Cell-type-specific drug-inducible protein synthesis inhibition demonstrates that memory consolidation requires rapid neuronal translation

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New protein synthesis is known to be required for the consolidation of memories, yet existing methods of blocking translation lack spatiotemporal precision and cell-type specificity, preventing investigation of cell-specific contributions of protein synthesis. Here we developed a combined knock-in mouse and chemogenetic approach for cell-type-specific drug-inducible protein synthesis inhibition that enables rapid and reversible phosphorylation of eukaryotic initiation factor 2α, leading to inhibition of general translation by 50% in vivo. We use cell-type-specific drug-inducible protein synthesis inhibition to show that targeted protein synthesis inhibition pan-neuronally and in excitatory neurons in the lateral amygdala (LA) impaired long-term memory. This could be recovered with artificial chemogenetic activation of LA neurons, although at the cost of stimulus generalization. Conversely, genetically reducing phosphorylation of eukaryotic initiation factor 2α in excitatory neurons in the LA enhanced memory strength but reduced memory fidelity and behavioral flexibility. Our findings provide evidence for a cell-specific translation program during consolidation of threat memories.
LA during consolidation of auditory threat memory. Such cued threat conditioning (cTC) is particularly amenable to studying the memory consolidation process because one-trial training is sufficient to form a persistent LTM, and a unimodal cue can be used for memory retrieval. We found that long-term threat memories are particularly labile to pan-neuronal and LA CamK2α+ cell-type-specific protein synthesis disruption in the first hour after training. We further found that the translation program regulated by phosphorylation status of eIF2α plays an important role in calibrating memory strength and fidelity. Our findings provide mechanistic insight into the nature of long-term threat memory consolidation.

Results
A chemogenetic resource for cell-type-specific protein synthesis inhibition. To induce phosphorylation of eIF2α, we engineered the kinase domain of the eIF2α kinase PKR, to harbor a recognition site (NS5A/B) for a recombinant nonstructural protein 3/4 (NS3/4) protease, which can be chemically inhibited with asunaprevir (ASV)18,19 (Fig. 1a). We first tested toxicity of ASV in amygdala slices from wild-type (WT) mice infused with an increasing dose of ASV (0, 10 and 100 nM and 1 μM) and determined that for all doses tested, there was no significant induction of either the immediate early gene c-Fos or the integrated stress response as assessed by examination of the level of p-eIF2α S51 (Extended Data Fig. 1a). The kinase domain of PKR is a caspase-generated fragment that is constitutively active and does not require either double-stranded RNA or dimerization for activation20. We designed our ciPSI multicistronic construct with NS3/4 protease21, enhanced green fluorescent protein (eGFP) and inducible PKR (iPKR) transgenes separated by self-cleaving 2A proteinase that allows translation of individual elements by ribosome skipping22 (Extended Data Fig. 1b). To test the inhibition of global translation in vitro, we metabolically labeled newly synthesized proteins with S35 methionine after starvation. In cells transfected with iPKR and NS3/4A, iPKR was degraded by NS3/4, and de novo translation was equivalent to the control cells, whereas cells transfected with iPKR plasmid alone in the absence of NS3/4A had substantially reduced translation by about 60%. Unmodified PKR kinase domain (PKRk) similarly reduced de novo translation relative to controls, but the PKRk levels were nonresponsive to NS3/4 protease (Extended Data Fig. 1c). As predicted, PKRk fragment was detected only in lysates from cells transfected with either unmodified PKRk or modified iPKR (Extended Data Fig. 1d).
We next knocked in the ciPSI multicistronic cassette into the first intron of the mouse *Eef1a1* genomic locus with two modifications (Fig. 1b, Supplementary Data 1 and Extended Data Fig. 2a,b). First, eGFP was substituted with an eGFP-L10 fusion to use as a fluorescent marker of cells expressing ciPSI and to eventually enable transcribing ribosome affinity purification profiling. Second, a STOP cassette flanked by loxP sites preceded the ciPSI cassette to allow cell-type-specific disruption of protein synthesis when combined with Cre-driver mouse lines and/or Cre-expressing viruses. These modifications had no effect on expression of iPKR (data not shown). A mouse Nestin (Nes) Cre-driver line was bred with the ciPSI line to generate transheterozygote Nes.iPKR pan-neuronal ciPSI knock-in mice, which were viable and fertile. The Nes-iPKR mice expressed eGFP-L10 in all neurons in the amygdala, as marked by complete overlap with neuronal nuclei (NeuN) staining (Fig. 1d), as well as in cortical areas, such as the anterior cingulate cortex and somatosensory cortex, hippocampal areas, CA1 and CA3, and the dentate gyrus (Extended Data Fig. 2c–g). Despite heterozygous deletion of *Eef1a1* allele and expression of the multicistronic cassette in Nes-iPKR mice, the mice displayed normal spontaneous locomotion and thigmotaxis in open field tests (Extended Data Fig. 3a–c).

To test the efficiency of ciPSI in blocking protein synthesis ex vivo, we subjected amygdala slices of Nes.iPKR and WT mice to bio-orthogonal noncanonical amino acid tagging (BONCAT) (ref. 22) of newly synthesized proteins. Nes.iPKR amygdala slices treated with 1 μM ASV exhibited a sharp decline in de novo translation (~80%) compared with controls (Fig. 2a). Because BONCAT uses azidohomoalanine (AIHA), a synthetic methionine analog, which can get outcompeted by endogenous methionine in vivo, this method does not sufficiently label de novo translation in vivo. Thus, an independent method of labeling de novo translation, surface sensing of translation (SUSET) (ref. 23), which measures translation elongation and is amenable for in vivo labeling, was used to test ciPSI efficiency in awake behaving mice. SUNSET immunoblotting showed that Nes.iPKR mice centrally infused with 150 μg ASV exhibited a robust decrease in protein synthesis (~50%) compared with controls (Fig. 2b). The inhibition of protein synthesis was concomitant with a specific increase in phosphorylation of eIF2α at 1 h after ASV treatment both ex vivo (Fig. 2c) and in vivo (Fig. 2d), with no effect on either the extracellular signal-regulated kinases 1/2 (ERK1/2) or mechanistic target of rapamycin (mTORC1) pathways (Fig. 2d). Next, we investigated the ASV pharmacokinetics in Nes.iPKR amygdala lysates by harvesting tissue at increasing time points after drug infusion. We found that peak expression of iPKR is reached at 0.5 h, which steadily declines at 1 and 3 h, and is completely degraded by 6 h (Fig. 2e). Subsequently, phosphorylation of eIF2α steadily increased at 0.5 and 1 h and then declined to baseline at 3 h. As a result of phosphorylation of eIF2α, proteins whose transcripts harbor uORFs, specifically ATF4 and GADD34, accumulated and remained at higher levels compared with control until 3 h (Fig. 2e), GADD34 is the regulatory subunit of the eIF2α S51 phosphatase, and the negative feedback caused by increased GADD34 levels combined with the degradation of iPKR by NS3/4 protease at the 3-h time point might be responsible for reduced phosphorylation of eIF2α below the control (Fig. 2e). We also probed for c-Fos to assess whether c-Fos levels change with ciPSI, and found that c-Fos levels significantly decrease below baseline at 3 and 6 h after ASV treatment (Fig. 2e), indicating general translation suppression.

Past studies have shown that the enduring L-LTP in amygdala requires protein synthesis in vivo24. To determine whether chemogenetic inhibition of pan-neuronal protein synthesis influences synaptic plasticity, we examined LTP of LA. We delivered three trains of high-frequency stimulation (HFS) to thalamo-amygdalar inputs and recorded the field excitatory postsynaptic potential (fEPSP) in the dorsal subdivision of LA while perfusing into the amygdala slices 5 nM ASV 10 min before and for 90 min after tetanus.

