Stimulation of mRNA translation rescues hippocampal synaptic plasticity and memory in mouse models of Alzheimer's disease

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Abstract

Impaired synaptic plasticity and progressive memory deficits are major hallmarks of Alzheimer's disease (AD). Hippocampal mRNA translation, required for memory consolidation, is defective in AD. Here, we show that genetic reduction of the translational repressors, Fragile X messenger ribonucleoprotein (FMRP) or eukaryotic initiation factor 4E (eIF4E)-binding protein 2 (4E-BP2), ameliorated the inhibition of hippocampal protein synthesis and memory impairment induced by AD-linked amyloid-β oligomers (AβOs) in mice. Furthermore, systemic treatment with (*2R,6R*)-hydroxynorketamine (HNK), an active metabolite of the antidepressant ketamine, prevented deficits in hippocampal mRNA translation, long-term potentiation (LTP) and memory induced by AβOs in mice. HNK activated hippocampal signaling by extracellular signal-regulated kinase 1/2 (ERK1/2), mechanistic target of rapamycin (mTOR), and p70S6 kinase 1 (S6K1)/ribosomal protein S6 (S6), which promote protein synthesis and synaptic plasticity. S6 phosphorylation instigated by HNK was mediated by mTOR in hippocampal slices, while rescue of hippocampal LTP and memory in HNK-treated AβO-infused mice depended on ERK1/2 and, partially, on mTORC1. Remarkably, treatment with HNK corrected LTP and memory deficits in aged APP/PS1 mice. RNAseq analysis showed that HNK reversed aberrant signaling pathways that are upregulated in APP/PS1 mice, including inflammatory and hormonal responses and programmed cell death. Taken together, our findings demonstrate that upregulation of mRNA translation corrects deficits in hippocampal synaptic plasticity and memory in AD models. The results raise the prospect that HNK could serve as a therapeutic to reverse memory decline in AD.

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent form of dementia, affecting over 55 million people worldwide [1]. Clinically, AD is characterized by progressive cognitive decline, caused by structural and functional deterioration of synapses [2]. Hippocampal mRNA translation (protein synthesis) is crucial for synapse function and plasticity, and for memory consolidation [3]. The initiation and elongation steps of mRNA translation are deregulated in the brains of AD patients and mouse models of AD [4, 5]. Both eukaryotic translation initiation factor 2α (eIF2α) and eukaryotic elongation factor 2 (eEF2) are hyperphosphorylated in AD brains due to the activation of cellular stress response mechanisms, including endoplasmic reticulum (ER) stress and the integrated stress response (ISR). This results in attenuation of global protein synthesis, changes in proteome composition [6] and memory failure [5, 7, 8]. We recently demonstrated that antagonizing the ISR through the use of the small molecule compound ISRIB corrects deficits in synapse function and memory in AD mice [9], demonstrating that rescuing brain mRNA translation and protein synthesis could be beneficial in AD treatment. These findings suggest that multiple pathways converge to suppress hippocampal mRNA translation to engender AD pathogenesis. The elucidation of pathways that contribute to defective brain mRNA translation thus emerges as key to identifying novel and effective therapeutic targets for memory impairments in AD.

Fragile X messenger ribonucleoprotein (FMRP, encoded by the *Fmr1* gene) is an X chromosome-linked translational repressor that blocks both initiation and elongation phases of mRNA translation [10]. Through interaction with cytoplasmic FMR1-interacting protein 1 (CYFIP1) [11], FMRP blocks formation of the eIF4F translation initiation complex. While numerous studies have implicated FMRP loss-of-function in

the pathogenesis of Fragile X syndrome and autism spectrum disorders [12], little is known on whether FMRP plays an important role in AD [13].

Eukaryotic initiation factors 4E, 4G and 4F (eIF4E, eIF4G and eIF4F, respectively) are central regulators of the initiation of mRNA translation [14]. When protein synthesis is stimulated, eIF4E associates with eIF4G in the eIF4F complex to promote translation. Eukaryotic initiation factor 4E binding protein 2 (4E-BP2, encoded by the *Eif4ebp2* gene) is the major 4E-BP isoform in the brain [15]. 4E-BP2 is a translational repressor that binds to eIF4E and inhibits its ability to interact with eIF4G, thereby preventing the assembly of the eIF4F complex [11]. 4E-BPs were recently found to mediate the antidepressant actions of ketamine and its active metabolite (*2R,6R*)-hydroxynorketamine (HNK) in mice [16], although complete mechanistic details remain to be determined.

We hypothesized that stimulation of brain mRNA translation via genetic reduction of translational repressors or by treatment with HNK, thought to promote protein synthesis, could rescue deficits in hippocampal protein synthesis, synaptic plasticity and memory in AD mouse models. Our findings suggest that rescuing brain protein synthesis is a potential approach to prevent cognitive deterioration and to restore memory in AD.

RESULTS

Genetic reduction of FMRP or 4E-BP2 rescues deficits in hippocampal protein synthesis and memory in AβO-infused mice

Fmr1$^{y/-}$ or *Eif4ebp2*$^{+/-}$ mice were used to test the hypothesis that alleviating the repression of mRNA translation could rescue memory in AD mice. We first investigated whether genetic reduction of FMRP or 4E-BP2 could prevent the inhibition of hippocampal protein synthesis induced by AβOs, soluble neurotoxins that accumulate in

the AD brain and trigger synapse failure and cognitive impairment [7, 17]. We infused 10 pmol AβOs (or vehicle) via intracerebroventricular (i.c.v.) injections in wildtype (WT), *Fmr1*$^{y/-}$ or *Eif4ebp2*$^{+/-}$ mice, and assessed global *de novo* protein synthesis in hippocampal slices using surface sensing of translation (SUnSET), a nonradioactive method based on puromycin incorporation [18]. In line with previous *in vitro* and *ex vivo* evidence [9, 19], AβOs triggered a reduction of approximately 30% in global hippocampal protein synthesis in WT mice **(Fig. 1A, B)**. In contrast, AβOs did not inhibit hippocampal protein synthesis in *Fmr1*$^{y/-}$ or in *Eif4ebp2*$^{+/-}$ mice **(Fig. 1A, B)**.

Next, we assessed the impairment of hippocampus-dependent long-term fear memory using a contextual fear conditioning paradigm in AβO-infused mice. Because *Fmr1* is an X-linked gene, we used both *Fmr1*$^{y/-}$ males and *Fmr1*$^{+/-}$ females in comparison to WT mice. Consistent with our previous reports [7, 19], i.c.v. infusion of AβOs impaired long-term fear memory in WT mice **(Fig. 1C, D)**. Significantly, however, AβOs did not inhibit fear memory in *Fmr1*$^{y/-}$ or in *Fmr1*$^{+/-}$ mice **(Fig. 1C, D)**.

We next examined whether genetic reduction of 4E-BP2 could prevent AβO-induced memory failure. *Eif4ebp2*$^{+/-}$ or WT mice received i.c.v. infusions of AβOs and were tested in the contextual fear conditioning paradigm 7 days later. 4E-BP2 haploinsufficiency *per se* led to memory impairment in vehicle-infused mice, consistent with the notion that translational control by 4E-BP2 plays an essential role in memory consolidation and must be tightly regulated. Importantly, 4E-BP2 haploinsufficiency prevented AβO-induced impairment of fear memory **(Fig. 1E)**. Of note, basal (pre-training) freezing responses were not altered in any of the genotypes investigated or by infusion of AβOs (**Fig. S1A-C**). These findings support the notion that alleviating brain translational repression prevents memory impairments induced by AβOs in mice.

