Correction of eIF2-dependent defects in brain protein synthesis, synaptic plasticity, and memory in mouse models of Alzheimer’s disease

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Neuronal protein synthesis is essential for long-term memory consolidation, and its dysregulation is implicated in various neurodegenerative disorders, including Alzheimer’s disease (AD). Cellular stress triggers the activation of protein kinases that converge on the phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which attenuates mRNA translation. This translational inhibition is one aspect of the integrated stress response (ISR). We found that postmortem brain tissue from AD patients showed increased phosphorylation of eIF2α and reduced abundance of eIF2B, another key component of the translation initiation complex. Systemic administration of the small-molecule compound ISRIB (which blocks the ISR downstream of phosphorylated eIF2α) rescued protein synthesis in the hippocampus, measures of synaptic plasticity, and performance on memory-associated behavior tests in wild-type mice cotreated with salubrinal (which inhibits translation by inducing eIF2α phosphorylation) and in both β-amyloid-treated and transgenic AD model mice. Thus, attenuating the ISR downstream of phosphorylated eIF2α may restore hippocampal protein synthesis and delay cognitive decline in AD patients.

INTRODUCTION
Alzheimer’s disease (AD) is the most prevalent form of dementia, affecting more than 35 million people worldwide. Pathological hallmarks of AD include brain accumulation of the amyloid-β peptide (Aβ) and of neurofibrillary tangles composed of hyperphosphorylated tau protein, neuroinflammation, synapse failure/loss, and neurodegeneration, ultimately culminating in memory failure (1). Despite major progress in the elucidation of mechanisms of pathogenesis in recent years, AD is still a disease in urgent need of effective therapeutics capable of preventing and/or blocking progressive cognitive deterioration. Given the complex nature of AD, identification of molecular pathways and targets that effectively improve cognition has been challenging.

De novo protein synthesis plays a key role in synaptic plasticity and long-term memory consolidation (2–7). Initiation of mRNA translation involves the formation of a ternary complex comprising methionyl transfer RNA (Met-tRNA), guanosine 5′-triphosphate (GTP), and the eukaryotic initiation factor 2 (eIF2) complex. Activity of eIF2B, a guanine exchange factor (GEF), allows continuous ribosome assembly and mRNA translation (8). This step is tightly regulated by phosphorylation of eIF2α on its α subunit (eIF2α-P), which inhibits the GEF activity of eIF2B and attenuates global translation (9, 10).

Various cellular stress stimuli trigger activation of eIF2α kinases, causing phosphorylation of eIF2α in a process termed the integrated stress response (ISR). The ISR attenuates global protein synthesis while favoring the translation of a specific subset of mRNAs that help to restore cellular homeostasis (11). Increased translation of activating transcription factor 4 [ATF4; also known as adenosine 3′,5′-cyclic monophosphate response element-binding protein 2 (CREB-2)] is thought to be a central mediator of the ISR (12).

Mounting evidence implicates aberrant ISR and eIF2α-P in brain disorders, including traumatic brain injury, prion disease, Down syndrome, and AD (13–23). ISR markers are increased in AD patient brains (16, 24, 25) as well as in the brains of mouse models of AD (15, 16, 26). We previously demonstrated that either genetic ablation or inhibition of eIF2α kinases rescues synapse function and memory defects in AD mouse models (15, 16). We thus hypothesized that rescuing brain protein synthesis downstream of eIF2α-P might be an attractive approach to correct cognitive impairment in AD.

Here, we report that AD brains exhibit increased eIF2α-P levels accompanied by reductions in eIF2B subunits. The ISR inhibitor, ISRIB, a small-molecule compound that stimulates eIF2B activity even in the presence of increased eIF2α-P (27–29), rescued reduced hippocampal protein synthesis and corrected impaired synaptic plasticity and memory in AD model mice. Our findings suggest that targeting dysregulated brain translational control by eIF2α may represent an effective approach to combat cognitive failure in AD.

RESULTS
Brain tissues from AD patients show increased eIF2α-P and reduced eIF2B subunit levels
We first confirmed that cortical extracts from brain tissue obtained postmortem from AD patients (demographics in table S1) displayed increased abundance of eIF2α-P relative to that in control samples (Fig. 1A; full blots in fig. S6 and full analysis in data file S1).