**Fig. 2** | Drug-induced neuronal protein synthesis inhibition. a, De novo translation labeled at the amino terminus (N terminus) using BONCAT in Nes.iPKR amygdala slices showed a robust decrease in protein translation in mutant amygdala treated with 1 μM ASV compared with VEH-treated mutant amygdala (***P < 0.0001), as well as ASV-treated WT amygdala (***P < 0.0001). n = 3 independent lysates from three mice per group. Two-way ANOVA with Bonferroni’s post hoc test was conducted. Genotype–drug interaction: F(1,8) = 34.6, P = 0.0004; drug: F(1,8) = 75.2, P < 0.0001; genotype: F(1,8) = 80.7, P < 0.0001. b, De novo translation labeled at the carboxy terminus (C terminus) in awake behaving mice using SUNSET also showed a significant decrease in translation in mutant mice infused with 150 μg ASV (100 nM in 2 μl) compared with VEH infusion (***P < 0.0001), as well as in WT mice infused with ASV (***P < 0.0001). n = 3 independent lysates from two mice per group. Two-way ANOVA with Bonferroni’s post hoc test was conducted. Genotype–drug interaction: F(1,8) = 19.49, P = 0.0022; drug: F(1,8) = 41.51, P = 0.0002; genotype: F(1,8) = 117.9, P < 0.0001. c, Bath application of 1 μM ASV caused a robust phosphorylation of eIF2α in mutant Nes.iPKR amygdala slices compared with ASV-treated WT slices and VEH-treated Nes.iPKR slices (***P < 0.0001). n = 3 independent lysates from three mice per group. Two-way ANOVA with Bonferroni’s post hoc test was conducted. Genotype–drug interaction: F(1,8) = 44.7, P = 0.0002; drug: F(1,8) = 53.5, P < 0.0001; genotype: F(1,8) = 51.7, P < 0.0001. d, ASV infusion in vivo (150 μg or 100 nM in 2 μl) also caused a significant increase in p-eIF2α in Nes.iPKR amygdala compared with controls (***P < 0.0001). n = 3 independent lysates from three mice per group. Two-way ANOVA with Bonferroni’s post hoc test was conducted. Genotype–drug interaction: F(1,8) = 70.87, P < 0.0001; drug: F(1,8) = 19.04, P < 0.0024; genotype: F(1,8) = 120.4, P < 0.0001. Major intracellular signaling pathways, ERK1/2, mitogen-activated protein kinase and mTORC1, assessed by examining p-ERK1/2 (T202/Y204) and p-S6 (S240/244) levels, were unchanged by ASV treatment in Nes.iPKR amygdala lysates. n(p/t-eIF2α) = 3 independent lysates from three mice per group; n(p/t-ERK1/2) = 3 independent lysates per group. Two-way ANOVA followed by post hoc Bonferroni’s test was conducted. e, Time course for c-Fos carried out by collecting amygdala lysate at different time points after ASV infusion (0, 0.5, 1, 3 and 6 h) shows peak expression of 36kDa iPKR at 0.5 h, which was undetectable at 6 h (**P < 0.0001). Endogenous PKR (68 kDa) remained unchanged after ASV treatment. Peak expression of p-eIF2α, normalized for t-eIF2α, was achieved at 0.5 h after ASV infusion followed by a steady decline from 3 h onward (**< P < 0.005). ATF4 and GADD34 proteins whose transcripts harbor a uORF were increased by ASV by 1 h and declined to baseline by 6 h (**P < 0.001, **P < 0.001). c-Fos levels significantly declined from baseline at 3 and 6 h postinfusion (**P < 0.01, ***P < 0.001). n = 3 independent lysates per group; repeated-measures one-way ANOVA with post hoc Bonferroni’s test. f, Schematic for L-LTP recording in the LA (top) and representative field potentials before (1) and 90 min after tetanus (2) for different groups of slices are shown (bottom). L-LTP evoked by three trains of HFS was impaired in Nes.iPKR amygdala slices treated with ASV (5 nM) after ASV application and perfused for 90 min after tetanus (***P < 0.0001; n(WT + VEH) = 12 slices, n(WT + ASV) = 12 slices, n(Nes.iPKR + VEH) = 13 slices and n(Nes.iPKR + ASV) = 15 slices). One-way ANOVA with Bonferroni’s post hoc test was performed. g, Mean fEPSPs at baseline (10 min) before ASV application at 30 min (that is, 10 min after ASV application), at 60 min (30 min after tetanus) and at 120 min (90 min after tetanus). ASV significantly reduced fEPSP slope in Nes.iPKR amygdala at 120 min compared with VEH treatment (**P < 0.01) and ASV-treated WT amygdala (***P < 0.001), but had no effect on baseline (n(WT + VEH) = 12 slices, n(WT + ASV) = 12 slices, n(Nes.iPKR + VEH) = 13 slices and n(Nes.iPKR + ASV) = 15 slices). Two-way ANOVA with Bonferroni’s post hoc test. Genotype–drug–time interaction: F(6,144) = 4.700, P = 0.0002; genotype–drug: F(2,144) = 172.5, P < 0.0001; time: F(3,144) = 5.999, P = 0.0007. Data are presented as mean ± s.e.m.
We found that L-LTP was significantly inhibited by ASV-induced release of iPKR (Fig. 2f). The drug had no effect on baseline fEPSP slope in both WT and Nes.iPKR slices (Fig. 2g). The inhibition had a rapid onset after tetanus and became more robust during L-LTP maintenance (Fig. 2g). Together, these results indicate that chemogenetic inhibition of protein synthesis impairs both induction and expression of L-LTP.

**Temporally structured protein synthesis is required for LTM consolidation.** Given our approach that bypasses the limitations and side effects of methods used to study protein synthesis in vivo, we could now study the role of de novo protein synthesis in memory consolidation. We trained Nes.iPKR and control mice in simple auditory threat conditioning and centrally infused ASV immediately after training (Fig. 3a). Nes.iPKR and WT mice were
Fig. 3 | Temporally structured protein synthesis is required for LTM consolidation. a, Locations of cannula implants for all experimental animals subjected to i.c.v. infusion were verified with post hoc histology. Schematic of the experimental paradigm (right) for simple cTC in Nes.iPKR mice pan-neuronally expressing the ciPSI system. b, Nes.iPKR animals learn the association between CS and US (CS2 versus CS1: **P < 0.05) similar to the WT mice (***P < 0.001). c, Repeat-measures two-way ANOVA with Bonferroni’s post hoc test was performed. CS: F(2,54) = 19.38, P < 0.0001. Nes.iPKR mice infused with ASV were comparable in STM performance as VeH-infused mice and WT controls (n(WT + VeH) = 5 animals, n(WT + ASV) = 9 animals, n(Nes.iPKR + VeH) = 4 animals and n(Nes.iPKR + ASV) = 5 animals). Ordinary one-way ANOVA was conducted. F(3,19) = 0.576, P = 0.638 (not significant). Representative motion traces for WT mice infused with VeH or ASV and for Nes.iPKR mice infused with VeH or ASV. cTC mean LTM tested 24 h after training was significantly impaired for Nes.iPKR mice infused with ASV compared with VeH (*P < 0.05) similar to the Nes.iPKR mice infused with ASV for all three CS presentations. Retraining the Nes.iPKR mice that previously underwent ciPSI-mediated amnesia fully recovered LTM for all three CS presentations (n(WT + VeH) = 5 animals, n(WT + ASV) = 10 animals, n(Nes.iPKR + VeH) = 8 animals, n(Nes.iPKR + ASV) = 5 animals and n(Nes.iPKR + ASV/retrain) = 7 animals). Repeat-measures two-way ANOVA with Bonferroni’s post hoc test was performed. Genotype: F(5,40) = 4.570, P = 0.0022. c, cTC mean LTM was significantly impaired for Nes.iPKR animals treated with ASV compared with VeH (**P < 0.0015). cTC mean LTM in Nes.iPKR mice was rescued with C16, compared with Nes.iPKR mice infused with ASV alone (**P < 0.0041). cTC mean LTM2 in retrained Nes.iPKR mice was comparable with WT mice (n(WT + VeH) = 7 animals, n(WT + ASV) = 10 animals, n(Nes.iPKR + VeH) = 8 animals, n(Nes.iPKR + ASV + C16) = 5 animals and n(Nes.iPKR + ASV/retrain) = 7 animals). One-way ANOVA with Bonferroni’s post hoc test was performed. F(5,41) = 4.890, P = 0.0013. g, The LTM deficit was present only for Nes.iPKR mice infused with ASV immediately after training (**P = 0.0002), but not 1 h (**P = 0.0004) or 4 h after training. One-way ANOVA with Bonferroni’s post hoc test was conducted. n(WT + VeH, 0 h) = 7 animals, n(WT + VeH, 1 h) = 10 animals, n(WT + ASV, 0 h) = 4 animals, n(WT + ASV, 1 h) = 4 animals, n(WT + ASV, 4 h) = 4 animals, n(WT + ASV, 4 h) = 4 animals, n(Nes.iPKR + ASV, 0 h) = 8 animals, n(Nes.iPKR + ASV, 1 h) = 7 animals and n(Nes.iPKR + ASV, 4 h) = 4 animals. Data are presented as mean ± s.e.m. LV, lateral ventricle; US, unconditioned stimulus. *P < 0.05, **P < 0.01, ***P < 0.001.
Fig. 4 | Cell-type-specific protein synthesis inhibition in principal neurons in the LA. a, CamK2α.iPKR mice were generated by bilaterally injecting AAV1.CamK2α.Cre (or AAV9.CamK2α.Cre.eGFP) into the LA of iPKR mice. ASV infusion caused a significant increase in p-eIF2α levels in GFP+ neurons in CamK2α.iPKR mice compared with GFP+ neighboring neurons in the LA (**P < 0.0001), as well as GFP+ neurons in CamK2α WT mice (**P < 0.0001). Both WT and iPKR mice were injected with AAV.CamK2α.Cre.GFP in the LA. n(CamK2α WT+ASV)=19 GFP+ neurons and 170 GFP+ neurons; n(CamK2α.iPKR + ASV) = 49 GFP+ neurons and 193 GFP+ neurons from three mice per group. Two-way ANOVA with Bonferroni’s post hoc test was conducted. Genotype–GFP interaction: F(1, 427) = 45.11, P < 0.0001; genotype: F(1, 427) = 28.53, P < 0.0001; GFP: F(1, 427) = 36.75, P < 0.0001. c, In vivo SUNSET assay: ASV-treated CamK2α.iPKR mice have significantly reduced translation in LA principal neurons compared with VEH treatment (**P < 0.0001) and ASV-treated WT mice (**P < 0.0001). One-way ANOVA followed by Bonferroni’s post hoc test was performed. n(CamK2α WT+ASV) = 36 GFP–neurons, n(CamK2α.iPKR+VEH) = 22 GFP–neurons and 78 GFP+ neurons, and n(CamK2α.iPKR+ASV) = 31 GFP–neurons and 58 GFP+ neurons from three mice per group. d, Schematic of experimental paradigm for cTC in CamK2α.iPKR mice. e, CamK2α.iPKR mice were comparable with WT in learning the CS-US association (*P = 0.07). Repeated-measures two-way ANOVA with Bonferroni’s post hoc test. n(CamK2α WT) = 5 animals and n(CamK2α.iPKR) = 5 animals. CS: F(2,24) = 24.93, P < 0.0001. f, Representative cTC LTM motion traces for CamK2α WT and CamK2α.iPKR. g, CamK2α.iPKR mice receiving ASV infusion immediately after training displayed a severe LTM deficit across all three CS presentations (**P < 0.0001) that was rescued with pretraining infusion of ISRIB. n(CamK2α WT+VEH) = 7 animals, n(CamK2α WT+ASV) = 9 animals, n(CamK2α WT+ISRIB) = 4 animals, n(CamK2α.iPKR+VEH) = 10 animals, n(CamK2α.iPKR+ASV) = 9 animals and n(CamK2α.iPKR+ASV+ISRIB) = 5 animals. Repeated-measures two-way ANOVA with Bonferroni’s post hoc test was performed. Genotype: F(5,35) = 9.767, P < 0.0001. h, cTC mean LTM was impaired in CamK2α.iPKR mice infused with ASV (**P < 0.0001) but was rescued by ISRIB administration (*P < 0.05). One-way ANOVA with Bonferroni’s post hoc test. n(CamK2α WT+VEH) = 7 animals, n(CamK2α WT+ASV) = 9 animals, n(CamK2α WT+ISRIB) = 4 animals, n(CamK2α.iPKR+VEH) = 10 animals, n(CamK2α.iPKR+ASV) = 9 animals and n(CamK2α.iPKR+ASV+ISRIB) = 5 animals. Data are presented as mean ± s.e.m.
comparable in learning the association of tone with footshock, as well as in short-term memory (STM) that is protein synthesis independent (Fig. 3b,c). However, when the animals were tested for LTM 24 h later, Nes.iPKR mice infused with ASV exhibited markedly reduced defensive behavior in response to the paired tone presentations, even though outside of tone presentations the mice had similar motion indices (Fig. 3d–f). Our findings are consistent with previous studies that showed deletion of genes encoding eIF2α kinases, Gcn2 and Pkr, led to an enhancement of long-term spatial and threat memories, and decreased the threshold for L-LTP induction\(^{14}\). To ensure that cisPsi did not permanently damage the memory system, we retrained Nes.iPKR mice previously infused with ASV in the auditory threat conditioning paradigm. Nes.iPKR mice fully recovered LTM and exhibited a species-appropriate defensive response to the conditioned tone (Fig. 3e,f). Because Nes. iPKR mice express viral NS3/4 protease in addition to the actualor iPKR, we tested the molecular specificity of cisPsi with the PKR inhibitor C16, which binds to PKR at the ATP binding site, thereby blocking its kinase activity\(^{15}\). Pretraining administration of C16 inhibited the activation of iPKR and prevented the LTM deficit in Nes.iPKR mice (Fig. 3e,f). Depending on the training paradigm, there are multiple critical periods during memory consolidation that are sensitive to protein synthesis inhibition\(^{16}\). Therefore, we assessed whether consolidation of associative threat memory in our paradigm requires one or more waves of de novo translation by inducing cisPsi at 0, 1 and 4 h after training (Fig. 3g). Only Nes. iPKR mice receiving ASV infusion immediately after training displayed LTM deficit (Fig. 3g), suggesting that there is only one wave of protein synthesis for up to 4 h after training in our paradigm.

