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Regional rates of brain protein synthesis are unaltered in dexmedetomidine sedated young men with fragile X syndrome: A L-[1-^{11}C]leucine PET study

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Abstract

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability. Fragile X mental retardation protein (FMRP), a putative translation suppressor, is absent or significantly reduced in FXS. One prevailing hypothesis is that rates of protein synthesis are increased by the absence of this regulatory protein. In accord with this hypothesis, we have previously reported increased rates of cerebral protein synthesis (rCPS) in the *Fmr1* knockout mouse model of FXS and others have reported similar effects in hippocampal slices. To address the hypothesis in human subjects, we applied the L[1-^{11}C]leucine PET method to measure rCPS in adults with FXS and healthy controls. All subjects were males between the ages of 18 and 24 years and free of psychotropic medication. As most fragile X participants were not able to undergo the PET study awake, we used dexmedetomidine for sedation during the imaging studies. We found no differences between rCPS measured during dexmedetomidine-sedation and the awake state in ten healthy controls. In the comparison of rCPS in dexmedetomidine-sedated fragile X participants ($n = 9$) and healthy controls ($n = 14$) we found no statistically significant differences. Our results from *in vivo* measurements in human brain do not support the hypothesis that rCPS are elevated due to the absence of FMRP. This hypothesis is based on findings in animal models and *in vitro* measurements in human peripheral cells. The absence of a translation suppressor may produce a more complex response in pathways regulating translation than previously thought. We may need to revise our working hypotheses regarding FXS and our thinking about potential therapeutics.

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The authors have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2020.104978.

Keywords

Fragile X syndrome; Protein synthesis; Positron emission tomography; Translation; Sedation; Dexmedetomidine; Leucine

Fragile X syndrome (FXS) is an inherited form of intellectual disability caused by loss of fragile X mental retardation protein (FMRP), an RNA-binding protein usually associated with polyribosomes. Numerous (800–6000) mRNA targets of FMRP have been reported (Darnell et al., 2011; Ascano Jr. et al., 2012) including mRNAs for about 30% of the synaptic proteome (Darnell and Klann, 2013), mRNAs for initiation and elongation factors regulating translation and for downstream components of the extracellular signal-regulated kinase pathway and mechanistic target of rapamycin complex 1 (Darnell et al., 2011). FMRP may act as a suppressor of translation (Laggerbauer et al., 2001; Li et al., 2001; Zhang et al., 2001). Proposed mechanisms of FMRP-mediated translation control include blocking initiation by interaction with cytoplasmic *FMR1*-interacting protein 1 and eukaryotic translation initiation factor 4E (Napoli et al., 2008), blocking elongation through recruitment of specific microRNA complexes to form RNA-induced silencing complexes (Plante et al., 2006; Ishizuka et al., 2002; Caudy et al., 2002), and stalling elongation by binding to actively translating ribosomal complexes (Darnell et al., 2011).

In adult *Fmr1* knockout (KO) mice, regional rates of cerebral protein synthesis (rCPS) were significantly elevated over those measured in wildtype (WT) mice, most remarkably in hippocampus (13% in CA1), thalamus (14% in lateral dorsal nuclei), hypothalamus (10% in supraoptic nuclei), and cortex (14% in frontal) (Qin et al., 2005). Studies of fibroblasts from a subset of subjects with FXS (Jacquemont et al., 2018) and of a human stem cell model (Utami et al., 2020) report elevated incorporation based on measurements with the SuNSET method. Based on findings in the mouse model, we hypothesized that dysregulation of protein synthesis is a core phenotype of this disease and that in participants with FXS we should also be able to detect increases in rCPS.

In our first study of participants with FXS we applied the L-[1-^{11}C] leucine positron emission tomography (PET) method for measurement of rCPS (Schmidt et al., 2005; Smith et al., 2005) to the study of 15 young adult subjects with FXS and 12 age-matched healthy controls. It was necessary to sedate FXS participants for these studies because they could not tolerate placement of an arterial catheter and were unable to remain motionless for the 90-min scan while we collected arterial samples. We used the hypnotic propofol because it is a widely used and safe drug, and we had already demonstrated that propofol did not affect rCPS in healthy control subjects (Bishu et al., 2009). When we compared rCPS measured in FXS participants with healthy controls, all propofol-sedated, we found that rCPS in FXS subjects were not increased in the brain as a whole or in any of the regions examined, but rather rCPS in FXS subjects were *reduced* in the whole brain (8%), cerebellum (8%), and cortex (9–10%) (Qin et al., 2013). We also found that propofol-sedation decreased rCPS in *Fmr1* KO mice (13% in hippocampus, 21% in frontal cortex, and 17% in parietal cortex), but it had only minor effects on rCPS in WT mice (Qin et al., 2013). These results suggest that propofol affects protein synthesis in the absence of FMRP, but not in its presence. Propofol

acts primarily through effects on $GABA_A$ receptors, and it is known that the $GABA_A$ system is affected in *Fmr1* KO mice (Olmos-Serrano et al., 2011; Olmos-Serrano et al., 2010; Curia et al., 2009; D'Hulst et al., 2006), in *dfmr1* flies (D'Hulst et al., 2006), and in subjects with FXS (D'Hulst et al., 2015). Selective effects of propofol on rCPS through an effect on the compromised $GABA_A$ system in FXS are in accord with our previous study in which we demonstrated that acute treatment with R-baclofen reversed increased rCPS in *Fmr1* KO mice with minimal effects on control mice (Qin et al., 2015).

Our present study was motivated by the concern that the selective effects of propofol on rCPS in FXS were interfering with our ability to uncover the effects of the absence of FMRP *per se*. We report here results of L-[1-^{11}C]leucine PET studies on subjects with FXS who were sedated with dexmedetomidine which, unlike propofol, does not work through the $GABA_A$ receptor. Rather, dexmedetomidine is an α_2-adrenergic agonist. In this study, we scanned participants with FXS with dexmedetomidine and controls both with and without dexmedetomidine. We hypothesized that rCPS would not be affected by dexmedetomidine-sedation and that, in FXS participants, rCPS would be higher than controls.

