International Journal of
Molecular Sciences

MDPI

Article

Cerebral Expression of Metabotropic Glutamate Receptor Subtype 5 in Idiopathic Autism Spectrum Disorder and Fragile X Syndrome: A Pilot Study

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Citation: Brašić, J.R.; Nandi, A.; Russell, D.S.; Jennings, D.; Barret, O.; Martin, S.D.; Slifer, K.; Sedlak, T.; Seibyl, J.P.; Wong, D.F.; et al. Cerebral Expression of Metabotropic Glutamate Receptor Subtype 5 in Idiopathic Autism Spectrum Disorder and Fragile X Syndrome: A Pilot Study. *Int. J. Mol. Sci.* **2021**, *22*, 2863. https://doi.org/10.3390/ijms22062863

Academic Editor: Kunio Yui

Received: 31 January 2021
Accepted: 4 March 2021
Published: 11 March 2021

Abstract: Multiple lines of evidence suggest that dysfunction of the metabotropic glutamate receptor subtype 5 ($mGluR_5$) plays a role in the pathogenesis of autism spectrum disorder (ASD). Yet animal and human investigations of $mGluR_5$ expression provide conflicting findings about the nature of dysregulation of cerebral $mGluR_5$ pathways in subtypes of ASD. The demonstration of reduced $mGluR_5$ expression throughout the living brains of men with fragile X syndrome (FXS), the most common known single-gene cause of ASD, provides a clue to examine $mGluR_5$ expression in ASD. We aimed to (A) compare and contrast $mGluR_5$ expression in idiopathic autism spectrum disorder (IASD), FXS, and typical development (TD) and (B) show the value of positron emission tomography (PET) for the application of precision medicine for the diagnosis and treatment of individuals with IASD, FXS, and related conditions. Two teams of investigators independently administered 3-[^{18}F]fluoro-5-(2-pyridinylethynyl)benzonitrile ([^{18}F]FPEB), a novel, specific $mGluR_5$ PET ligand to quantitatively measure the density and the distribution of $mGluR_5$s in the brain regions, to participants of both sexes with IASD and TD and men with FXS. In contrast to participants with TD, $mGluR_5$ expression was significantly increased in the cortical regions of participants with IASD and significantly reduced in all regions of men with FXS. These results suggest the feasibility of this protocol as a valuable tool to measure $mGluR_5$ expression in clinical trials of individuals with IASD and FXS and related conditions.

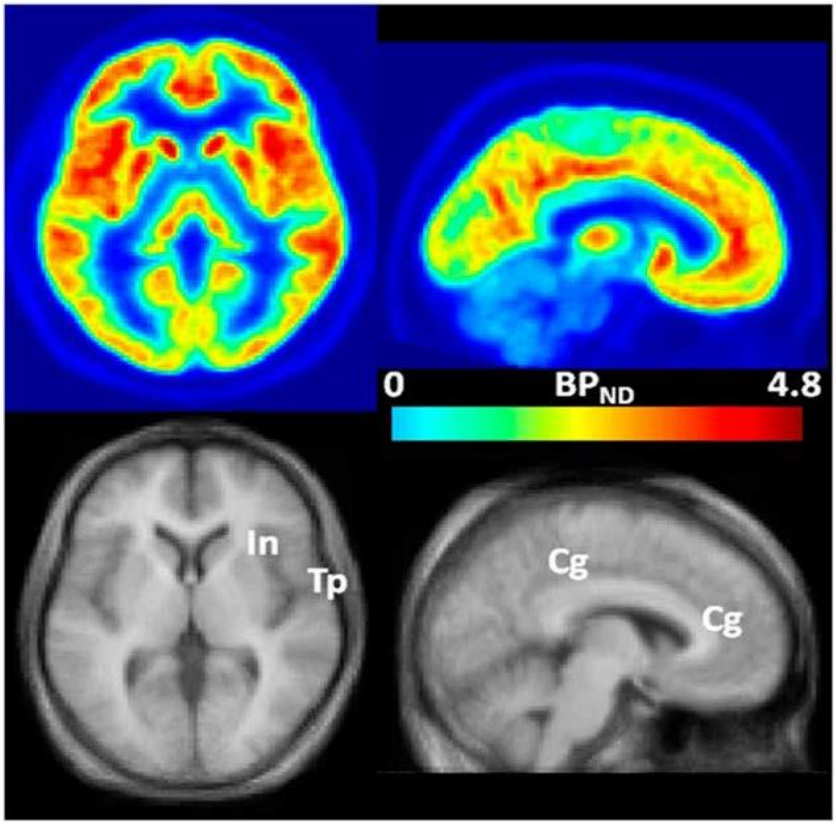
Keywords: binding potential; cortex; caudate nucleus; cingulate; fragile X mental retardation 1 gene (*FMR1*); neurodevelopmental disorders; positron emission tomography (PET); putamen; radiotracer; thalamus