Cell-type-specific de novo translation in LA CamK2α-positive neurons is necessary for LTM consolidation. Lesioning and functional inactivation studies have shown that the LA is an integral brain structure for the formation and storage of conditioned aversive memories\(^{34,35}\). Learned threat elicits persistent cortical and thalamic input-specific synaptic potentiation in principal excitatory neurons within the LA\(^{36,37}\). Thus, we asked whether de novo protein synthesis in CamK2α-positive principal neurons in LA is necessary for aversive memory consolidation. We virally delivered CamK2α.Cre.eGFP into the bilateral LA of iPKR mice and control mice (Fig. 4a). Phosphorylation of eIF2α was specifically increased in neurons from CamK2α.iPKR mice (expressing iPKR from the CamK2α promoter) compared with WT mice and non-eGFP-positive neurons in the same animals (Fig. 4b) with a corresponding decrease in de novo translation (Fig. 4c) upon ASV administration. CamK2α. iPKR mice were threat conditioned as described previously and centrally infused with ASV immediately after training (Fig. 4d). CamK2α.iPKR mice were comparable with WT controls in memory acquisition (Fig. 4e), but LTM tested 24 h later was significantly impaired (Fig. 4f–h). This LTM consolidation deficit with cisPsi was prevented by pretraining administration of ISRIB (integrated stress response inhibitor), a drug that rescues the p-eIF2α-mediated constraint on general translation by enhancing the activity of eIF2B (Fig. 4g,h)\(^{16}\). We also tested ASV-treated CamK2α.iPKR mice in an open field test and elevated plus maze to gauge anxiety-related behaviors, and found that although spontaneous locomotion was normal in these animals, there was a significant increase in the percentage of time spent in the open arm, indicating reduced anxiety-related behavior (Extended Data Fig. 4a–e).

It is evident that the translation program regulated by eIF2α is necessary for LTM consolidation; however, the physiological accumulation of ATF4 as a result of increased eIF2α phosphorylation raises the question of whether it is either decreased protein synthesis or increased ATF4 levels causing the memory deficit. Indeed, a previous study has reported that even in the absence of translation inhibition, the p-eIF2α-mediated increase in ATF4 levels can cause memory deficit by transcriptional repression of CREB-regulated genes\(^{17}\). Dissociating the effects of general

