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14. ABSTRACT

We hypothesize that excessive cap-dependent translation is a causative factor in autism spectrum disorder (ASD). To test this hypothesis, we have been studying transgenic mice that overexpress eIF4E have been testing the following specific aims: 1) to determine whether eIF4E transgenic mice display behaviors consistent with ASD, 2) to determine whether ASD-like behaviors displayed by eIF4E transgenic mice can be reversed by novel cap-dependent translation inhibitors, and 3) to determine whether eIF4E transgenic mice display cellular and molecular abnormalities due to excessive cap-dependent translation. mice. Our studies will provide information concerning whether overexpression of eIF4E is a *biological risk factor* for ASD. Our studies also will provide important information concerning the role of upregulated cap-dependent translation in ASD, and could link ASD mechanistically at the level of cap-dependent translational control to fragile X syndrome (FXS), tuberous sclerosis complex (TSC), and autistic patients with *PTEN* and *EIF4E* mutations. Moreover, the results of these studies would provide information for the design and use of compounds to *therapeutically target* eIF4E-eIF4G interactions and eIF4A for treating patients with ASD.

15. SUBJECT TERMS
autism spectrum disorder, cap-dependent translaiton, eIF4E, repetitive behaviors, perseverative behaviors, social behaviors

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Introduction

We hypothesize that excessive cap-dependent translation is a causative factor in autism spectrum disorder (ASD). To test this hypothesis, we have been studying transgenic mice that overexpress eIF4E have been testing the following specific aims: **1) to determine whether eIF4E transgenic mice display behaviors consistent with ASD, 2) to determine whether ASD-like behaviors displayed by eIF4E transgenic mice can be reversed by novel cap-dependent translation inhibitors, and 3) to determine whether eIF4E transgenic mice display cellular and molecular abnormalities due to excessive cap-dependent translation.** We have been determining whether eIF4E transgenic mice display behaviors consistent with ASD by examining their social behaviors, anxiety-related behaviors, perseverative/repetitive behaviors, and their responses to sensory stimuli. In previous studies we have used a novel compound termed 4EGI-1 that selectively disrupts interactions between translation factors eIF4E and eIF4G to inhibit translation in the brain. Therefore, we have been determining whether 4EGI-1 reverses ASD-like behaviors displayed by eIF4E transgenic mice. Moreover, because eIF4E/eIF4G interactions promote the helicase activity of eIF4A to stimulate cap-dependent translation, we will determine whether the eIF4A inhibitor hippuristanol can reverse ASD-like behaviors displayed by the eIF4E transgenic mice. Finally, we have been conducting studies to determine whether eIF4E transgenic mice have increased translation, altered neuronal morphology, and altered synaptic plasticity due to excessive cap-dependent translation. Our studies will provide information concerning whether overexpression of eIF4E is a ***biological risk factor*** for ASD. Our studies also will provide important information concerning the role of upregulated cap-dependent translation in ASD, and could link ASD mechanistically at the level of cap-dependent translational control to fragile X syndrome (FXS), tuberous sclerosis complex (TSC), and autistic patients with *PTEN* and *EIF4E* mutations. Moreover, the results of these studies would provide information for the design and use of compounds to ***therapeutically target*** eIF4E-eIF4G interactions and eIF4A for treating patients with ASD.

Body

Herein I will describe the research accomplishments associated with each task that was outlined in the approved Statement of Work.

The first task in the Statement of Work was to obtain regulatory approval for the use of mice by New York University (NYU) IACUC Committee and USAMRMC Office of Research Protections. The animal protocol was approved the NYU IACUC and Committee as well as the USAMRMC Office of Research Protections.

The second task in the Statement of Work was to determine whether the eIF4E transgenic mice exhibit behaviors consistent with ASD. This included subtask 1, which was to measure the social behaviors of the eIF4E transgenic mice and subtask 2, which was to measure anxiety-related behaviors, perseverative/ repetitive behaviors, and sensorimotor gating of the eIF4E transgenic mice. We have completed these studies, which are included in a manuscript that was published earlier this year in the prestigous journal *Nature* (Santini et al., 2013).

Perseverative and repetitive behaviors are one of the domains required for the diagnosis of ASD (Lewis et al., 2007). These behaviors include stereotypies (purposeless repetitive movements and activities) as well as cognitive inflexibility (inability to disengage from a previously learned behavior and adopt a new behavioral strategy). We employed a marble burying test to quantify repetitive digging behavior (Thomas et al., 2009) and found that the eIF4E transgenic mice buried significantly more marbles compared to their wild-type littermates (Figure 2a in Santini et al., 2013). We also examined self-grooming, which is a stereotypic repetitive behavior that has been observed in other mouse models of ASD (Peca et al., 2011; McFarlane et al., 2008) and discovered that the eIF4E transgenic mice

exhibited increased self-grooming (Figure 2b in Santini et al., 2013). We next evaluated whether eIF4E transgenic mice exhibited cognitive inflexibility by examining choice arm reversal in a water-based Y-maze task (Hoeffer et al., 2008). eIF4E transgenic mice showed intact learning abilities during the acquisition phase of the task and normal memory for the escape arm when tested 24 hours later. However, when the position of the escape arm was changed in the reversal phase of the task, eIF4E transgenic mice required significantly more trials to satisfy the same success criterion compared to their wild-type littermates (Figure 2c in Santini et al., 2013). Taken together, these experiments suggest that increased eIF4E expression and consequently, exaggerated cap-dependent protein synthesis, results in repetitive and perseverative behaviors.

Abnormalities in social interaction skills are another behavioral defect displayed by individuals with ASD (Rapin and Tuchman, 2008). Thus, we also tested social behavior in eIF4E transgenic mice with two well-established behavioral paradigms, the three-chamber arena and the reciprocal social interaction task (Moy et al., 2004). eIF4E transgenic mice exhibited reduced preference for a nonspecific stranger as indicated by an equal amount of time spent interacting with the mouse stranger and a novel object (Figure 2f in Santini et al., 2013). Similarly, the eIF4E transgenic mice displayed a reduced preference for the chamber where the stranger mouse was located (Figure 2g in Santini et al., 2013). Moreover, eIF4E transgenic mice also exhibited diminished reciprocal interactions with a freely moving stranger mouse (Figure 2h in Santini et al., 2013), further supporting deficits in social behavior. The deficits in social behavior of the eIF4E transgenic mice are unlikely to be caused by a generalized increased anxiety since the mice did not display anxiety-like traits when tested in other paradigms (i.e. center/total distance ratio in the open field test (Supplementary Figure 2c in Santini et al., 2013) and elevated plus maze (Supplementary Figure 2j in Santini et al., 2013). Moreover, the eIF4E transgenic mice exhibited a mild hyperactivity (first 10 min of novelty and open field tests (Supplemenatry Figure 2a and 2b in Santini et al., 2013) but no impairments in motor coordination, motor learning and sensorimotor gating abilities (Supplementary Figure 2i and 2k in Santini et al., 2013). All together, the behavioral analysis of the eIF4E transgenic mice indicates that increased cap-dependent protein synthesis in the brain results in a distinct pattern of behavioral abnormalities consistent with ASD.

The third task in the Statement of Work was to determine whether the ASD-like behaviors displayed eIF4E transgenic mice could be reversed by novel cap-dependent translation inhibitors. Subtask 1 was to determine whether ICV infusion of 4EGI-1 could reverse ASD-like behaviors exhibited by eIF4E transgenic mice. Subtask 2 was to determine whether ICV infusion of hippuristanol can reverse ASD-like behaviors by eIF4E transgenic mice.

We have largely completed the experiments in first subtask and these studies were published in the Nature manuscript (Santini et al., 2013). We employed a subthreshold dose of 4EGI-1 previously described in our laboratory (Hoeffer et al., 2011) to normalize the behavioral abnormalities in eIF4E transgenic mice without impairing their wild-type littermates. We infused either 4EGI-1 or vehicle directly into the lateral ventricle of cannulated eIF4E transgenic mice and their wild-type littermates. eIF4E transgenic mice treated with 4EGI-1 exhibited a decrease in repetitive behavior in the marble burying task starting on day four and persisted throughout day five, whereas the wild-type mice treated with 4EGI-1 behaved similarly to the vehicle-treated wild-type mice (Figure 4a in Santini et al., 2013). We then tested the ability of 4EGI-1 to correct the behavioral inflexibility displayed by eIF4E transgenic mice in the Y-maze. We found that blockade of eIF4E/eIF4G interactions with 4EGI-1 significantly improved the performance of eIF4E transgenic mice by decreasing the number of trials required to reach the success criterion in the reversal phase of the task (Figure 4b in Santini et al., 2013). These findings indicate that chronic treatment of eIF4E transgenic mice with 4EGI-1 reverses their repetitive and perseverative behaviors.

Finally, we determined whether infusions of 4EGI-1 also rescued the social behavior deficits displayed by the eIF4E transgenic mice. We found that eIF4E transgenic mice infused with 4EGI-1 for four days exhibited a preference for a non-specific stranger as indicated by an increased amount of

time spent in interacting with the stranger mouse over the novel object (Figure 4c in Santini et al., 2013). This result suggests that chronic treatment with 4EGI-1 also corrects social behavior deficits displayed by eIF4E transgenic mice.

We have just begun the second subtask to determine whether ICV infusion of hippuristanol can reverse ASD-like behaviors by eIF4E transgenic mice. We plan on finishing these experiments in the third year of funding.