1. Materials and methods

Procedures on human subjects were carried out as described in a protocol approved by the National Institutes of Health Combined Neurosciences Institutional Review Board (06-M-0214, NCT00362843), the National Institutes of Health Radioactive Drug Research Committee, and the National Institutes of Health Radiation Safety Committee. All participants or, in the case of subjects with FXS, their legal guardians gave written informed consent prior to enrollment.

1.1. Subjects

Male full mutation FXS subjects 18–24 years of age who had taken no psychotropic medication during the previous year with no recent history of seizures were recruited to participate. Diagnosis was confirmed by DNA analysis (Mayo Clinic, Rochester, MN). Control subjects were healthy males 18 to 24 years old. Pre-enrollment screening was clinical history, physical examination and the abbreviated Structured Clinical Interview (SCID-I/NP) to rule out the possibility of Axis I disorders in DSM-IV-TR. Subjects were tested for drugs of abuse at screening and again on the day of PET scanning; none of our subjects had any evidence of drug use. Twenty healthy volunteers and twelve FXS subjects met the inclusion criteria and were enrolled in the study. One of the fragile X subjects withdrew immediately before the PET study and one was too anxious to undergo sedation, so his study was terminated. In one fragile X subject, our genetic testing failed to confirm the diagnosis of fragile X, so his data were not included. Of the twenty controls, two could not be scheduled for sedated PET scans, one withdrew before the PET scan, and one was withdrawn because he was unable to tolerate arterial line placement. Of the remaining sixteen healthy controls, two were not included due to technical problems with the PET scanning. Good quality data sets were obtained for 14 healthy controls and 9 subjects with FXS. All PET studies were performed between 8:30 AM and 1:00 PM.

1.2. Sedation regimen

Anesthesiologists administered sedation for imaging studies. Standard monitors and practice standards as recommended by the American Society of Anesthesiologists were used. For this study, we chose dexmedetomidine (Hospira Inc., Lake Forest, IL), an α_2-adrenergic agonist, with the goal of achieving motionlessness and minimal respiratory depression during the studies. For studies with sedation, an intravenous line was inserted in one arm after placement of topical anesthesia. Dexmedetomidine was administered with a loading infusion of 1 μg/kg over 10 min, which was followed by a continuous infusion titrated (1–3 μg/kg/h) as necessary to achieve motionlessness. Subjects maintained spontaneous ventilation and received supplemental oxygen *via* nasal cannula throughout the study. After study completion, subjects were transferred to the post-anesthesia care unit for monitoring.

1.3. Brain magnetic resonance imaging (MRI)

All subjects underwent a noncontrast T1-weighted MRI of the brain for region of interest (ROI) placement. MRI examinations were performed on a 3.0 Tesla scanner (Phillips Healthcare, Cleveland, OH, USA). Images were reconstructed with voxel dimensions of 0.87 × 0.87×1mm or 0.94 × 0.94 × 1 mm and interpolated to isotropic voxel dimensions of $(0.87\ mm)^3$ or $(0.94\ mm)^3$. One control subject's MRI was reconstructed with isotropic voxel dimensions of $(1.0\ mm)^3$. Mean voxel dimensions in FXS and control subjects were $(0.87\ mm)^3$ and $(0.92\ mm)^3$, respectively.

1.4. Brain regions of interest

Regions of interest (ROIs) were manually drawn with custom software written in Matlab (Mathworks, Natick, MA, USA) on each MRI by visually identifying anatomic landmarks. Parietal cortex was drawn in the coronal plane, hippocampus and amygdala in the sagittal plane and all other regions in the transverse plane.

1.5. PET studies

L-[1-^{11}C]Leucine was prepared from H^{11}CN with a modified Strecker-Bucherer reaction as previously described (Bishu et al., 2008). The acquisition protocol was identical to that previously described (Veronese et al., 2010) except for the addition of venous blood sampling in some subjects. Briefly, an arterial catheter was placed in the radial artery of the non-dominant arm for sampling arterial blood and a venous catheter in the antecubital fossa of the dominant arm for injection of tracer. In a subset of the subjects, an intravenous catheter was also placed in the contralateral antecubital fossa for venous sampling. Venous blood data were acquired for comparison with arterial blood data in other analyses; they were not used in the current study. Studies were performed on the ECAT High Resolution Research Tomograph (CPS Innovations, Knoxville, TN). After optimal positioning of the subject within the field of view, a six-minute transmission scan was obtained for attenuation correction. The emission scan was initiated coincident with the intravenous infusion of 629–1036 MBq (17–28 mCi) of L-[1-^{11}C]leucine administered by a computer-controlled infusion pump (MedFusion 3500, Harvard Apparatus, Inc., Holliston, MA, USA) at a constant rate over 2 min.

PET data were acquired over a 90-min scan interval as originally validated (Schmidt et al., 2005; Smith et al., 2005), but the data were analyzed over a 60-min scan interval, as the two analysis intervals produce almost identical results (Tomasi et al., 2018). Data were acquired in list mode and reconstructed by means of the motion-compensated 3D ordinary Poisson ordered subset expectation maximization (OSEM) algorithm (30 subsets, 2 iterations) (Carson et al., 2003). Spatial resolution after reconstruction was approximately 2.6 mm full-width-at half-maximum in radial and transverse directions (Carson et al., 2003). Three-dimensional data were reconstructed to 207 slices 1.23 mm thick with a pixel size of 1.21 × 1.21 mm. Images were reconstructed as 36 frames of data (16 × 15 *sec*, 4 × 30 sec, 4 × 60 sec, 4 × 150 sec, 8 × 300 sec). With this reconstruction, motion-correction was based on position data collected throughout the scan with the Polaris system (Bloomfield et al., 2003), and the data were corrected for attenuation based on a single attenuation-correction map (MuMap) applied to all frames of data. Some subjects, particularly those who made large abrupt movement(s) during scanning, displayed evidence of incomplete motion correction in the reconstructed data. To correct for residual motion we applied the following procedure: 1.) Summed Frames 1–17 (0–4.5 min) and aligned the summed image to the MuMap; (2.) Determined 3D rigid body spatial transformation matrices for Frames 18–36 (4.5–60 min post injection) by aligning each frame to the summed image; (3.) Applied the inverse of each transformation from the previous step to the original MuMap to obtain individual MuMaps for Frames 18–36; 4.) Reconstructed the data again utilizing the original motion-correction together with individual MuMaps for each frame; 5.) Aligned Frames 18–36 with the sum of the first 17 frames using the transformations defined in Step 2. These steps assured not only that the data from each frame were aligned to the initial frames, but also that an accurate attenuation correction was applied.

