SUPPLEMENTAL MATERIAL Schmidt et al. 3/29/2022

SUPPLEMENTAL TABLE 1. rCPS (nmol/g/min) †

	Cohort I		Cohort II	
	Healthy Controls (10)	Fragile X Subjects (5)	Healthy Controls (6)	Fragile X Subjects (5)
Whole brain**	1.43 ± 0.18	1.14 ± 0.13	1.46 ± 0.13	1.26 ± 0.15
Frontal cortex**	1.69 ± 0.21	1.29 ± 0.15	1.67 ± 0.15	1.40 ± 0.14
Parietal cortex**	1.78 ± 0.24	1.31 ± 0.15	1.75 ± 0.16	1.47 ± 0.15
Cingulate cortex**	1.53 ± 0.19	1.20 ± 0.13	1.54 ± 0.14	1.21 ± 0.14
Hippocampus*	1.38 ± 0.16	1.16 ± 0.12	1.35 ± 0.13	1.23 ± 0.10
Amygdala	1.21 ± 0.14	1.19 ± 0.23	1.22 ± 0.20	1.10 ± 0.19
Thalamus**	1.36 ± 0.20	1.07 ± 0.13	1.41 ± 0.08	1.19 ± 0.14
Caudate**	0.93 ± 0.08	0.73 ± 0.10	0.93 ± 0.06	0.81 ± 0.11
Putamen**	1.16 ± 0.13	0.93 ± 0.10	1.14 ± 0.07	1.02 ± 0.13
Corona radiata	0.61 ± 0.06	0.51 ± 0.13	0.62 ± 0.04	0.61 ± 0.10

Values are the means ± SD for the number of subjects indicated in parentheses.

† We assumed a tissue density of 1.0 g/mL.

All procedures for PET scanning were identical in all subjects. We added a few extra venous blood draws in Cohort II. Data from the regional analysis were subjected to a mixed model ANOVA with region as a within subject variable and diagnosis and cohort as between subject variables. The region x diagnosis x cohort ($F_{8,176}$=1.463, p=0.217) interaction was not statistically significant. Neither the region x cohort ($F_{8,176}$=0.875, p=0.494) nor the diagnosis x cohort ($F_{1,22}$=1.152, p=0.338) interactions were statistically significant, but the region x diagnosis ($F_{8,176}$=12.068, p<0.001) interaction was statistically significant. *Post-hoc* t-tests for region x diagnosis indicate that, regardless of cohort, rCPS were statistically significantly lower in participants with FXS in most regions as indicated in the table: *, $0.01 \geq p \geq 0.001$; **, $p < 0.001$. Whole brain rCPS were

analyzed by means of a two-way ANOVA with diagnosis and cohort as between subject variables. Neither the cohort x diagnosis ($F_{1,22}$=0.520, p=0.478) interaction nor the main effect of cohort ($F_{1,22}$=1.238, p=0.278) was statistically significant, but the main effect of diagnosis ($F_{1,22}$=14.70, p=0.0009) was. Regardless of cohort, whole brain rCPS was 17% lower in subjects with FXS compared with healthy controls.

