Rapid Communication

Dysregulated *COMT* expression in fragile X syndrome

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Abstract

Transcriptional and proteomics analyses in human fragile X syndrome (FXS) neurons identified markedly reduced expression of *COMT,* a key enzyme involved in the metabolism of catecholamines, including dopamine, epinephrine and norepinephrine. FXS is the most common genetic cause of intellectual disability and autism spectrum disorders. *COMT* encodes for catechol-o-methyltransferase and its association with neuropsychiatric disorders and cognitive function has been extensively studied. We observed a significantly reduced level of *COMT* in in FXS human neural progenitors and neurons, as well as hippocampal neurons from *Fmr1* null mice. We show that deficits in *COMT* were associated with an altered response in an assay of dopaminergic activity in *Fmr1* null mice. These findings demonstrate that loss of FMRP downregulates *COMT* expression and affects dopamine signaling in FXS, and supports the notion that targeting catecholamine metabolism may be useful in regulating certain neuropsychiatric aspects of FXS.

Keywords:

RNAseq, proteomics, fragile X syndrome, isogenic stem cell model, neurons*, COMT*

Background

Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability and autism, affecting 1 in 4,000 males and 1 in 8,000 females(1,2). It is caused by a CGG trinucleotide repeat expansion at the promoter region of the *FMR1* gene, leading to hypermethylation and silencing of its protein product, Fragile X Messenger Ribonucleoprotein 1 (FMRP)(2). Apart from cognitive impairment, patients with FXS present with several behavioral abnormalities including hyperactivity, impulsivity, anxiety, and aggression(2,3). Although the loss of FMRP is the primarily mechanism underlying FXS, there is a range of clinical severity among individuals with the disorder. Recent studies have investigated the role of secondary genes in the behavioral abnormalities associated with FXS. Among the genes studied, single nucleotide polymorphisms (SNPs) in *COMT* were found to be associated with reduced risk for compulsive and stereotyped behavior(4). *COMT* encoding for cathecol-o-methyltransferase, plays a role in the metabolism of catecholamines including dopamine to regulate their levels in the brain. In humans, *COMT* is located at chromosome 22 and contains functional polymorphisms that have been shown to affect its activity. One such polymorphism is a guanine-to-adenine (G>A) substitution which results in a valine-to-methionine (Val>Met) at amino acid 158 of the translated protein. The Val158Met substitution in COMT has been reported to reduce its enzymatic activity, resulting in slower degradation and higher dopamine levels in the prefrontal cortex(5). Recent meta-analyses showed an association between the Val158Met substitution and psychiatric disorders including schizophrenia(6–8). Variations in *COMT* have also been associated with a range of behavioral abnormalities, including ADHD and aggressive behavior(9,10). Here, we show that the loss of FMRP leads to lower levels of COMT expression in both human and mouse neurons, and is associated with altered dopaminergic neurotransmission in a mouse model of FXS.

Results

Reduced expression of COMT in FMRP-deficient neurons

To investigate whether *COMT* expression is altered in FXS, we examined RNA-seq analysis performed on both isogenic and human pluripotent stem cell (hPSC)-derived FXS neurons(11), referred as *FMR1*KO and FXS, respectively. Collectively, due to their absence of FMRP expression, we refer both *FMR1*KO and FXS hPSCs as FMRP-deficient hPSCs throughout the text (11,12). We found a significant reduction in *COMT* expression in FMRP-deficient neurons (**Fig. 1a**), which we further validated by qRT-PCR (**Fig.1b**). Proteomics analysis similarly showed significant reduction of COMT protein expression in neurons (**Fig. 1c)**(11). We also found that this reduction in *COMT* expression is not limited to mature neurons but also present in FMRP-deficient neural progenitor cells (NPCs) (**Fig. 1d**).

To examine the translational status of *COMT* mRNA, we performed polysome profiling on FMRP-deficient NPCs. Cycloheximide-treated NPCs were separated through sucrose gradient fractionation, and *COMT* mRNA present in polysome (translating) fractions was quantified by qRT-PCR. We observed that the levels of *COMT* mRNA in translating fractions was markedly reduced in FMRP-deficient NPCs (**Fig. 1e**). Consistent with this finding, COMT protein expression was completely absent in FXS neurons (**Fig. 1f**).