1.6. Analysis of blood samples

Arterial blood sampling was initiated concurrently with the start of the [^{11}C]leucine infusion. Arterial blood samples were hand drawn continuously (õne sample every 9–10 s) for the first 4 min, and at increasing intervals thereafter for a total of ~40 samples per study. Concentrations of unlabeled and labeled leucine in arterial plasma and total ^{11}C and $^{11}CO_2$ activities in arterial blood were measured according to the methods detailed previously (Bishu et al., 2008).

1.7. PET data analysis

For each scan, a 3D volume was constructed from the average of the emission data acquired between 30 and 60 min. This volume was isotropically smoothed with a Gaussian filter (full-width-at-half-maximum 3 mm) and aligned to the MRI volume by use of the Flexible Image Registration Toolbox (Fischer and Modersitzki, 2004) with a 3D rigid body transformation. The resliced average 30 to 60 min PET image was visually reviewed for correct alignment with the MRI by use of Vinci (Volume Imaging in Neurological Research, CoRegistration and ROIs Included; the Max Planck Institute for Neurological Research, Cologne, Germany). The transformation parameters were then applied to each frame of the PET study (without prior smoothing) to effect their alignment with the MRI volume.

The kinetic model for the behavior of leucine in brain (Suppl. Fig. 1) has been described previously (Tomasi et al., 2009). The parameters of the model were estimated for each voxel in the whole brain volume by means of the Basis Function Method (BFM) (Tomasi et al., 2009) with a slightly modified algorithm to avoid negative parameter estimates (Veronese et al., 2018). PET data beginning at the time of tracer injection and continuing for 60 min were used in all analyses. Images of each parameter were constructed, and ROIs drawn on MRIs were transferred to parametric images to compute average values of all parameters of the model.

1.8. Power analysis

Power analysis was based on the data in our published study in which rCPS in controls (n = 12) and subjects with FXS (n = 14), all studied under propofol anesthesia, were compared. We used measurements of whole brain rCPS. In that study the difference between groups was 10% with a statistical significance (p-value) of 0.004. The effect size (Cohen's d) was 0.9. We calculated that at an α error probability of 0.05 we should be able to detect a 10% difference with 8/group (power of 0.5), 10/group (power of 0.6), 13/group (power of 0.7), 16/group (power of 0.8). We aimed for 10–15/group to detect a similar difference to that seen in the study of subjects under propofol anesthesia.

1.9. Statistical analyses

Values are reported as means ± SEM. Differences between groups in physiologic variables and regional estimates of rCPS were tested for statistical significance by means of repeated measures ANOVA (rmANOVA). For this analysis we substituted groups mean values for missing values in the hippocampus and amygdala, and we did not include the whole brain region. In the case of a statistically significant interaction we further probed by Bonferroni-corrected *post-hoc t*-tests. Healthy controls were studied twice, once fully conscious and once under dexmedetomidine sedation. The order of and the interval between the two studies varied among subjects (interval mean ± SD, 61 ± 64 days, range 0.2–190 days). We used SPSS, Version 21 (IBM, Armonk, NY, USA) for statistical computations.

2. Results

2.1. Subjects

We studied 14 healthy controls and nine subjects with FXS (Table 1). Whereas mean values for weight were similar for the two groups, the variance was greater in the FXS group. CGG repeat numbers were evaluated in all FXS participants and in 11 of the healthy controls. In healthy controls, values ranged from 20 to 37; in subjects with FXS, all had only alleles with CGG repeat lengths exceeding 200 except two. These two subjects were mosaic with alleles in the premutation range and in the > 200 range. In one of these subjects the alleles in the premutation range were methylated (Hayward et al., 2019).

2.2. Effects of dexmedetomidine-sedation in healthy controls

Ten of the controls were scanned twice with L-[1-^{11}C]leucine, awake and sedated with dexmedetomidine (Fig. 1, 2; Suppl. Table 1). Comparison of rates of protein synthesis in the whole brain in awake and sedated subjects indicates that there was no statistically

significant difference between the two conditions in control subjects (Fig. 1). Rates of protein synthesis under the two conditions in the nine brain regions were compared by means of rmANOVA with region and condition as within subject variables (Table 2A). The condition x region interaction was statistically significant ($F_{8, 72} = 2.95$, $p = .019$), but *post hoc t*-tests indicate that only in the amygdala were differences in rCPS measured under the two conditions statistically significantly different (mean difference 14%, $p = .035$). In all other regions, rCPS were very similar under the two conditions. Our results indicate that dexmedetomidine-sedation has, at most, minor effects on rCPS in healthy young men.

2.3. Rates of cerebral protein synthesis in sedated fragile X subjects

Next, we compared rCPS in dexmedetomidine-sedated controls ($n = 14$) and subjects with FXS ($n = 9$) (Fig. 3&4). Mean values of physiological variables measured during dexmedetomidine sedation (Table 3) were similar for the two groups of subjects except for pCO_2 which was slightly higher in fragile X subjects, but values in both groups were in the normal range. Arterial blood pressure remained stable; one subject in the FXS group received glycopyrrolate to treat bradycardia (a known side-effect of dexmedetomidine). The total doses of dexmedetomidine administered in the two groups were not statistically significantly different, but the range in the subjects with FXS (1.0–14.5 μg/kg) was much greater than in controls (2.7–5.8 μg/kg). Median total doses in the two groups were 5.03 and 3.44 μg/kg, respectively.

Next, we compared measurements of rCPS in these two groups of dexmedetomidine-sedated subjects. In the whole brain, rCPS were not significantly different from each other ($t = 1.540$, $df = 21$, $p = .1384$) (Fig. 3). In nine brain regions, rCPS in the two groups (Fig. 4) were compared by means of rmANOVA with region as a within subject variable and diagnosis as a between subject variable (Table 2B). The diagnosis x region interaction approached statistical significance ($p = .066$). Mean values of rCPS in subjects with FXS tended to be lower compared to controls in cortical regions, but differences were not statistically significant in any region examined. Region of interest volumes (Table 4) for controls and participants with fragile X syndrome were similar except in frontal cortex in which volumes were statistically significantly smaller in fragile X participants by 9%, and in caudate in which volumes were statistically significantly larger in fragile X participants by 21%.