1. Introduction

Autism spectrum disorder (ASD) [1] comprises a heterogeneous group of neurodevelopmental disorders including (A) a subtype characterized by a behavioral phenotype with no known etiology [idiopathic autism spectrum disorder (IASD)] [2] and (B) medical disorders with known genetic etiologies [3], such as fragile X syndrome (FXS) [4]. All subtypes of ASD are characterized by impaired social communication and repetitive and restricted behaviors and interests [1–5]. Additionally, FXS and some other subtypes of ASD are also characterized by intellectual disability (ID) [6]. Dysfunction of protein synthesis mediated by abnormal pathways including metabotropic glutamate receptors (mGluR) plays a role in the pathometabolism of IASD [7,8] and FXS [9–12]. Despite the evidence for dysfunction of $mGluR_5$ expression in IASD, conflicting findings include the decreased expression in the dorsolateral prefrontal cortex [13] and increased expression in the post-central gyrus and the cerebellum [14].

The confusion about $mGluR_5$ expression in IASD may be resolved utilizing techniques that have provided convergent validity to studies of $mGluR_5$ expression in FXS, the most common single-gene cause of ASD and ID. FXS results from the presence of the fragile X mental retardation 1 (*FMR1*) gene leading to deficits of Fragile X Mental Retardation Protein (FMRP). Dysregulated activation of group I metabotropic glutamate receptors [metabotropic glutamate receptors subtypes 1 and 5 ($mGluR_{1/5}$)] causing metabotropic glutamate receptor dependent long-term depression (mGluR-LTD) plays a role in the pathogenesis of FXS [15,16]. The mechanisms of $mGluR_{1/5}$ dysregulation leading to the neurobehavioral symptoms of FXS have been elucidated by the study of *fmr1* knockout (KO) mouse models. The deficits of FMRP in *fmr1* KO mouse models result in dysfunction of crucial group 1 metabotropic glutamatergic pathways leading to dysregulated downstream signaling cascades including the mammalian target of rapamycin (mTOR) and the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK) pathways [17]. The correction of mGluR-LTD and behavioral symptoms in *fmr1* KO mouse models suggests that the a biomarker to measure $mGluR_5$ expression in the living human brain represents a means to apply precision molecular medicine to ameliorate behavioral symptoms of FXS and possibly other subtypes of ASD [9,10,17–24].

Clinical trials of FXS have been flawed by several limitations, including the absence of a tool to measure the expression of $mGluR_5$ in the living brains of participants with FXS [9,12,17,24]. We showed that 3-[^{18}F]fluoro-5-(2-pyridinylethynyl)benzonitrile ([^{18}F]FPEB), a novel, specific $mGluR_5$ ligand to quantitatively measure the density and distribution of $mGluR_5$s in the brain regions of humans through PET (Figure 1) [25] may be a promising means to obtain quantitative measurements of $mGluR_5$ expression in individuals with IASD or FXS for clinical trials and other investigations [14,17,23,26]. We seek to expand our investigations to compare and contrast $mGluR_5$ expression for participants of both sexes with IASD [14,23] and typical development (TD) and men with FXS [17,24,26].



(A) **(B)**

Figure 1. Transaxial (**A**) and sagittal (**B**) non-displaceable binding potential (BP_{ND}) [27] images of 3-[^{18}F]fluoro-5-(2-pyridinylethynyl)benzonitrile ([^{18}F]FPEB) (top) and matching magnetic resonance (MR) images (bottom) in statistical parametric mapping (SPM) [25] standard space. Regions with high BP_{ND} values, name.ly insular (In), temporal (Tp), and cingulate (Cg) cortices, are indicated on co-registered MRimages [25]. This research was originally published in *JNM*. Wong DF, Waterhouse R, Kuwabara H, Kim J, Brašić JR, Chamroonrat W, Stabins M, Holt DP, Dannals RF, Hamill TG, Mozley PD. ^{18}F-FPEB, a PET radiopharmaceutical for quantifying metabotropic glutamate 5 receptors: a first-in-human study of radiochemical safety, biokinetics, and radiation dosimetry. J Nucl Med. 2013;54:388-396. © SNMMI [25].

Development of interventions to ameliorate the specific molecular deficits of individuals with IASD and FXS with and without ASD will facilitate the utilization of precision medicine to target the unique needs of each person [9,24].