Next, we investigated whether COMT expression is preferentially affected in excitatory versus inhibitory neurons. Immunofluorescence analysis for COMT showed that it colocalized with both GABA- and TBR1-positive cells in control neurons (Figure 2a). In contrast, COMT expression was completely absent from both GABA- and TBR1-positive FMRP-deficient neurons. This demonstrates that the deficiency of COMT in FMRP-deficient neurons is not restricted to either neuronal subtype (**Fig. 2a**).

We next examined whether COMT expression is reduced in a rodent model of FXS. We measured COMT expression in primary hippocampal neurons derived from *Fmr1*KO mice and found significantly reduced levels compared to control neurons (**Fig. 2b**), consistent with our observations in human FMRP-deficient neurons. Because COMT plays an important role in the metabolism of dopamine, changes in COMT expression may affect dopaminergic signaling activity. To test whether reduced COMT expression correlates with abnormal dopaminergic function in FXS, we subjected *Fmr1*KO mice to methamphetamine-induced hyperactivity testing as a measure of dopaminergic neurotransmission(13) (**Fig. 2c**). While the distance travelled during the 30-min habituation period is similar between the groups, the distance travelled in 90 min is modestly but significantly higher in saline-treated *Fmr1*KO mice compared to WT controls, consistent with previous reports(14). Following methamphetamine treatment, this excessive activity appears to be further enhanced in *Fmr1*KO mice compared to wildtype controls (**Fig. 2d**), supporting possible dysregulation of dopaminergic neurotransmission in *Fmr1*KO mice.

Discussion

In this study, we investigated the dysregulation of COMT expression in human and mouse models of FXS. Our data showed that COMT expression was significantly reduced in FMRP-deficient cells. This reduction was observed at the transcript, protein, and translating mRNA levels in human neural progenitor cells and neurons, as well as in primary mouse hippocampal neurons. COMT plays a role in the degradation of catecholamines, including dopamine, epinephrine, and norepinephrine, and L-dopa (15). Thus, we hypothesized that disrupted COMT function may lead to an altered dopaminergic neurotransmission. Consistent with this idea, *Fmr1* knockout mice showed increased locomotor activity in the methamphetamine-induced hyperactivity test, a commonly used assay of dopaminergic neurotransmission.

Since FMRP is an RNA binding protein with more than 1000 mRNA targets that are increasingly being characterized, we checked whether *COMT* is an FMRP target. Previous studies employing cross-linking immunoprecipitation experiments coupled with sequencing (CLIP-seq) to identify substrates of FMRP have been performed on mouse brain, cell lines and hPSC-derived NPCs and neurons (22–25). Interestingly, *COMT* was found to be bound by FMRP in two studies. Ascano et al showed that *COMT* is only bound by FMRP encoded by isoform 7 of *FMR1,* the most abundantly expressed isoform; and Maurin et al showed that COMT is specifically bound by FMRP in the hippocampus but not the cerebellum or cortex (23,24). In contrast, a recent CLIP-seq study performed in hPSC-derived NPC and neurons did not identify *COMT* as an FMRP target, a trend towards reduced *COMT* expression was reported (22). This discrepancy may reflect variability in *COMT* expression across cell lines used in the Li et al study where 2 hESC lines (H1 and H13) and the GM1-iPSC line were used.

Polymorphisms in *COMT* have been linked to several neuropsychiatric conditions, including schizophrenia, obsessive-compulsive disorder, substance abuse, and attention deficit hyperactivity disorder(15). Furthermore, animal studies have shown that disruptions in COMT function lead to phenotypes that resemble symptoms seen in FXS, such as changes in aggressiveness(16), anxiety-like behavior and stress sensitivity(16,17), working memory performance(17,18), social interactions(19), and increased acoustic startle response(17,19).

A recent study has shown that the Val158Met SNP in *COMT*, specifically AA genotype, is associated with improved behavioral symptoms in FXS, such as reduced risk for property destruction and compulsive behavior(4). It is interesting to note that the same AA genotype is associated with increased risk of depression in the general population, indicating that the effect of this SNP may be dependent on genetic background. In addition, Val158Met SNP, which is also associated with schizophrenia, has been found to cause decreased expression of COMT mRNA level in post-mortem human brain tissue (20). Reduced expression of *COMT* has also been observed in the lymphoblasts of 22q11 deletion syndrome patients, who are at risk for psychosis

and behavioral issues(21).