HNK corrects deficits in hippocampal protein synthesis, synaptic plasticity and memory induced by AβOs in mice

We sought to determine whether pharmacological activation of mRNA translation could correct synapse and memory impairments induced by AβOs. Effective pharmacological approaches to stimulate brain protein synthesis remain elusive. Ketamine is a fast-acting antidepressant at subanesthetic doses, and it appears to act via pathways controlling mRNA translation [16, 20]. However, potential side effects of ketamine, including addictive/dissociative actions, may limit its widespread clinical application [21]. Importantly, the ketamine metabolite, (*2R,6R*)-hydroxynorketamine (HNK), engages protein synthesis-related pathways to elicit antidepressant responses [16] in the absence of significant detrimental side-effects in mice [22, 23].

We first investigated whether HNK could rescue AβO-induced deficits in hippocampal protein synthesis. We treated acute hippocampal slices from WT mice with HNK (20 μM) for 20 min prior to exposure to AβOs (1 μM; 2 h) and assessed global mRNA translation using SUnSET. Whereas AβOs decreased protein synthesis by ~ 20% in saline-treated hippocampal slices, pre-treatment with HNK blocked the attenuation of protein synthesis caused by AβOs **(Fig. 2A, B)**.

To determine whether HNK could counteract AβO-induced inhibition of hippocampal synaptic plasticity, we treated hippocampal slices with HNK for 20 min prior to exposure to AβOs and assessed long-term potentiation (LTP) at Schäffer collateral-CA1 synapses. Whereas slices exposed to AβOs failed to maintain LTP following tetanic stimulation, slices treated with HNK prior to stimulation showed induction and maintenance of LTP even in the presence of AβOs (**Fig. 2C, D**).

We further investigated the effects of HNK on AβO-induced memory impairment in mice. We treated AβO-infused mice with saline or HNK (20 mg/kg, i.p.) 1 h prior to

training in the contextual fear conditioning task. Basal freezing responses were not altered by AβOs or HNK (**Fig. S1D**). Significantly, treatment with HNK *per se* had no impact on memory but prevented long-term fear memory deficits engendered by AβOs **(Fig. 2E)**.

HNK activates ERK/RSK and promotes mTOR-dependent S6 phosphorylation

To gain insight into the mechanisms of action of HNK in the brain, we used acute hippocampal slices to examine the impact of HNK on signaling pathways that mediate translational control. We assessed the activation of mechanistic target of rapamycin (mTOR), extracellular signal-regulated kinase 1/2 (ERK1/2), p70S6 kinase (S6K1), p90S6 kinase (RSK) and ribosomal protein S6 (S6), all of which have been implicated in translational control and synaptic plasticity **(Fig. 3A)** [15, 24, 25]. We found that HNK triggered rapid (5 min) and transient phosphorylation (activation) of mTOR at Ser2448 (**Fig. 3B, Fig. S2A)**, and a sustained increase (10-60 min) in ERK1/2 phosphorylation **(Fig. 3C)**. This was accompanied by an approximately 50% increase in phosphorylation of S6K1 at Thr389 **(Fig. 3D),** and by a marked (250%) increase in phosphorylation of RSK at Ser380 (**Fig. 3E**) 10 min after HNK treatment.

Because S6 is a direct target of both S6K1 and RSK, we determined the phosphorylation status of S6 upon HNK treatment. HNK instigated sustained (10-60 min) phosphorylation of S6 at Ser235/236 **(Fig. 3F)**, residues targeted by both S6K1 and RSK [26], but not at Ser240/244 **(Fig. S2B)**. Treatment with HNK did not affect FMRP levels or the phosphorylation of 4E-BP1/2 or eIF2α in hippocampal slices (**Fig. S2C-E**).

We next investigated whether ERK1/2 or mTOR mediated HNK-induced activation of S6. We treated hippocampal slices with HNK in the presence of PD98059 (MEKi), a MEK1/2 inhibitor that blocks ERK1/2 activation, or in the presence of rapamycin (Rapa), an mTOR complex 1 (mTORC1) inhibitor. Blocking mTORC1

activity, but not ERK1/2, blocked the increase in S6 phosphorylation by HNK **(Fig. 3G)**. We further found that neither MEKi or rapamycin alone reduced HNK-induced Ser380 phosphorylation of RSK (**Fig. 3H**), suggesting that both ERK1/2 and mTOR may be involved in RSK activation by HNK. As expected, MEKi attenuated (by ~ 60%) HNK-stimulated ERK1/2 phosphorylation, but caused no changes in HNK-induced mTOR activation **(Fig. S2F)**. Conversely, rapamycin robustly blocked mTOR, but not ERK phosphorylation **(Fig. S2G)**. Collectively, these results demonstrate that HNK engages ERK1/2 and mTOR to phosphorylate RSK, and mTOR to phosphorylate S6, defining an important signaling pathway involved in the regulation of neuronal protein synthesis.

Correction of hippocampal synaptic plasticity and memory by HNK is mediated by ERK signaling

We next investigated whether HNK-induced restoration of synaptic plasticity and memory upon exposure to AβOs was mediated by ERK1/2 or mTOR signaling. We treated acute hippocampal slices from WT mice with MEKi (20 μM), rapamycin (100 μM) or vehicle for 20 min, then added HNK (20 μM) or vehicle for 20 min prior to exposure to AβOs (1 μM), and assessed LTP at Shaffer collateral-CA1 synapses.

Consistent with our findings above, AβOs inhibited CA3-CA1 LTP, which was prevented by pre-treatment with HNK. Inhibition of MEK1/2, but not of mTOR, abolished HNK-mediated rescue of LTP **(Fig. 4A, B)**. Importantly, we assessed whether ERK1/2 signaling was necessary for memory rescue by HNK. We treated AβO-infused mice with MEKi (10 mg/kg, i.p.) or vehicle, followed by HNK (20 mg/kg, i.p.) or vehicle 1 h prior to training in the contextual fear conditioning paradigm. While treatment with HNK rescued memory in AβO-infused mice, pre-treatment of mice with MEKi abolished

the protective action of HNK **(Fig. 4C)**. Collectively, these data indicate that HNK-induced rescue of LTP and memory is mediated by ERK1/2 signaling.

Transcriptional signature of HNK treatment in the mouse hippocampus

To further dissect the impact of HNK in the mouse hippocampus, we determined whether treatment with HNK promoted global transcriptional changes *in vivo*. Mice were treated with HNK (0.5 mg/kg, i.p.) for 14 days, and hippocampal gene expression was assessed by RNAseq (**Supplementary Tables 1 and 2**). Among differentially expressed genes, HNK promoted the upregulation of 469 genes and downregulation of 433 genes (**Fig. 5A**). Gene ontology (GO) analyses revealed that HNK induced the expression of genes associated with mitochondrial metabolism and transcription. At the same time, it downregulated genes linked to GTPase activity (**Fig. 5B**). As revealed by network analysis, HNK upregulated several mitochondrial genes involved in the electron transport chain (e.g., mt-Cytb, mt-Co1, mt-Co2, mt-Nd4), RNA metabolism and ribogenesis (e.g., Wdr42, Ddx10, Ddx18, Esf1) In contrast, other genes such as Src and eIF6 were downregulated (**Fig. 5C, D**). Altogether, these results suggest that HNK promotes a transcriptional signature that modulates mitochondrial energy metabolism, transcription and ribosomal biogenesis in the hippocampus., which could mediate enhanced neuronal metabolism and protein synthesis.