The fourth task in the Statement of Work was to determine whether the eIF4E transgenic mice display cellular and molecular abnormalities due to excessive cap-dependent translation. Subtask 1 was to determine whether cap-dependent protein synthesis is increased in the brains of eIF4E transgenic mice. Indeed, we found that protein synthesis was elevated in the brains of the eIF4E transgenic mice (Figure 1d in Santini et al., 2013). Moreover, we found that ICV infusion of 4EGI-1 blocked the increase in protein synthesis in the prefrontal cortex of the eIF4E transgenic mice (Supplementary Figure 4f and 4g in Santini et al., 2013). Subtask 2 was to determine whether eIF4E transgenic mice exhibit altered dendritic spine morphology and if so, whether 4EGI-1 and hippuristanol reversed the alterations. We found that there was an increase in spine density in pyramidal neurons in layer 2/3 of the prefrontal cortex (Figure 3c and 3d in Santini et al., 2013). We have just begun to determine whether ICV infusion of 4EGI-1 and hippuristanol can reverse the increased spine density in the pyramidal neurons in layer 2/3 of the prefrontal cortex in the eIF4E transgenic mice. We will complete these experiments in the third year of funding. Subtask 3 was to determine whether eIF4E transgenic exhibit abnormal protein synthesis-dependent synaptic plasticity and if so, whether 4EGI-1 and hippuristanol reverse the abnormalities. We found that the eIF4E transgenic mice exhibited enhanced metabotropic glutamate receptor-dependent long-term depression (LTD) in the striatum and the hippocampus (Figure 3e and 3f in Santini et al., 2013). Moreover, we found that 4EGI-1 reversed the enhanced striatal LTD (Figure 3h in Santini et al., 2013). We are now determining whether hippuristanol can reverse the enhanced striatal LTD, and whether 4EGI-1 and hippuristanol can reverse the enhanced hippocampal LTD displayed by the eIF4E transgenic mice. We will complete these studies in the third year of funding.

The fifth task is data analysis and reporting. As evidenced in this report, we have been analyzing the data throughout the performance of the experiments. Final data analysis and summaries will be prepared for reporting at the end of the performance period in year three of funding.

Please note that a detailed description of the behavioral tests is provided in the Appendix.

Key Research Accomplishments

- Demonstration that eIF4E transgenic mice display repetitive and perseverative behaviors that are consistent with ASD.

- Demonstration that eIF4E transgenic mice display impaired social behaviors that are consistent with ASD.

- Demonstration that repetitive/perseverative behaviors and impaired social behaviors exhibited by eIF4E transgenic mice can be reversed by the 4EGI-1, which inhibits eIF4E-eIF4G interactions and cap-dependent translation.

- Demonstration that the eIF4E transgenic mice exhibit increased cap-dependent synthesis, altered dendritic spine morphology, and altered synaptic plasticity in the striatum and hippocampus.

- The key findings described above were published in the prestigious journal *Nature*.

Reportable Outcomes (Year 2, 1 August 2012 - 31 July 2013)

1) Invited speaker at Conference entitled "Frontiers in Stress and Cognition: From Molecules to Behavior", Ascono, Ticino, Switzerland

2) Invited seminar, Riken Brain Science Institute, Wako-shi, Japan

3) Invited seminar, Department of Neurochemistry, Tokyo University Graduate School of Medicine, Tokyo, Japan

4) Invited seminar, Biotechnology Research Institute and Molecular Neuroscience Center, Division of Life Sciences, The Hong Kong University of Science and Technology, Clearwater Bay, Kowloon, Hong Kong

5) Invited seminar, Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan

6) Invited seminar, Department of Biological Sciences, Seoul National University, Seoul, South Korea

7) Invited speaker, Symposium entitled "Recent Advances in Biosciences", Sponsored by Institute of Biomedical Sciences, Academia Sinica and College of Medicine, Tzu Chi University, Hualien, Taiwan

8) Invited seminar, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

9) Invited seminar, Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD

10) Invited speaker, The International Meeting for Autism Research, Symposium entitled "Reversibility", San Sebastian, Spain

11) Invited seminar, Department of Biological Sciences, Korea Advanced Institute for Science and Technology, Daejeon, South Korea

Conclusion

In the first two years of funding we have demonstrated that increased eIF4E expression and consequently, exaggerated cap-dependent protein synthesis results in the appearance of ASD-like behaviors in mice. We speculate that exaggerated cap-dependent protein synthesis, which is sufficient for the generation of synaptic alterations leading to ASD-like behaviors, results in enhanced translation of a specific subset of mRNAs. Importantly, our results also indicate that a pharmacological intervention that targets the formation of the eIF4F initiation complex (eIF4E+eIF4G+eIF4A) is sufficient to correct ASD-like endophenotypes displayed by the eIF4E transgenic mice. These experiments directly demonstrate that dysegulated translational control at the initiation phase of protein synthesis causes behavioral abnormalities in several domains consistent with ASD. Moreover, these results suggest that the eIF4F initiation complex is a viable therapeutic target for the treatment of individuals with ASD.

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Santini, E., Huynh, T.N., MacAskill, A.F., Carter, A.G., Pierre, P., Ruggero, D., Kaphzan, H., and Klann, E. (2013) Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature* **493:** 411-415.

Thomas, A. *et al.* Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology* **204**, 361–373 (2009).

Appendices

List of items in appendices:

1) Detailed description of behavioral paradigms.

2) Santini, E., Huynh, T.N., MacAskill, A.F., Carter, A.G., Pierre, P., Ruggero, D., Kaphzan, H., and Klann, E. (2013) Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature* **493:** 411-415.

Social Interaction Tests:

Social Approach: Mice will be placed in a three-chambered testing arena, where two areas are novel and the central area familiar to the mouse. One novel chamber will contain a caged stranger mouse (social target), the middle section the neutral starting location, and the other novel chamber identical to the first except that it does not contain a mouse (inanimate target). Time spent in each chamber will be measured and mice tested for propensity to approach and engage with a stranger mouse. ASD-like phenotypes would manifest as low social approach scores to the caged stranger mouse.

Social Novelty: This test will be conducted in the same testing apparatus as the social approach test, except that a familiar mouse will be added to the previously empty novelty chamber. In this case, preference for interaction between the familiar mouse and the stranger mouse will be tested. Under normal conditions, mice habituate to the conspecific mouse and rapidly move to investigate a novel conspecific mouse. Mice exhibiting ASD-like phenotypes may demonstrate the opposite behavior and spend more time with the familiar conspecific mouse.

Anxiety-related Behaviors:

Open Field Analysis: Mice normally remain low to the surface and near the edges of novel environments and venture to more exposed areas, i.e. the center of a space or upward by rearing, with less frequency. Measuring these parameters gives a reflection of the anxiety a mouse exhibits when exposed to a novel environment. Mice will be placed in a bright open testing arena, enclosed by transparent material. Mice will be placed in the center of the arena and allowed to explore the novel environment for 15 min. Mice with an ASD-related anxiety phenotype would be expected to spend less time in the center of the arena and show a reduced frequency to engage in rearing exploratory behaviors. Additionally, stereotypic (repetitive) movements can be captured by the tracking system used for this task, thereby allowing the measurement of this phenotype with general anxiety testing.

Elevated Plus Maze: The mice will be tested in a plus-shaped maze. Two arms are enclosed on the sides by non-transparent materials (closed) while two arms are completely exposed (open). Mice will be placed in the center of the maze and the time spent in each arm recorded. This test of anxiety and exploratory behavior (novelty) will measure the tendency of the mouse to leave the “protected” closed arms and venture into the exposed area in the open arms of the maze. ASD individuals generally display enhanced anxiety. If the eIF4E transgenic mice display phenotypes similar to those in ASD, then they would display reduced time in the open arms of the maze.

Repetitive/Perseverative Behaviors:

Marble Burying: Mice often “bury” or otherwise conceal glass marbles placed in their home cages. Burying behavior is thought to involve reward pathways associated with either anxiety relief or compulsiveness, and is considered an animal model of anxiety and obsessive-compulsive disorder (OCD), but also has been employed in the examination of ASD mouse models. Increased number of marbles buried by eIF4 transgenic mice might reflect enhanced anxiety or a tendency to exhibit repetitive behavior.

Arm Reversal in Y-Maze: Mice will be trained in a simple Y-water maze based escape task. Visual cues are located at the either arm of the Y-maze. Mice will be trained to locate a submerged escape platform in one arm denoted by a specific visual cue. This version of the Y-maze does not require food restriction to enforce appetitive acquisition. Then the location of the platform will be changed. The ability of the mice to change their arm choice will be measured. In this way, resistance to change (perseveration) can be observed by measuring the latency to choose the new arm compared to the original escape location. This task will permit us to more specifically separate perseverance phenotypes from phenotypes derived from search strategies. This task models the propensity for ritualistic and repetitive behaviors exhibited by ASD-afflicted individuals. Mice with an ASD-like phenotype would be expected to display an increase in the time spent investigating previous escape locations.

Prepulse Inhibition: This test measures the hearing and reflex startle response of the animals. The animal will be removed from its home cage and is place into a sound proof chamber. The startle response tone (120 decibels for 20 ms) will be given and startle response scored by an automated system. Then a series of mild prepulse tones will be paired with the 120 decibel tone and the response scored. The prepulse tones will be 74, 78, 82, 86, and 90 decibels and 20 ms in duration. Each prepulse tone will be paired with the 120 decibel tone, with the prepulse tone increasing with each

pairing. Four trials of each pairing will be performed. The startle response decreases with increasing prepulse tone in wild-type mice.