Conclusion

Our findings suggest that COMT may play a role in the pathophysiology of FXS and that catecholamine modulation as a therapeutic approach for FXS warrants further exploration. Further research is also needed to fully understand the mechanisms by which FMRP regulates COMT and the impact of reduced COMT levels on catecholamine metabolism in FXS.

List of abbreviations

FXS	Fragile X Syndrome
hPSC	human pluripotent stem cells
hESC	human embryonic stem cells
NPC	neural progenitor cells
SNPs	single nucleotide polymorphism

Declaration

Ethics approval and consent approval

All animal procedures were in compliance with the Institutional Animal Care and Use Committee (IACUC) at the Biomedical Science Institute (A*STAR) and in accordance to their approved guidelines.

Consent for publication

N.A.

Availability of data and materials

The datasets used and/or analyzed during the current study are obtained from Utami et al., *Biol Psych,* 2020 (GEO (Gene Expression Omnibus) ID GSE117248 and ProteomeXchange consortium ID: PXD011630.

Competing interests

The authors declare that they have no competing interests

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Authors contributions

M.A.P. and K.H.U. designed the study, interpreted data and wrote the manuscript; N.A.B.M.Y., K.H.U., N.S. and M.G.M performed the experimental work, analyzed and interpreted data. N.A.B.M.Y and K.H.U performed molecular analyses, N.S. performed proteomics experiments, M.G.M was responsible for animal work experiments and data interpretation. S.R.L. supervised the study and interpreted RNAseq analysis. P.S. and S.N. designed, performed and analyzed the polysome-fractionation experiments. All the authors edited or commented the manuscript.

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References

1. Turner G. Fragile X Syndrome: Diagnosis, Treatment and Research. J Med Genet. 1997;
2. Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB, Moine H, Kooy RF, et al. Fragile X syndrome. Nature reviews. Disease primers. 2017.
3. Davidson M, Sebastian SA, Benitez Y, Desai S, Quinonez J, Ruxmohan S, et al. Behavioral Problems in Fragile X Syndrome: A Review of Clinical Management. Cureus. 2022;14(2).
4. Crawford H, Scerif G, Wilde L, Beggs A, Stockton J, Sandhu P, et al. Genetic modifiers in rare disorders: the case of fragile X syndrome. Eur J Hum Genet [Internet]. 2021;29(1):173–83. Available from: http://dx.doi.org/10.1038/s41431-020-00711-x
5. Bowers ME, Buzzell GA, Salo V, Troller-Renfree S V., Hodgkinson CA, Goldman D, et al. Relations between catechol-O-methyltransferase Val158Met genotype and inhibitory control development in childhood. Dev Psychobiol. 2020;62(2):181–90.
6. Taylor S. Association between COMT Val158Met and psychiatric disorders: A comprehensive meta-analysis. Am J Med Genet Part B Neuropsychiatr Genet. 2018;177(2):199–210.
7. Ma J, Zhao M, Zhou W, Li M, Huai C, Shen L, et al. Association Between the COMT Val158Met Polymorphism and Antipsychotic Efficacy in Schizophrenia: An Updated Meta-Analysis. Curr Neuropharmacol. 2020;19(10):1780–90.
8. González-Castro TB, Hernández-Díaz Y, Juárez-Rojop IE, López-Narváez ML, Tovilla-Zárate CA, Fresan A. The Role of a Catechol-O-Methyltransferase (COMT) Val158Met Genetic Polymorphism in Schizophrenia: A Systematic Review and Updated Meta-analysis on 32,816 Subjects. NeuroMolecular Med. 2016;18(2):216–31.
9. Pálmason H, Moser D, Sigmund J, Vogler C, Hänig S, Schneider A, et al. Attention-deficit/hyperactivity disorder phenotype is influenced by a functional catechol-O-methyltransferase variant. J Neural Transm. 2010;117(2):259–67.
10. Soeiro-De-Souza MG, Stanford MS, Bio DS, Machado-Vieira R, Moreno RA. Association of the COMT met158 allele with trait impulsivity in healthy young adults. Mol Med Rep. 2013;7(4):1067–72.
11. Utami KH, Skotte NH, Colaço AR, Yusof NABM, Sim B, Yeo XY, et al. Integrative Analysis Identifies Key Molecular Signatures Underlying Neurodevelopmental Deficits in Fragile X Syndrome. Biol Psychiatry. 2020;88(6):500–11.
12. Utami KH, Yusof NABM, Kwa JE, Peteri UK, Castrén ML, Pouladi MA. Elevated de novo protein synthesis in FMRP-deficient human neurons and its correction by metformin treatment. Mol Autism. 2020;11(1):1–11.
13. Araki H, Yamamoto T, Kobayashi Y, Futagami K, Kawasaki H, Gomita Y. Effect of methamphetamine and imipramine on cerebral ischemia-induced hyperactivity in Mongolian gerbils. Jpn J Pharmacol. 2002;88(3):293–9.
14. Ding Q, Sethna F, Wang H. Behavioral analysis of male and female Fmr1 knockout mice on C57BL/6 background. Behav Brain Res [Internet]. 2014;271:72–8. Available from: http://dx.doi.org/10.1016/j.bbr.2014.05.046
15. Craddock N, Owen MJ, O'Donovan MC. The catechol-O-methyl transferase (COMT) gene as a candidate for psychiatric phenotypes: Evidence and lessons. Mol Psychiatry. 2006;11(5):446–58.
16. Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, et al. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc Natl Acad Sci U S A. 1998;95(17):9991–6.
17. Papaleo F, Crawley JN, Song J, Lipska BK, Pickel J, Weinberger DR, et al. Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. J Neurosci. 2008;28(35):8709–23.