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**Fig. 5** | Bidirectional control of memory strength by phosphorylation of eIF2α S51 in LA principal neurons. **a** Heterozygous CamK2α.eIF2α (A/+) and homozygous CamK2α.eIF2α (A/A) were generated by bilaterally injecting AAV1.CamK2α.Cre into the LA of eIF2α (S51A) CAG Prf/eIF2αGFP mice. **b** In vivo SUNSet assay: homozygous CamK2α.eIF2α (A/A) mice displayed a significant increase in de novo translation compared with the CamK2α WT mice and heterozygous CamK2α.eIF2α (A+/ +) mice (**P < 0.0001), n(CamK2α WT) = 101 neurons from three mice, n(CamK2α eIF2α (A+/ +)) = 93 neurons from three mice and n(CamK2α.eIF2α (A/A)) = 93 neurons from three mice. One-way ANOVA followed by Bonferroni’s post hoc test was performed. F(2, 284) = 52.01, P < 0.0001. **c** Double-transgenic iPKR eIF2α (A/+) and iPKR eIF2α (A/A) mice were bilaterally injected with AAV1.CamK2α.Cre in the LA to generate CamK2α.iPKR eIF2α (A+/+) and CamK2α.iPKR eIF2α (A/A) mice, respectively. All groups of mice (CamK2α WT, CamK2α eIF2α (A/+), CamK2α.eIF2α (A/A), CamK2α.eIF2α/eIF2α (A/+), and CamK2α.eIF2α/eIF2α (A/A)) learned the CS-US association; however, CamK2α.eIF2α (A/A) and CamK2α.eIF2α/eIF2α (A/A) learned better than CamK2α WT mice (CS2. CamK2α WT versus CamK2α.eIF2α (A/A); ***P < 0.0009; CamK2α eIF2α (A/A) versus CamK2α.eIF2α/eIF2α (A/A), P = 0.0740). Repeated-measures two-way ANOVA with Bonferroni’s post hoc test was conducted. CS: f(1,131) = 44.25, P < 0.0001. n(CamK2α WT) = 6 animals, n(CamK2α.eIF2α (A/+)) = 4 animals, n(CamK2α.eIF2α (A/A)) = 6 animals, n(CamK2α.eIF2α/eIF2α (A/+)) = 4 animals and n(CamK2α.eIF2α/eIF2α (A/A)) = 7 animals. **d** Freeze-frame motion traces for CamK2α WT mice treated with VEH, CamK2α.iPKR mice treated with ASV and CamK2α.eIF2α (A/A) mice. **e** In the cTC LTM test, homozygous CamK2α.eIF2α (A/A) mice displayed enhanced memory compared with CamK2α WT mice (**P < 0.01), whereas as shown previously, CamK2α iPKR mice treated with ASV have impaired memory (**P < 0.01). Heterozygous CamK2α.eIF2α (A/+), mice displayed comparable LTM as WT mice. Heterozygous CamK2α.iPKR eIF2α (A/A) mice treated with ASV exhibited comparable memory deficit as CamK2α iPKR mice treated with ASV. However, the memory deficit in CamK2α.iPKR mice was fully rescued by introducing nonphosphorylatable eIF2α in homozygous CamK2α.iPKR.eIF2α (A/A) mice (**P < 0.0001). n(CamK2α WT) = 7 animals, n(CamK2α.eIF2α (A/+)) = 7 animals, n(CamK2α.eIF2α (A/A)) = 10 animals, n(CamK2α.eIF2α/eIF2α (A/A)) = 5 animals and n(CamK2α+iPKR.eIF2α (A/A) + ASV) = 5 animals. Repeated-measures two-way ANOVA with Bonferroni’s post hoc test was performed. Genotype: f(5,35) = 12.79, P < 0.0001. **f** Bar graphs for mean cTC LTM for all groups. One-way ANOVA with Bonferroni’s post hoc test. f(5,36) = 23.30, P < 0.0001. n(CamK2α WT) = 6 animals, n(CamK2α.eIF2α (A/+)) = 8 animals, n(CamK2α.eIF2α (A/A)) = 9 animals, n(CamK2α.eIF2α/eIF2α (A/A) + ASV) = 8 animals, n(CamK2α.eIF2α/eIF2α (A/A) + ASV) = 5 animals and n(CamK2α+iPKR.eIF2α (A/A) + ASV) = 6 animals. **g** Memory enhancement in CamK2α.eIF2α (A/A) mice was accompanied by cognitive inflexibility at the offset of CS. During ITIs, CamK2α.eIF2α (A/A) freeze at a much greater rate compared with WT (**P < 0.0001) and CamK2α.eIF2α (A/A) mice (**P < 0.01). Similarly, CamK2α.iPKR.eIF2α (A/A) + ASV mice displayed a higher freezing rate during the ITIs compared with CamK2α.eIF2α (A/A) treated with ASV (**P < 0.05), n(CamK2α WT) = 6 animals, n(CamK2α.eIF2α (A/+)) = 10 animals, n(CamK2α.eIF2α (A/A)) = 10 animals, n(CamK2α.iPKR + ASV) = 5 animals, n(CamK2α.eIF2α (A/+)+ ASV) = 5 animals and n(CamK2α.iPKR.eIF2α (A/A)+ ASV) = 6 animals. One-way ANOVA with Bonferroni’s post hoc test. f(5,36) = 8.569, P < 0.0001, h, During pre-CS, locomotor activity was equivalent across groups. n(CamK2α WT) = 6 animals, n(CamK2α.eIF2α (A/+)) = 10 animals, n(CamK2α.eIF2α (A/A)) = 10 animals, n(CamK2α.eIF2α/eIF2α (A/A) + ASV) = 5 animals, n(CamK2α.eIF2α/eIF2α (A/A) + ASV) = 5 animals and n(CamK2α+iPKR.eIF2α (A/A) + ASV) = 6 animals. Repeated-measures two-way ANOVA was performed. f(5,26) = 1.335, not significant. Data are presented as mean ± s.e.m. **P < 0.05, ***P < 0.01, ****P < 0.001, *****P < 0.0001.**
translation inhibition from that of ATF4 expression on memory processes is not trivial because a pharmacological inhibitor of ATF4 does not exist; moreover, long-term knockdown of ATF4 causes deficits in synaptic plasticity and LTM\(^{40}\). Therefore, using the IPKR system, we achieve a spatiotemporally limited inhibition of general translation initiation that is likely accompanied by translationally regulated transcriptional inhibition of CREB-regulated genes. Notably, ATF4 also acts as a transcriptional inducer for Ppp1r15a (also known as Gadd34)\(^{40}\), whose gene product GADD34 is a key component of eIF2\(\alpha\) dephosphorylating complex GADD34–PP1. Thus, the increase in ATF4 constrains the temporal window for eIF2\(\alpha\) phosphorylation mediated by cPSSI, thereby enabling a temporally stringent control of translation initiation. To further ascertain the effect of blocking protein synthesis on memory processes, we devised an alternate chemogenetic strategy that does not lead to ATF4 increase by blocking cap-dependent translation initiation in LA CamK2\(\alpha\) neurons with a knock-in mouse line for expressing tet-dependent synthetic microRNA specific for eIF4E, and injected a cocktail of adeno-associated virus (AAV).CamK2\(\alpha\).Cre and AAV.DIO.tTA into the bilateral LA\(^{31}\) (Extended Data Fig. 5a) to achieve eIF4E knockdown. eIF4E is a component of the eIF4F complex that binds the 5’ m\(^7\)GTP cap found on the majority of cellular mRNAs, and the availability of free eIF4E is tightly regulated in mammalian cells by eIF4E-binding proteins such as 4E-BP and CYFIP1 (ref. \(^{16}\)). Knocking down eIF4E in LA CamK2\(\alpha\) neurons did not impair learning in the auditory threat conditioning paradigm (Extended Data Fig. 5b), but strongly impaired LTM (Extended Data Fig. 5c,d). Together, the LTM deficit observed using two independent approaches for blocking translation initiation in CamK2\(\alpha\).IPKR and CamK2\(\alpha\).4Ekd, respectively, strongly supports our hypothesis that de novo translation in LA CamK2\(\alpha\) neurons is necessary for consolidation of long-term threat memories.

**Bidirectional control of memory strength by phosphorylation of eIF2\(\alpha\) S51 in LA principal neurons.** Constitutive heterozygous phosphomutant eIF2\(\alpha\) S51A (A/+)) mice have enhanced LTM in several memory paradigms including contextual and auditory threat conditioning and conditioned taste aversion\(^{12}\), indicating that phosphorylation of eIF2\(\alpha\) S51 is a malleable constraint on
Notably, the memory enhancement in the CamK2α.eIF2α (A/A) mice came with a cost. We found that the dysregulated increase in translation tone in CamK2α.eIF2α (A/A) mice resulted in impaired behavioral flexibility during the tone offset in the LTM test. CamK2α.eIF2α (A/A) mice freeze starting at tone onset but continue to freeze after the tone offset during intertrial interval (ITI) epochs (Extended Data Fig. 6b and Fig. 5g). This failure to switch off defensive state is triggered only after the onset of first tone, whereas at preconditioned stimulus (pre-CS) the animals have normal motor behavior, indicating absence of generalized anxiety or contextual threat response (Fig. 5h). We next tested CamK2α.eIF2α (A/A) animals in open field arena and elevated plus maze, and found normal locomotion in open field (Extended Data Fig. 6c–e), but a decrease in the percentage of time spent in the open arm in the elevated plus maze, indicative of increased anxiety-like behavior (Extended Data Fig. 6f,g). These data suggest that a dysregulated increase in the translation program regulated by eIF2α leads to a failure in risk assessment in nontreating environments and epochs. These findings also are consistent with the anxiolytic effect of iPKR-mediated eIF2α phosphorylation on exploratory behavior in the open arm of elevated plus maze, and thus support the relevance of an eIF2α-dependent translation program in eliciting anxiety-related behaviors.

**Dysregulated protein synthesis in LA CamK2α+ neurons compromises the precision of a complex memory.** Finally, we addressed whether lost memories can be rescued with artificial reactivation of LA CamK2α+ principal neurons. Designer drug activated by designer drug (DREADD) hM3Dq-mediated neuronal activation engages the mitogen-activated protein kinase ERK1/2 and mTORC1 pathways that are positively associated with protein synthesis<sup>64,65</sup>. We injected a cocktail of AAV.CamK2α.Cre together with AAV.hSyn.DIO.hM3Dq.mCherry into the LA of iPKR knock-in mice (Fig. 6a). DREADD agonist C21 (ref. 46) significantly increased de novo translation in CamK2α+ neurons of CamK2α.iPKR hM3Dq mice compared with the vehicle (VEH)-treated group (Fig. 6b). We then trained CamK2α.eIF2α (A/A), CamK2α.hM3Dq and WT control mice in a differential threat conditioning paradigm that involves three interleaved presentations of a paired tone (CS+) and an unpaired tone (CS−) in a single session (Fig. 6c,d). Consistent with earlier data, posttraining ASV infusion impaired LTM and led to a significant decline in freezing response to paired tone (Fig. 6f,g). Forty-eight hours after LTM1 test, we retested the animals for LTM2 in a different context after chemogenetic activation of hM3Dq receptors in the CamK2α+ neurons using agonist C21. We found that although artificial reactivation of CamK2α+ neurons recovered CS+LTM, it led to stimulus generalization and resulted in generalized defensive freezing response to CS− (Fig. 6h) that reflected in a significant decline in threat discrimination index (Fig. 6i). CamK2α+ WT and CamK2α.hM3Dq mice exhibited no change in discrimination index between the two LTM tests. Besides stimulus generalization, freezing during ITI was also increased by artificially boosting protein synthesis in translation-inhibited CamK2α+ neurons (Extended Data Fig. 7a,b).