In another set of experiments, C57BL/6 mice were given daily injections of salubrinal (1 mg/kg, intraperitoneally, for five consecutive days; Fig. 2B), an inhibitor of eIF2α dephosphorylation. Long-term memory was then assessed using the novel object recognition (NOR) and contextual fear conditioning (CFC) tasks. Treatment with salubrinal impaired long-term memory in both NOR (Fig. 2C) and CFC (Fig. 2D) tests. Systemic treatment of mice with ISRIB (0.25 mg/kg, intraperitoneally) prevented memory impairments induced by salubrinal in both NOR and CFC tests (Fig. 2C and D). ISRIB did not prevent the increase in hippocampal eIF2α-P caused by salubrinal administration in mice (Fig. 2E) but blocked the accumulation of ATF4, a central mediator of the ISR (Fig. 2F). These findings are consistent with the notion that ISRIB acts downstream of eIF2α-P to restore memory, and indicate that systemically administered ISRIB reaches the brain and prevents memory impairments induced by the accumulation of eIF2α-P.

**ISRIB prevents eIF2α-P-mediated impairment in long-term memory in an acute model of AD**

We next determined whether ISRIB could counteract the activation of ISR and memory deficits that are induced by β-amyloid oligomers (AβOs), toxins that accumulate in AD brains and cause eIF2α-P-mediated synapse and memory failure (1, 15, 30).

To investigate this possibility, C57BL/6 mice received a single intracerebroventricular infusion of AβOs [10 pmol, expressed as Aβ monomers; (15, 31, 32)] followed by administration of ISRIB (0.25 mg/kg, daily, intraperitoneally) for the duration of the experiment (Fig. 3A). Because ISRIB has been reported to trigger pro-mnemonic effects (27), we did not inject ISRIB between training and test sessions of memory tasks. Intracerebroventricular infusion of AβOs induced hippocampal eIF2α-P (Fig. 3B) and increased ATF4 levels (Fig. 3C). Treatment with ISRIB counteracted the increase in hippocampal ATF4 protein levels (but not mRNA; Fig. 3C and Fig. S1A) without affecting eIF2α-P levels (Fig. 3B).

We then hypothesized that ISRIB could correct translation defects induced by AβOs. To test this hypothesis, we exposed ex vivo mouse hippocampal slices to AβOs (1 μM) in the absence or presence of ISRIB (0.2 μM) and assessed de novo protein synthesis using SUnSET (33). We found that ISRIB prevented hippocampal translational repression induced by AβOs (Fig. 3D). Similar results were obtained in primary neuronal cultures using BONCAT (bio-orthogonal non-canonical amino acid tagging) (Fig. 3E) (34).

We further found that dendritic spine density was reduced in hippocampal CA1 in mice that received an intracerebroventricular infusion of AβOs and that systemic treatment with ISRIB restored spine density in AβO-infused mice (Fig. 3F). Intracerebroventricular infusion of

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**Fig. 1. AD brains display increased eIF2α-P and reduced levels of eIF2B subunits.** (A to E) Western blotting for translation initiation factors eIF2α-P (A; ratio of variance test F = 3.125), total eIF2α (B; F = 1.319), eIF2γ (C; F = 1.547), eIF2βα (D; F = 1.375), and eIF2βε (E; F = 2.823) in cortical tissue from AD subjects and age-matched controls. N = 7 to 8 cases in each group. eIF2α-P was normalized to total eIF2α, eIF2Bα (D; F = 1.547), and assessed by eIF2α-P and reduced levels of eIF2B subunits. Because mRNA translation is central to long-term memory consolidation, these findings suggest that rescuing brain eIF2-mediated translation could be a target to treat memory failure in AD.

**ISRIB prevents eIF2α-P–induced impairment of long-term memory in mice**

We performed a proof-of-concept study to determine whether the ISR inhibitor, ISRIB, could prevent memory impairment caused by brain accumulation of eIF2α-P. ISRIB has been shown to bind to and stabilize the eIF2 complex in its active form, thereby bypassing eIF2α-P to stimulate the free eIF2B pool (29). We first determined whether systemic (intraperitoneal) administration of ISRIB resulted in its accumulation in the mouse brain. To this end, we administered ISRIB (0.25 mg/kg, intraperitoneally) to mice for six consecutive days and measured ISRIB concentrations in plasma and brain extracts by mass spectrometry (MS). Results showed that ISRIB could be detected in both plasma and brain at 4 and 24 hours after drug administration (Fig. 2A).
AβOs caused reductions in hippocampal synaptophysin, postsynaptic density protein 95 (PSD-95), and brain-derived neurotrophic factor (BDNF), and treatment with ISRIB had no effect on these proteins (fig. S1, B to E).