LETTER

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Exaggerated translation causes synaptic and behavioural aberrations associated with autism

Emanuela Santini[1], Thu N. Huynh[1], Andrew F. MacAskill[1], Adam G. Carter[1], Philippe Pierre[2,3,4], Davide Ruggero[5]*, Hanoch Kaphzan[1]†* & Eric Klann[1]

Autism spectrum disorders (ASDs) are an early onset, heterogeneous group of heritable neuropsychiatric disorders with symptoms that include deficits in social interaction skills, impaired communication abilities, and ritualistic-like repetitive behaviours[1,2]. One of the hypotheses for a common molecular mechanism underlying ASDs is altered translational control resulting in exaggerated protein synthesis[3]. Genetic variants in chromosome 4q, which contains the *EIF4E* locus, have been described in patients with autism[4,5]. Importantly, a rare single nucleotide polymorphism has been identified in autism that is associated with increased promoter activity in the *EIF4E* gene[6]. Here we show that genetically increasing the levels of eukaryotic translation initiation factor 4E (eIF4E) in mice[7] results in exaggerated cap-dependent translation and aberrant behaviours reminiscent of autism, including repetitive and perseverative behaviours and social interaction deficits. Moreover, these autistic-like behaviours are accompanied by synaptic pathophysiology in the medial prefrontal cortex, striatum and hippocampus. The autistic-like behaviours displayed by the eIF4E-transgenic mice are corrected by intracerebroventricular infusions of the cap-dependent translation inhibitor 4EGI-1. Our findings demonstrate a causal relationship between exaggerated cap-dependent translation, synaptic dysfunction and aberrant behaviours associated with autism.

eIF4E-transgenic mice (βT-*Eif4e*)[7] exhibited increased levels of eIF4E across brain regions (Fig. 1a) without compensatory changes in levels of other translational control proteins (Fig. 1b). We investigated whether eIF4E was bound preferentially to either eIF4E-binding protein (4E-BP) or eIF4G, which repress and promote, respectively, the initiation of cap-dependent translation[8,9]. We found significantly higher levels of eIF4E–eIF4G interactions in the brains of eIF4E-transgenic mice (Fig. 1c and Supplementary Fig. 1a), with no alterations in the interaction between eIF4E and 4E-BP (Fig. 1c, left, and Supplementary Fig. 1a). To confirm that the increased eIF4E–eIF4G interactions resulted in increased protein synthesis, we infused puromycin into the lateral ventricle of cannulated mice and labelled newly synthesized proteins using SUnSET[10,11], and observed increased *de novo* cap-dependent translation (Fig. 1d and Supplementary Fig. 1b–g). Overall, our results indicate that overexpression of eIF4E results in exaggerated cap-dependent translation in the brains of eIF4E-transgenic mice.

We then determined whether eIF4E-transgenic mice display repetitive and perseverative behaviours, which are behavioural domains required for ASD diagnosis[2]. eIF4E-transgenic mice exhibited repetitive digging behaviour in the marble-burying test[12] and increased self-grooming[13] compared with wild-type littermate controls (Fig. 2a, b). eIF4E-transgenic mice also displayed cognitive inflexibility in both a water-based Y-maze task and a modified version of the Morris water maze[14,15]. Learning ability in the acquisition and memory phases of these tasks was intact; however, in the reversal phases, eIF4E-transgenic mice were impaired in locating the new platform positions (Fig. 2c, d and Supplementary Fig. 2e–h). We tested an additional form of behavioural inflexibility by examining the eIF4E-transgenic mice for extinction of cued fear conditioning and found that they did not exhibit a significant reduction in freezing responses after extinction training (Fig. 2e). These experiments suggest that excessive cap-dependent translation in the brain affects the ability to suppress previously codified response patterns and the ability to form new behavioural strategies in response to changed environmental circumstances.

Abnormalities in social interaction skills are another behavioural defect displayed by individuals with ASDs[2]. In tests to examine social behaviour[16–18], the eIF4E-transgenic mice did not show a preference for a nonspecific stranger versus a new, inanimate object (Fig. 2f, g). Moreover, eIF4E-transgenic mice exhibited diminished reciprocal interactions with a freely moving stranger mouse (Fig. 2h), further evidence of deficits in social behaviour. The deficits in social behaviour of the eIF4E-transgenic mice are unlikely to be caused by a generalized increase in anxiety (Supplementary Fig. 2c, d, j). Moreover, the eIF4E-transgenic mice exhibited mild hyperactivity (Supplementary Fig. 2a, b), but no impairments in motor coordination, motor learning and sensorimotor gating (Supplementary Fig. 2i, k, l). Taken together, our behavioural analysis of the eIF4E-transgenic mice indicates that increased cap-dependent translation in the brain results in a distinct pattern of behavioural abnormalities consistent with ASDs.

Previous studies suggest that ASD symptoms such as cognitive inflexibility and deficits in social behaviour are generated by abnormalities in prefrontal and/or striatal circuits[19]. Consistent with this idea, the medial prefrontal cortex (PFC) is implicated in the modulation of social behaviours and social skills[20], whereas motor, social and communication impairments in boys with ASDs are associated with anatomical abnormalities in the striatum[21]. Therefore, we next examined whether the eIF4E-transgenic mice exhibited specific synaptic pathophysiologies in the medial PFC and striatum.

In the eIF4E-transgenic mice, examination of spontaneous synaptic 'mini' events in layers 2/3 of acute medial PFC slices revealed an increase in the frequency but not amplitude of excitatory events (miniature excitatory postsynaptic currents (mEPSCs); Fig. 3a), and an increase in the amplitude, but not frequency, of inhibitory events (miniature inhibitory postsynaptic currents (mIPSCs); Fig. 3b). No changes were observed in layer 5 (Supplementary Fig. 3a, b). Thus, our data suggest an enhancement of excitatory input and postsynaptic sensitivity for inhibitory events onto layer 2/3 pyramidal neurons, consistent with the hypothesis that autism may arise from an imbalance between excitatory and inhibitory synaptic transmission[22].

To determine whether the increased frequency of spontaneous mEPSCs might result from an enhanced number of synaptic contacts, we imaged dendritic spines using two-photon laser-scanning

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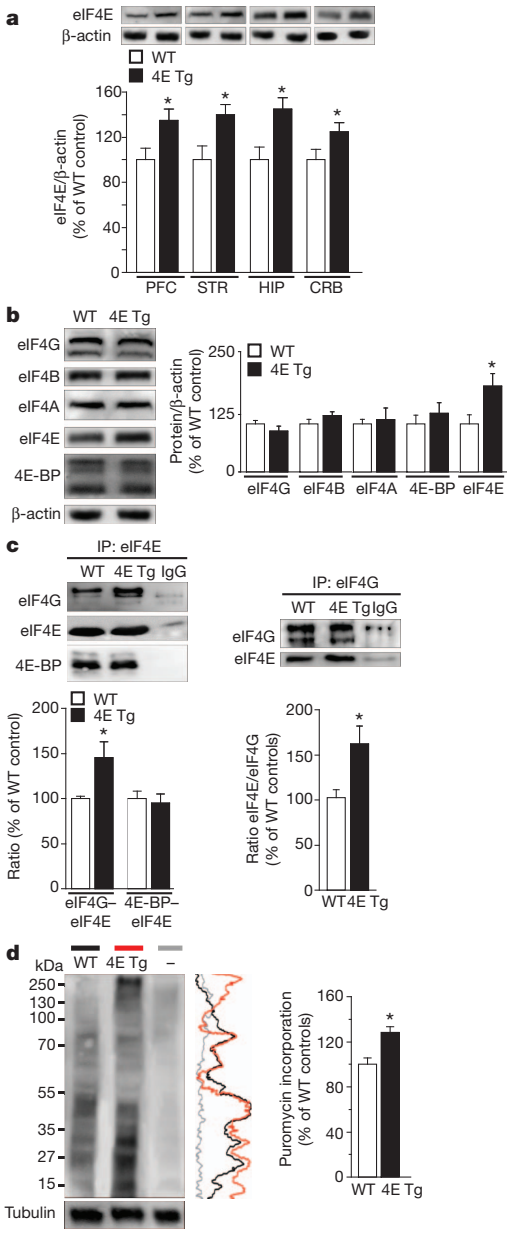

Figure 1 | eIF4E-transgenic mice exhibit increased eIF4E/eIF4G interactions and exaggerated cap-dependent translation. **a**, eIF4E-transgenic mice (4E Tg) exhibit increased eIF4E expression in multiple brain regions. $n = 4$ mice per genotype; $^*P < 0.05$ versus wild type (WT), Student's *t*-test. CRB, cerebellum; HIP, hippocampus; PFC, prefrontal cortex; STR, striatum. **b**, eIF4E-transgenic mice exhibit normal expression of other translational control proteins. $n = 4$ mice per genotype; $^*P < 0.05$, Student's *t*-test. **c**, eIF4E-transgenic mice exhibit increased eIF4E–eIF4G interactions. Immunoprecipitation (IP) of eIF4E (left) and eIF4G (right). $n = 3$ mice per genotype; $^*P < 0.05$, Student's *t*-test. **d**, eIF4E-transgenic mice exhibit increased translation as measured with SUnSET (see Methods). Vertical line traces of each autoradiogram are shown on the right. $n = 3$ mice per genotype; $^*P < 0.05$, Student's *t*-test. '–' represents a control sample without puromycin. All data are shown as mean and s.e.m.