HNK corrects synaptic plasticity and memory deficits in aged APP/PS1 mice

Our finding that HNK prevented synapse and memory deficits induced by AβOs prompted us to investigate whether HNK could reverse pre-existing impairments in synaptic plasticity and memory in aged (19-24 month-old) APP/PS1 mice, used as a transgenic model of late-stage AD. HNK (0.5 mg/kg, i.p.) or saline were administered

daily for 12-14 days, and hippocampus-dependent spatial memory was tested 8-9 days after the onset of treatment using the two-day Radial Arm Water Maze (RAWM) paradigm, a memory task for which aged APP/PS1 are impaired [27]. Following the RAWM, hippocampal slices were obtained, and LTP at Schäffer collaterals was measured. Remarkably, HNK reversed both the inhibition of hippocampal LTP **(Fig. 6A, B)** and spatial memory deficits **(Fig. 6C)** in aged APP/PS1 mice. Control measurements showed no differences in input/output (I/O) curves between WT and APP/PS1 mice, or between animals treated with vehicle or HNK (**Fig. S3A**). Additional control experiments revealed that treatment with HNK had no impact on body weight **(Fig. S3B)** or hippocampal Aβ load in APP/PS1 mice **(Fig. S3C)**.

HNK rescues hippocampal transcriptional alterations in APP/PS1 mice

Finally, we investigated hippocampal transcriptional alterations induced by HNK in aged APP/PS1 mice. Mice were treated with HNK (0.5 mg/kg, i.p.) or saline for 14 days, and hippocampal transcriptomic changes were assessed by RNAseq. GO analyses revealed significantly upregulated pathways in APP/PS1 mice (compared to WT mice) that were corrected by HNK treatment. This included regulation of programmed cell death and response to hormones (**Fig. 6D**). Reactome pathway analyses further implicated the innate immune system and, notably, three pathways associated with RNA metabolism and translation, namely nonsense-mediated RNA decay, co-translational protein targeting to membranes and formation of a pool of free 40S subunits, as aberrantly regulated pathways in APP/PS1 mice that are rescued by HNK (**Fig. 6D**). These pathways are consistent with previous observations of neuroinflammation, deregulated hormonal signaling, TNF-α-dependent neuronal stress responses, and memory impairment in AD models [7, 28–30].

By performing Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) analysis, we further identified transcriptional regulators known to control gene networks that HNK rescued in APP/PS1 mice. Among those regulators, we found molecules involved in inflammatory responses (Nfκb1, PPARα), transcriptional repression (HDAC1, HDAC2) and, importantly, integrated stress response effectors (ATF3, ATF4) (**Fig. 6E**). Network analysis on STRING revealed a key cluster of ribosomal genes whose aberrant expression in APP/PS1 mice was corrected by HNK treatment (**Fig. 6F**).

In addition, we analyzed differentially expressed genes that were upregulated in APP/PS1 mice and were corrected by HNK treatment. Network analysis on STRING and GO revealed that HNK rescued transcriptional programs involved in cytosolic ribosomes and regulation of immune system process (**Fig. S4A, B**). Downregulated genes in APP/PS1 mice that were corrected by HNK were also analyzed. We found that HNK rescued mRNA levels for genes coding for RNA polymerase II holoenzyme subunits, calcium channel complex and synaptic proteins in APP/PS1 mice (**Fig. S5A, B**). Altogether, these results demonstrate that HNK rescues transcriptional programs associated with inflammation, impaired proteostasis, calcium signaling and synaptic proteins in aged APP/S1 mice.

DISCUSSION

Our findings demonstrate that genetic and pharmacological approaches to alleviate the repression of hippocampal mRNA translation and stimulate protein synthesis rescue synaptic plasticity and memory in mouse models of AD. Findings using mice lacking FMRP or with reduced 4E-BP2 support the concept that correcting defective brain protein synthesis counteracts AD-mediated synapse and memory impairment.

The exact mechanisms by which FMRP contributes to AD have not been entirely determined. Of note, the FMRP partner, CYFIP1, has been found to be upregulated in adult human brain cortical slices exposed to AβOs [18], suggesting that increased FMRP-CYFIP1 activity might underlie the inhibition of brain mRNA translation in AD. Future studies appear warranted to provide insight into the impact of FMRP and its interaction with CYFIP1 in AD.

Fmr1$^{+/-}$ mice have been reported to develop memory impairments in the CFC paradigm [31]. We note, however, that *Fmr1*$^{+/-}$ mice did not present altered CFC memory in our study, in line with additional evidence [32]. These apparent discrepancies may result from different strains and protocols used to assay memory.

Both insufficient and excessive brain protein synthesis can be detrimental to cognition and memory [33]. Indeed, loss-of-function mutations in *Fmr1* cause neurodevelopmental disorders and intellectual disability in humans, likely due to aberrant mRNA translation [34]. In agreement with our current findings in 4E-BP2 haploinsufficient mice, 4E-BP2 ablation has been reported to promote cognitive impairment in mice [15, 35]. This underscores the need for fine-tuned control of protein synthesis for optimal brain function, and may pose limitations to genetic manipulation of FMRP or 4E-BP2 for AD treatment. Thus, pharmacological strategies to restore mRNA translation to an optimal level appear attractive to preserve or restore cognition.

As an approach to correct defective mRNA translation in AD models, we have investigated the actions of HNK, a ketamine metabolite whose mechanism of action is thought to involve activation of protein synthesis pathways [26]. We demonstrate that HNK prevents the inhibition of hippocampal protein synthesis, LTP and memory induced by AβOs in mice. We further found that HNK triggers phosphorylation of mTOR, ERK1/2, S6K1, RSK and S6, central mediators of translational control [26, 36]. Notably,

HNK-induced phosphorylation of S6 depended on the activity of mTOR, a key inducer of translation and ribosome biogenesis. On the other hand, ERK is a key signaling effector that stimulates translation of immediate early genes necessary for synaptic plasticity [37, 38], and we found that HNK-induced rescue of LTP and memory in AβO-infused mice is dependent on ERK1/2 activity.

HNK treatment upregulated hippocampal genes involved in oxidative phosphorylation. AD is associated with reduced energy metabolism and expression of mitochondrial electron transport chain subunits [39], suggesting that rescuing mitochondrial function could be beneficial in AD. Of note, a very recent study reports that treatment with trazodone corrects translational attenuation of mitochondrial proteins in prion-diseased mice, and that this is accompanied by improved mitochondrial and synaptic function [40]. Moreover, treatment with HNK decreased the expression of a gene network that includes Src and eIF6. Src is a tyrosine kinase involved in inflammatory response [41] and eIF6 is an initiation factor that attenuates translation by maintaining the 60S ribosome subunit in an inactive state [42]. Therefore, the downregulation of these genes by HNK may reduce neuroinflammation and promote translation initiation.

Remarkably, HNK reversed pre-existing impairments in synaptic plasticity and memory in aged (19–24-month-old) APP/PS1 mice. HNK further corrected aberrant transcriptional signatures related to inflammation and translation/proteostasis in APP/PS1 mice, suggesting that enduring changes in brain gene expression may contribute to the neuroprotective mechanisms of HNK. Our findings on hippocampal transcriptional changes *in vivo* further suggest that HNK impacts translation by upregulating translation-associated pathways, which may promote ribosome biogenesis and increase translational capacity. Collectively, these findings support a potential therapeutic action of HNK even in the presence of substantial brain deposition of Aβ.