SUPPLEMENTAL FIGURES

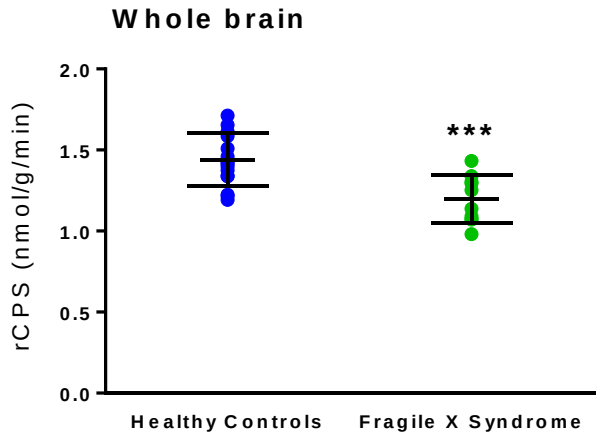
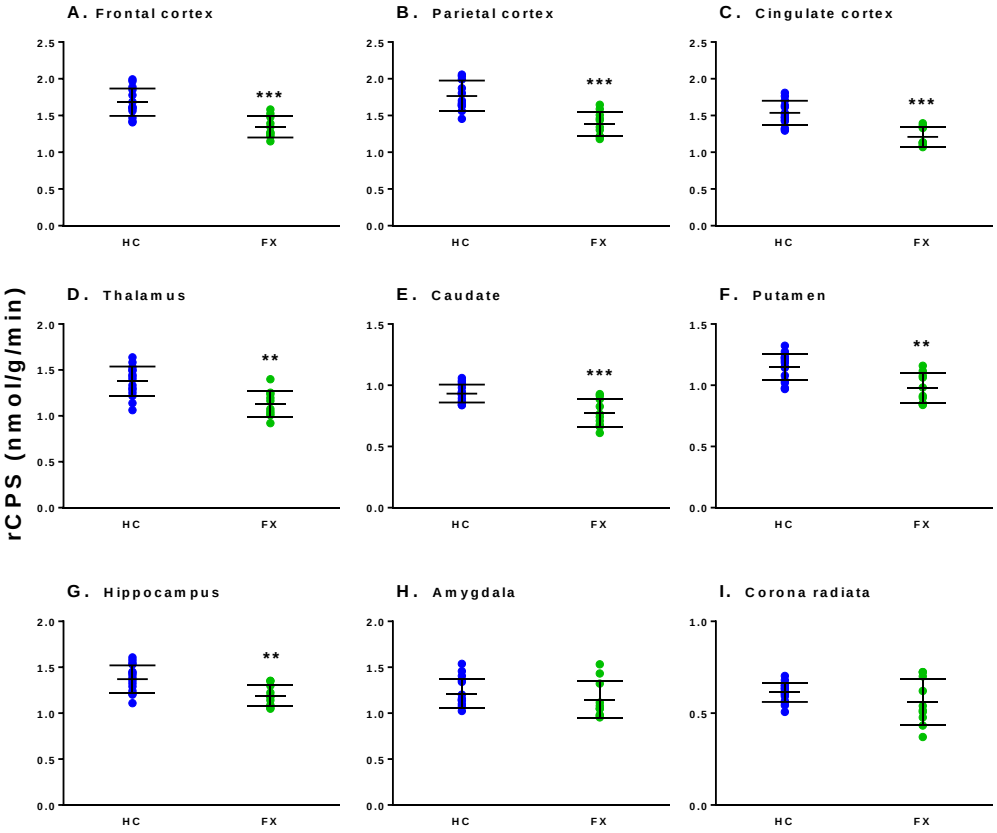


Fig. S1 rCPS in whole brain in healthy controls and participants with fragile X syndrome

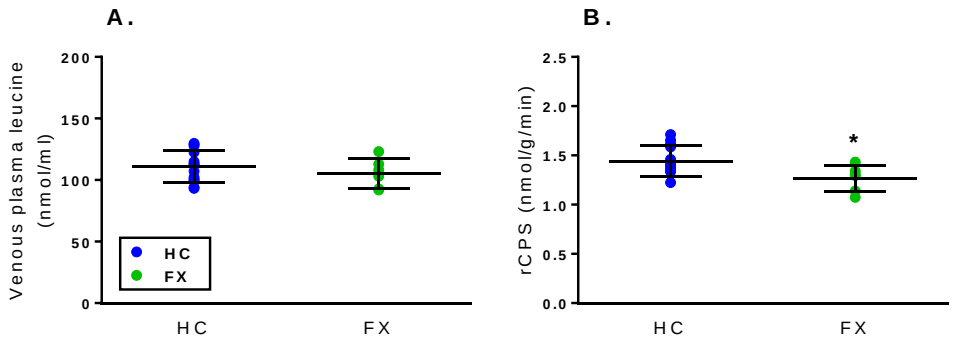
Each point represents the measurement in a single subject. Horizontal lines are the means and SDs for 16 healthy controls and 10 participants with FXS. Statistical significance noted on the figure refers to the unpaired t-test result (t=3.872, df=24, p=0.0007) indicating that mean rCPS in whole brain is 17% lower in participants with FXS.