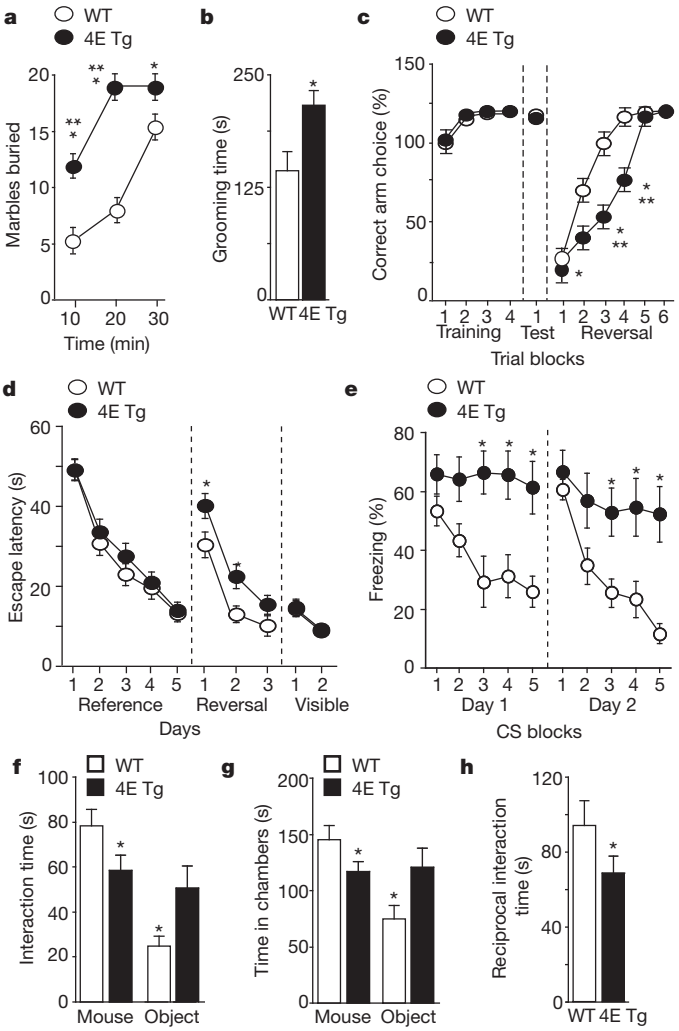

Figure 2 | eIF4E-transgenic mice exhibit ASD-like behaviours. eIF4E-transgenic mice were compared to wild-type littermates. **a**, Marble-burying test. $n = 21$–22 mice per genotype; $^*P < 0.05$, $^{***}P < 0.001$, repeated-measures analysis of variance (ANOVA) (time × genotype, $F_{(2,46)} = 31.62$, $P < 0.001$) followed by Bonferroni–Dunn post-hoc test. **b**, Self-grooming test. $n = 12$ mice per genotype; $^*P < 0.05$, Student's *t*-test. **c**, Y-maze reversal task. $n = 21$–22 mice per genotype; $^*P < 0.05$, $^{***}P < 0.001$, repeated-measures ANOVA (time × genotype, $F_{(5,138)} = 16.74$, $P < 0.001$) followed by Bonferroni–Dunn post-hoc test. **d**, Morris water maze reversal learning. $n = 12$–13 mice per genotype; $^*P < 0.05$, repeated-measures ANOVA (time × genotype, $F_{(3,92)} = 6.1$, $P < 0.001$) followed by Bonferroni–Dunn post-hoc test. **e**, Extinction of cued fear memory (15 conditioned stimuli (CS) per day represented as three CS blocks). $n = 6$ mice per genotype; $^*P < 0.05$, repeated-measures ANOVA (day 1: time × genotype, $F_{(4,40)} = 5.73$, $P < 0.001$; day 2: time × genotype, $F_{(4,40)} = 4.81$, $P < 0.01$) followed by Bonferroni–Dunn post-hoc test. **f**, **g**, Social behaviour test. The time spent either interacting with a stranger mouse (**f**) or in the chambers (**g**). $n = 6$ mice per genotype; $^*P < 0.05$, repeated-measures ANOVA (stimulus × genotype, $F_{(1,10)} = 6.04$, $P < 0.05$ (**f**); stimulus × genotype, $F_{(1,10)} = 6.12$, $P < 0.05$ (**g**)) followed by Bonferroni–Dunn post-hoc test. **h**, Reciprocal social interaction task. $n = 6$ mice per genotype; $^*P < 0.05$, Student's *t*-test. All data are shown as mean and s.e.m.

microscopy (Fig. 3c, d and Supplementary Fig. 3c, d). We found a significant increase (~12%) in spine density and observed a significantly smaller spine volume in the eIF4E-transgenic mice than in wild-type littermates (wild type = $0.123 \pm 0.004\ \mu m^3$ (mean ± s.e.m.) and eIF4E-transgenic = $0.110 \pm 0.004\ \mu m^3$, $P = 0.01$ versus wild type, Student's *t*-test).

Next, we examined whether increased expression of eIF4E also resulted in synaptic pathophysiology in the striatum. We used high-frequency stimulation to induce long-term depression (LTD) in acute striatal slices[23], and found that eIF4E-transgenic mice exhibited enhanced LTD compared to wild-type littermates (Fig. 3e and Supplementary Fig. 3e, f). We propose that the enhanced LTD in eIF4E-transgenic mice results in altered efficiency of striatal information storage and processing, culminating in the inability to form new motor patterns and/or to disengage from previously learned motor behaviours.

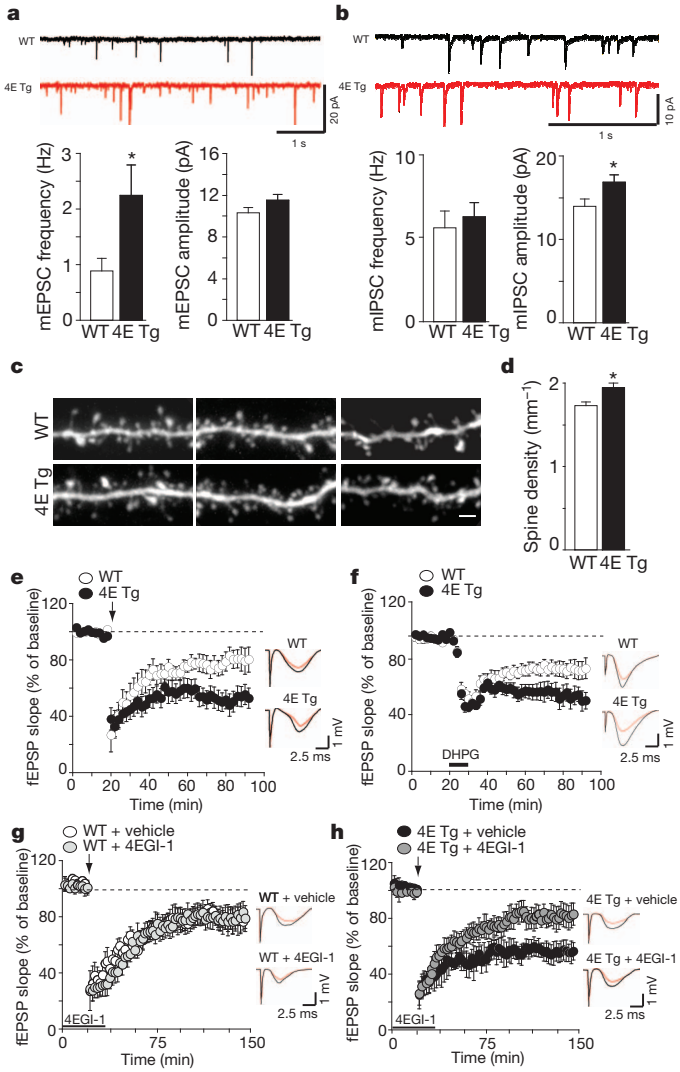

Figure 3 | eIF4E-transgenic mice exhibit alterations in synaptic function, dendritic spine density and synaptic plasticity. **a**, **b**, eIF4E-transgenic mice exhibit increased mEPSC frequency (**a**) and increased mIPSC amplitude (**b**) in layer 2/3 medial PFC pyramidal neurons. $n = 27–30$ neurons per genotype; $^*P < 0.05$, Student's t-test. **c**, **d**, eIF4E-transgenic mice exhibit increased dendritic spine density in layer 2/3 medial PFC pyramidal neurons. High-magnification images (**c**) and quantification (**d**) of spiny dendrites. $n = 12$ neurons per genotype; $^*P < 0.05$, Student's t-test. Scale bar, 2 μm. **e**, eIF4E-transgenic mice exhibit enhanced striatal LTD. $n = 13$ slices from 8 mice per genotype. **f**, eIF4E-transgenic mice exhibit enhanced hippocampal mGluR-LTD. DHPG denotes the mGluR agonist 3,5-dihydroxyphenylglycine. $n = 15$ slices from 8 mice per genotype. **g**, **h**, 4EGI-1 normalizes enhanced striatal LTD shown by eIF4E-transgenic mice (**h**), without affecting LTD in wild-type mice (**g**). $n = 18$ slices from 9 mice per genotype and treatment. All field recordings were analysed with repeated-measures ANOVA. Arrows indicate delivery of high-frequency stimulation. Solid bars indicate the duration of bath application of DHPG (10 μM, 10 min) and 4EGI-1 (100 μM, 45 min). Representative traces (right) showing field excitatory postsynaptic potentials (fEPSPs) before (black) and 60 min after (red) high-frequency stimulation. All data are shown as mean and s.e.m.

To determine whether the synaptic alterations described in the eIF4E-transgenic mice were selective for the frontostriatal circuit, we examined synaptic plasticity in the hippocampus[24]. We found that eIF4E-transgenic mice exhibited enhanced metabotropic glutamate receptor-dependent LTD (mGluR-LTD) compared to wild-type littermates (Fig. 3f and Supplementary Fig. 3g, h), consistent with previous studies showing that changes in brain protein synthesis are accompanied by altered (enhanced or reduced) hippocampal mGluR-LTD[25,26]. Thus, consistent with the ubiquitous increase in brain expression of eIF4E, the eIF4E-transgenic mice display altered synaptic function and plasticity in several brain regions (medial PFC, striatum and hippocampus) implicated in behavioural abnormalities associated with ASDs.