To further understand what happens when eIF2α-controlled general translation in LA principal neurons is boosted during consolidation of a complex memory, we explored differential threat conditioning in CamK2α.eIF2α S51A mice (Extended Data Fig. 7c). We found that whereas homozygous CamK2α.eIF2α (A/A) mice can discriminate between CS+ and CS− during LTM1, they have a significantly increased freezing response to CS−. In contrast,
CamK2α WT mice robustly discriminated CS+ from CS− and displayed negligible freezing responses to CS− (Extended Data Fig. 7d). The enhanced CS− response in the CamK2α.eIF2α (A/A) mice resulted in a poor discrimination index (Extended Data Fig. 7e). We next examined the freezing during ITI and found that similar to earlier results with simple threat conditioning, CamK2α.eIF2α (A/A) exhibited a significantly increased freezing response after tone offset, indicating behavioral inflexibility (Extended Data Fig. 7f,g). These findings indicate that the precision of a memory trace is contributed by the finely regulated translation program in LA CamK2α+ neurons during memory consolidation.

**Discussion**

Protein synthesis is metabolically expensive and thus is tightly regulated at the level of initiation, which has limited investigation of cell-autonomous protein synthesis in physiological processes, but until now an effective chemogenetic tool to block protein synthesis has been lacking. To address this issue, we have bioengineered a spatiotemporally precise chemogenetic resource for rapidly and reversibly blocking cell-autonomous protein synthesis via phosphorylation of eIF2α. Phosphorylation of eIF2α is a tightly regulated molecular event that acts as a master effector of the integrated stress response. Using our chemogenetic iPKR mouse resource, we made several notable discoveries. First, temporally structured pan-neuronal protein synthesis is required for LTM consolidation. Recent LTM examined 24 h after training is most sensitive to protein synthesis disruption in the first hour after training. Second, blocking de novo translation in CamK2α+ principal neurons within the LA disrupts LTM consolidation. This block of memory consolidation can be rescued either by using the eIF2B activator ISRIB or...
by dephosphorylating both alleles of eIF2α. Third, expressing a biallelic phosphomutant eIF2α in CamK2α+ principal neurons results in enhanced strength of the memory, but introduces behavioral inflexibility and a generalized defensive response for an unpaired or safe tone. Fourth, artificial reactivation of LA CamK2α+ neurons 24 h after protein synthesis inhibition recovers lost memory for the paired tone but causes stimulus generalization.

Protein synthesis dependence during memory consolidation. We found that too little or too much phosphorylation of eIF2α causes aberrant memory storage and expression. A parsimonious interpretation of our data is that state-dependent modulation of eIF2α phosphorylation is critical for a finely tuned translation program supporting the associative memory. eIF2α phosphorylation itself is a homeostatic process wherein the phosphorylation event triggers the synthesis of the eIF2α dephosphorylating enzyme GADD34, bringing the system back to a lower state of eIF2α phosphorylation. Our data are consistent with Batista et al.11, showing that phosphorylation of eIF2α causes a block of general translation. In contrast, Jhang et al.12 reported that chemogenomic dimerization of FK506-binding protein (FKBP)-PKR (iPKR) induced phosphorylation of eIF2α by 1.5-fold without blocking general translation; thus, they attributed the memory deficit in iPKR-expressing animals to ATF4 expression. It is possible that because these investigators injected the drug inducer of iPKR (AP20187) 2 h before radioisotope S35 methionine injection intraperitoneally, the sensitive time window for protein synthesis disruption was missed during labeling. Compared with iPKR, PKRk is more effective in suppressing translation because it not only directly phosphorylates eIF2α, but it also interacts with and phosphorylates endogenous full-length PKR, further boosting eIF2α S51 phosphorylation10. In our system, central infusion of ASV resulted in a 2-fold increase in p-eIF2α, which resulted in a robust 50% decrease of general translation as assessed with in vivo SUNSET that involved local infusion of puromycin directly into the LA. Our convergent data from a parallel strategy of cell-autonomous protein synthesis inhibition in LA CamK2α+ neurons using a synthetic microRNA against eIF4E provides further support for the requirement of de novo protein synthesis in LTM consolidation. Nonetheless, we also observed an increase in ATF4 levels after phosphorylation of eIF2α and cannot exclude the possibility that the memory deficit in the iPKR transgenic mice is in part due to upregulated translation of transcripts containing uORFs, such as ATF4, and the ensuing repression of CREB-regulated genes.

Memory generalization and behavioral inflexibility as a result of dysregulated translation. Several studies have used immediate early gene-based engramp cell targeting approaches to interrogate the nature of memory formation and recall. Artificial reactivation of conditioned threat engramp cells in the LA using optogenetics has been shown to recover memories previously lost by protein synthesis inhibition using anisomycin8,9,10. Our chemogenetic iPKR mouse resource is not amenable for the engramp cell targeting approach because the engramp cells by definition need to be tagged during the training event for iPKR expression, a process dependent on de novo transcription and translation. Nonetheless, our results show that artificial reactivation of previously translation-inhibited CamK2α+ neurons in the LA results in memory recovery, but at the cost of stimulus generalization. We propose that learning-induced somatic and synaptic protein synthesis functions to stabilize the connections between the pathway-specific afferents from conditioned tone processing brain regions (auditory cortex and auditory thalamus) to amygdalar engramp cells in such a way that the conditioned tone can access and activate the downstream neuronal network in the LA during recall. This natural cue-elicted neuronal reactivation is completely recapitulated by artificial reactivation of LA neurons, such that there is imprecise restoration of the memory, perhaps because of a lack of reinsertion of synapse specificity of the original memory trace. Phosphomutant eIF2α mice, with a rigid increase in translation in LA principal neurons, also exhibit stimulus generalization and behavioral inflexibility at tone offset. Taken together, our data indicate that a finely tuned translation program in the amygdala is required to coordinate the stability and precision of the LTM trace.

Model of protein synthesis regulation during LTM consolidation. Based on our data, we propose the following cellular/molecular model of protein synthesis regulation in the LA during long-term consolidation. CTC leads to a coordinated increase in protein synthesis in CamK2α+ neurons in the soma and in local subcellular compartments, such as dendritic spines and axons. This results in robust memory strength and precision (Extended Data Fig. 8a). Chemogenetic inhibition of protein synthesis using iPKR blocks general translation in LA CamK2α+ neurons, resulting in memory loss (Extended Data Fig. 8b). Expression of biallelic phosphomutant eIF2α in LA CamK2α+ neurons, although insensitive to iPKR-mediated eIF2α phosphorylation, results in an aberrant increase in basal translation. This leads to robust memory strength, but low memory precision (Extended Data Fig. 8c). Artificial chemogenetic reactivation of CamK2α+ neurons following inhibition of protein synthesis leads to an increase in protein synthesis but does not recapitulate learning-induced local translation, thus causing memory generalization. This is manifested as normalized memory strength and low memory precision (Extended Data Fig. 8d).

We have used multipronged chemogenetic approaches to investigate the translational control of long-term threat memories. Future studies will be required to elucidate how protein synthesis regulation occurs in genetically defined and functionally coherent cell types within the memory network during LTM consolidation.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-019-0568-z.

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Methods

Cloning of recombinant plasmids. iPKR was produced by adding the NSSA/B junction sequence (DEEMEECAFLPY) within the PPKR. N53/4 protease, iPKR and GFP were amplified using Phusion polymerase (NEB) and cloned into pEG-F (Invitrogen) using appropriate restriction enzymes. All constructs were verified by sequencing. Purifications were done using a PCR extraction kit (Qiagen). The ligations were performed with Quick Ligate kit (NEB), and the products were transformed into chemically competent TOP10 cells (Invitrogen) that were grown in Luria–Bertani medium containing 55 mg/ml kanamycin (Sigma). The mammalian expression constructs were subcloned into the gene-targeting plasmids as previously reported. The final construct was extracted with phenol/chloroform:isoamyl alcohol (25:24:1), precipitated in 70% ethanol and dissolved in Tris–EDTA buffer.

Cell culture and transfection. 293FT cells were grown in a 24-well plate in growth medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) to have 90% confluency on the day of transfection. Transfection was carried out with 1 μg of a reference plasmid using 2 μl 293fectin reagent (Invitrogen). The amounts of the remaining plasmids were adjusted to have the same molarity per well. Cells were lysed on ice in phosphate-buffered saline supplied with 1% Triton X-100 (Sigma) and inhibitors against proteases (78437; Pierce), phosphatases (78420; Pierce) and translation machinery (100 μg/ml cyclineximide; Sigma); the cytosolic extracts were isolated after precipitating the insoluble fraction. The protein concentrations were measured using bicinchoninic acid protein assay (Pierce).