We next tested whether systemic treatment with ISRIB could prevent AβO-induced cognitive impairment. We found that ISRIB prevented AβO-induced long-term memory failure in both NOR (Fig. 3G) and CFC (Fig. 3H) tasks. Control measurements showed no differences in locomotor or exploratory activities in mice that received an AβO infusion and/or were treated with ISRIB (fig. S2, A to C) and no differences in the training phase in either NOR or CFC tests (fig. S2, D and E). These findings indicate that ISRIB corrects AβO-instigated eIF2α-P–dependent hippocampal translational repression, attenuates dendritic spine loss, and rescues memory impairments in mice.

**ISRIB restores synapse function and memory in a transgenic mouse model of AD**

Next, we investigated whether ISR inhibition could reverse deficits in synaptic plasticity and memory in 10- to 13-month-old APPswe/PS1ΔE9 mice, a transgenic mouse model of AD characterized by age-dependent brain accumulation of Aβ (35). We initially investigated the effect of ISRIB on long-term potentiation (LTP) in hippocampal slices from APPswe/PS1ΔE9 mice. Whereas slices from APPswe/PS1ΔE9 mice failed to maintain LTP at CA1 Schäffer collateral synapses after tetanic stimulation, treatment with ISRIB restored LTP (Fig. 4, A and B). We further found that ISRIB restored spine density in pyramidal neurons from hippocampal CA1 region of APPswe/PS1ΔE9 mice (Fig. 4C).

To determine the effect of ISRIB on long-term spatial memory, we treated APPswe/PS1ΔE9 mice [or wild-type (WT) littermates] with ISRIB (0.25 mg/kg, intraperitoneally) daily for 2 weeks before testing in the Morris water maze (MWM). This protocol was found to alleviate the ISR in the hippocampus, as assessed by total levels of GADD34 (fig. S3). ISRIB improved learning in APPswe/PS1ΔE9 mice, as indicated by faster learning curves compared to saline-treated APPswe/PS1ΔE9 mice (Fig. 4D), but did not improve memory retention assessed in the MWM probe trial (Fig. 4E). However, ISRIB improved long-term contextual memory of APPswe/PS1ΔE9 mice in the CFC test (Fig. 4F). Control experiments showed that APPswe/PS1ΔE9 (or WT) mice treated with ISRIB had no changes in locomotor or exploratory activities, and their weights were similar to control animals (fig. S4, A to D). Thus, treatment with ISRIB restores synaptic plasticity and memory in APPswe/PS1ΔE9 mice.

We proceeded to determine whether treatment with ISRIB affected amyloid deposition in the brains of APPswe/PS1ΔE9 mice. We found that ISRIB-treated APPswe/PS1ΔE9 mice had reduced mean amyloid plaque size (Fig. 4, G to I) but increased plaque density in the hippocampal formation (Fig. 4, J and K). Total Aβ42 in the hippocampus (Fig. 4L) and cortex (Fig. 4M) was unchanged by treatment. We hypothesized that the reduced mean plaque size in APPswe/PS1ΔE9 mice treated with ISRIB could be due to altered plaque phagocytosis by glial cells. APPswePS1ΔE9 mice exhibited increased hippocampal immunoreactivities for Iba-1 (microglial marker) and glial fibrillary acidic protein (GFAP) (astrocytic marker) compared to WT mice (fig. S5). Treatment with ISRIB had no effect on either Iba-1 (fig. S5, A to C) or GFAP (fig. S5, D to F) immunoreactivities in the hippocampi of APPswe/PS1ΔE9 mice. Collectively, our findings indicate that ISRIB attenuates translational repression and restores synaptic plasticity and memory independently of amyloid burden in AD models.
DISCUSSION

Activation of the ISR and attenuation of brain protein synthesis have been implicated in memory deficits in AD (15, 16, 24–26) and in other neurodegenerative diseases (13–23), supporting the notion that correcting defective brain protein synthesis might comprise an effective therapeutic target in AD. Because three of four known eIF2α kinases have been shown to play pathogenic roles in AD (15, 16, 24, 25, 36, 37), identifying approaches that act downstream of eIF2α-P may comprise a more viable strategy to alleviate cognitive impairment than simultaneously targeting individual kinases. Here, we show that treatment with ISRIB, a small-molecule compound that targets ISR downstream of eIF2α, restores synaptic plasticity and long-term memory defects in AD mouse models.