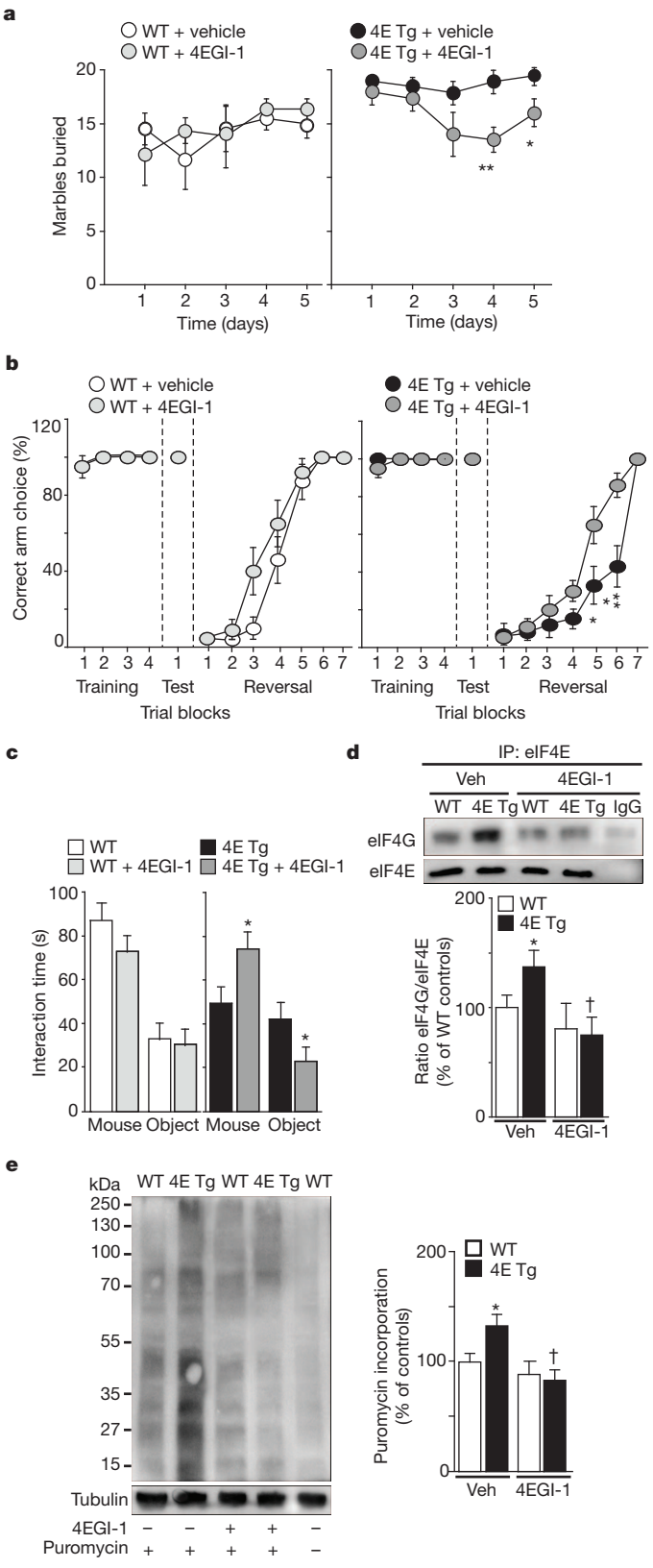

Figure 4 | The cap-dependent translation inhibitor 4EGI-1 reverses ASD-like behaviours shown by eIF4E-transgenic mice. **a**, Treatment of eIF4E-transgenic mice with 4EGI-1 reduces the marble-burying behaviour. $n = 6$ mice per genotype and treatment; $^{*}P < 0.05$, $^{**}P < 0.01$, two-way repeated-measures ANOVA (treatment × genotype, $F_{(1,20)} = 4.21$, $P < 0.05$) followed by Bonferroni–Dunn post-hoc test. **b**, 4EGI-1 improves the cognitive flexibility of eIF4E-transgenic mice in the Y-maze test. $n = 6$–7 mice per genotype and treatment; $^{*}P < 0.05$, $^{**}P < 0.01$, two-way repeated-measures ANOVA (treatment × genotype, $F_{(1,21)} = 4.61$, $P < 0.05$) followed by Bonferroni–Dunn post-hoc test. **c**, 4EGI-1 improves social behaviour of eIF4E-transgenic mice in the three-chamber arena test. $n = 6$ mice per genotype and treatment; $^{*}P < 0.05$, two-way repeated-measures ANOVA (treatment × genotype, $F_{(1,20)} = 6.26$, $P < 0.05$) followed by Bonferroni–Dunn post-hoc test. **d**, 4EGI-1 decreases the enhanced eIF4E–eIF4G interactions in eIF4E-transgenic mice. Immunoprecipitation of eIF4E in the striatum. $n = 4$ mice per genotype; $^{*}P < 0.05$ (versus vehicle-treated wild type), $^{\dagger}P < 0.05$ (versus 4EGI-1-treated wild type), two-way ANOVA, followed by Bonferroni–Dunn post-hoc test. **e**, 4EGI-1 normalizes the exaggerated cap-dependent translation in eIF4E-transgenic mice as measured with SUnSET. The last wild-type sample represents a control without puromycin. $^{*}P < 0.05$, $^{\dagger}P < 0.05$, two-way ANOVA followed by Bonferroni–Dunn post-hoc test. All data are shown as mean and s.e.m.

Finally, we asked whether exaggerated cap-dependent translation was responsible for the synaptic alterations and ASD-like behaviours shown by the eIF4E-transgenic mice. We took advantage of 4EGI-1, an inhibitor of eIF4E–eIF4G interactions[8,11], to block the synaptic and behavioural consequences of increased eIF4E expression. Bath application of 4EGI-1 normalized the enhanced striatal LTD observed in the eIF4E-transgenic mice (Fig. 3g, h), suggesting that exaggerated striatal LTD (Fig. 3h) is a direct consequence of increased binding of eIF4E to eIF4G (Supplementary Fig. 3i–k).

Next, we used a subthreshold dose of 4EGI-1 (ref. 11) to normalize the behavioural abnormalities in eIF4E-transgenic mice without impairing their wild-type littermates. eIF4E-transgenic mice treated with 4EGI-1 exhibited a decrease in repetitive behaviour during the marble-burying task, which started on day four and persisted throughout day five (Fig. 4a). Moreover, we found that 4EGI-1 maintained the behavioural effects observed in the marble-burying task (Supplementary Fig. 4a, b). We also found that blockade of eIF4E–eIF4G interactions with 4EGI-1 significantly improved the performance of eIF4E-transgenic mice in the reversal phase of the Y-maze test (Fig. 4b). These findings indicate that chronic treatment of eIF4E-transgenic mice with 4EGI-1 reverses their repetitive and perseverative behaviours. We also found that infusions of 4EGI-1 rescued the social behaviour deficits shown by the eIF4E-transgenic mice in the three-chamber arena test, as they exhibited an increased preference for a nonspecific stranger compared to a new object (Fig. 4c).

At the completion of the behavioural studies with 4EGI-1, we performed co-immunoprecipitation experiments, confirming that 4EGI-1 reduced the increased eIF4E–eIF4G interactions exhibited by the eIF4E-transgenic mice (Fig. 4d and Supplementary Fig. 4c–e). Furthermore, puromycin-labelling of newly synthesized proteins was reduced to wild-type levels, indicating that 4EGI-1 was effective in attenuating the increased cap-dependent translation in the eIF4E-transgenic mice (Fig. 4e and Supplementary Fig. 4f, g). Together, these results indicate that repeated treatment of eIF4E-transgenic mice with 4EGI-1 reverses the increased binding of eIF4E to eIF4G, exaggerated cap-dependent translation, and reversal of ASD-like behaviours.

Here we have demonstrated that increased eIF4E expression and, consequently, dysregulated translational control at the initiation phase of protein synthesis in mice results in the appearance of synaptic dysfunction and aberrant behaviours consistent with ASDs. On the basis of our observations, we propose that exaggerated cap-dependent protein synthesis in the eIF4E-transgenic mice and fragile X syndrome model mice[27,28] results in enhanced translation of a specific subset of messenger RNAs. Thus, the identity of both these mRNAs and the *cis*-acting elements in the 5′ untranslated region responsible for eIF4E-dependent protein synthesis and their possible overlap with fragile X mental retardation protein target mRNAs will be important investigations in future studies.

Our studies with eIF4E-transgenic mice indicate that ASD-like behaviours can be induced by exaggerated cap-dependent translation in the brain. Moreover, we demonstrated that aberrant repetitive, perseverative and social behaviours shown by eIF4E-transgenic mice are reversed by reducing eIF4E–eIF4G interactions, thereby restoring translational homeostasis. Thus, our findings establish a causal link between exaggerated cap-dependent translation and behaviours associated with autism. Finally, our findings indicate that behavioural defects caused by exaggerated cap-dependent translation, which also occurs in fragile X syndrome[29,30], a disorder with a high incidence of autism, are not irrevocable and can be corrected well into adulthood.

METHODS SUMMARY

All procedures involving animals were approved by the New York University Animal Care and Use Committee and followed the National Institutes of Health Guidelines for the use of animals in research. For a detailed description of all the techniques used in this study, please see the Methods. All the experiments were performed with the examiners blinded to genotype.

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions The study was directed by E.K. and conceived and designed by E.S. and E.K. E.S. performed the molecular, behavioural and electrophysiological experiments. T.N.H. performed behavioural experiments. A.F.M. and A.G.C. performed the dendritic spine-density experiments. P.P. contributed the anti-puromycin (12D10) antibody. D.R. contributed with reagents and expertise concerning translation control by eIF4E. H.K. performed the cortical whole-cell electrophysiological experiments. The manuscript was written by E.S. and E.K. and edited by all of the authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to E.K. (eklann@cns.nyu.edu).

METHODS

Housing. Generation of βT-*Eif4e* transgenic mice (eIF4E-transgenic mice) has been described previously[7].

For all the experiments, we made use of littermates derived from crossing heterozygotes. Mice were backcrossed to the N10 generation in C57BL/6J mice. Overall, eIF4E-transgenic mice were viable, fertile and showed no gross anatomical abnormalities in the age range used for this study. eIF4E-transgenic mice and their wild-type littermates were housed in groups of 3–4 animals per cage and kept on a regular 12 h light/dark cycle (7:00–19:00 light period). Food and water were available ad libitum.

Surgery and drug infusion. Mice were anaesthetized (ketamine (100 mg kg^{-1}) and xylazine (10 mg kg^{-1})) and mounted onto a stereotaxic apparatus. Cannulae (26-gauge) were implanted unilaterally at the following coordinates: −0.22 mm anterioposterior, +1 mm mediolateral, and −2.4 mm dorsoventral[31]. Mice were allowed 1 week to recover after the surgery.

The infusions of the eIF4E–eIF4G inhibitor 4EGI-1 were performed as described previously[11]. In brief, 4EGI-1 dissolved in 100% dimethylsulphoxide (DMSO) was diluted in vehicle (0.5% (2-hydroxypropyl)-β-cyclodextrin and 1% DMSO in artificial cerebrospinal fluid (ACSF)). Vehicle or 4EGI-1 (20 μM) was infused over 1 min (0.5 μl min^{-1}; Harvard Apparatus). On the last day of treatment, mice received infusion of 4EGI-1 alone or puromycin (25 μg in 0.5 μl) before 4EGI-1 infusions. All behaviour and tissue dissection occurred 1 h after 4EGI-1 infusions.

Behaviour. The following behavioural tests were performed on male eIF4E-transgenic mice and their wild-type littermates (2–6 months of age) as described previously: novelty induced locomotor activity[32], open field[33], elevated plus maze[33], rotarod[34], prepulse inhibition[33], marble[14], social behaviour[16], direct social interaction[35,36], Y-maze and the Morris water maze[7,35].