Generation of iPKR knock-in animals. 129S6/SvEvTac embryonic stem cells from the W4 parental cell line were used for homologous recombination at Eef1a1 genomic locus. Gene targeting, generation of knock-in mice and Southern blotting of the W4 parental cell line were used for homologous recombination at Eef1a1 (Pierce).

Gene targeting, generation of knock-in mice and Southern blotting were conducted by the Gene Targeting Facility at The Rockefeller University (New York, NY, USA), as previously described. The following primers were used for genotyping: β-actin forward, 5′-GCC TGT ATT CTC CAT CG-3′ and β-actin reverse, 5′-CCA GTT GGT AAC AAT GCC ATG T-3′; eGFP forward, 5′-CA GAA GAA CGG CAT CAA GTG-3′ and eGFP reverse, 5′-ACG AAC TCC AGC AGG ACC ATG-3′; iPKR forward, 5′-CAC GTG CAG CAA GCC CCA CCA GCA TCT GTA TG-3′ and iPKR reverse, 5′-TCC TGG CCC TGC AGT ACC TCA TGC TGC ACG-3′.

Animals. Mice were provided with food and water ad libitum and were maintained in a 12–12 light–dark cycle at New York University at stable temperature (78°F) and humidity (40–50%). All mice were backcrossed to the C57Bl/6 strain for at least five generations. Nestin Cre transgenic mice (stock no. 003771) were obtained from Jackson Laboratory as previously described. Nestin Cre mice were bred to the floxed iPKR knock-in mouse line to generate transheterozygote Nes.iPKR mice. Transgenic homozygous Eif2α (S51A); CAG PezKedGFP (mouse that is, eIF2α (A/A)) were a gift from Dr Randal Kaufman. Double-transgenic floxed iPKR/eIF2α mice, respectively. Control WT littermates were obtained by breeding floxed iPKR mice with eIF2α (A/A) mice, respectively. Control WT littermates were injected with AA V9.Camk2α.Cre.eGFP and AA V9.DIO.Tg1 in the LA. For eIF4E knockdown experiments, 100 nl each of AA V1.Camk2α.Cre and AA V9.CAG.DIO.Tg1 (1 × 1011 GCs per ml; Vigene) was injected into the LA of control WT littermates with 100 nl each of AA V1.Camk2α.Cre and AA V9.CAG.DIO.Tg1 (1 × 1011 GCs per ml; Vigene) (Penn Vector Core). For artificial reactivation studies involving CamK2α/iPKR hM3Diq mouse, a mixture of 100 nl each of AA V1.Camk2α.Cre and AA V9.CAG.DIO.Tg1 was injected into the LA of the iPKR mice. Double-transgenic floxed iPKR/eIF2α (A/A) mice, respectively. Control WT littermates were injected with AA V9.Camk2α.Cre.eGFP.

Electrophysiology. The electrophysiology experiments were performed as previously described, and mice were between 2 and 4 months of age at the time of the experiments. In brief, transverse slices (300 μm) containing the amygdala were isolated and transferred to recording chambers (preheated to 32°C). For each experiment, the brains were perfused with oxygenated aCSF (125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 2.5 mM NaHCO₃, 25 mM d-glucose, 2 mM CaCl₂, and 1 mM MgCl₂) at least 1 hr before recordings began at a rate of 2 ml/min. Most of the experiments, picrotoxin (75 μM; Tocris) was present in the perfusion solution. For bath application, the drugs were made and stored as concentrated stock solutions and diluted 1:100-fold when applied to the perfusate. EPSPs from the internal capsule pathway were recorded using microelectrodes filled with aCSF (resistance 1–4 MΩ). A bipolar Teflon-coated platinum electrode was placed in the thalamic afferent fiber to the LA, which is located in the ventral part of the striatum just above the central nucleus of the amygdala. The test stimuli for basal synaptic response were at 0.05 Hz. In all experiments, basal EPSPs were stable for at least 20 min before the start of each experiment. L-LTP was induced with three 1-s 100-Hz HFS trains, with an intertrain interval of 60 s. After induction of L-LTP, we collected EPSPs for an additional 120 min. A total of 5 μM ASV or VEH was bath applied 10 min before L-LTP induction and lasted for all of the recording. Slope values were compared from the Nex.iPKR transgenic mice, and their control littermates were treated with either ASV or VEH. Synthetic efficacy was monitored at 0.05 Hz and averaged every 2 min. EPSPs were amplified and digitized using the A-M Systems Model 1800 and Digidata 1440 (Molecular Devices).

Behavior. All behavior sessions were conducted during the light cycle. Mice were randomly assigned for experimental conditions including drug or VEH infusions and for the order of testing in any given experimental paradigm. All behavior data were collected by experimenters blind to the genotype and experimental conditions.

Open field activity. Mice were placed in the center of an open field (27.31 × 27.31 × 20.32 cm³) for 15 min during which a computer-operated open field system (Activity monitor system 6.02; Med Anim) was used to detect the spontaneous movement of the animals as they explored the arena. The parameters tested were distance traveled and the ratio of center to total distance at three epochs.

Elevated plus maze. The plus maze consisted of two open arms (30 × 5 cm²) and two enclosed arms of the same size with 14-cm-high sidewalls and an endwall. The arms extended from a common central square (5×5 cm²) perpendicular to each other, making the shape of a plus sign. The entire plus maze apparatus was elevated to a height of 30 cm. Testing began by placing an animal on the central platform.
conditioning chambers housed inside sound attenuated cubicles (Coulbourn (Noldus) was used to record the time spent on open arms and closed arms, total the maze was wiped with 30% ethanol in between trials. Ethovision XT13 software
floor and white house light. For simple threat conditioning, mice were placed in the
50 mM NaF, 1 mM EDTA, 1 mM EGTA, 10 mM Na4P2O7, 1% Triton X-100, and sonicated in ice-cold homogenization buffer (10 mM HEPES, 150 mM NaCl, 0.1% SDS and 10% glycerol) that was freshly supplemented with 10
VT1200S vibratome (Leica). Amygdala was microdissected from the brain slices
sample buffer (0.25 M homogenization buffer. Protein concentrations were measured using bicinchoninic
- tubulin (T8328, 1:5,000; Sigma) and mouse
anti-puromycin (MABE343, 1:1,000; Millipore), rabbit anti-Gadd34 (10449-1-AP, (5364, 1:500; Cell Signaling), mouse anti-S6 (2317, 1:500; Cell Signaling), mouse
(N31, 1:500; Sigma) and l-cysteine-free DMEM media (Invitrogen). Equal amounts of protein from
imaging data were acquired using an SP8 confocal microscope (Leica) with 10× and 20× objective lenses with Leica LAAX software and analyzed with ImageJ 2.0.0 using the Bio-Formats importer plug-in. To quantify the p-eIF2α S51 and puromycin signal intensity of each GFP-immunoreactive cell, we collected z stacks (10 optical sections with 0.563-μm step size) for three cortical sections per mouse (n = 3 mice) with 20× objective with 2x zoom. All compared samples were processed using the same protocol, and images were taken with equal microscope settings. Images were analyzed using ImageJ software. To compare across groups, we normalized all measures to the average intensity of the control group.

Statistics. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications.51,52. No animals were excluded from the data analyses. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software). Data are expressed as mean ± standard error of the mean (±SEM). Data distribution was assumed to be normal, but this was not formally tested. Data from two groups were compared using two-tailed unpaired Student’s t-test. Multiple group comparisons were conducted using one-way analysis of variance (ANOVA) or repeated-measures two-way ANOVA, with post hoc tests as described in the appropriate figure legend. Statistical analysis was performed with an α level of 0.05. The P values <0.05 were considered significant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Sequence information for the targeting vector used to generate the iPKR knock-in mouse line is provided in Supplementary Data 1. Further data that support the findings of this study are available from the corresponding authors upon reasonable request.

References
51. Trinh, M. A. et al. The eIF2α kinase PERK limits the expression of hippocampal recombinant glutathione receptor dependent long-term depression. Learn. Mem. 21, 298–304 (2014).
52. Santini, E. et al. Exaggerated translation causes synaptic and behavioural aberrations associated with autism. Nature 493, 411–416 (2013).

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Andrews) for the 2A plasmid, A. Klinakis (Biomedical Research Foundation Academy of Athens), A. Domingos and J. Friedman (The Rockefeller University) for the plasmid containing the STOP sequence and the Eef1a1 targeting plasmid, P. Vandenabeele (Ghent University) for the PKR plasmid, R. Kaufman (Sanford Burnham Prebys Medical Discovery Institute) for the Eif2sa1 (S51A); CAG Prltd4kDIO.A.GFP mouse line and J. Pelletier (McGill University) for the Col1a1TRE GFP.shmir4E.389 mouse line. We thank the Allen Brain Institute for providing AAV.CAG Pr.DIO.tTA. We thank all members of the Klann laboratory for critical feedback and discussions. This study was supported by National Institute of Health Grants (nos. NS034007 and NS047384) to E.K., a Howard Hughes Medical Investigator grant to N.H. and a Brain and Behavior Research Foundation NARSAD Young Investigator grant to P.S.