HNK has been implicated as a mediator of the antidepressant actions of ketamine without relevant dissociative or addictive effects [43], and is currently in clinical trials in treatment-resistant depression (NCT04711005). If HNK is proven safe and effective, like the related FDA-approved drug Esketamine, this would raise the prospect that HNK could be repurposed for treatment of cognitive decline and, potentially, of non-cognitive manifestations in AD, such as AD-linked depression.

In conclusion, our findings show that restoring brain mRNA translation by either releasing translational brakes or stimulating pathways which promote protein synthesis alleviates synaptic deficits and memory impairment in AD models. Thus, HNK has the potential to prevent and, notably, to reverse synaptic plasticity defects and memory impairments in AD. The notion that impaired control of mRNA translation is a druggable target may pave the road towards effective therapeutics in AD.

MATERIALS AND METHODS

Animals

Adult male or female mice (3 month-old) were obtained from the animal facility at McGill University and were housed in groups of 2-5 mice per cage, with food and water *ad libitum*, in rooms with controlled temperature (21°C) and humidity (~55%) on a 12 h light/dark cycle. Male and female APPswe/PS1ΔE9 mice (Jackson Laboratories, strain code #005864), *Eif4ebp2*$^{+/-}$, *Fmr1*$^{+/-}$ and *Fmr1*$^{y/-}$ mice on a C57BL/6J background (obtained from The Jackson Laboratories) were bred at the animal facilities of McGill University and the Federal University of Rio de Janeiro. All procedures followed the Canadian Council on Animal Care guidelines and were approved by McGill University Committees and by the Institutional Animal Care and Utilization Committee of the Federal University of Rio de Janeiro.

Biochemistry in hippocampal slices

Hippocampal slices (400 μm) were obtained and allowed to recover in artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 4.4 mM KCl, 1 mM Na_2HPO_4, 25 mM $NaHCO_3$, 2 mM $MgCl_2$, 2mM $CaCl_2$, 10 mM glucose) for 2 h. For time course experiments, slices were treated with (*2R,6R*)-hydroxynorketamine (HNK) (Tocris Biosciences, #1430202-69-9; 20 μM) or saline for the time intervals indicated. Newly synthesized polypeptides were detected using surface sensing of translation (SUnSET), as described [44]. *Eif4ebp2*$^{+/-}$, *Fmr1*$^{y/-}$ or corresponding littermate WT mice received i.c.v. infusions of 10 pmol AβOs (or vehicle) and hippocampal slices were prepared 7 days later. For SUnSET experiments with HNK, hippocampal slices from WT mice were treated with HNK (20 μM; 20 min) and then exposed to AβOs (1 μM; 2 h). Slices were then exposed to puromycin (5 μg/mL; 45 min) and collected for Western blotting. Puromycin incorporation was evaluated as a measure of newly synthesized polypeptides, and β–actin was used as loading control. A negative control lacking puromycin was added to each experiment.

For mechanistic studies using HNK, hippocampal slices from WT mice were pre-exposed to vehicle, PD98059 (MEK inhibitor; Sigma Aldrich, #P215; 20 μM) or rapamycin (mTORC1 inhibitor; Sigma Aldrich, R0395; 100 μM) for 30 min and then exposed to HNK (20 μM) for 10 min.

Western blotting and ELISA

Hippocampal slices were dissociated using Bio-Plex Cell Lysis Kit (Bio-Rad), centrifuged for 10 min at 10,000 g at 4ºC, and the supernatant was collected for Western blot analysis. Protein concentration was determined using Bradford Protein Assay Kit, and samples were prepared to a final concentration of 3 μg/μL. After boiling for 5 min,

50 μg of total protein were loaded per lane and resolved in 12% Tris-glycine SDS-PAGE gels. Proteins were then transferred to a nitrocellulose membrane and incubated with respective primary antibodies. Primary antibodies used were: puromycin (#MABE343, clone 12D10, 1:1,000; EMD Millipore), phospho-ERK1/2 Thr202/Tyr204 (#4370, 1:1,000; Cell Signaling), ERK1/2 (#9102, 1:1,000; Cell Signaling); phospho-p70S6K Thr389 (#9205, 1:1,000; Cell Signaling), p70S6K (#9206, 1:1,1000; Cell Signaling), phospho-S6 Ser235/236 (#4858, 1:1,000; Cell Signaling), phospho-S6 Ser240/244 (#5364, 1:1,000; Cell Signaling),S6 (#2217, 1:1,000; Cell Signaling), FMRP (#4317, 1:1,1000; Cell Signaling), phospho-4E-BP1 Ser65 (#9451, 1:1,000; Cell Signaling), 4E-BP1/2 (#2845, 1:1,1000; Cell Signaling), phospho-mTOR Ser2448 (#2971, 1:1,1000; Cell Signaling), mTOR (#2983, clone 7C10, 1:1,1000; Cell Signaling), phospho-eIF2α Ser51 (#3597, 1:1,000; Invitrogen); eIF2α (#9722, 1:1,000; Cell Signaling) and β–actin (#A2228, 1:10,000; Sigma Aldrich). Immunoblots were developed by HRP-conjugated secondary antibodies (anti-mouse or anti-rabbit, #31430 or #31460, 1:5,000; Thermo Fisher) followed by ECL plus exposure, or by IR dye-conjugated fluorescent secondary antibodies (1:5,000; LiCor). When phosphorylated and total proteins were detected, membranes were initialy probed for the phosphorylated epitope, then stripped with Restore stripping buffer (Thermo Fisher, 21059), and finally probed for total protein with the antibodies listed above. Quantification was performed using ImageJ (NIH). ELISA for human $A\beta_{42}$ (Thermo Fisher; KHB3441) was performed according to manufacturer's instructions, as previously described [9, 19].

AβO preparation and intracerebroventricular (i.c.v.) infusion

$A\beta_{1-42}$ peptide was purchased from California Peptide (Salt Lake, CA). Aβ oligomerization and quality control were performed as previously described [7, 19]. I.c.v. infusion of AβOs (10 pmol) in mice were performed as described [7, 45].

Contextual Fear Conditioning Task

Mice were gently handled for 1 min/day for 3 days prior to behavioral experiments. To assess contextual fear memory, a two-phase protocol was used as described [46]. In the training phase, mice were placed in the apparatus, in which they received a foot shock (0.35 mA for 1 s) at 2 min. After 30 s, they received another foot shock (0.35 mA for 1 s), were kept in the apparatus for an additional 30 s, and then placed back in their home cages. For the test phase, carried out 24 h later, mice were placed in the same apparatus for 5 min, with no shock. Freezing behavior was recorded automatically on Freeze Frame (Harvard Apparatus, Holliston, MA) and was used as a memory index. For experiments using HNK, 20 mg/kg HNK (or saline) was injected i.p. 1 h prior to training. PD98059 (10 mg/kg, i.p.) or saline was injected 1h prior to HNK injection.

Two-day Radial Arm Water Maze

Radial Arm Water Maze (RAWM) was performed in WT or APP/PS1 mice as described [20, 22]. Briefly, spatial memory was assessed by the ability to find a platform located in a defined arm of a 6-arm maze placed inside a circular pool. On day 1, five training blocks were performed with alternation between visible and hidden platform. On day 2, all five blocks had the platform hidden. A training block comprises 3 trials of 60 s each. The number of entries into incorrect maze arms (errors) was recorded for each session and the number of errors per block was averaged.