For all experiments, mice were acclimated to the testing room 30 min before behavioural training and all behaviour apparatuses were cleaned between each trial with 30% ethanol. The experimenter was blinded to genotype and drug treatment while performing and scoring all behavioural tasks. All behavioural tests were performed starting with the least aversive task first (locomotor activity) and ending with the most aversive (either water-based mazes or extinction of fear memory).

Western blots. Mice were killed by decapitation 1 h after the infusion with either 4EGI-1 alone or 4EGI-1 plus puromycin. The striatum and prefrontal cortex were rapidly dissected, placed on an ice-cold surface, and sonicated in 1% SDS and boiled for 10 min. Aliquots (2 μl) of the homogenate were used for protein determination with a BCA (bicinchoninic acid) assay kit (Pierce, Thermo Scientific). Equal amounts of protein (20 μg) for each sample were loaded onto 10% polyacrylamide gels. Proteins were separated by SDS–PAGE and transferred overnight to polyvinylidene difluoride membranes (Immobilon-Psq, Millipore Corporation). The membranes were immunoblotted with antibodies against eIF4E (1:1,000), eIF4G (1:1,000), eIF4B (1:1,000), eIF4A (1:1,000) and 4E-BP (1:1,000) (Cell Signaling Technology). Antibodies against β-actin and tubulin (1:5,000, Cell Signaling Technology) were used to estimate the total amount of proteins. Detection was based on a horseradish peroxidase (HRP)-conjugated secondary antibody (Promega) and chemiluminescence reagent (ECL or ECL plus; GEHealthcare), and visualized using a Kodak 4000MM imager to obtain pixel density values for the band of interest (Carestream). All images were obtained using maximum sensitivity settings with no binning (0–65 K signal range). No images analysed presented saturating signals for the bands of interest (>65 K greyscale value). The amount of each protein was normalized for the amount of the corresponding β-actin or tubulin detected in the sample.

Immunoprecipitation. Tissue was homogenized in ice-cold lysis immunoprecipitation buffer containing (in mM): 40 HEPES, pH 7.5, 150 NaCl, 10 pyrophosphate, 10 glycerophosphate, 1 EDTA and 0.1% CHAPS, protease inhibitor II, phosphatase inhibitor mixture I, II (Sigma-Aldrich). Cleared homogenate (500 μg) was incubated with either anti-eIF4G (2.5 μg) or anti-eIF4E (2.5 μg) (Bethyl Laboratories) and gently shaken overnight at 4 °C. The antibody–lysate mix was incubated with 75 μl IgG bound to agarose beads (Thermo Scientific). The bead–sample slurry was incubated while rocking at 4 °C overnight. Supernatant was removed and saved, and immunoprecipitates were washed three times in lysis buffer, and once in wash buffer (50 mM HEPES, pH 7.5, 40 mM NaCl, 2 mM EDTA). SDS–PAGE buffer was added to the washed immunoprecipitates, which then were resolved on 4 to 12% gradient gels. Efficiency of the immunoprecipitation was determined by examining the supernatant and wash fractions obtained from the procedure on images obtained from Kodak 4000MM imager (see western blots section). Band density values for coimmunoprecipitated eFI4E, eIF4G and 4E-BP were normalized to immunoprecipitated eIF4G or eIF4E.

SUnSET. A protein synthesis assay was performed as previously described using the SUnSET method[11]. Puromycin-treated samples were identified on blots using the mouse monoclonal antibody 12D10 (1:5,000 from a 5 mg ml^{-2} stock). Because only a small fraction of the brain proteins were labelled, signal from blots was identified using ECL-Advance (GEHealthcare).

Electrophysiology. Hippocampal (400 μm), prefrontal and striatal slices (300 μm) for electrophysiology were prepared as described previously[24].

Solution to maintain slices. Cutting solution (in mM): 110 sucrose, 60 NaCl, 3 KCl, 1.25 NaH_2PO_4, 28 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 5 glucose and 0.6 ascorbate. ACSF (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 D-glucose, 2 $CaCl_2$ and 1 $MgCl_2$. Slices were incubated at room temperature and then were placed in the recording chamber for additional recovery time of 60 min at 33 °C.

Extracellular recordings. Extracellular fEPSPs were recorded as described previously[23,24]. In all the experiments, baseline synaptic transmission was monitored for at least 20 min before LTD induction. Three trains of high-frequency stimulation (3 s duration, 100 Hz frequency at 20 s intervals) were used to induce LTD in striatal slices[23], and 10 min of incubation with DHPG (50 μM) was used to induce mGluR-dependent LTD in hippocampal slices[24]. The slope of fEPSPs was expressed as a percentage of the baseline average before LTD induction.

Intracellular recordings. Medial prefrontal pyramidal cells were illuminated and visualized using a ×60 water-immersion objective mounted on a fixed-stage microscope (BX61-WI, Olympus), and the image was displayed on a video monitor using a charge-coupled device camera (Hamamatsu). Recordings were amplified by multiclamp 700B and digitized by Digidata 1440 (Molecular Devices). The recording electrode was pulled from a borosilicate glass pipette (3–5 MΩ) using an electrode puller (P-97, Sutter Instruments), filled with an internal solution according to the specific experimental requirement, and patched onto the soma. The series resistance of the patch pipette was compensated ~70% and re-adjusted before each experiment. A measured liquid junction potential was corrected by adjusting the pipette offset. All voltage–clamp recordings were low-pass filtered at 10 kHz and sampled at 50 kHz.

Internal solution for mEPSCs (in mM): 120 caesium-methane-sulphonate, 10 HEPES, 10 EGTA, 4 $MgCl_2$, 0.4 NaGTP, 4 MgATP, 10 phosphocreatine and 5 QX-314 (pH adjusted to 7.3 with CsOH, 290 mOsm). Bicuculline 50 μM and tetrodotoxin 1 μM (Tocris) were added to the external ACSF bath solution.

Internal solution for mIPSCs (in mM): 140 CsCl, 10 EGTA, 10 HEPES, 2 $MgCl_2$, 2.0 Mg-ATP, 4 Na_2-ATP, 0.4 Na_2-GTP and 5 QX-314 (pH adjusted to 7.3 with CsOH, 290 mOsm), thus yielding a chloride reversal potential of around 2 mV for the chloride currents. Tetrodotoxin (1 μM), 6,7-dinitroquinoxaline-2,3-dione (DNQX) (40 μM) and D-2-amino-5-phosphonopentanoate (AP5) (50 μM) were added to the ACSF bath solution.

In these conditions, mEPSCs and mIPSCs were recorded in voltage clamp at −70 mV and measured for 120 s and 60 s, respectively.

Dendritic spine morphology. Dendritic spine density experiments were performed as previously described[37,38]. In brief, two-photon imaging was accomplished with a custom microscope and high-resolution stacks ($x = 0.13$ μm, $y = 0.13$ μm, $z = 0.2$ μm per voxel) of dendritic segments throughout the entire cell were taken for morphological analysis in NeuronStudio. Spine-head volume was calculated using a rayburst algorithm. Images were deconvolved before volume measurements using custom routines written in MATLAB (Mathworks).