We looked for correlations between total dose of dexmedetomidine and rCPS in both groups of participants. In healthy controls, neither the correlations in whole brain nor in any of the nine regions were statistically significant (Supplemental Figs. 2A&3). In FXS subjects the correlation between total dose of dexmedetomidine and rCPS was statistically significant only in caudate nucleus (Pearson $r = 0.696$, $p = .04$) (Supplemental Figs. 2B&4) indicating that rCPS were higher with higher doses of dexmedetomidine.

In summary, we found no statistically significant differences in rCPS between controls and fragile X subjects studied under dexmedetomidine-sedation. Parametric images of rCPS at the level of frontal cortex in typical subjects are shown in Fig. 5.

We compared our results in participants sedated with dexmedetomidine with our previous results in participants sedated with propofol (Qin et al.2013) (Table 5). We analyzed the whole brain separately because it encompassed all the individual regions. The complete results for whole brain and the regions examined are in Table 5. For the whole brain neither the diagnosis x anesthetic interaction ($p = .551$) nor the main effect of anesthetic ($p = .823$) were statistically significant but the main effect of diagnosis was ($p = .002$) indicating that protein synthesis is 8% lower in anesthetized participants with fragile X syndrome compared to anesthetized controls. For the seven regions, we analyzed results by means of a rmANOVA with regions as a within subject variable and diagnosis and anesthetic as between subject variables. The region x diagnosis x anesthetic interaction was statistically significant ($F = 2.880$, $p = .031$; degrees of freedom (with sphericity assumed) [6, 276]). Of the regions we measured, diagnosis-dependent changes in rCPS were primarily in the cortex in both studies. In frontal cortex, rCPS were 10% lower in fragile X participants compared to controls under propofol and 5% under dexmedetomidine. In parietal cortex, rCPS were 9% lower in fragile X participants compared to controls in propofol study and 7% lower in dexmedetomidine study. In subcortical regions in which variability was greater, diagnosis-dependent differences were not statistically significant. The ROI volumes (Supplemental Table 2) differed between the studies of the two anesthetics likely due to changes in procedures over the seven-year interval in which the PET scans were administered. Despite these differences, rCPS in both studies were lower in the fragile X participants compared to controls.

3. Discussion

Contrary to our expectation, the main finding of this study is that rCPS are not increased in adult subjects with FXS compared with age-matched controls. Our results have bearing on our understanding of the pathophysiology of fragile X syndrome. Whereas current evidence supports a role of FMRP as a suppressor of translation of specific messages *in vitro*, in the intact and functioning human brain we do not find evidence of increased rates of translation that would be predicted in the absence of FMRP. Understanding the underlying pathological processes in this disease is critical for developing a rationale for treatment strategies in this disease.

We studied subjects between the ages of 18 and 24 years. Whereas it would be most interesting to study this neurodevelopmental disorder at younger ages this was not permissible in human subjects for a study that requires exposure to radiation. In our previous studies of the *Fmr1* KO mouse model we have studied adult mice at 3, 4 and 6 months of age, and in all cases, we found higher rCPS in *Fmr1* KO mice compared with WT. The ages studied in the mouse are equivalent to a mature adult human (28–40 years). In the mouse, studies at younger ages are not feasible because of limitations in our ability to implant catheters in mice weighing less than 20 g. Perhaps in the *Fmr1* KO rat we will be able to study the effects of loss of FMRP during brain development.

In the present study, determinations of rCPS were conducted while subjects were deeply sedated with dexmedetomidine. The need to sedate subjects adds a level of complexity to the interpretation of the results. We showed that rCPS in most regions are unaffected by

dexmedetomidine sedation in healthy young men, but we cannot rule out the possibility that dexmedetomidine could have selective effects on rCPS in subjects with FXS. We attempted to study the effects of dexmedetomidine in *Fmr1* KO mice to test this idea, but these experiments were not possible. To sedate mice with dexmedetomidine it was necessary to administer about 100 times the human dose. This high dose resulted in an effect on pancreatic α_2 adrenergic receptors and an inhibition of insulin release. Under these conditions, arterial plasma glucose concentrations rose to over 400 mg/100 ml. These unphysiological conditions are problematic for the measurement of rCPS, so we abandoned these experiments.

In our previous study of rCPS in subjects with FXS we had used propofol for sedation. In that study we found statistically significant decreases in rCPS in the whole brain, cerebellum, and cortex (Qin et al., 2013). Further investigation of rCPS in mice indicated that propofol had a selective effect on rCPS in *Fmr1* KO mice. With the use of a sedating agent that did not act on the GABA system, as propofol does, we hoped to be able to reveal how protein synthesis is affected in the human disease. Our strategy of using an agent that acts on α_2-adrenergic receptors for sedation seemed a reasonable approach. We note, however, that treatment with an α_2-adrenergic agonist such as clonidine in FXS boys is reported by a parent survey to be effective in addressing hyperactivity and hyperarousal (Berry-Kravis and Potanos, 2004; Hagerman et al., 1995).