ISRIB has been shown to improve symptoms in rodent models of traumatic brain injury, vanishing white matter disease, Down syndrome, superoxide dismutase 1 (SOD1)–linked amyotrophic lateral sclerosis (38), and prion disease (18, 19, 22, 23, 39). However, two previous studies have reported no beneficial effects of ISRIB in AD model mice (40). We note that, in contrast to our approach, the previous studies treated AD model mice with a single, high-dose, intraperitoneal injection of ISRIB (2.5 to 5 mg/kg). Briggs et al. (40) further reported that prolonged treatment of Tg2576 mice with

Fig. 3. ISRIB prevents memory impairments, dendritic spine loss, and defective hippocampal protein synthesis induced by AβOs. (A) Experimental timeline of treatments, behavioral tests, and brain tissue collection in C57BL/6 mice to assess the effect of ISRIB on memory in the presence of AβOs. (B and C) Western blotting analysis for eIF2α-P (B) and ATF4 (C) in the hippocampi of mice that received an intracerebroventricular (i.c.v) infusion of AβOs (or vehicle) and were treated with ISRIB (0.25 mg/kg, intraperitoneally) or saline for 12 days. N = 8 to 9 (B) and 10 to 12 (C) mice per group. n.s., not significant. (D) Analysis of protein synthesis by a SUnSET assay in mouse hippocampal slices exposed to AβOs (1 μM) in the absence or presence of ISRIB (0.2 μM) for 3 hours, with the addition of puromycin during the last 30 min. N = 15 to 18 slices from a total of 15 mice per experimental condition. Representative images shown had brightness linearly adjusted for clearer visualization. (E) Analysis of the abundance of newly synthesized proteins, detected by BONCAT assay (labeling with AHA followed by Click chemistry for biotinylation of AHA-containing polypeptides; see Materials and Methods) followed by streptavidin-conjugated resin pulldown and Western blotting, in primary hippocampal cultures exposed to AβOs (0.5 μM) in the absence or presence of ISRIB (0.2 μM) for 3 hours. Symbols represent experiments with independent hippocampal cultures and independent AβO preparations; N = 3 independent primary cultures. (F) Dendritic spine density in apical dendrites of neurons from the CA1 region of the hippocampus from mice treated daily with ISRIB (0.25 mg/kg, intraperitoneally) or saline, as described in (A). Each symbol corresponds to the mean of three independent 20-μm segments per neuron, five neurons per mouse, four to five mice per group. (G and H) Discrimination index in the NOR test (G; F = 5.468) and freezing time in the CFC test (H; F interaction = 5.683) in mice treated and tested as described in (A). Data are from n = 14 to 17 (G) and 11 to 13 (H) mice per group; * P < 0.05 and ** P < 0.01, two-way ANOVA followed by Dunnett's post hoc test for all experiments, except those in (D) and (E), which were analyzed by one-way ANOVA followed by Dunnett's post hoc test. Each dot represents an individual mouse.


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ISRIB (5 mg/kg) led to increased mortality, which differs from our approach to treat mice with a low-dose of ISRIB (0.25 mg/kg) for several days, which resulted in its detection in the plasma and brains of mice [as shown by liquid chromatography–MS/MS (LC-MS/MS)]. Using this dosing regimen, we demonstrate beneficial effects of ISRIB on synapse function and cognitive performance in two different mouse models of AD. These results are in line with a recent report showing that subchronic administration of ISRIB successfully restored spine density and electrophysiological properties of aged mice (41).

ISRIB was originally described as a memory-enhancing compound (27), and its mechanism of action has now been extensively investigated. ISRIB binds to a regulatory site and stabilizes eIF2B, stimulating its GEF activity independently of eIF2α-P (29), thus favoring...
translational derepression. To the best of our knowledge, no off-target effect of ISRIB has been reported.