18. Babovic D, O'Tuathaigh CM, O'Sullivan GJ, Clifford JJ, Tighe O, Croke DT, et al. Exploratory and habituation phenotype of heterozygous and homozygous COMT knockout mice. Behav Brain Res. 2007;183(2):236–9.
19. Tammimäki A, Käenmäki M, Kambur O, Kulesskaya N, Keisala T, Karvonen E, et al. Effect of S-COMT deficiency on behavior and extracellular brain dopamine concentrations in mice. Psychopharmacology (Berl). 2010;211(4):389–401.
20. Dempster EL, Mill J, Craig IW, Collier DA. The quantification of COMT mRNA in post mortem cerebellum tissue: Diagnosis, genotype, methylation and expression. BMC Med Genet. 2006;7:1–7.
21. Gothelf D, Law AJ, Frisch A, Chen J, Zarchi O, Michaelovsky E, et al. Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. Biol Psychiatry [Internet]. 2014;75(5):406–13. Available from: http://dx.doi.org/10.1016/j.biopsych.2013.07.021
22. Li M, Shin J, Risgaard RD, Parries MJ, Wang J, Chasman D, et al. Identification of FMR1-regulated molecular networks in human neurodevelopment. Genome Res. 2020;30(3):361–74.
23. Maurin T, Lebrigand K, Castagnola S, Paquet A, Jarjat M, Popa A, et al. HITS-CLIP in various brain areas reveals new targets and new modalities of RNA binding by fragile X mental retardation protein. Nucleic Acids Res. 2018;46(12):6344–55.
24. Ascano M, Mukherjee N, Bandaru P, Miller JB, Nusbaum JD, Corcoran DL, et al. FMRP targets distinct mRNA sequence elements to regulate protein expression. Nature. 2012;492(7429):382–6.
25. Darnell JC, Van Driesche SJ, Zhang C, Hung KYS, Mele A, Fraser CE, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell. 2011;