It is possible that sedation itself may affect protein synthesis differently in the presence and absence of FMRP. In several previous studies of protein synthesis in animal models of FXS increased rates of rCPS have been reported. In our studies in which *in vivo* measurements of rCPS in awake adult *Fmr1* KO mice were made, we found regionally selective increases in rCPS, particularly in cortex, hippocampus, and parts of the thalamus and hypothalamus (Qin et al., 2005; Qin et al., 2015; Liu et al., 2012). It has also been reported that incorporation of labeled methionine is elevated in hippocampal slices from *Fmr1* KO mice (Dolen et al., 2007; Osterweil et al., 2010; Henderson et al., 2012; Osterweil et al., 2013) and rats (Till et al., 2015). In all these experiments, animals had received anesthesia prior to measurements. In the *in vivo* experiments, mice had been anesthetized with isoflurane, a GABA agonist, for insertion of vascular catheters 24 h prior to the measurement. In the *in vitro* studies on hippocampal slices, animals were overdosed with Nembutal, also a GABA agonist, prior to preparation of hippocampal slices. In our recent studies of animals that did not receive anesthesia prior to preparation of the slices, however, we did not see a statistically significant difference in incorporation of labeled leucine into protein in hippocampal slices between WT and *Fmr1* KO mice (Cooke et al., 2019). Our PET data on the effects of propofol-anesthesia (Bishu et al., 2009) and dexmedetomidine-sedation in human subjects indicate that these drugs have no effects on rCPS in controls. Our work in mice indicates that propofol has little effect on rCPS in WT but decreases rCPS in *Fmr1* KO mice (Qin et al., 2013). We have not studied the effects of isoflurane, Nembutal, or dexmedetomidine on rCPS in *Fmr1* KO mice. It is possible that it is the sedation-induced diminution of neuronal activity *per se* and not an effect on a specific receptor that is responsible for the change in rCPS in subjects with FXS. The only way to answer this question is to devise a way to measure rCPS in subjects with FXS in the awake state. We recently validated a modification to the original L-[1-^{11}C]leucine PET protocol in which we shortened the study duration and

substituted venous for arterial blood sampling (through use of venous-calibrated population-derived input functions) in order to make it easier for subjects to tolerate the PET scanning procedure (Tomasi et al., 2018; Tomasi et al., 2019). This should enable some subjects with FXS to complete a L-[1-^{11}C]leucine PET study in the awake state and help address this question.

4. Conclusions

Our results indicate that the absence of the translation suppressor, FMRP, may produce a more complex response in pathways regulating translation than previously thought. Our findings call for a revision of our working hypotheses and our thinking about potential therapeutics for FXS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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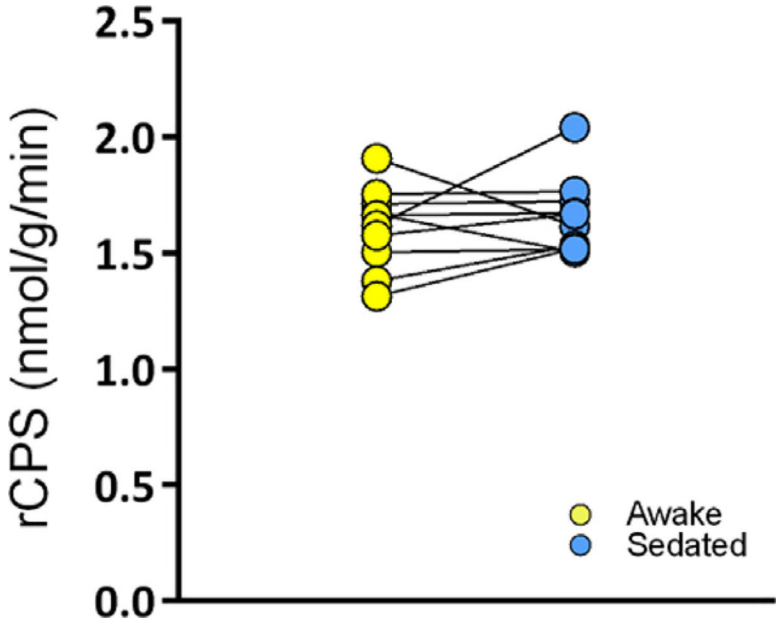

Fig. 1.

rCPS in whole brain in ten controls each studied awake and under dexmedetomidine-sedation. Each point is an individual value, and values determined in the awake and sedated conditions in each subject are connect by a line. Rates of protein synthesis in the whole brain in the two conditions were compared by paired *t*-test ($t = 0.7543$, df = 9, $p = .470$).