Author contributions
P.S., P.A. and N.H. conceptualized the iPKR system. P.S. and E.K. created the conceptual design of all in vivo work. P.A. designed and characterized the iPKR system in vitro and generated the iPKR mouse model under the supervision of N.H. P.S. carried out behavior training, and collected and analyzed in vivo and ex vivo data. P.H.-V. carried out behavior training. F.L. carried out and analyzed slice electrophysiology. A.G. helped with mouse behavior training. J.E.L. provided critical advice on behavioral design. P.S. and E.K. wrote the paper. All authors read and commented on the paper.

Competing interests
The authors declare no competing interests.

Additional information
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Supplementary information is available for this paper at https://doi.org/10.1038/s41593-019-0568-z.
Correspondence and requests for materials should be addressed to N.H. or E.K.
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Extended Data Fig. 1 | Characterization of iPKR system. a) The toxicity of NS3/4 inhibitor ASV was determined by central i.c.v. administration of varying doses of the drug. Representative Western blots (top) shows the levels of cFos, phospho-eIF2α, total eIF2α, and control β-Actin band in response to the administration of ASV at the doses: 0, 10 nM, 100 nM (1 μM in 2 μl saline). Bar graph with individual data points shows quantification of cFOS (left) and phospho/total eIF2α (right) normalized to β-Actin. n=3 independent Western blots, 3 mice per group. One-way ANOVA. b) Schematic of the engineering approach for chemogenetic protein synthesis inhibitor plasmid construct consisting of NS3/4 protease, eGFP and iPKR kinase domain separated by 2A ribosome skipping sites under CMV promoter. Control plasmids harbored iPKR without NS3/4 protease or unmodified PKR kinase domain (PKRk). c) Metabolic S35 labeling of de novo translation in vitro showed significantly decreased translation in the presence of PKRk and iPKR (**p<0.01) but the translation block was lifted by the co-expression of NS3/4 protease that degrades iPKR (**)p<0.01). n=2 biological replicate lysates per group; One way ANOVA followed by Bonferroni’s post-hoc test. F(5,6)=19.01, **p=0.0013. d) iPKR expression is correspondingly regulated by NS3/4 protease (**p<0.01), whereas unmodified PKRk levels were unaltered by NS3/4 protease. n=2 per group; One way ANOVA followed by Bonferroni’s post-hoc test. F(5,6)=22.21, ***p=0.0008. Data are presented as ± SEM.
Extended Data Fig. 2 | Generation of iPKR mouse. a) Schematic showing the subcloning and targeting strategy of the multicistronic cassette containing loxP-flanked STOP cassette, NS3/4 protease, EGFP-L10, and iPKR kinase domain, which were separated by 2A ribosome skipping sites. The entire cassette was inserted between exon1 and exon 2 of Eef1a1 genomic locus in mouse eS cells. Recognition site for the Southern blot probe is indicated. b) Southern blot after BamHI restriction enzyme-digested DNA isolated from embryonic stem cells using the probe indicated in a). Modified (6.2 kB kb) and unmodified (10.3 kB) DNA bands are indicated with arrows. In Nes iPKR brains, EGFP-L10 is expressed in the soma of neurons in the anterior cingulate cortex (c), somatosensory cortex (d), CA1 (e), CA3 (f) and dentate gyrus (g) consistent with NeuN expression. Insets show the corresponding brain areas at higher magnification.
Extended Data Fig. 3 | Nes.iPKR mice display normal locomotor and anxiety related behavior. a) Nes.iPKR mice acclimated to the novel environment equivalent to the wildtype and exhibited c) normal locomotor activity in the open field test. d) Nes.iPKR animals displayed normal thigmotaxis as assessed by % distance traveled in the center compared to total distance. n = 5–7 per group: RM One-way ANOVA for (a) and Unpaired t-test for (c) and (d).
Extended Data Fig. 4 | General translation inhibition in CamK2α principal neurons. Blocking protein synthesis in CamK2α principal neurons in LA did not affect acclimation to a novel environment (a), total locomotor activity (b) or thigmotaxis, assessed by % distance traveled in center compared to total distance (c). d) In the elevated plus maze, however, animals with protein synthesis blocked in CamK2α principal neurons exhibited reduced anxiety i.e. increased %open arm duration (*p<0.05) compared to vehicle treated CamK2α/IPKR mice and CamK2α wildtype mice even though they make equivalent entries to the open arm (e). n = 4-5 per group. RM Two-way ANOVA for a), One-way ANOVA followed by Bonferroni’s post-hoc test for (b), (c), (d) and (e).
Extended Data Fig. 5 | Blocking cap-dependent translation in LA CamK2α principal neurons. a) Alternate strategy of blocking translation in CamK2α principal neurons in LA using cre-tet regulated synthetic micro-RNA targeted against eIF4E. Col1a1.TRE.GFP.shmir4E mice were bilaterally injected in the lateral amygdala with AAV1.CamK2α.Cre and AAV9.DIO.tTA, and placed off dox diet for 10 days before training. b) eIF4E protein level was significantly decreased in GFP+ neurons that express shmir4E. Two-way ANOVA with Bonferroni’s post-hoc test. Genotype X GFP interaction: F(1,311) = 32.29, ****p < 0.0001; GFP: F(1,311) = 45.32, ****p < 0.0001. c) CamK2α 4Ekd mice learned the association between CS and US during training. RM Two-way ANOVA with Bonferroni’s post-hoc test. CS: F(2,14) = 17.54, ***p = 0.0002. d) Cued LTM was severely impaired across all three CS presentations. n=8 per group; RM Two-way ANOVA with Bonferroni’s post-hoc test. F(10,20) = 15.65, **p = 0.0027. d) Mean cTC LTM was significantly impaired in CamK2α 4Ekd mice compared to wildtype (**p < 0.001). n = 8 per group; Unpaired t-test. Data are presented as ± SEM.
Extended Data Fig. 6 | Characterization of mice expressing phosphomutant eIF2α in LA CamK2α principal neurons. a) eIF2α phosphorylation at S51 was significantly reduced in GFP+ neurons in CamK2α.eIF2α(A/A) mice compared to GFP− neurons, as well as GFP+ neurons in CamK2α.WT mice (**p < 0.001). (n = 60–74 per group, 3 animals); One-way ANOVA with Bonferroni’s post-hoc test. F (2, 154) = 1055, ****p < 0.0001. b) Representative motion traces from the open field test for CamK2α.WT, CamK2α.eIF2α(A/+) and CamK2α.eIF2α(A/A) mice. c) In the open field test, CamK2α.eIF2α (A/A) mice acclimated to the novel environment and had comparable spontaneous locomotion compared to the CamK2α.WT mice and CamK2α.eIF2α (A/+) mice. RM One-way ANOVA. d) Bar graphs representing thigmotaxis, i.e. %time spent in center compared to total distance traversed in the open field arena for the three groups. One-way ANOVA. e) In the elevated plus maze, CamK2α.eIF2α(A/A) mice spent a significantly higher duration in the open arm compared to CamK2α.WT mice (g) (*p < 0.05) indicating anxiety like behavior, even though they made equivalent entries to the open arm. One-way ANOVA with Bonferroni’s post-hoc test. F(2,17) = 3.775, *p=0.0440. Data are presented as +/- SEM.
Extended Data Fig. 7 | Artificial chemogenetic activation of LA CamK2α principal neurons. a) All groups of mice - CamK2α, iPKR hM3Dq, CamK2α, hM3Dq and CamK2α WT, exhibited low freezing during ITI in LTM1. XY plots showing %freezing during individual ITIs and Post-CS (left; RM Two-way ANOVA) and bar graphs showing mean %freezing during ITI (right; One-way ANOVA). n = 6–9 per group. b) During LTM2, administration of DREADD agonist C21 caused an increase in freezing during ITI for both CamK2α hM3Dq and CamK2α iPKR hM3Dq groups compared to CamK2α WT mice. XY plots showing %freezing during individual ITIs and Post-CS (left). n = 5–7 per group. RM Two-way ANOVA genotype: F(2,15) = 12.63, ***p < 0.0006. Bar graphs showing mean %freezing during ITI (right). One-way ANOVA with Bonferroni’s post-hoc test. *p < 0.05 and **p < 0.01. c) CamK2α eIF2α (A/A) mice displayed comparable learning in the differential threat conditioning training for both CS+ (right) and CS− (left). d) However, in the LTM test, they displayed significant increase in CS− response compared to CamK2α WT mice (**p < 0.01). Two-way ANOVA with Bonferroni’s post-hoc test. CS: F(1,28) = 49.18, ****p < 0.0001; Genotype: F(1,28) = 15.26, ***p = 0.0005. e) The cTD discrimination index was significantly lower for CamK2α eIF2α (A/A) mice (**p < 0.01) relative to controls. n=7-10 per group; Unpaired t-test. f) Besides stimulus generalization, CamK2α eIF2α (A/A) mice also displayed cognitive inflexibility and could not stop freezing after the tone offset, and thus had significantly higher freezing rate during the ITIs. RM Two-way ANOVA with Bonferroni’s post-hoc test. Genotype: F(1,10) = 16.70, **p = 0.0022. g) Mean freezing response during ITI is significantly increased in CamK2α eIF2α (A/A) mice (**p = 0.0097). n=7-10 per group; Unpaired t-test. Data are presented as mean +/- SEM.
Extended Data Fig. 8 | Model for protein synthesis regulation during long-term memory consolidation. **a** In wild-type mice, threat conditioning leads to a spatiotemporally regulated increase in somatic and dendritic protein synthesis that stabilizes the memory trace. **b** Application of ciPSI system prevents the coordinated increase in cell-wide translation leading to impaired LTM. **c** Dephosphorylation of eIF2α enhances general translation, but it is dysregulated and unable to coordinate the cell-wide translation program to store a complex memory trace, resulting in memory generalization. **d** Artificial reactivation of the amygdala principal neurons after protein synthesis inhibition-mediated amnesia leads to an increase in translation but does not restore synapse specificity and thus leads to memory generalization.
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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- **n/a**
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- □ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- □ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- □ The statistical test(s) used AND whether they are one- or two-sided
- □ Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- □ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
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- □ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- □ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- □ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