RNA sequencing

Total RNA was extracted using the RNeasy Micro kit (Qiagen), following manufacturer's instructions. The purity of RNA preparations was checked using the 260/280 nm absorbance ratio. Only preparations with 260/280 nm absorbance ratios higher than 1.8 were used. RNA concentrations were determined by absorption at 260 nm. Messenger RNA was purified from total RNA using poly-T oligo-attached magnetic beads. After fragmentation, the first strand cDNA was synthesized using random hexamer primers, followed by second strand cDNA synthesis using either dUTP for directional library or dTTP for non-directional library. Library sequencing was performed on an Illumina platform and paired-end reads were generated. Quality control was performed; the index of the reference genome was built using Hisat2 v2.0.5 and paired-end clean reads were aligned to the reference genome using Hisat2 v2.0.5. featureCounts v1.5.0-p3 was used to count read numbers mapped to each gene. Differential expression analysis of two conditions/groups (two biological replicates per condition) was performed using the DESeq2 R package (1.20.0). The resulting p-values were adjusted using the Benjamini and Hochberg's approach to control for false discovery rate (FDR). Genes with an adjusted FDR p-value ≤ 0.05 found by DESeq2 were considered differentially expressed. DEGs were analyzed using Gene Ontology (GO) (http://geneontology.org) using the mouse database. Pathway enrichment was analyzed by Fisher's Exact test and corrected by FDR. For STRING analyses, DEGs were analyzed for all interaction sources except text mining, and limited to high confidence scores. DEG detected in saline-treated APP/PS1 but not in HNK-treated APP/PS1 mice (compared to control) were also used for STRING, GO and TRRUST analyses. DEGs were loaded onto the mouse Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) database (https://www.grnpedia.org/trrust) and used for identification of

putative master transcription factors. Results were considered significant with FDR values ≤ 0.05.

Electrophysiology

Transverse hippocampal slices (400 μm) were obtained from 3-month-old C57BL/6 mice and placed on a perfusion chamber on aCSF (124 mM NaCl, 4.4 mM KCl, 1 mM Na_2HPO_4, 25 mM $NaHCO_3$, 2 mM $MgCl_2$, 2mM $CaCl_2$, 10 mM glucose). Slices were allowed to recover for at least 90 min, and were perfused with 20 μM HNK or saline for 20 min, followed by AβOs (400 nM) or vehicle for another 20 min. Stimulation was performed in CA3, and field excitatory postsynaptic potentials (fEPSPs) were recorded from hippocampal CA1 region as described [19]. For mechanistic studies, hippocampal slices were pre-exposed to vehicle, PD98059 (Sigma Aldrich, #P215; 20 μM) or rapamycin (Sigma Aldrich, R0395; 100μM) for 20 min and then exposed to HNK or vehicle (20μM), followed by AβOs (1 μM) for 20 min prior to tetanic stimulation.

fEPSPs were also recorded in hippocampal slices from 19–24-month-old WT or APP/PS1 mice that were daily treated with vehicle or HNK (0.5 mg/kg, i.p.) for 12-14 days prior to slice preparation.

Statistical analysis

Data are expressed as means ± S.E.M. and were analyzed using GraphPad Prism 8 software (La Jolla, CA). Sample sizes and statistical tests for each experiment are indicated in the corresponding Figure Legends. p-values for relevant comparisons are indicated in Figures or Figure Legends.

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Author contributions

F.C.R., D.C., A.A.-.V. and S.T.F. designed the study. F.C.R., D.C., E.K.A, A.S., S.B., S.W., J.D.C., R.S-N., M.C., E.A. and A.A.-.V. performed research. F.C.R., D.C., O.A. and A.A.-.V. analyzed data. K.N., M.V.L., J.C.-.L., O.A., F.G.D.F., A.A.-.V., N.S. and S.T.F. contributed reagents, materials, animals and analysis tools. F.C.R., D.C.,

F.G.D.F.., M.V.L., A.A.-.V., N.S. and S.T.F. analyzed and discussed results. F.C.R., D.C., M.V.L. and S.T.F. wrote the manuscript with inputs from the other authors, notably by N.S. and A.A.-V.

Competing interests

The authors declare no competing interests.

Data and materials availability

All data supporting the conclusions are presented in the manuscript and main Figures, or in Supplementary Materials.

FIGURES LEGENDS

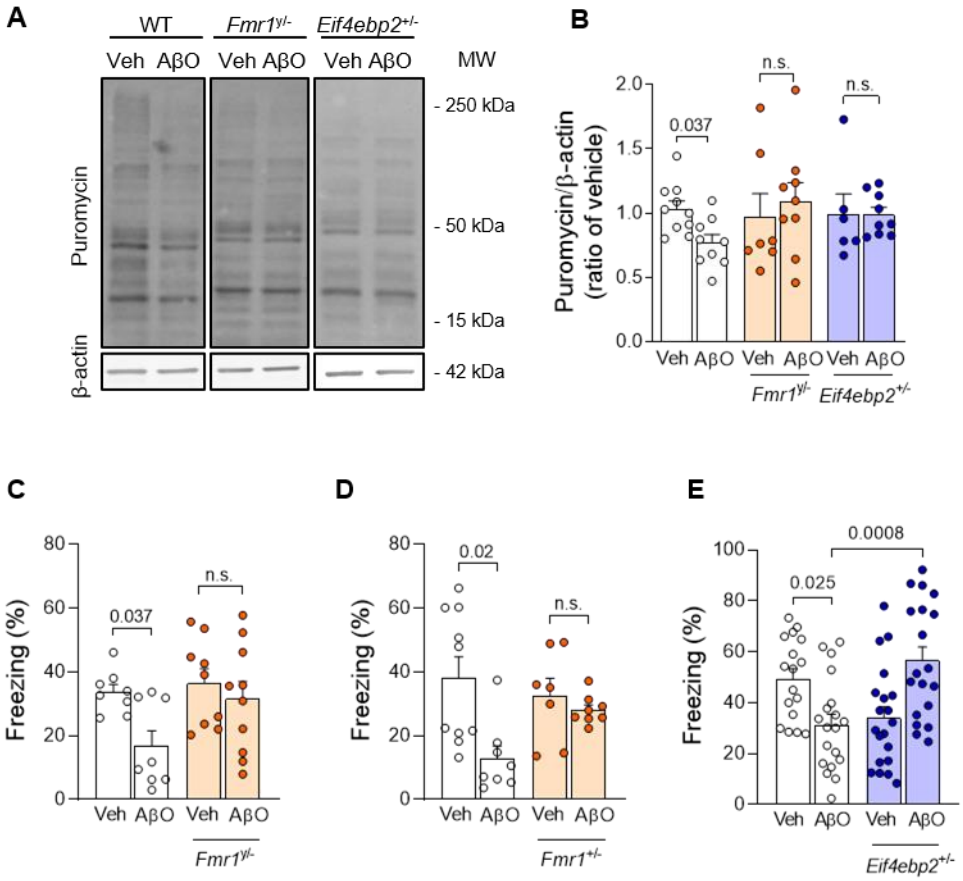


Figure 1. Genetic reduction of FMRP or eIF4E-BP2 prevents defective hippocampal protein synthesis and memory in AβO-infused mice. (A, B) Male *Eif4ebp2*$^{+/-}$ or *Fmr1*$^{y/-}$ mice (or corresponding WT littermates) received an i.c.v. infusion of 10 pmol AβOs (or vehicle) (N = 10 Veh, 9 AβOs, 6 Fmr1$^{y/-}$ Veh, 9 Fmr1$^{y/-}$ AβOs, 7 Eif4ebp2$^{+/-}$ Veh, 9 Eif4ebp2$^{+/-}$ AβOs). Hippocampal slices were obtained 7 days later and were exposed to puromycin (5 µg/mL; 45 min) for SUnSET. Puromycin levels were evaluated by Western blotting and normalized by β-actin levels. (C-E) 3 month-old *Fmr1*$^{y/-}$ (N = 8 Veh, 9 AβOs, 9 Fmr1$^{/-}$ Veh, 10 Fmr1$^{y/-}$ AβOs), *Fmr1*$^{+/-}$ (N = 10 Veh, 8 AβOs, 7 *Fmr1*$^{+/-}$ Veh, 8 *Fmr1*$^{+/-}$ AβOs) or *Eif4ebp2*$^{+/-}$ mice (N = 17 Veh, 19 AβOs, 20 *Eif4ebp2*$^{+/-}$ Veh, 19 *Eif4ebp2*$^{+/-}$ AβOs) (or corresponding WT littermates) received an i.c.v. infusion of 10 pmol AβOs (or vehicle) and memory was assessed using the contextual fear conditioning (CFC) test 7 days later.