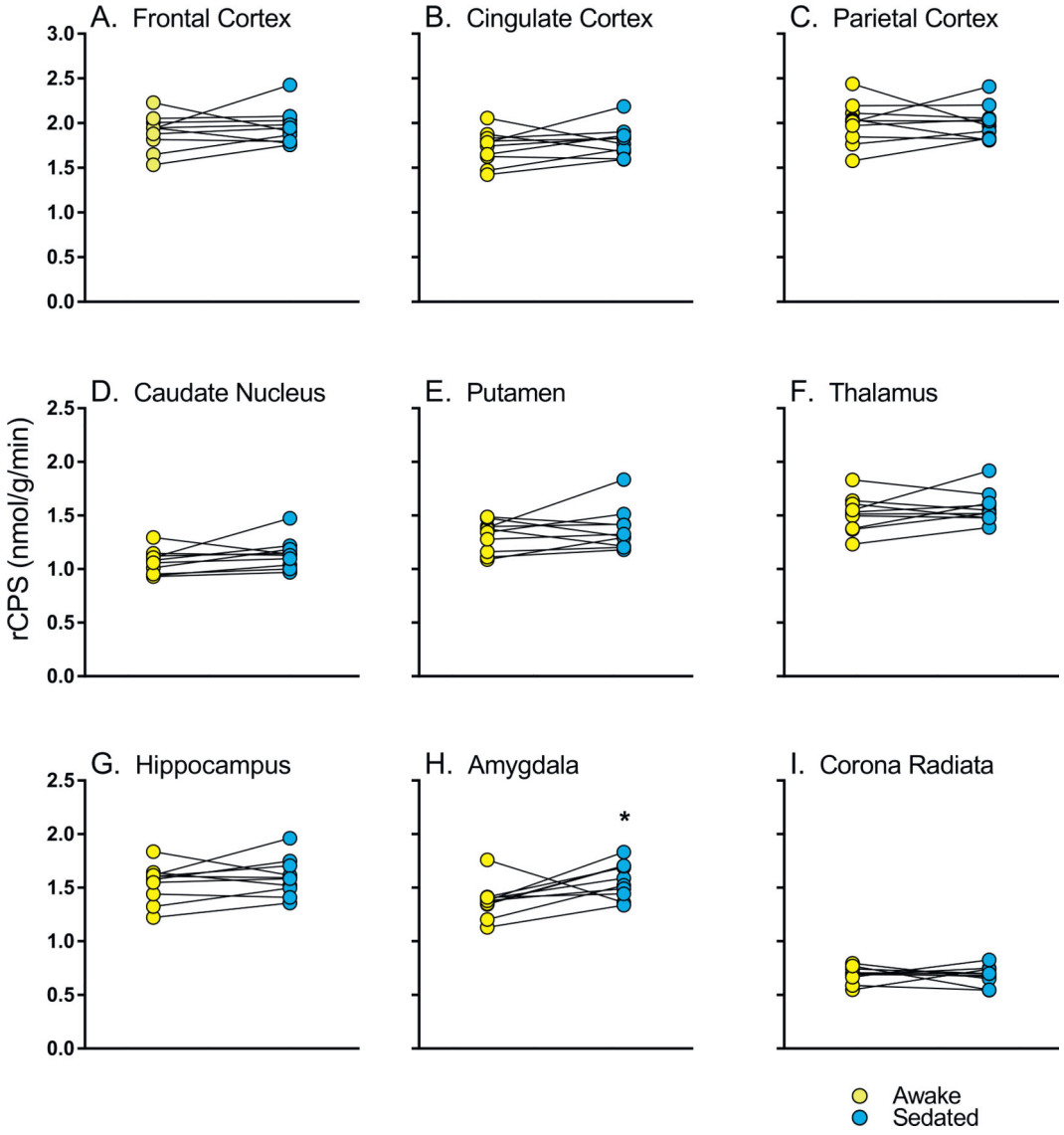

Fig. 2.

rCPS in ten controls each studied awake and under dexmedetomidine-sedation. Each point is an individual value, and values determined in the awake and sedated states in each subject are connected by a line. The two conditions were compared by means of rmANOVA with both region and condition as within subject variables. The condition x region interaction was statistically significant ($p = .019$), but Bonferroni-corrected *post hoc t*-tests indicate that only in the amygdala were differences in rCPS measured under the two conditions statistically significantly different (*, mean difference 14%, $p = .035$). In all other regions mean rCPS were very similar under the two conditions.

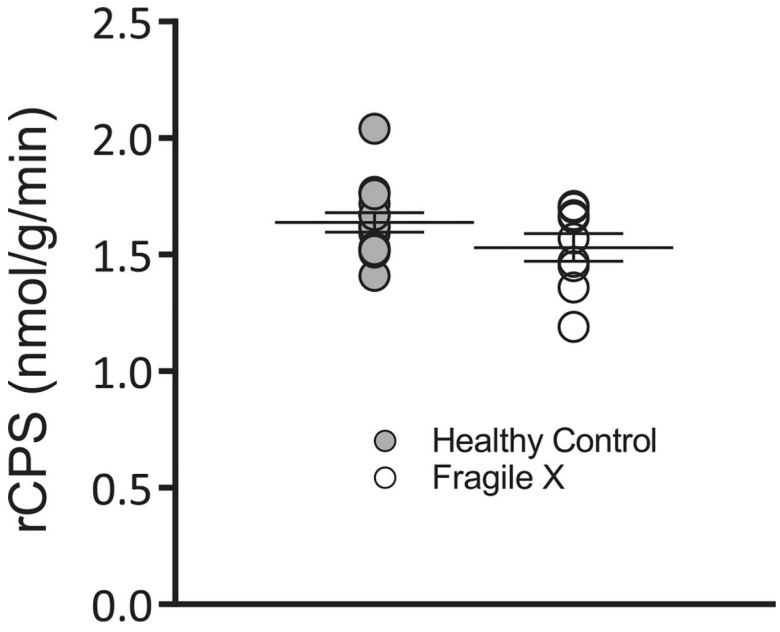

Fig. 3.

rCPS in whole brain in nine FXS subjects and 14 controls all studied under dexmedetomidine sedation. Each point is an individual value. The lines represent the means ± SEMs. Values compared by means of unpaired *t*-test ($t = 1.540$, df = 21, $p = .138$) were not statistically significantly different.