Fig. S2 rCPS in nine brain regions in healthy controls and participants with fragile X syndrome



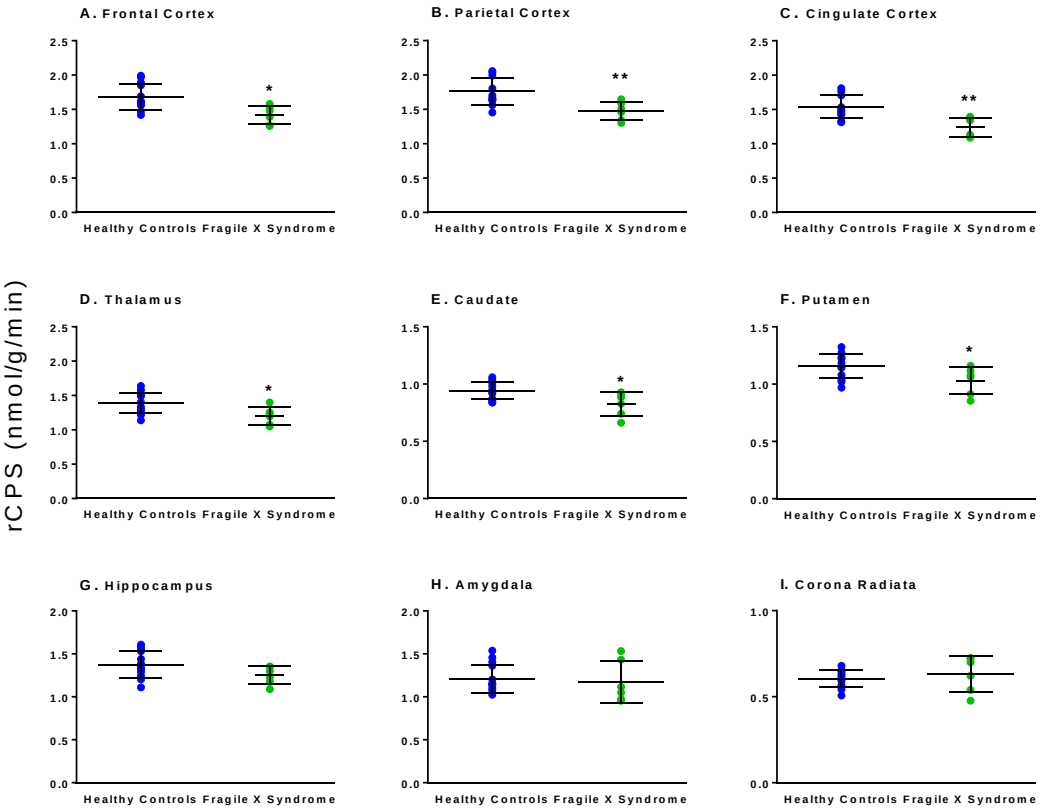
Each point represents the measurement in a single subject. Horizontal lines are the means and SDs for 16 healthy controls and 10 participants with FXS. Results of statistical analysis are presented in Table 3. Statistical significance noted on the panels refers to the results of *post-hoc* t-tests following a mixed model ANOVA with diagnosis as a between subject variable and region as a within subject variable. *, $0.05 \geq p \geq 0.01$; **, $0.01 \geq p \geq 0.001$; ***, $0.001 \geq p$.

Fig S3. rCPS in whole brain in plasma leucine-matched healthy controls and participants with fragile X syndrome



Time-weighted plasma leucine concentrations (A) and whole brain rCPS (B) in healthy controls and participants with FXS with weighted average venous plasma leucine concentrations between 90 and 130 nmol/ml. Each point represents the measurement in a single subject. Horizontal lines are the means and SDs for the following number of subjects: healthy controls, n=13; FX subjects, n=6. Legend in A. applies to both panels. Plasma leucine data were subjected to a two tailed t-test (t=0.9428, df=17; p = 0.359) indicating no statistically significant difference between the diagnostic groups. Whole brain rCPS data were subjected to a two-tailed t-test (t=2.401, df=17; p = 0.028) indicating that rCPS was 12% lower in participants with FXS compared to healthy controls. *, $0.05 \geq p \geq 0.01$.

Fig. S4. rCPS in nine brain regions in plasma leucine-matched healthy controls and participants with fragile X syndrome