Figures

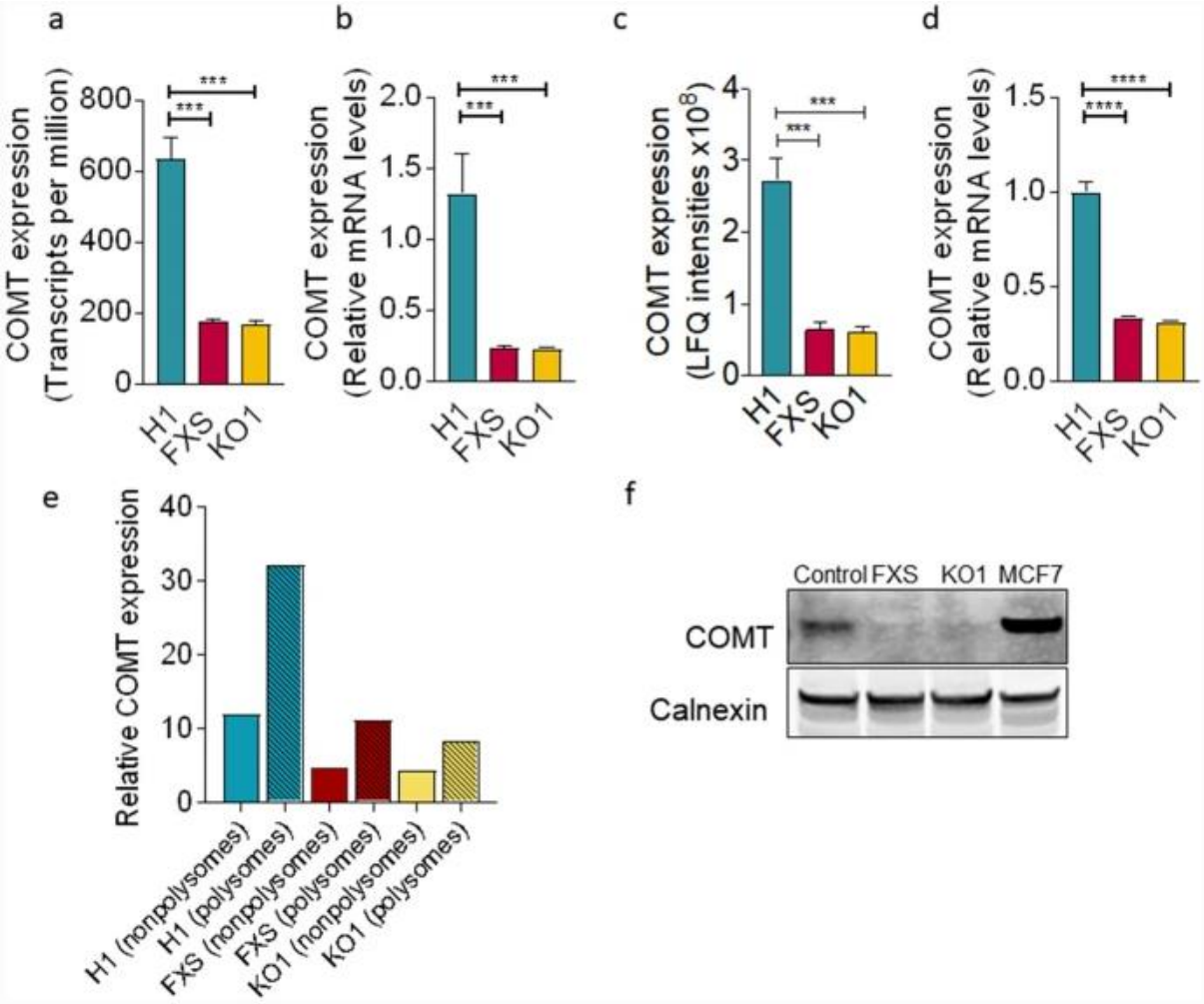

Figure 1. *COMT* expressions are markedly reduced in FXS stem cell-derived neuron and NPCs a-f) Reduced *COMT* expression identified by RNA-Seq analysis of *FMR1*KO and FXS neurons (**a**), qRT-PCR (**b**), and protein mass spectrometry (**c**). H1 hESC, the parental line used to generate *FMR1*KO, is used as a control. *COMT* expression is also reduced at the NPC stage, as shown by qRT-PCR of total RNAs (**d**) and translating mRNAs on polysome-bound fractionated lysates of *FMR1*KO and FXS NPCs (**e**). COMT protein expression is reduced in FXS and *FMR1*KO neurons as shown by immunoblot quantification (**f**); uncropped images are

provided in Figure S1. For RNA-Seq analysis, TPM values were shown as mean ± SEM based on n = 3 biological replicates per genotype, *** p = 1.38 x 10^{-75} for FXS and 2.46 x 10^{-82} for *FMR1*KO. For qRT-PCR, values were shown as mean ± SEM based on n = 3 biological replicates per genotype; ***p < 0.001 was determined by one-way ANOVA with Tukey's post-hoc test. For mass spectrometry, LFQ intensity values were shown as mean ± SEM based on n = 3 biological replicates per genotype, *** p = 0.05 for FXS and 3.78 x 10^{-5} for *FMR1*KO. For polysome fractionated samples, fraction 1-4 are pooled as non-polysome sample, and fraction 5-8 are pooled as polysomes.