Our ISRIB dosing regimen (low dose for several days) effectively restored eIF2α-dependent translational control in AD mice, without noticeable biochemical or cognitive effects in WT control mice. Differences between our results and those of Sidrauski et al. (27) may be due to the fact that, in their case, a single and higher dose of ISRIB (5 mg/kg) was administered within a critical window for memory consolidation, during which de novo protein synthesis is essential for the persistence of newly formed memories (42). This is in line with previous reports showing that ISRIB modifies the ISR under conditions of increased eIF2α-P, but not under basal conditions (43, 44).

We found that ISRIB had a mild effect on aberrant hippocampal ATF4 mRNA expression in AβO-infused mice. Although not a canonical ISR event, increased ATF4 mRNA expression has been described as a consequence of ISR under some circumstances (45). mRNA expression has been decreased in AD brains, consistent with a recent single-nucleus transcriptomic study showing that ATF4 expression is reduced in AD brains (22). This is in line with previous reports showing that ISRIB modifies the ISR under conditions of increased eIF2α-P, but not under basal conditions (43, 44).

increase eIF2B activity by using novel eIF2B activators (22) or repurposed compounds (49) may prove effective to target brain protein synthesis and ward off cognitive decline in AD.

MATERIALS AND METHODS

Ethics

All experiments with mice were carried out in accordance with the “Principles of Laboratory Animal Care” (U.S. National Institutes of Health) guidelines and performed under protocols approved and supervised by the Institutional Animal Care and Use Committees of the Federal University of Rio de Janeiro (protocol number IBQM039/16) and New York University (NYU) (protocol number 09-1332).

Postmortem human brain tissue

Brain samples (prefrontal cortex) from noncognitively impaired and AD patients (defined by neuropathological criteria) were obtained from the Emory University Brain Bank. Experimental procedures involving human tissue were in compliance with the NYU Institutional Review Board. Specimen information can be found in table S1. Postmortem interval (PMI) varied among donors, but average PMI was similar in AD versus control brains.

Animals

For the intracerebroventricular AβO-infusion model, we used 3-month-old male and female C57Bl/6 mice, equally divided among experimental groups. To assess any potential sex bias, we analyzed all groups divided by sex. Mice were obtained from the animal facility at the Federal University of Rio de Janeiro and were maintained on a 12-hour light/dark cycle with food and water ad libitum, with five mice per cage. Transgenic APPswePS1ΔE9 mice (B6/C3 background, The Jackson Laboratory #34829) and WT littermates were obtained from the NYU animal facility. Male and female mice (10 to 13 months old) were used, and results were analyzed grouping both sexes together. Mice (four to five per cage) were kept on a 12-hour light/dark cycle with food and water ad libitum. For every experiment, mice were pseudo-randomized into the different experimental groups, and allocation of animals of the same cage in one experimental condition was avoided.

Reagents

ISRIB (>98% purity) was from Sigma–Aldrich. Aβ1–42 was purchased from California Peptide. Culture media and infrared (IR) dye–conjugated secondary antibodies were from Li-Cor. Streptavidin–horseradish peroxidase (HRP), streptavidin–AF594, ProLong anti-fade reagent, tris-glycine gels, and Aβ42 enzyme-linked immunosorbent assay (ELISA) kits were from Invitrogen. Antibody to puromycin (12D10) was from EMD Millipore. Antibodies to ATF4, GFAP, eIF2Bα, eIF2Bε, eIF2γ, and β-actin were from Abcam. BDNF ELISA kits were from Abcam. Antibodies to eIF2α-P(Ser51), eIF2α, and β-tubulin were from Cell Signaling Technology. 6E10 antibody was from Enzo Life Sciences. Biotin–conjugated antibody to Iba-1 was from Wako Fujifilm. Bicinchoninic acid (BCA) protein assay kit was from Thermo Fisher Scientific. Laemmli buffer (4×) was from Bio-Rad. Alkyne-conjugated biotin and Protein Reaction Buffer kit were from Abcam. BDNF ELISA kits were from Abcam. BDNF ELISA kits were from Abcam. BDNF ELISA kits were from Abcam. BDNF ELISA kits were from Abcam. BDNF ELISA kits were from Abcam.
Pregnant mice were euthanized on the 19th day of gestation, and primary neuronal cultures were used within 48 hours of preparation.