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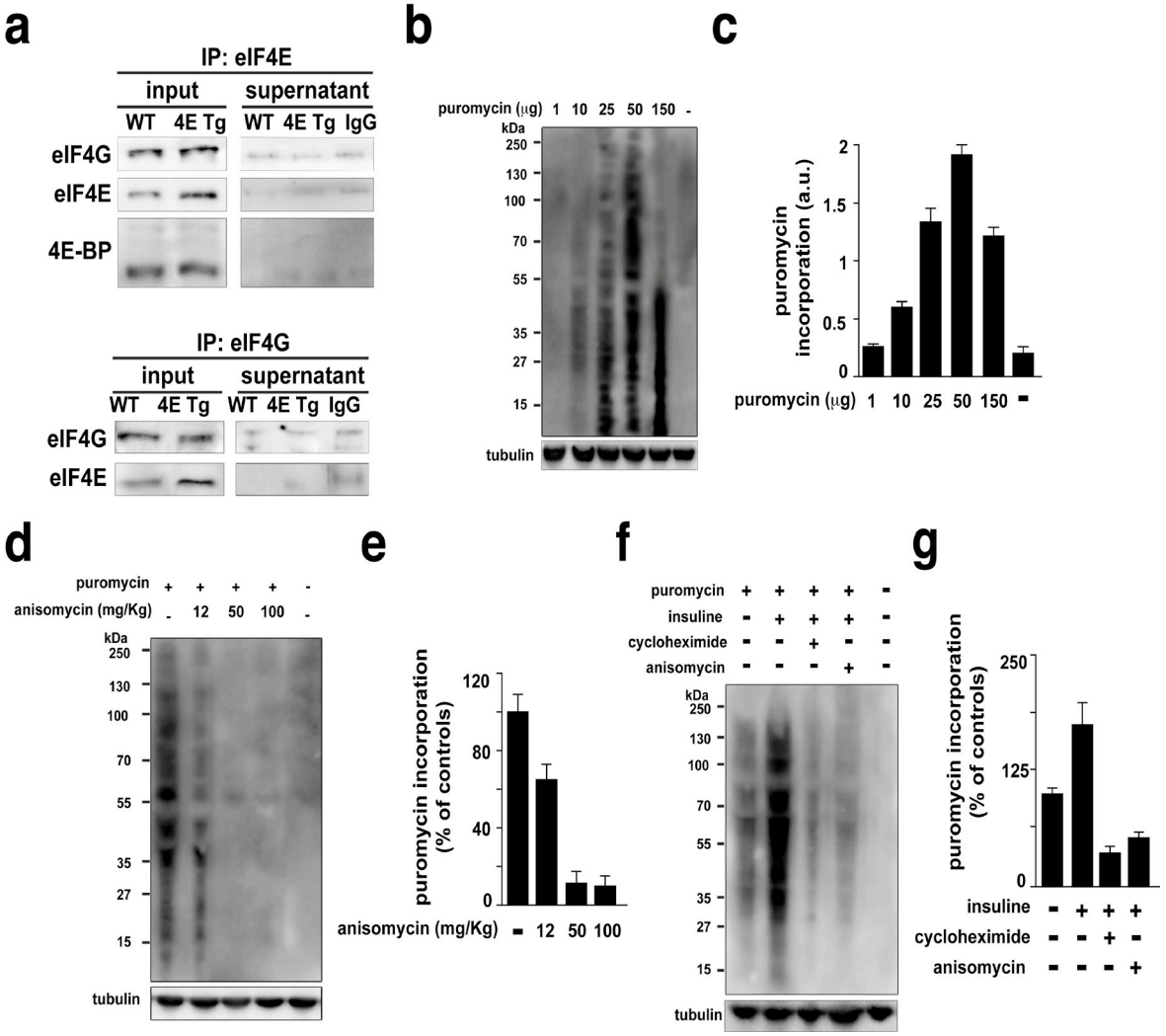
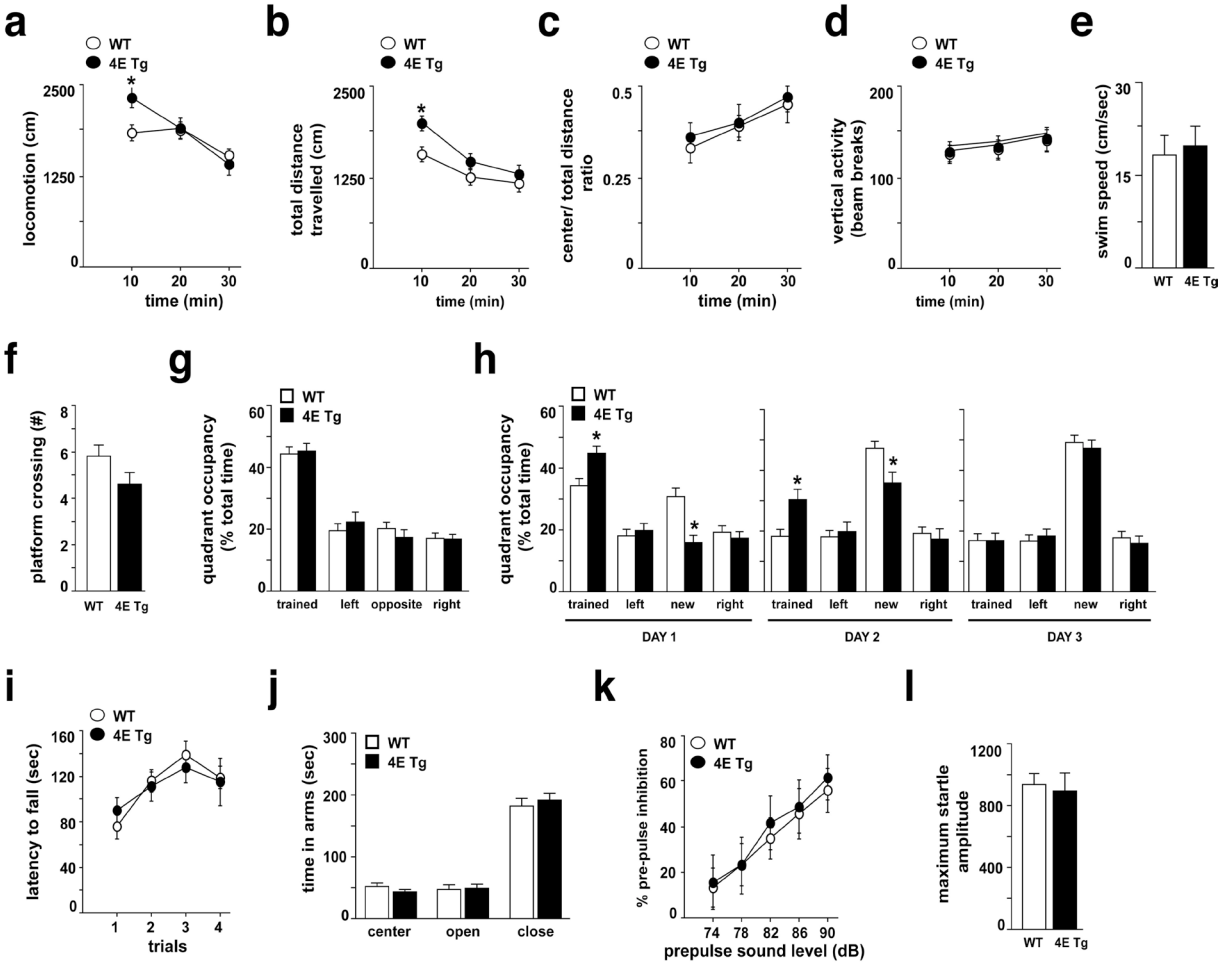
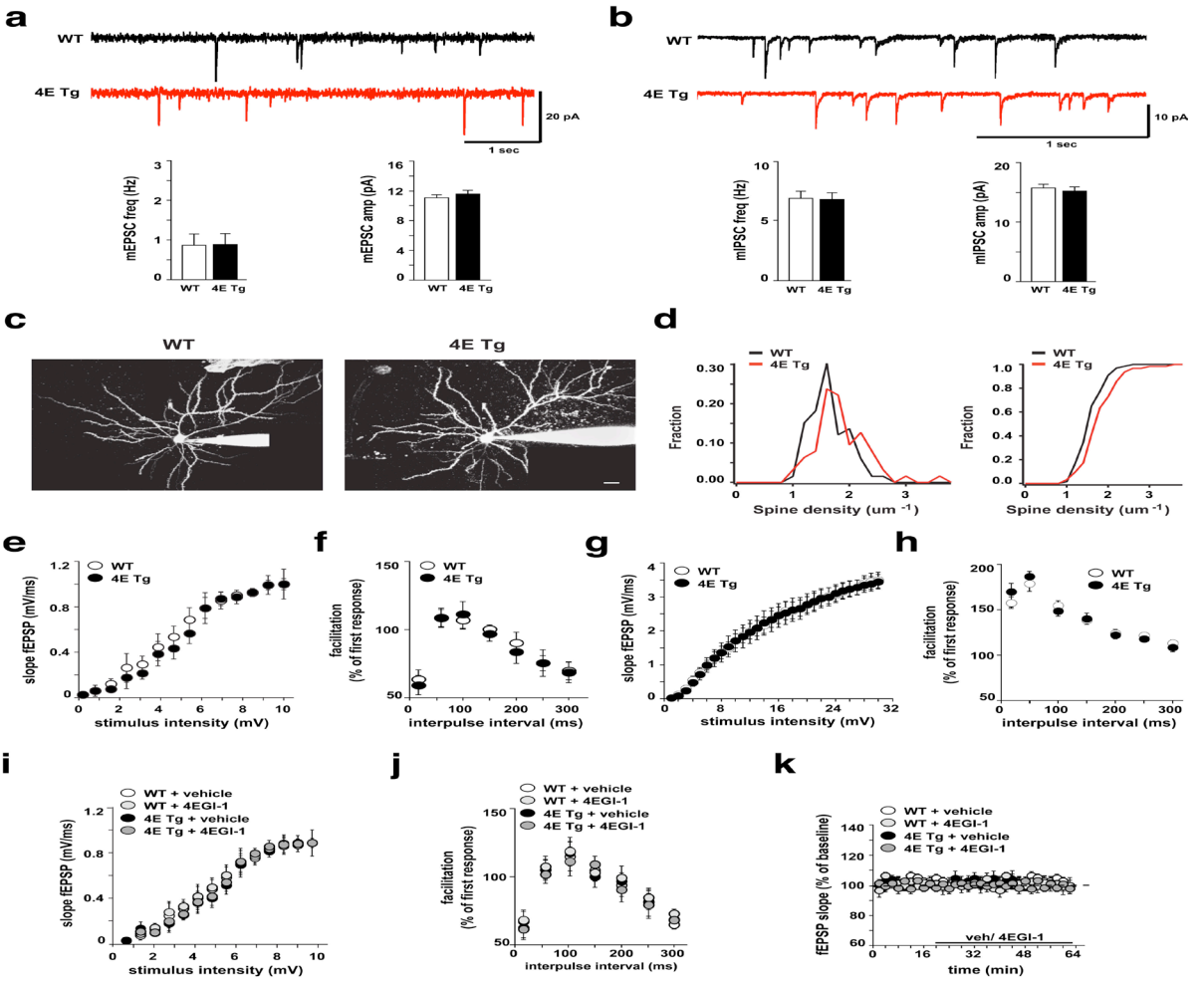