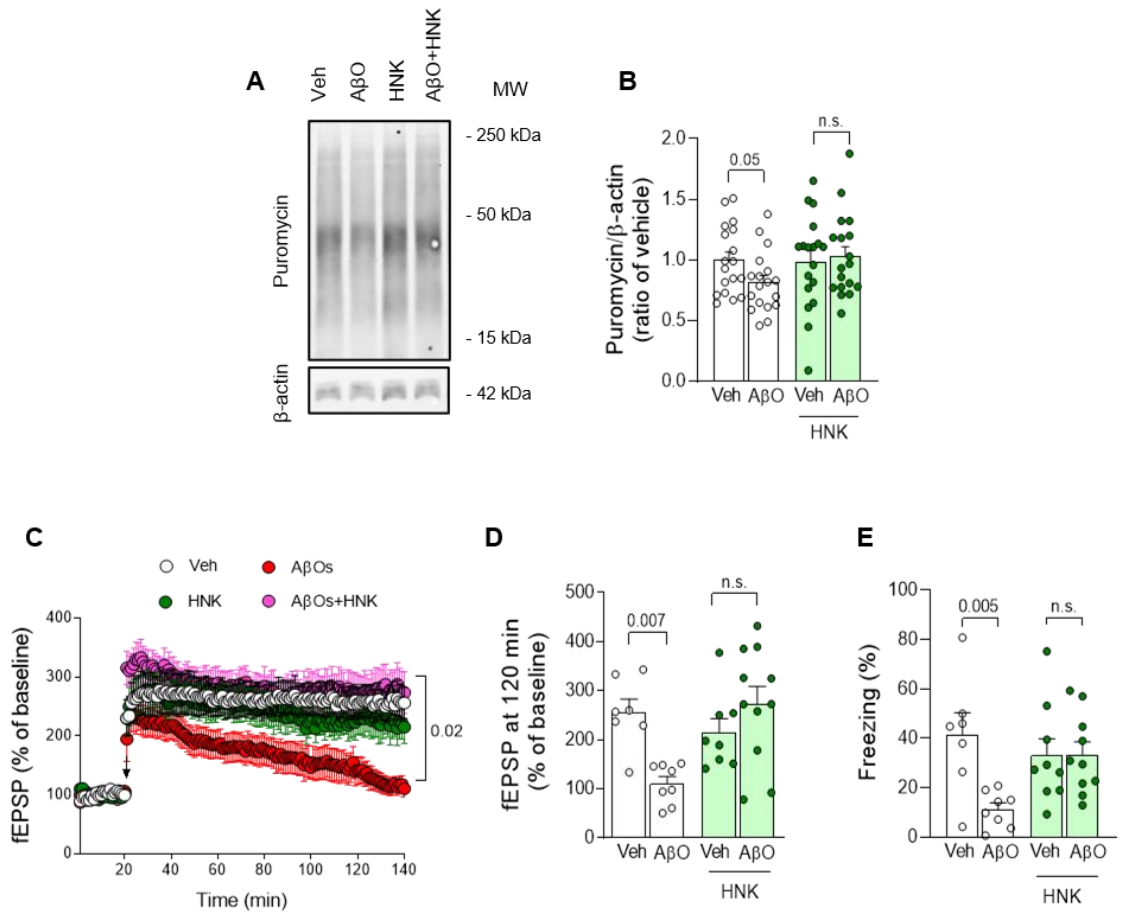


Figure 2. HNK rescues defective hippocampal protein synthesis, LTP and memory in AβO-infused mice (A, B) Acute hippocampal slices were treated with HNK (20 μM; 20 min) prior to AβOs (1 μM; 2 h), and were then exposed to puromycin (5 μg/mL; 45 min) for SUnSET. Puromycin levels were evaluated by Western blotting and normalized by β-actin levels (N = 18 slices per experimental group). (C) Field excitatory post-synaptic potentials (fEPSP) recorded in acute hippocampal slices (from C57BL/6J mice) exposed to saline or HNK (20 μM; 20 min) prior exposure to vehicle or AβOs (400 nM; 20 min) (N = 7 Veh, 8 AβOs, 9 Veh/HNK, 11 AβOs/HNK). Arrows indicate tetanic stimulation. (D) fEPSP slope measured 120 min after tetanic stimulation. (E) Six days after i.c.v. infusion of AβOs (10 pmol) or vehicle, mice were treated with saline or HNK (20 mg/kg, i.p.) 1 h prior to contextual fear conditioning (CFC) training. The CFC test phase was performed 24 h later (N = 7 Veh, 8 AβOs, 9 Veh/HNK, 10 AβOs/HNK). p-values are shown for appropriate comparisons; Two-way ANOVA followed by Holm-Sidak's post-hoc test.