Reverse transcription polymerase chain reaction
Total RNA was extracted from cultures using the SV Total RNA Isolation System (Promega), following the manufacturer’s instructions. RNA concentration and purity were determined by absorption at 260 and 280 nm, respectively. For quantitative reverse transcription polymerase chain reaction (qRT-PCR), 1 μg of total RNA was used for complementary DNA (cDNA) synthesis using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Quantitative expression analysis of targets was performed on a 7500 Fast Real-Time PCR system (Thermo Fisher Scientific) with the Power SYBR Green PCR Master Mix. β-Actin was used as an endogenous reference gene for data normalization. qRT-PCR was performed in 15-μl reaction volumes. Primer sequences used for Atf4 and Actb amplification were as follows: Atf4, CCACCATGGGCTT ATTAGAGG (forward) and CTGGATCGAAGAATGTGCT (reverse); Actb, GTGAG- GTTGACATC CCGTAAA (forward) and GTACTTGGCCTCAG- GAGGAG (reverse). Cycle threshold (CT) values were used to calculate fold changes in gene expression using the 2^-ΔΔCT method (52).

Enzyme-linked immunosorbent assay
Tissue samples were homogenized in 100 mM tris, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, and 1% Triton X-100, supplemented with protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). ELISAs for BDNF and Aβ42 were performed according to the manufacturer's instructions.

Bio-orthogonal noncanonical amino acid tagging
BONCAT was used for de novo protein synthesis assessment (53). Primary neuronal cultures had their medium replaced by methionine-free RPMI supplemented with 2% B27, 1% Glutamax, and 2% BSA. Cells were incubated for 30 min to allow for methionine starvation, and azidohomoalanine (AHA), a methionine analog, was added at 1 mM final concentration to label newly synthesized proteins. AβOs (0.5 μM) and/or ISRIB (0.2 μM) were added, and cells were incubated for 2 hours at 37°C. Cellular content was harvested using RIPA buffer (Pierce) supplemented with Halt protase/phosphatase inhibitor cocktail. Protein levels were determined using the BCA method (Thermo Pierce). AHA-conjugated proteins were biotinylated using the Protein Reaction Buffer Kit (Thermo Fisher Scientific), following the manufacturer’s instructions. Newly synthesized proteins were precipitated using streptavidin-conjugated resin and detected by Western blotting using streptavidin-conjugated HRP (1:2000).

SUnSET
Hippocampal slices were prepared as previously described (16). Briefly, 400-μm hippocampal slices were obtained using a chopper and recovered in artificial cerebrospinal fluid (ACSF) for 1 hour. Slices were then exposed to 1 μM AβOs (or vehicle) in the absence or presence of 0.2 μM ISRIB for 3 hours. Puromycin was added to the media to a final concentration of 5 μg/ml during the last hour of the experiment. After incubation, slices were flash-frozen and processed for Western blotting. Puromycin incorporation was quantified as a measure of newly synthesized proteins, as previously described.
described (33). Puromycin, when used at low concentrations (as in our experiments), cannot compete with the aminoacyl-tRNA, rather binding stochastically to the C terminus of nascent peptides (33, 54–56). The representative image shown in Fig. 3J had its brightness level linearly increased solely for visualization purposes, but quantification of incorporated puromycin was performed in raw images without any manipulation.

**Animal treatments**

Intracerebroventricular infusions of AβOs were performed as described previously (15, 31, 32, 57). Mice were anesthetized briefly with 2% isoflurane, and AβOs were injected 1 mm to the right of the midline point equidistant of each eye and 1 mm posterior to a line drawn through the anterior base of the eyes. Mice were then placed back in their cages.

Mice were injected daily intraperitoneally with ISRIB (0.25 mg/kg) or vehicle (200 μl per injection). Treatment regimen is described in each section of the results and followed a protocol known to have a beneficial effect in a murine model of prion disease and previously established in the literature (18), wherein Halliday et al. showed that substantial amounts of ISRIB reached the brain and persisted for at least 24 hours. For salubrinal experiments, mice were concomitantly injected daily with ISRIB (0.25 mg/kg) and salubrinal (1 mg/kg). Results obtained in this study were used as a template for subsequent studies using AD mouse models.