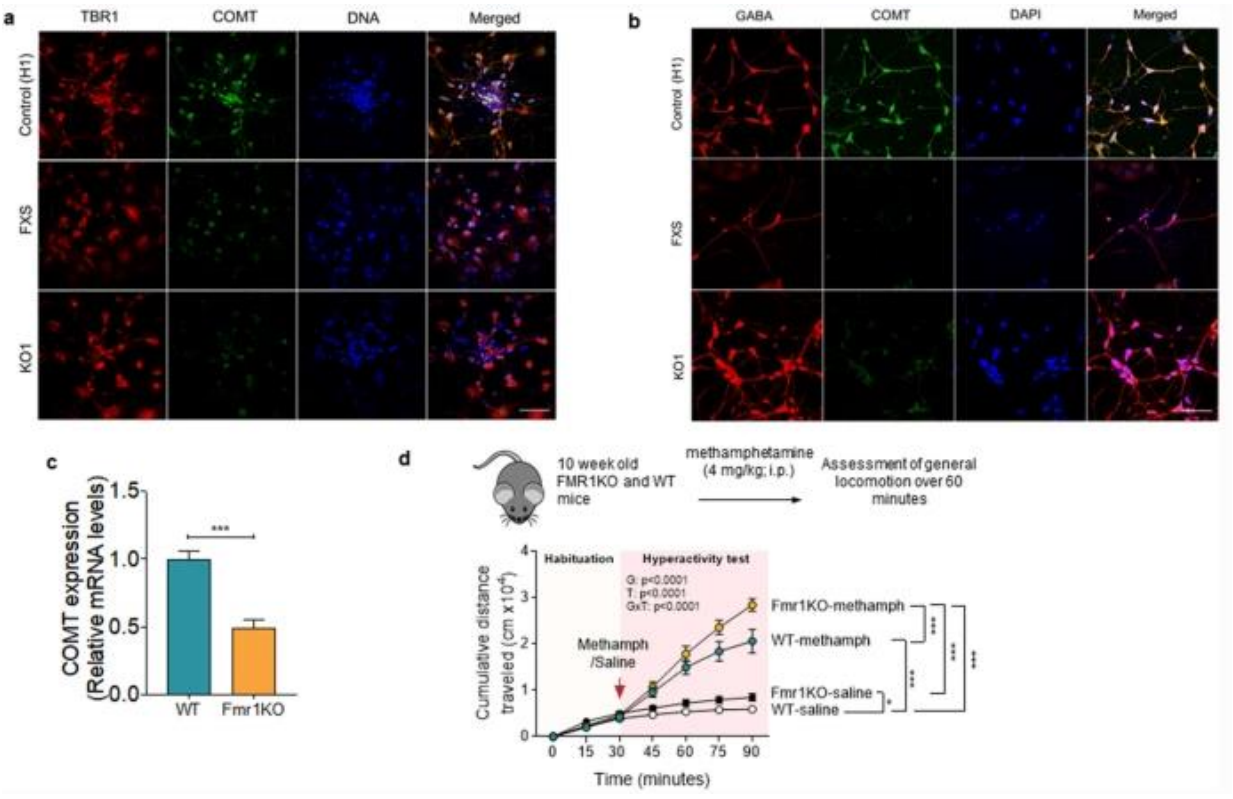

Figure 2. Dysregulated *COMT* expression is associated with abnormal dopamine-linked metamphetamine response in Fmr1KO mice a-d) Immunostaining showing the expression of COMT in (**a**) excitatory (TBR1-positive) and (**b**) inhibitory (GABA-positive) neurons. (**c**) Expression of COMT in primary hippocampal neurons from *Fmr1*KO mice. Values shown as

mean ± SEM based on n = 3 biological replicates per genotype; ***p < 0.001 was determined by a two-tailed t-test. (**d**) Increased locomotion in the methamphetamine-induced hyperactivity test suggests altered dopaminergic neurotransmission in *Fmr1*KO mice. Values shown as mean ± SEM based on n = 3-4 males per group; ***p < 0.001 was determined by two-way ANOVA with Tukey's post-hoc test. G= group, T= time, GxT= interaction.

Tables

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