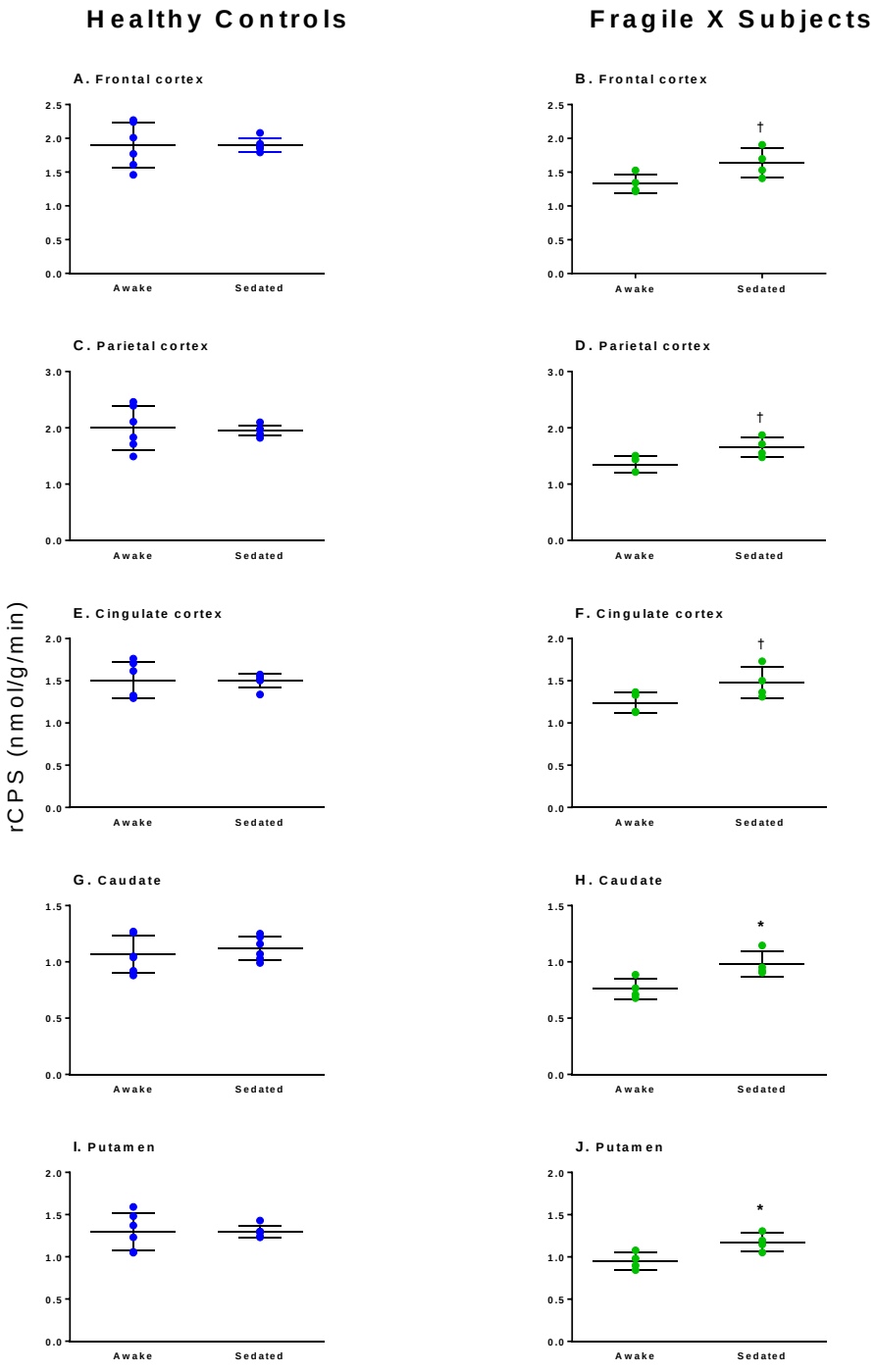


Regional rCPS in subjects with time-weighted average venous plasma leucine concentrations between 90 and 130 nmol/ml. Each point represents the measurement in a single subject. Horizontal lines are the means and SDs for the following number of subjects: healthy controls, n=13; participants with FXS, n=6. Data from the regional analysis were subjected to a mixed model ANOVA with region as a within subject variable and diagnosis as a between subject variable. The region x diagnosis ($F_{8,136}$=7.59, $p < 0.001$) interaction was statistically significant. *Post-hoc* t-tests indicate that rCPS were statistically significantly lower in subjects with FXS in most regions as indicated on the figure: *, $0.05 \geq p \geq 0.01$; **, $p < 0.01$. Statistically significant percent differences were: frontal cortex, 16%; parietal cortex, 16%; cingulate cortex, 20%; thalamus, 14%; caudate, 12%; putamen, 11%. Mean value in hippocampus was 9% lower in hippocampus in subjects with FXS, but this effect did not reach statistical significance (p=0.096). In amygdala and corona radiata mean values were similar in both groups.