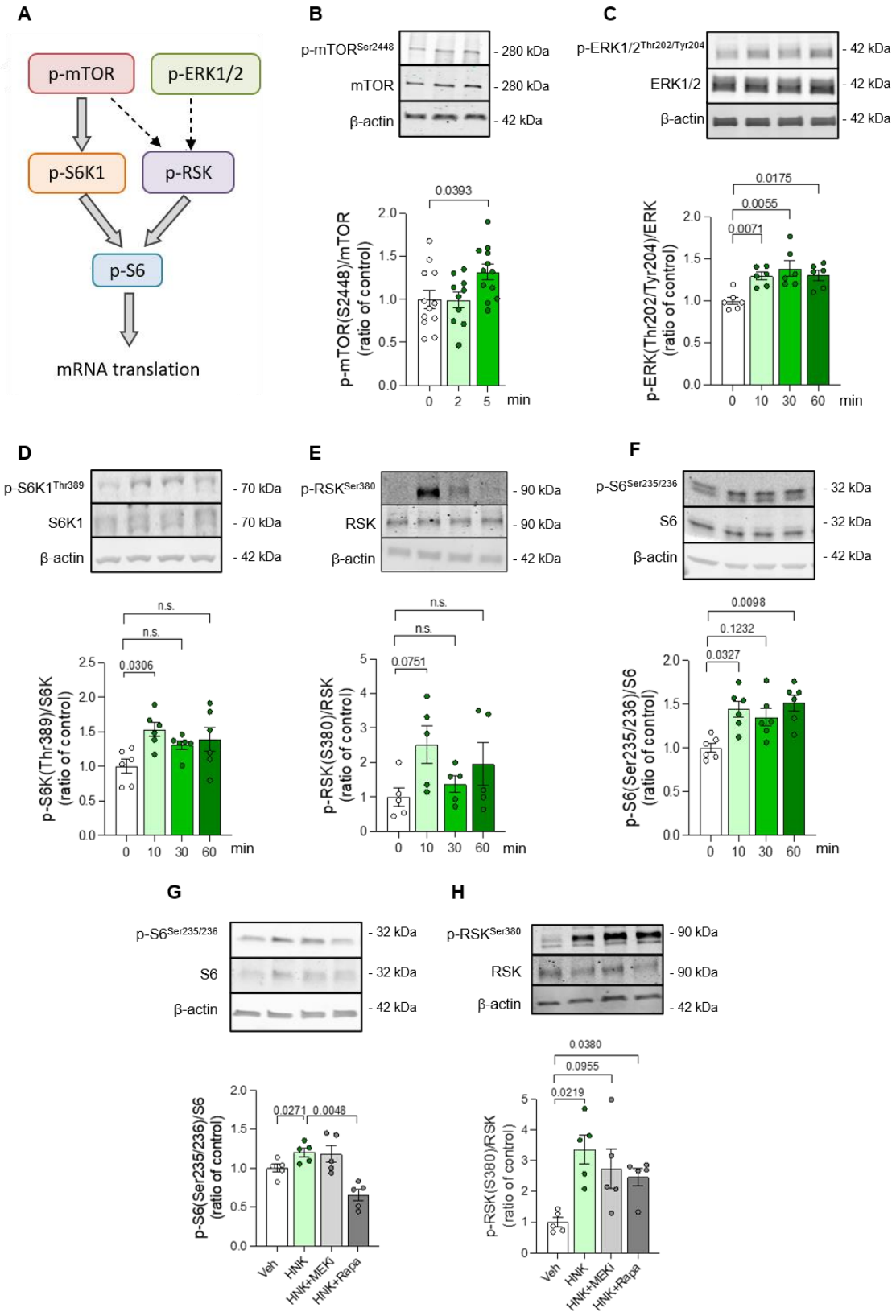


Figure 3. HNK activates hippocampal signaling pathways associated with translational control and protein synthesis. (A) Schematic diagram of signaling pathways controlling translation. (B-F) Acute hippocampal slices were treated with

vehicle or HNK (20 μM) for the indicated time periods. Phosphorylated mTOR, ERK S6K1, RSK, S6 were evaluated by Western blotting and normalized by respective total protein levels (N = 6-12 slices/experimental group). (G, H) Acute hippocampal slices were treated with vehicle, rapamycin (Rapa; 100 μM) or PD98059 (MEKi; 20 μM) for 30 min and were then exposed to vehicle or HNK (20 μM) for 10 min. Phosphorylated S6 and RSK were evaluated by Western blotting (N= 5 slices/experimental group). p-values are shown for the indicated comparisons; One-way repeated measures ANOVA followed by Holm-Sidak's post-hoc test.

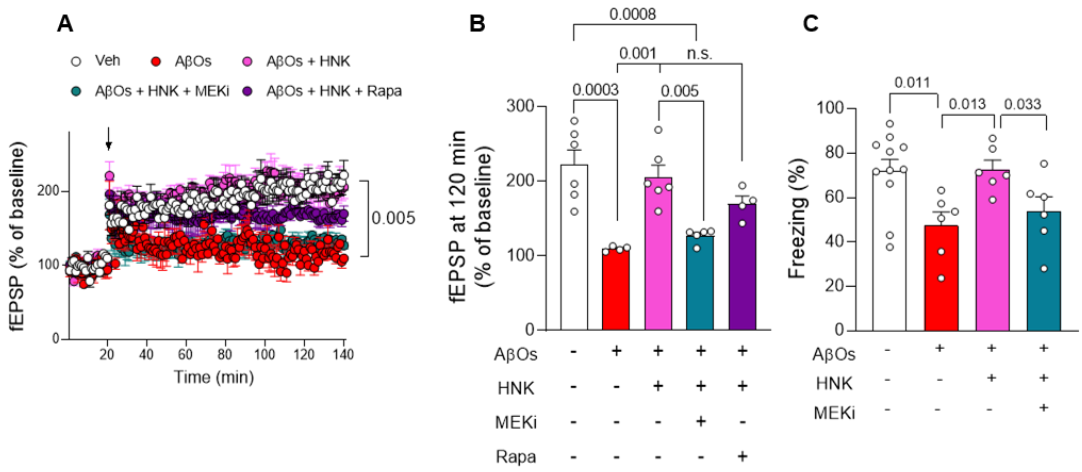
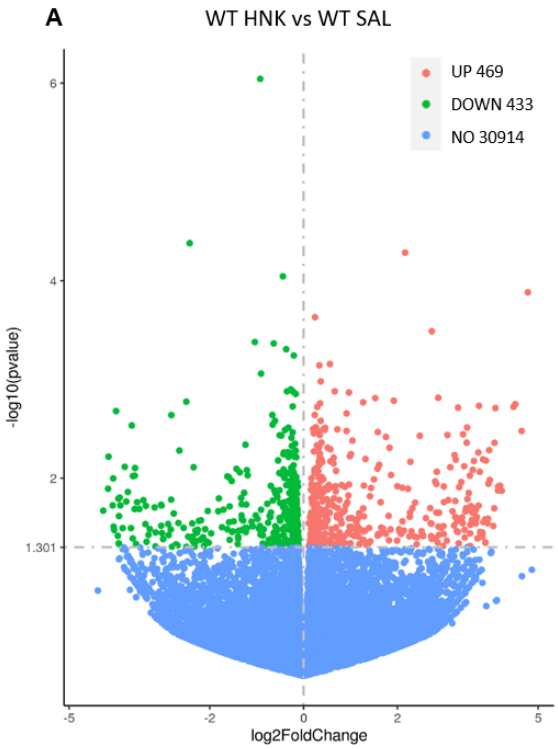
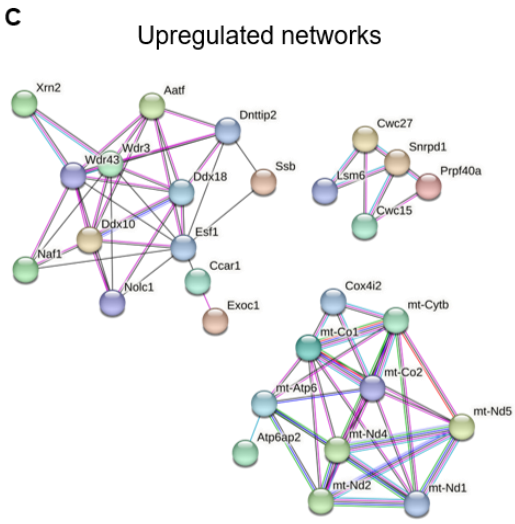


Figure 4. Correction of hippocampal synaptic plasticity and memory by treatment with HNK is mediated by ERK signaling. (A) Acute hippocampal slices were treated with PD98059 (MEKi; 20 μM), rapamycin (Rapa; 100 μM) or vehicle for 20 min, then HNK (20 μM) or vehicle was added 20 min prior to AβOs (1 μM). Field excitatory post-synaptic potentials (fEPSP) were recorded in hippocampal slices (N = 6 Veh, 4 AβOs, 6 AβOs+HNK, 5 AβOs+HNK+MEKi, 4 6 AβOs+HNK+ Rapa). Arrows indicate tetanic stimulation. (B) fEPSP slope measured 120 min after tetanic stimulation. (C) Six days after i.c.v. infusion of AβOs (10 pmol) or vehicle, mice were treated with MEKi (10 mg/kg, i.p.) or saline for 1 h, and then treated with HNK (20 mg/kg, i.p.) for 1 h prior to contextual fear conditioning (CFC) training. The CFC test phase was performed 24 h later (N = 11 Veh, 6 AβOs, 6 AβOs+HNK, 6 AβOs+HNK+MEKi). p-values are shown for appropriate comparisons: Student t-test comparison between Veh and AβOs, and One-way ANOVA followed by Holm-Sidak's post-hoc test with AβOs+HNK as reference.