**Behavioral analyses**

Long-term memory was assessed using the NOR, CFC, and MWM tests, as described below. Locomotor activity was assessed in an open-field arena (OFA). In all behavioral experiments, the experimenter was blinded to the groups tested. Animals that had biased exploration during training in the NOR (100% exploration of a specific object during training phase) were excluded from analysis. In the MWM, mice that did not swim during trials were excluded from both the learning analysis and the probe trial. Last, in the CFC task, mice with unaltered behavior after being shocked were excluded from the analysis.

**Novel object recognition**

Tests were carried out using a NOR box (30 cm by 30 cm by 50 cm). In the training phase, mice were exposed to two identical objects, which they were allowed to freely explore for 5 min. Time spent exploring each object was recorded, and 24 hours later, in the test phase, one of the objects was replaced by a novel object. Mice again were exposed to the objects for 5 min, and the total exploration time of both old (familiar) and new (novel) objects was determined. Discrimination index was determined by \((T_{\text{novel}} - T_{\text{familiar}})/(T_{\text{novel}} + T_{\text{familiar}})\). After the task was completed, mice were placed in a different arena for 5 min, where total distance, average velocity, and total time spent at the periphery were recorded and determined using ANALY-maze software (Stoelting Co.).

**Contextual fear conditioning**

To assess contextual fear memory, a two-phase protocol was used, as described (15, 58) with minor modifications. In the training phase, mice were presented to the conditioning cage (40 cm by 25 cm by 30 cm), which they were allowed to freely explore for 2 min, followed by application of a single foot shock (0.35 mA) for 2 s. Mice were kept for another 30 s in the cage and removed. On the next day, mice were presented to the same cage for 5 min without receiving a foot shock. Freezing behavior was recorded automatically using the Freezing software (Panlab) and was used as a memory index. For APPswe/PS1ΔE9 mice, because of the extensive memory impairment in these mice, protocol included 2 min of free exploration, followed by two foot shocks (0.8 mA), 2 s each, spaced by 30 s. Mice were then kept for another 2 min in the cage and removed.

**Morris water maze**

The water maze test was carried out as previously described (16, 59). Briefly, three training sessions per day were carried out for 5 days, except in the first day, when mice were trained in four sessions. Time to reach the platform was recorded as a measure of learning capacity. Average velocity and total distance traveled in the pool were recorded as control locomotor parameters. Probe trials were performed for 1 min 24 hours after the last training session. Time spent in the target quadrant was recorded as a memory output. Recording was performed using EthoVision software.

**Open-field arena**

Locomotor activity in APPswePS1ΔE9 mice was assessed using OFA (Med Associates Inc.). Mice were allowed to freely explore the box for 15 min, during which their movement was recorded using Activity MDB software (Med Associates Inc.). Total distance traveled, average velocity, and total time spent at the periphery were recorded.

**Golgi staining**

Mice were euthanized, and whole brains were carefully harvested, rinsed in phosphate-buffered saline (PBS), and prepared for Golgi staining using the FD Rapid Golgi Kit (FD Neurotechnologies) following the manufacturer’s instructions. Briefly, brains were immersed in a mixture containing solutions A and B (1:1) for 14 days protected from light, with gentle swirling twice a week. Brains were then incubated in solution C for 72 hours before sectioning. After staining, brains were sliced on a Leica vibratome in 200-μm-thick sections and developed for 5 min in solutions D and E, followed by dehydration. Slices were mounted on gelatin-coated slides and imaged on a Nikon Eclipse TE2000-U microscope under bright-field illumination. For dendritic spine quantification, three dendrite segments of 10 to 20 μm that were at least 50 μm from the cell soma were selected per neuron; five neurons were analyzed per brain. Total dendritic spines in each segment were counted and normalized by total length of the dendritic segment. For image analysis, the experimenter was blinded to the conditions analyzed. For visualization purposes, representative images shown in Fig. 3E had their brightness and contrast linearly altered, but spine counting was performed in the absence of any image manipulation.

