, consistent with mTORC1-independent LTP.

LTP consolidation requires both protein synthesis and actin cytoskeletal dynamics in dendritic spines (Honkura et al., 2008; Panja and Bramham, 2014; Rex et al., 2007). Recent work of De Rubeis et al. (2013) demonstrated a dual role for CYFIP1 in translation and actin regulation in cultured neurons. Upon treatment with BDNF, CYFIP1 is released from eIF4E to interact with the WAVE complex involved in actin cytoskeletal remodeling and spine plasticity. In DG LTP, Arc synthesis is required for stabilization of nascent F-actin at perforant path synapses (Messaoudi et al., 2007). This raises the intriguing possibility that CYFIP1 promotes Arc synthesis and converges with Arc in actin cytoskeletal regulation. In the context of LTP, it is tempting to speculate that 4E-BP2 regulation emerges as a consequence of spine cytoskeletal dynamics. The synapse-specific regulation of 4E-BP2 is consistent with this notion and evidence from axon growth cones suggests that the actin cytoskeleton can serve as a platform for local protein synthesis (Van Horck and Holt, 2008).

The present work reveals a dynamic contribution of BDNF-TrkB signaling to MNK in translational control of LTP consolidation in the dentate gyrus. mTORC1 signaling is essential for translation-dependent plasticity in many brain regions but is not required for DG LTP. It will therefore be important to determine how these different mechanisms of translation contribute to shaping cell-specific behavior and information processing.

EXPERIMENTAL PROCEDURES

See also the Supplemental Experimental Procedures.

Electrophysiology and Intrahippocampal Infusion

All procedures were performed according to NIH Guidelines for the Care and Use of Laboratory Animals Norway (FOTS 20124922). Protocols for intrahippocampal drug infusion and in vivo electrophysiological recording of DG LTP in urethane anesthetized mice and rats are detailed in the Supplemental Experimental Procedures.

Synaptoneurosome Isolation and In Vitro Stimulation

Synaptoneurosomes for ex vivo analysis or in vitro stimulation were prepared as described by Napoli et al. (2008) with minor modifications. See the Supplemental Experimental Procedures.

Sucrose Gradient Fractionation and Polysome Assay and RNA Extraction

Lysates from synaptoneurosomes and whole DG were loaded on a 10%–50% linear sucrose gradient, centrifuged at 200,000 × g_{av}, and gradients were fractionated into 12 fractions to which luciferase spike-in control RNA and linear polyacrylamide were added. Total RNA for each fraction was isolated using Isol-RNA Lysis Reagent (5 PRIME) and reverse transcribed using Superscript. See the Supplemental Experimental Procedures for details.

Puromycin Labeling of Newly Synthesized Proteins

Puromycin (1 μl, 12.5 min, 50 μM, dissolved in 0.1% DMSO-PBS) was infused at 2 hr after HFS, and DG tissue was collected 1 hr later. Puromycin-tagged nascent polypeptides were detected by immunoblotting with anti-puromycin. See the Supplemental Experimental Procedures for details.

Statistics

Pairwise comparisons of means were evaluated with a two-tailed Student's t test. ANOVA was used when evaluating more than two groups, with the Tukey honest significant difference (HSD) test used for specific comparisons. Data are presented as mean ± SEM. Data value summary and statistical results are shown in Table S1.

See the Supplemental Experimental Procedures for drugs and antibodies, western blotting and RT-qPCR, and immunohistochemistry.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, eight figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.10.016.

AUTHOR CONTRIBUTIONS

D.P. performed electrophysiological experiments, biochemical assays, qPCR, and immunohistochemical staining for the LTP experiments in rats and mice. Synaptoneurosome stimulation and biochemical analysis were performed by D.P. and J.W.K in Bergen and by L.D. and F.Z. in Rome. A.V. contributed to the polysome sedimentation assays and analysis. K.W. contributed to the qPCR analysis of polysome fractions. C.G.P. and R.F. provided the *Mnk* knockout mice. All authors contributed to the design of the study and the interpretation of the data. D.P and C.R.B wrote the paper with contributions from all authors.

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