B

Upregulated GO terms	FDR
Electron transport-coupled proton transport	5.43×10^{-3}
Oxidative phosphorylation	1.95×10^{-2}
Positive regulation of transcription	4.65×10^{-2}
Downregulated GO term	**FDR**
Activation of GTPase activity	3.31×10^{-2}



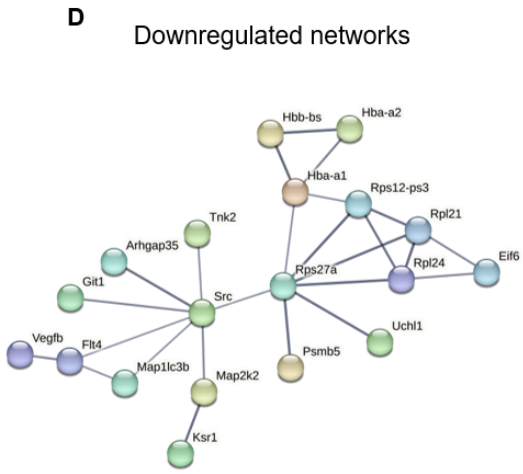
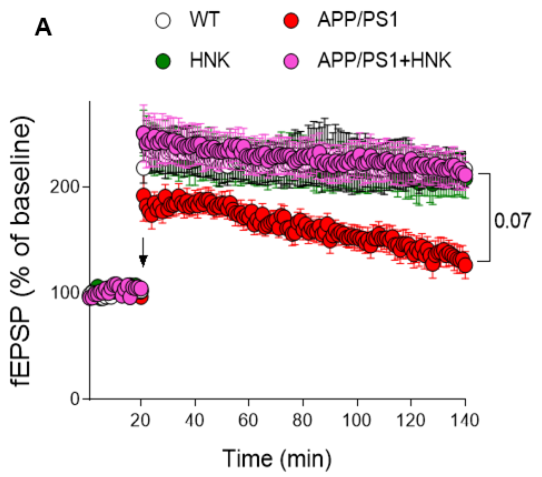
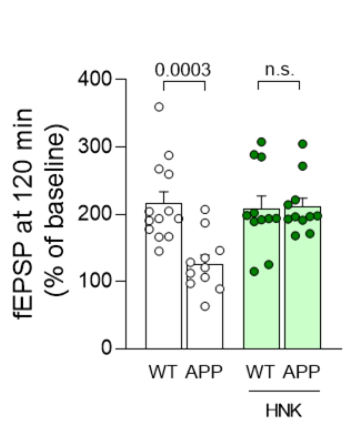
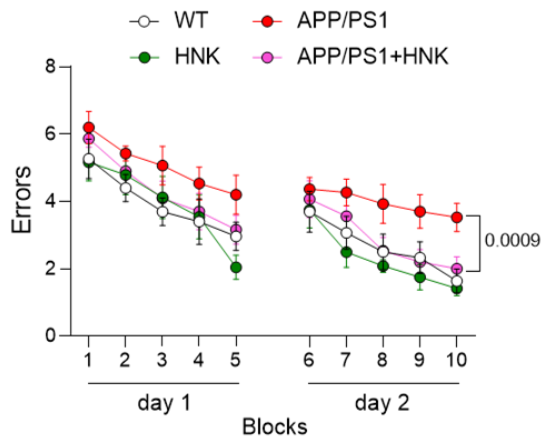


Figure 5. Treatment with HNK modifies the hippocampal transcriptional profile in mice. (A) Volcano plot from RNA-seq analysis of HNK-treated vs saline-treated WT mice. Downregulated genes are shown in green and upregulated genes in red ($p < 0.05$). (B) Gene ontology analysis (molecular function) for upregulated and downregulated genes (FDR < 0.05). (C, D) STRING analysis of upregulated and downregulated networks. The most connected nodes are shown (N = 6 mice per experimental group).







D

Upregulated pathways in APP/PS1 mice rescued by HNK	
GO term	**FDR**
Regulation of tumor necrosis factor production	2.49×10^{-2}
Response to hormone	2.41×10^{-2}
Inflammatory response	3.73×10^{-2}
Positive regulation of programmed cell death	2.49×10^{-2}
Response to stress	1.46×10^{-2}
Reactome pathway	**FDR**
Innate immune system	1.22×10^{-2}
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	3.81×10^{-2}
SRP-dependent cotranslational protein targeting to membrane	3.83×10^{-2}

E

Upregulated key TR in APP/PS1 mice rescued by HNK	
Key transcriptional regulator (TR)	**p-value**
Nuclear factor of kappa light polypeptide gene enhancer in B cells 1, p105 (Nfkb1)	2.67×10^{-7}
Peroxisome proliferator activated receptor alpha (Ppara)	2.35×10^{-5}
Histone deacetylase 1 (Hdac1)	6.54×10^{-4}
Activating transcription fator 3 (Atf3)	4.23×10^{-3}
Histone deacetylase 2 (Hdac2)	6.69×10^{-3}
Activating transcription fator 4 (Atf4)	1.13×10^{-2}

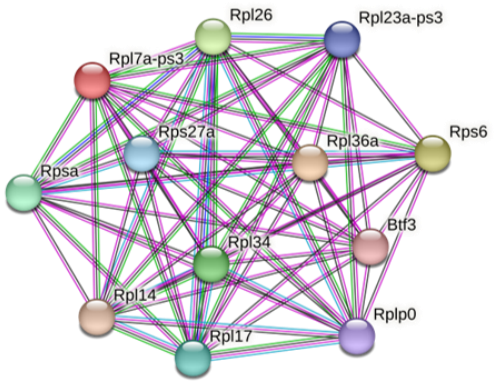


Figure 6. Treatment with HNK reverses LTP and memory deficits, and corrects hippocampal transcriptional alterations in APP/PS1 mice. (A) fEPSP recording in hippocampal slices from 19–24-month-old APP/PS1 (or WT) mice treated daily with HNK (0.5 mg/kg, i.p.) or vehicle for 12-14 days (N = 13 WT, 11 APP/PS1, 11 WT/HNK,

11 APP/PS1/HNK). N = number of slices (derived from at least 3 mice) per experimental condition. Arrows indicate tetanic stimulation. (B) fEPSP slope measured 120 min after tetanic stimulation. (C) Spatial memory assessed using the Radial Arms Water Maze (RAWM) test on 19–24-month-old APP/PS1 (or WT) mice treated daily with HNK (0.5 mg/kg, i.p.) or vehicle for 8-10 days (N = 10 WT, 10 APP/PS1, 8 WT/HNK, and 10 APP/PS1/HNK). p-values for appropriate comparisons are indicated; Two-way ANOVA followed by Holm-Sidak's post-hoc test. (D-F) RNA-seq analyses of genes that were differentially expressed in saline-treated APP/PS1 (compared to WT mice) mice and whose expression was corrected by treatment with HNK. (D) Gene ontology (molecular function and reactome pathway). (E) Key transcription factor analysis by TRRUST (FDR < 0.05). (F) Representative STRING cluster of ribosome-linked genes that were differentially expressed in APP/PS1 (compared to WT mice) and were normalized by treatment with HNK. (N = 6 mice per experimental group).