**Electrophysiology**

Acute 400-μm transverse hippocampal slices from WT or APPswe/PS1ΔE9 mice were prepared using a Leica VT1200 S vibratome as described previously (60). Slices were maintained at room temperature for at least 2 hours in ACSF containing 118 mM NaCl, 3.5 mM KCl, 2.5 mM CaCl₂, 1.3 mM MgSO₄, 1.25 mM NaH₂PO₄, 5.0 mM NaHCO₃, and 15 mM glucose, bubbled with 95% O₂/5% CO₂. For electrophysiology, monophasic, constant-current stimuli (100 μs) were delivered with a bipolar silver electrode placed in the stratum radiatum of area CA3. Field excitatory postsynaptic potentials (fEPSPs) were recorded using a glass microelectrode from the stratum radiatum.
of area CA1. Late-LTP (L-LTP) was induced using high-frequency stimulation consisting of two 1-s 100-Hz trains separated by 60 s, each delivered at 70 to 80% of the intensity that evoked spkEPSPs. Slices were perfused with ISRIB (0.2 μM in ACSF) or vehicle during the whole recording process.

Immunohistochemistry
WT and APPsw/PS1E9 treated with ISRIB (as described above) were anesthetized with a ketamine/xylazine mixture. Mice were first perfused with PBS for 45 s and then with 4% paraformaldehyde (PFA) for 90 s. Brains were carefully removed from the skull and post-fixed in 4% PFA for 48 hours at 4°C, under agitation. Brains were blocked on 3% agarose and sectioned using a VT1200 S vibratome (Leica Biosystems) to 40-μm sections. Sections were kept at 4°C until use. For Aβ (6E10), Iba-1, and GFAP staining, free-floating sections were initially permeabilized using 0.5% Triton X-100 solution for 15 min. Sections were then blocked for 1 hour with 5% normal goat serum in PBS containing 0.1% Triton X-100 and incubated overnight with 6E10 (mouse, 1:200) and anti-GFAP (chicken, 1:1000) solution. Sections were washed three times using PBS containing 0.1% Triton X-100 and incubated with anti-rabbit and anti-chicken secondary antibodies (1:500) for 90 min. Sections were then washed three times with PBS containing 0.1% Triton X-100 and subsequently incubated overnight with biotin-conjugated anti-Iba-1 (rabbit, 1:500). Sections were washed three times using 0.1% Triton X-100 and incubated with Alexa Fluor 594–conjugated streptavidin (1:500) and anti-α-syn (chicken, 1:1000) solution. Sections were washed three times using PBS containing 0.1% Triton X-100 and mounted using ProLong with DAPI (4′,6-diamidino-2-phenylindole). Imaging was performed on a Leica SP8 confocal microscope. Images were acquired and analyzed using identical parameters across the experiment. Total fluorescence was determined by generating a mask in Fiji. The mask was used to determine the threshold separating signal and noise. Total pixel intensity (determined by the “raw integrated density” variable) was then obtained using the whole image as region of interest (ROI). For glial content surrounding plaques, 20× magnification images were used. A region containing the plaque and an ~30-μm radius surrounding area was used as ROI. Total Iba-1 or GFAP fluorescence was determined as above. For image analysis, the experimenter was blinded to the conditions.

Statistical analysis
Pilot experiments were performed to allow calculations of sample and effect sizes by power analysis. G Power software (Düsseldorf University) was used for power analysis calculations after determination of the statistical analysis that would be used to analyze each experiment. Statistical analyses were performed using GraphPad Prism 6 software. Of the two independent groups were analyzed using Student’s t tests, whereas comparison of three or more independent experimental groups was performed with analysis of variance (ANOVA) followed by appropriate post hoc tests, as stated in the figure legends and shown for all analyses in data file S1.

SUPPLEMENTARY MATERIALS

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Correction of eIF2-dependent defects in brain protein synthesis, synaptic plasticity, and memory in mouse models of Alzheimer's disease

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Restoring protein synthesis and cognition

The loss of protein synthesis caused by chronic activation of the integrated stress response (ISR) is observed in hippocampal neurons and correlates with cognitive loss in mouse models of Alzheimer's disease. The compound ISRIB blocks the translation inhibition arm of the ISR but failed to confer cognitive benefit and caused mortality in mouse models of Alzheimer's disease in previous studies. However, Oliveira et al. found that daily systemic administration of low-dose ISRIB, which did not cause mortality or obvious side effects, rescued protein synthesis and synaptic plasticity in the hippocampus and restored performance on long-term memory tests both in wild-type mice in which translation had been pharmacologically inhibited and in two mouse models of Alzheimer's disease, although there was no effect on amyloid plaque load. The findings suggest that restoring protein synthesis in the brain may delay cognitive deficits in Alzheimer's disease patients.