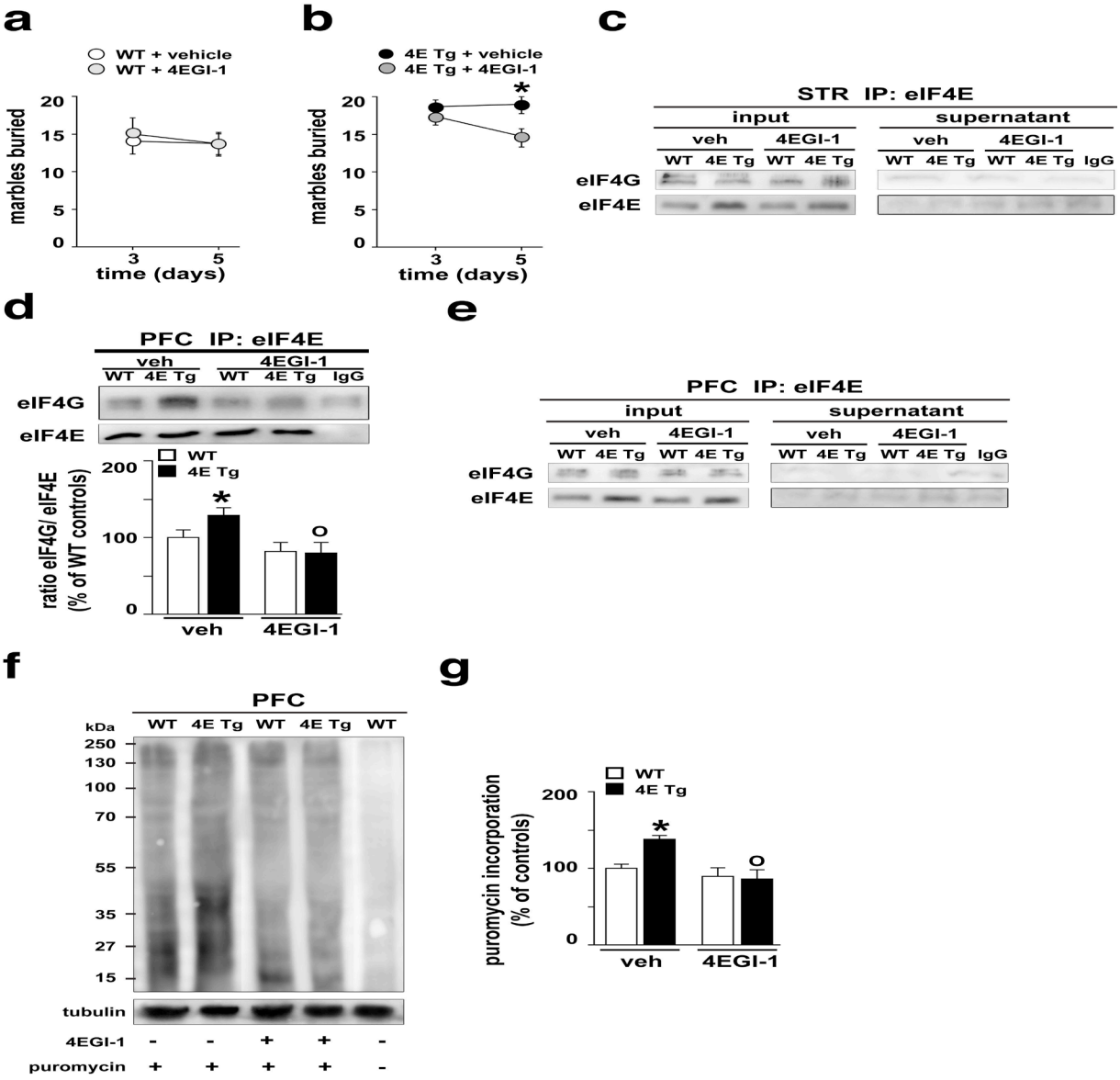
Supplementary Figure 1. (**a**) Representative Western blots showing brain levels of eIF4E, eIF4G and 4E-BP (input) and the supernatant after immunoprecipitation with eIF4E and eIF4G. All data are shown as mean ± SEM. (**b, c**) Dose response of puromycin infused ICV. Representative Western blots (**b**) and quantification (**c**) of newly synthesized brain proteins labeled with increasing doses of puromycin using the SUnSET method (see Supplementary Methods). n=3 mice/dose. One-way ANOVA [treatment effect, $F_{(5,17)}$=78.07, p<0.001] (**d, e**) Anisomycin blocks the incorporation of puromycin into newly synthesized brain proteins in a dose-dependent manner. Puromycin (25 μg in 0.5 μl; IVC) was infused either with or without anisomycin (12, 50, 100 mg/Kg; i.p.). Representative Western blots (**d**) and quantification (**e**) of proteins labeled with puromycin using the SUnSET method (see Supplementary Methods). n=3 mice/treatments. One-way ANOVA [treatment effect, $F_{(3,12)}$=35.52, p<0.001]. (**f, g**) Protein labeling with puromycin using the SUnSET is sensitive to stimulation and inhibition of protein synthesis. Hippocampal slices incubated with puromycin (10 mg/ml) and stimulated with either insulin (1 μM) alone or in combination with either cycloheximide (300 μM) or anisomycin (40 μM). Representative Western blots (**f**) and quantification (**g**) of proteins labeled puromycin using SUnSET (see Supplementary Methods). n=3 slices from 3 mice/treatments. One-way ANOVA [treatment effect, $F_{(3,11)}$=16.62, p<0.001] .In all the SUnSET experiments (panels **b-g**): – represents a control sample without puromycin. Lower panel shows the levels of tubulin, which was used as loading control. All data are shown as mean ± SEM.



Supplementary Figure 2. (**a**) Novelty-induced locomotor activity shown in 10 min intervals. *p<0.05 vs WT, repeated measures ANOVA [genotype X time, $F_{(5,100)}$=3.69, p<0.01] followed by Bonferroni-Dunn test. (**b, c, d**) Open field test. Total distance (**b**), ratio center/total distance (**c**) and vertical activity (**d**) shown in 10 min intervals. *p<0.05 vs WT, repeated measures ANOVA [genotype X time, $F_{(5,100)}$=3.82, p<0.01] followed by Bonferroni-Dunn test. (**e**) Swim speed (cm/sec) in the MWM. n.s., Student's t-test. (**f, g**) Number of platform crossings (**f**) and quadrant occupancy (**g**) during the probe test of the MWM. . n.s., repeated measure ANOVA. (**h**) Quadrant occupancy for each day of the reversal learning phase of the MWM. *p<0.05 vs WT, repeated measures ANOVA [DAY1: genotype X time, $F_{(3,92)}$=6.12, p<0.001; DAY2: genotype X time, $F_{(3,92)}$=5.93, p<0.01; DAY3: n.s.] followed by Bonferroni-Dunn test. (**i**) Accelerating rotarod test. Latency to fall from the accelerating rod (sec) is shown for four test trials. n.s., repeated measure ANOVA. (**j**) Elevated plus maze test. Time spent in the arms and in the center of the maze. n.s., two-way ANOVA. (**k, l**) Prepulse inhibition (PPI) of the acoustic startle response is represented as % of PPI of the startle response (**k**). n.s., repeated measures ANOVA. Acoustic startle response is expressed as maximum startle amplitude to the 120-dB stimulus (**l**). n.s., Student's t-test. In all the experiments n=12-13 mice/genotype. All data are shown as mean ± SEM.



Supplementary Figure 3. (**a**) mEPSC traces obtained from layer 5 mPFC pyramidal neurons (upper panel). n=22-25 neurons/genotype. n.s., Student's *t*-test. (**b**) mIPSC traces obtained from layer 5 mPFC pyramidal neurons (upper panel). n=34-35 neurons/genotype. n.s., Student's *t*-test. (**c**) Two-photon images showing layer 2/3 mPFC pyramidal neuron filled with Alexa Fluor-594 through the whole-cell recording pipettes. Scale bar= 20 µm. (**d**) Histograms (left) and cumulative histograms (right) of spine density in WT (black) and Tg (red) mice, showing a significant difference between the two populations. (**e**) Input versus output plot representing the slope of the striatal fEPSP in response to increasing stimulus intensity. n=10 slices from 8 mice/genotype. n.s., repeated measures ANOVA. (**f**) Percent facilitation in the striatum, determined by the ratio of the second fEPSP to the first fEPSP shown at interpulse intervals from 10 to 300 ms (right panel). n=13 slices from 8 mice/genotype. n.s., repeated measures ANOVA. (**g**) Input versus output plot representing the slope of the hippocampal fEPSP in response to increasing stimulus intensity (left panel). n=12 slices from 9 mice/genotype. n.s., repeated measures ANOVA. (**h**) Percent facilitation in the hippocampus, determined by the ratio of the second fEPSP to the first fEPSP is shown at interpulse intervals from 10 to 300 ms (right panel). n=13 slices from 8 mice/genotype. n.s., repeated measures ANOVA. (**i**) Input versus output plot representing the slope of the striatal fEPSP in response to increasing stimulus intensity. n=15 slices from 9 mice/genotype/treatment. n.s., repeated measures ANOVA. (**j**) Percent facilitation in the striatum, determined by the ratio of the second fEPSP to the first fEPSP is shown at interpulse intervals from 10 to 300 ms (right panel). n=13 slices from 9 mice/genotype/treatment. n.s., repeated measures ANOVA. 4EGI-1 (100 µM) was bath applied for 45 min prior and during the duration of the experiments. (**k**) 4EGI-1 does not alter basal synaptic transmission in striatal slices. Baseline fEPSP was recorded for 20 min prior and during the application of 4EGI-1 (100 µM). n=13 slices from 9 mice/genotype/treatment. n.s., repeated measures ANOVA. All data are shown as mean ± SEM.



Supplementary Figure 4. (**a, b**) Marble-burying behavior of WT (**a**) and eIF4E transgenic mice (**b**) tested five hours after the infusion of 4EGI-1. Data are shown as mean ± SEM (n=6 mice/genotype/treatment). *p<0.05 vs vehicle-treated 4E Tg mice, two-way repeated measures ANOVA [genotype X treatment, $F_{(1,20)}$=6.16, p<0.05] followed by Bonferroni-Dunn test. (**c**) Representative Western blots showing the levels of eIF4E and eIF4G in the striatum (**c**, STR, input,) and in the supernatant after immunoprecipitation with eIF4E also is shown (**c,** STR, supernatant). (**d**) Representative Western blots and quantification of proteins recovered after immunoprecipitation (IP) of eIF4E in the prefrontal cortex (PFC). n=4 mice/genotype. *p<0.05 and °p<0.05 vs vehicle-treated WT and 4E Tg, respectively, two-way ANOVA, followed by Bonferroni-Dunn test. (**e**) Representative Western blots showing the levels of eIF4E and eIF4G in the striatum (**e**, STR, input,) and in the supernatant after immunoprecipitation with eIF4E also is shown (**e,** STR, supernatant). (**f, g**) Representative Western blots and quantification of newly synthesized proteins labeled with puromycin using the SUnSET method in WT and 4E Tg mice infused with either vehicle or 4EGI-1 and puromycin in prefrontal cortex (PFC). The last sample represents a control without puromycin. Lower panel shows the levels of tubulin, used as loading control. *p<0.05 and °p<0.05 vs vehicle-treated WT and 4E Tg, respectively, two-way ANOVA, followed by Bonferroni-Dunn test. All data are shown as mean ± SEM.

Supporting Data

All figures and figure legends are contained in the Santini et al. manuscript, which is attached in the preceding pages.