EFFECTS OF DEXMEDETOMIDINE SEDATION ON rCPS IN HEALTHY CONTROLS AND FRAGILE X SUBJECTS

In our previous study in which subjects were sedated with dexmedetomidine, we found no statistically significant difference between sedated healthy controls and participants with FXS (*1*). Comparison of the present results with those previous studies suggests that dexmedetomidine sedation has effects on rates of translation in FXS subjects. In healthy controls, we have shown previously it does not (*1*). In four of the subjects with FXS and six of the healthy controls in the present study we had also measured rCPS in the same subject under dexmedetomidine sedation. Conditions of sedation were described previously. We compared rCPS under the two conditions (awake, sedated) in this limited number of subjects from the two diagnostic groups (healthy controls, FXS subjects). For this analysis we used only PET data analyzed by means of the venous calibrated population derived input function method used in the present study and as validated previously (*2*). We subjected these paired studies for each diagnostic group to ANOVA with both brain region (frontal cortex, parietal cortex, cingulate cortex, thalamus, caudate, putamen, hippocampus, amygdala, and corona radiata) and condition (awake, sedated) as within subject variables. In healthy controls neither the region x condition interaction [$F_{8,40} = 1.033$, $p=0.405$] nor the main effect of condition [$F_{1,40} = 0.004$, $p=0.951$] was statistically significant, but as expected the main effect of region was [$F_{8,40} = 255.213$, $p < 0.001$] (Fig. S5 A,C,E,G,I). In the comparison, in participants with FXS, both the region x condition interaction [$F_{8,24} = 2.688$, $p=0.084$] and the main effect of condition [$F_{1,24} = 4.533$, $p=0.123$] approached statistical significance (fig. S5 B,D,F,H,J). In the healthy controls the mean rCPS are almost identical under both conditions confirming our previous results (*1*). In contrast, in the FXS subjects, rCPS were 20-30% higher under dexmedetomidine sedation compared to awake.

Fig. S5. rCPS in healthy controls and fragile X subjects: paired comparisons awake v dexmedetomidine sedation



Effects of sedation with dexmedetomidine on rCPS in healthy controls (n=6) and participants with FXS (n=4). Each point represents the measurement in a single subject. Horizontal lines are the means and SDs. Each subject was studied twice, once awake and a second time under deep dexmedetomidine sedation.

Conditions of sedation were described previously (*1*). In this analysis we used only PET data analyzed by means of the venous calibrated population derived input function method as previously validated. Values for rCPS in nine brain regions were analyzed by means of ANOVA with both region (frontal cortex (A, B), parietal cortex (C, D), cingulate cortex (E, F), thalamus, caudate (G, H), putamen (I, J), hippocampus, amygdala, and corona radiata) and condition (awake, sedated) as within subject variables. Healthy volunteers (A, C, E, G, I) and subjects with FXS (B, D, F, H, J) were analyzed separately. ANOVA results for healthy controls indicate that neither the region x condition interaction [$F_{8,40} = 1.033$, $p=0.405$] nor the main effect of condition [$F_{1,40} = 0.004$, $p=0.951$] was statistically significant, but the main effect of region was [$F_{8,40} = 255.213$, $p < 0.001$]. ANOVA results for subjects with FXS indicate that the region x condition interaction [$F_{8,24} = 2.688$, $p=0.084$] and the main effect of condition [$F_{1,24} = 4.533$, $p=0.123$] approached statistical significance. The main effect of region was [$F_{8,24} = 107.019$, $p < 0.001$] statistically significant. *Post-hoc* paired t-tests were explored and results are indicated as follows: †, $0.10 \geq p \geq 0.05$; *, $0.05 \geq p \geq 0.01$. Paired t-test results in FXS subjects for regions not shown were: thalamus, p=0.110; hippocampus, p=0.241; amygdala, p=0.965; corona radiata. p=0.131. Mean ± SD percent effects were frontal cortex, 24 ± 20%; cingulate cortex, 20 ± 16%; parietal cortex 24 ± 16%; caudate 30 ± 18%; and putamen 24 ± 12%.

SUPPLEMENTAL REFERENCES

1. K. C. Schmidt, I. Loutaev, Z. Quezado, C. Sheeler, C. B. Smith, 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. *Neurobiology of disease* **143**, 104978 (2020).
2. G. Tomasi, M. Veronese, A. Bertoldo, C. B. Smith, K. C. Schmidt, Substitution of venous for arterial blood sampling in the determination of regional rates of cerebral protein synthesis with L-[1-C-11]leucine PET: A validation study. *J Cerebr Blood F Met* **39**, 1849-1863 (2019).