- Data collection: FreezeFrame 4, Activity Monitor 6.02, Ethovision XT13, Leica LASX, Protein Simple - Alpha Imager 3.4
- Data analysis: We used GraphPad Prism 8 to analyze behavior data. We used ImageJ 2.0.0-rc-69/1.52p to analyze confocal images and Western Immunoblots.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All data generated or analyzed during this study are included in this article and supplementary files.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size were estimated based on existing published research. |
|-------------|------------------------------------------------------------------|
| Data exclusions | None |
| Replication | Each experiment represents several independent cohorts, as described in the methods. |
| Randomization | Mice were randomly allocated to experimental groups. |
| Blinding | Data was analyzed by researchers blind to genotypes/ experimental manipulations. |

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq |
| ☑   | Flow cytometry |
| ☑   | MRI-based neuroimaging |

### Antibodies

**Antibodies used**

- **Biotin** (abcam ab3394, 1:500), **p-eIF2alpha** (Cell Signaling 9721, 1:300), **p-eIF2alpha Ser51** (Cell Signaling 3398, 1:300), **t-eIF2alpha** (Cell Signaling 9722, 1:500), **PKR** (Cell Signaling 3072, 1:300), **ATF4** (Santa Cruz sc-200, 1:300), **p-ERK1/2 Thr202/Tyr204** (Cell Signaling 9101, 1:1000), **ERK1/2** (Cell Signaling 9102, 1:1000), **p-S6 (Ser240/244)** (Cell Signaling 2317, 1:500), **t-S6** (Cell Signaling 2317, 1:500), **Pumonicin** (Millipore MABE343, 1:1000), **beta-Tubulin** (Sigma-Aldrich T8328, 1:5000), **beta-Actin** (Sigma-Aldrich A5316, 1:5000), **cFos** (Santa Cruz sc-253, 1:300), **GFP** (Abcam ab13970, Thermo Fisher G10362, 1:300), **mCherry** (Abcam ab205402, 1:500), **Gadd34** (Proteintech #10449-1-AP, 1:1000)

**Validation**

- **Biotin**: synthetic peptide against KLH (biotin), website states specific to free biotin and biotinylated antibody and proteins. Primary literature citing use of this antibody: Stypulkowski, E et al. Sci Signal 2018. PMID: 29295957
- **p-eIF2alpha Ser51**: synthetic phosphopeptide corresponding to residues surrounding Ser51 of eIF2alpha. website states the antibody detects endogenous eIF2alpha only when phosphorylated at Ser51 and does not recognize eIF2alpha phosphorylated at other sites. Primary literature citing use of this antibody: Jiang et al. Nat Comm 2016. PMID: 27416896
- **t-eIF2alpha**: synthetic peptide against carboxy terminal sequence of eIF2alpha. websites states the antibody is specific to total eIF2alpha protein; Primary literature citing use of this antibody: Jiang et al. Nat Comm 2016. PMID: 27416896
- **PKR**: synthetic peptide against PKR carboxy terminal (aa 500-600), website states antibody was knockout validated and does not cross-react with other eIF2alpha kinases. Primary literature citing use of this antibody: Ikegami et al. PLoS Pathogens 2009. PMID: 19197359
- **ATF4**: synthetic peptide against C terminus of CREB-2 (ATF4), website states antibody was knockout validated and is specific to ATF4 protein. Primary literature citing use of this antibody: Lin et al. Mol Neurobiol 2015. PMID: 24915969
- **p-ERK1/2 Thr202/Tyr204**: synthetic phosphopeptide corresponding to residues surrounding Thr202/Tyr204 of p44 ERK1 protein. website states antibody is specific for endogenous levels of p44 and p42 MAP Kinase (ERK1 and ERK2) when phosphorylated either individually or dually at Thr202 and Tyr204 of ERK1 (Thr185 and Tyr187 of ERK2), and does not cross-react with the non-phosphorylated ERK1/2. Primary literature citing use of this antibody: Beirowski et al. PNAS 2017. PMID: 28484008
- **p-S6 (Ser240/244)**: monoclonal antibody generated using a synthetic phosphopeptide corresponding to residues surrounding Ser240 and Ser244 of human ribosomal protein S6. Primary literature citing use of this antibody: Beirowski et al. PNAS 2017. PMID: 28484008
| t-S6: mouse monoclonal antibody generated using a recombinant fusion protein corresponding to full-length human S6 ribosomal protein. Primary literature citing use of this antibody: Beirowski et al. PNAS 2017. PMID: 28484008 |
| Puromycin: monoclonal antibody generated using synthetic peptide against puromycin from Streptomyces alboniger. website states antibody detects puromycin-incorporated neosynthesized proteins treated with puromycin only and has been evaluated by western blotting in HEK293 cell lysates treated with puromycin. Primary literature citing use of this antibody: Primary literature citing use of this antibody: Beirowski et al. PNAS 2017. PMID: 28484008 |
| beta-Tubulin: monoclonal antibody derived from the hybridoma AA2 produced by fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with purified bovine tubulin. website states antibody reacts with beta-tubulin, types I, II, III and IV. Primary literature citing use of this antibody: Santini et al. Science Signal 2017. PMID: 29114037 |
| beta-actin: monoclonal antibody derived from hybridoma AC-74 produced by fusion of mouse myeloma cells and splenocytes from mice immunized with synthetic beta-cytoplasmic actin N-terminal peptide, conjugated to biotin (KLH). website states antibody is specific for beta-actin. Primary literature citing use of this antibody: Yu et a. Nat Comm 2015. PMID: 25984442 |
| cFos: polyclonal antibody generated using epitope mapped within an internal region of c-Fos of human origin. Primary literature citing use of this antibody: Yu, H et al. J Neuroscience 29(13): 4056-64. PMID:19339601 |
| Chicken anti-GFP: synthetic full length protein corresponding to GFP. website states antibody was validated using western blot and transgenic animals. Primary literature citing use of this antibody: Doyle et al. Cell 2008. PMID: 19013282; Rabbit anti-GFP: Monoclonal antibody generated using full length GFP. Viswanathan S et al. Nat Methods 2015. PMID: 25915120 |
| mCherry: synthetic full length protein corresponding to mCherry. website states antibody was validated using western blot and transgenic mice. Primary literature citing use of this antibody: Moreno et al. Curr Biol 2019. PMID: 30554899 |
| Gadd34: Knock-out validated polyclonal antibody, generated in rabbits using GADD34 fusion protein Ag0578. Primary literature citing use of this antibody: Dalton et al. Br J Cancer 2013. PMID: 23412101 |

### Eukaryotic cell lines

**Policy information about cell lines**

| Cell line source(s) |
|---------------------|
| 293T cells from ATCC, 129S6/SvEvTac embryonic stem cells |

| Authentication |
|----------------|
| 293T cells from ATCC: website states that routine cell line authentication is carried out with short tandem repeat profiling, cellular morphology, karyotyping and cytochrome C oxidase I assay testing. 129S6/SvEvTac embryonic stem cells: These cells were generated, and routinely authenticated with karyotyping and immunostaining for pluripotency markers by the Rockefeller University Gene targeting center. |

| Mycoplasma contamination |
|--------------------------|
| Cell lines were not contaminated with mycoplasma. |

| Commonly misidentified lines (See ICLAC register) |
|--------------------------------------------------|
| None |

### Animals and other organisms

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| Laboratory animals |
|--------------------|
| Mus musculus, iPKR mice 5x backcrossed to C57Bl6J, male and female, 10-15 weeks old |

| Wild animals |
|--------------|
| None |

| Field-collected samples |
|-------------------------|
| None |

| Ethics oversight |
|------------------|
| University Animal Welfare Committee and Institutional Biosafety Committee approved and provided guidance on the study protocol. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.