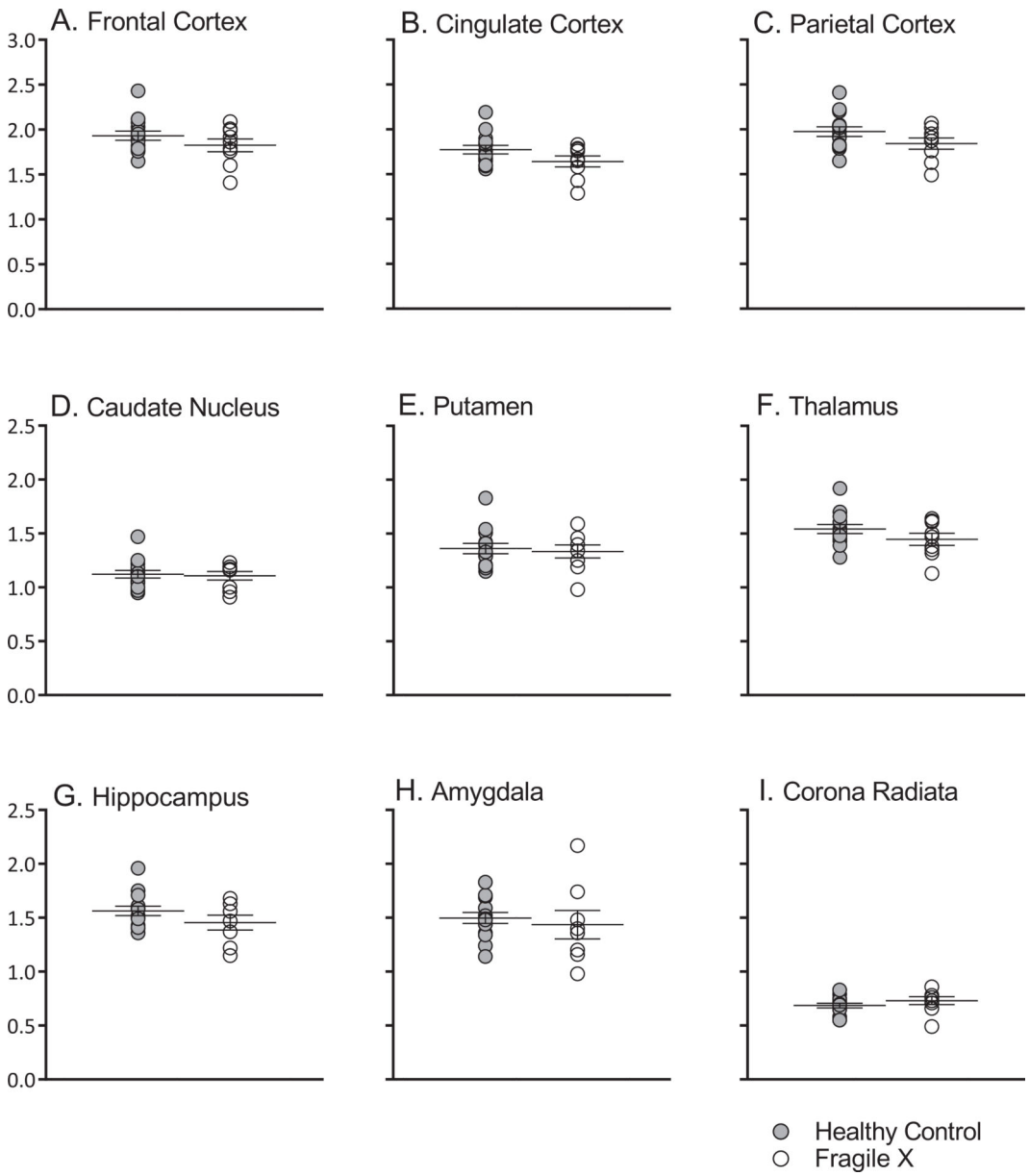

Fig. 4.

rCPS in nine brain regions in nine FXS subjects and 14 controls all studied under dexmedetomidine sedation. Each point is an individual value. The lines represent the means ± SEMs. The effects of diagnosis were compared by means of rmANOVA with region a within subject variable and diagnosis a between subject variable. The diagnosis x region interaction was not statistically significant ($p = .066$).

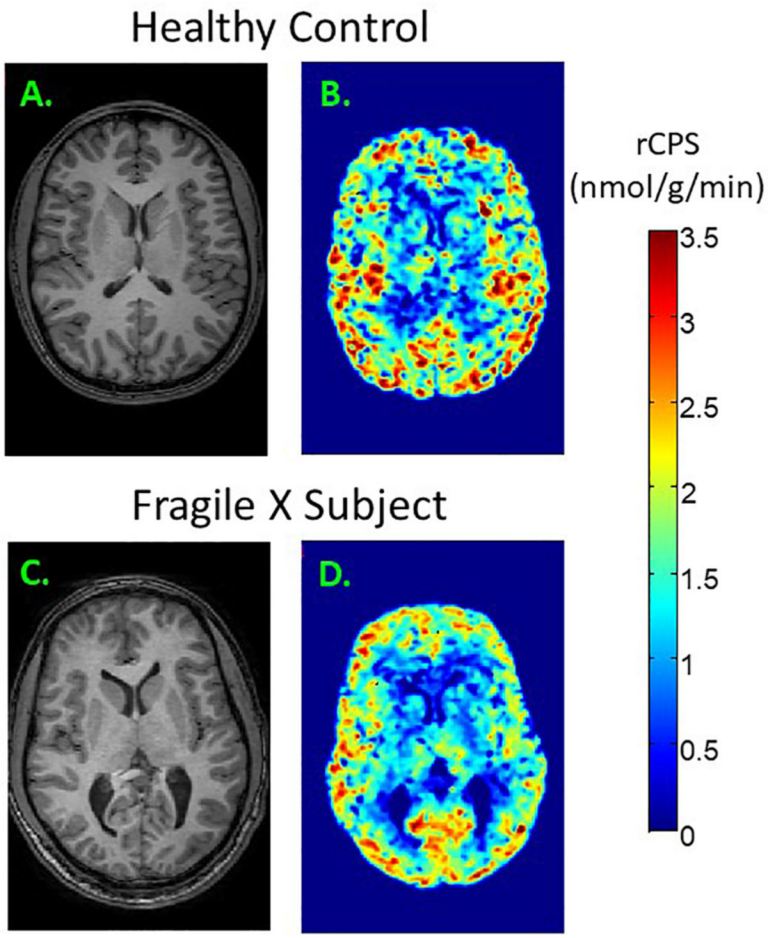

Fig. 5.

Representative parametric images of rCPS at the level of the frontal cortex in a typical control (B.) and FXS subject (D.) both studied under dexmedetomidine sedation. Magnetic resonance images on the left (A., C.) correspond to rCPS images on the right. Parametric images were smoothed with a 3D Gaussian filter (kernel 2.6 mm full-width-at half-maximum) and color-coded for rCPS (colorbar). Images are in the transverse plane.

Table 1

Subjects.

	Healthy controls (14)	Fragile X subjects (9)
Age (yr)	21.8 ± 0.4	21.2 ± 0.9
Height (m)[a]	1.76 ± 0.02	1.76 ± 0.03
Weight (kg)	78.2 ± 2.5	77.8 ± 4.7
FMR1 CGG repeat number[b]	30 ± 1 (11)	> 200 (7)[c]

Values are means ± SEM.

[a]Height was not measured in two controls, so $n = 12$.

[b]FMR1 CGG repeat number was analyzed in all FXS participants and eleven controls.

[c]Two of the nine subjects with FXS were mosaic with one allele with > 200 CGG repeats and the others in the premutation range. All other subjects were full mutation FXS with > 200 CGG repeats.

Table 2

ANOVA results for rCPS measurements.

Interaction and main effects	Degrees of freedom[a]	F	p-value
A. Controls awake *versus* sedated with dexmedetomidine			
Region x condition	[8, 72]	2.947	.019
Region	[8, 72]	450.177	< .001
Condition	[1, 9]	1.439	.261
B. Dexmedetomidine-sedated controls *versus* subjects with fragile X syndrome			
Region x diagnosis	[8, 168]	2.310	.066
Region	[8, 168]	354.144	< .001
Diagnosis	[1,21]	1.090	.308

[a]Degrees of freedom reported assume sphericity. Values of F were computed with the Huynh-Feldt correction.

Table 3

Physiological variables during dexmedetomidine anesthesia.

