Exaggerated translation causes synaptic and behavioural aberrations associated with autism

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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 behaviours1,2. One of the hypotheses for a common molecular mechanism underlying ASDs is altered translational control resulting in exaggerated protein synthesis3. Genetic variants in chromosome 4q, which contains the *EFT4E* locus, have been described in patients with autism4,5. Importantly, a rare single nucleotide polymorphism has been identified in autism that is associated with increased promoter activity in the *EFT4E* gene6. Here we show that genetically increasing the levels of eukaryotic translation initiation factor 4E (eIF4E) in mice7 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 4EPI1. 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 translation8,9. We found significantly higher levels of eIF4E–eIF4G interactions in the brains of eIF4E-transgenic mice (Fig. 1c and Supplementary Fig. 1a, 1b) 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 SUNSET10,11, 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. eIF4E-transgenic mice exhibited repetitive digging behaviour in the marble-burying test12 and increased self-grooming13 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 maze14,15. 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 deficit displayed by individuals with ASDs. In tests to examine social behaviour16–18, the eIF4E-transgenic mice did not show a preference for a non-specific 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 circuits19. Consistent with this idea, the medial prefrontal cortex (PFC) is implicated in the modulation of social behaviours and social skills20, whereas motor, social and communication impairments in boys with ASDs are associated with anatomical abnormalities in the striatum21. Therefore, we next examined whether the eIF4E-transgenic mice exhibited specific synaptic pathophysiologicals 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, 3b). 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 transmission22.

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...
All data are shown as mean and s.e.m. increased translation as measured with SUnSET (see Methods). Vertical line genotypes; n = 21–22 mice per genotype; *P < 0.05, Student’s t-test. CRB, cerebellum; HIP, hippocampus; PFC, prefrontal cortex; STR, striatum. We used type littermates (wild type canta smaller spine volume in the eIF4E-transgenic mice than in wild-type littermates (wild type > 0.05 versus wild type; Student’s t-test. WT, Student’s t-test; eIF4E-transgenic mice exhibit normal expression of other translational control proteins. n = 4 mice per genotype; *P < 0.05, Student’s t-test. 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 exhibited increased translation as measured with SUnSET (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. 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 ± 0.004 μm^3 (mean ± s.e.m.) and eIF4E-transgenic = 0.110 ± 0.004 μm^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^{29}, 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 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. eIF4E-transgenic mice exhibited 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 (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(2,40) = 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(3,138) = 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 < 0.001) followed by Bonferroni–Dunn post-hoc test. f, g, Social behaviour test. 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.
mGluR-LTD\textsuperscript{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.

![Graphs and images related to synaptic function and plasticity in different brain regions.](image)

**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, f, 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\textsuperscript{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...
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 interactions8,9, 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 occurs in eIF4E-transgenic mice.

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 J/T-EIf4E transgenic mice (eIF4E-transgenic mice) has been described previously.

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 doroventral. Mice were allowed 1 week to recover after the surgery.

The infusions of the eIF4E–eIF4G inhibitor 4EGI-1 were performed as described previously. 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, open field, elevated plus maze, social behaviour, direct social interaction, prenatoposterior, prefrontal and striatal slices (300 μm) for electrophysiology were prepared as described previously. Solution to maintain slices. Internal solution for mEPSCs (in mM): 120 csam-methane-sulphonate, 10 HEPS, 10 EDTA, 4 MgCl₂, 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.

External solution for mIPSCs (in mM): 140 CsCl, 10 MgCl₂, 2.4 mm Ca²⁺, 1 Mg²⁺, 0.5 dithiothreitola and 5 QX-314 (pH adjusted to 7.3 with CsOH, 290 mOsm). Tetrodotoxin 1 μM (Tocris) 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. In brief, two-photon imaging was accompanied with a custom microscope and high-resolution spectral imaging (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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