	Healthy controls (14)	Fragile X subjects (9)
Mean arterial blood pressure (mm Hg)[§]	88 ± 2	89 ± 5
Heart rate (bpm)[§]	54 ± 2	58 ± 4
Respiration rate (bpm)[§§]	18 ± 2	17 ± 2
Arterial blood pCO_2 (mm Hg)	42 ± 1	45 ± 1[*]
Arterial blood pO_2 (mm Hg)	164 ± 10	145 ± 12
Arterial blood pH[§]	7.40 ± 0.01	7.38 ± 0.01
Hematocrit %	50 ± 1	52 ± 1
Arterial blood glucose (mM)	5.2 ± 0.2	5.6 ± 0.3
Arterial plasma leucine (nmol/ml)	126.0 ± 6.5	113.6 ± 5.1
Dexmedetomidine total dose (μg/kg)	3.9 ± 0.3	6.1 ± 1.4

Values are the means ± SEM. Measurements reported were taken just before injection of tracer.

In several cases we were unable to take measurements as indicated.

[§] $N = 13$ for controls

[§§] $N = 12$ for controls.

[*] Statistically significantly higher than control value, $p = .03$, Student's t-test.

Table 4

Region of interest volumes (cc).

	Control (14)	Fragile X (9)
Whole brain	1383.7 ± 20.6	1350.4 ± 42.8
Frontal cortex	175.6 ± 3.9	159.1 ± 3.4**
Parietal cortex	27.8 ± 0.6	27.8 ± 0.6
Cingulate cortex	17.2 ± 0.7	15.5 ± 1.0
Hippocampus[a]	7.8 ± 0.6	7.1 ± 0.5†
Amygdala[a]	1.1 ± 0.1	1.0 ± 0.1
Thalamus	15.7 ± 0.6	15.5 ± 0.6
Caudate	6.8 ± 0.4	8.2 ± 0.6*
Putamen	7.8 ± 0.3	7.3 ± 0.4
Corona radiata	5.2 ± 0.2	4.9 ± 0.2

Values are the means ± SEM for the number of subjects indicated in parentheses except where indicated.

[a]14 controls and 8 subjects with fragile X syndrome. In one subject with fragile X syndrome, the quality of the MRI was not sufficient to accurately identify the hippocampal and amygdala volumes. For the ANOVA we substituted the mean values of the group for the missing values. Data were analyzed by means of a rmANOVA with region as a within subject factor and diagnosis as a between subject factor. We excluded the whole brain ROI from the ANOVA. The region x diagnosis interaction was statistically significant ($F = 8.026$, $p = .005$; degrees of freedom (with sphericity assumed) [8, 168]). The main effect of region was statistically significant as expected ($F = 2970.055$, $p < .001$). The main effect of diagnosis was also statistically significant ($F = 6.938$, $p = .016$; degrees of freedom (with sphericity assumed) [1, 21]). Statistical significance for regional differences between groups is indicated as follows:

*, $.01 \leq p \leq .05$

**, $.001 \leq p \leq .01$

†, $.05 \leq p \leq .10$.

Table 5

Comparison of effects of fragile X diagnosis on rCPS in subjects sedated with propofol and dexmedetomidine.

Region	rCPS (nmol/g/min)			
	Propofol		Dexmedetomidine	
	Control (12)	Fragile X (15)	Control (14)	Fragile X (9)
Whole brain[b]	1.67 ± 0.03	1.52 ± 0.03	1.64 ± 0.04	1.53 ± 0.06
Frontal cortex	2.07 ± 0.04	1.86 ± 0.04**	1.93 ± 0.05	1.83 ± 0.07
Parietal cortex	2.01 ± 0.04	1.82 ± 0.04**	1.98 ± 0.05	1.84 ± 0.06†
Hippocampus[a]	1.55 ± 0.05	1.56 ± 0.04	1.56 ± 0.04	1.46 ± 0.07
Amygdala[a]	1.49 ± 0.09	1.45 ± 0.05	1.50 ± 0.05	1.44 ± 0.13
Thalamus	1.65 ± 0.05	1.68 ± 0.04	1.54 ± 0.04	1.45 ± 0.06
Caudate	1.13 ± 0.04	1.09 ± 0.03	1.12 ± 0.04	1.11 ± 0.04
Putamen	1.43 ± 0.06	1.31 ± 0.03†	1.36 ± 0.05	1.33 ± 0.06

Values are the means ± SEM for the number of subjects indicated in parentheses except where indicated.

Data from subjects sedated with propofol were published previously (Qin et al., 2013).

Data were analyzed by means of a 3-way ANOVA with region as a within subject factor and diagnosis and anesthetic as between subject factors.

The region x diagnosis x anesthetic interaction was statistically significant (F = 2.880, p = .031; degrees of freedom (with sphericity assumed) [6, 276]). The region x anesthetic interaction was statistically significant (F = 5.195, $p = .001$), the region x diagnosis interaction was statistically significant (F = 4.127, $p = .005$) and the main effect of region was statistically significant (F = 437.177, $p < .001$). The diagnosis x anesthetic interaction was not statistically significant (F = 0.000, $P = .985$); neither were the main effects of anesthetic (F = 1.123, $P = .295$) nor diagnosis (F = 3.152, $p = .082$). We analyzed for individual regional differences between controls and subjects with FXS by means of *t*-tests corrected for multiple comparisons. Levels of statistical significance are denoted as follows:

*, $0.01 \leq p \leq .05$

**, $0.001 \leq p \leq .01$

†, $0.05 \leq p \leq .10$.

[a] 11 controls and 15 subjects with fragile X syndrome sedated with propofol and 15 controls and 8 subjects with fragile X syndrome sedated with dexmedetomidine. In one control sedated with propofol and one subject with fragile X syndrome sedated with dexmedetomidine, the quality of the MRI was not sufficient to accurately identify the hippocampal and amygdala volumes.

[b] Whole brain was not included in the statistical analysis. A separate ANOVA of the whole brain rCPS indicates that neither the diagnosis x anesthetic interaction (F = 0.362, $p = .551$; degrees of freedom were 1, 46) nor the main effect of anesthetic (F = 0.051, $p = .823$) were statistically significant, but the main effect of diagnosis was (F = 10.850, $p = .002$).