We aimed to (A) compare and contrast $mGluR_5$ expression for participants of both sexes with IASD [14,23] and typical development (TD) [14,25] and men with FXS [17,26] and (B) show the value of PET with ([^{18}F]FPEB) for the application of precision medicine for the diagnosis and treatment of individuals with IASD, FXS, and related conditions [9,17,24,28].

2. Results

The clinical characteristics of all participants {group (IASD, FXS, or TD), institution [Institute for Neurodegenerative Disorders (IND) or Johns Hopkins University (JHU)], sex (female or male), age in years, and basal metabolic index (BMI)} are tabulated in Table 1 [26,29].

Table 1. Clinical characteristics of participants [26,29].

Participant	Group	Institution	Sex	Age in Years	BMI
INDTD01	TD	IND	Male	44	.
INDTD02	TD	IND	Male	57	.
INDTD07	TD	IND	Female	62	.
INDTD08	TD	IND	Female	62	.
INDTD14	TD	IND	Male	28	25.8
INDTD16	TD	IND	Male	31	29.8
INDTD17	TD	IND	Male	38	25.0
INDTD30	TD	IND	Female	28	.
INDTD35	TD	IND	Female	56	42.3
INDTD47	TD	IND	Female	22	.
INDTD48	TD	IND	Female	29	.
INDTD49	TD	IND	Female	20	.
JHUTD4	TD	JHU	Female	19	28.4
JHUTD6	TD	JHU	Female	19	.
JHUTD14	TD	JHU	Male	24	21.7
JHUTD105	TD	JHU	Male	26	.
JHUTD1001	TD	JHU	Male	32	27.1
JHUTD1002	TD	JHU	Male	27	28.6
JHUTD1005	TD	JHU	Male	39	29.3
JHUASD3	IASD	JHU	Male	18	28.8
JHUASD4	IASD	JHU	Male	18	.
JHUASD5	IASD	JHU	Male	19	22.2
JHUASD7	IASD	JHU	Female	18	22.3
JHUASD8	IASD	JHU	Male	23	28.5
JHUASD9	IASD	JHU	Male	20	19.4
JHUASD12	IASD	JHU	Male	22	20.7
INDFXS34	FXS	IND	Male	23	36.6
INDFXS38	FXS	IND	Male	24	30.9
INDFXS40	FXS	IND	Male	22	33.2
INDFXS41	FXS	IND	Male	27	25.8
INDFXS42	FXS	IND	Male	34	.
INDFXS44	FXS	IND	Male	26	24.1
INDFXS45	FXS	IND	Male	33	22.0
INDFXS-M50	FXS	IND	Male	57	34.1
JHUFXS2	FXS	JHU	Male	24	34.9
JHUFXS4	FXS	JHU	Male	27	28.3

BMI: Basal metabolic index; FXS: Fragile X syndrome; IASD: idiopathic autism spectrum disorder; IND: Institute for Neurodegenerative Disorders; JHU: Johns Hopkins University; TD: Typical development; (Period): Missing data.

The $mGluR_5$ uptake of participants in the regions of interest (ROI) [caudate nucleus (CN), medial temporal cortex (mTp), occipital cortex (Oc), parietal cortex (Pa), posterior cingulate cortex (pCg), putamen (Pu), thalamus (Th), and temporal lobe (Tp)] of all participants are recorded in Table 2 [26,29].

Table 2. Metabotropic glutamate receptor subtype 5 uptake in regions of interest of participants [26,29].

Participant	CN	mTp	Oc	Pa	pCg	Pu	Th	Tp
INDTD01	3.01	1.94	1.41	1.72	1.92	2.52	1.08	2.37
INDTD02	3.03	2.05	1.63	1.76	1.04	2.35	1.30	2.35
INDTD07	3.31	2.04	1.50	1.76	2.07	2.70	1.60	2.56
INDTD08	3.01	2.02	1.57	1.83	1.97	2.64	1.32	2.50
INDTD14	2.12	1.41	1.16	1.43	1.72	1.85	1.06	1.68
INDTD16	2.68	1.61	1.20	1.26	1.42	2.03	2.24	1.84
INDTD17	2.63	1.82	1.37	1.36	1.55	2.03	1.16	2.04
INDTD30	2.42	1.73	1.26	1.14	1.26	1.87	1.08	2.17
INDTD35	3.82	1.90	1.74	1.86	2.15	2.48	1.43	2.39
INDTD47	2.09	1.29	2.17	1.41	1.86	1.95	1.02	2.74
INDTD48	3.30	1.76	1.94	1.95	2.25	2.49	1.51	2.51
INDTD49	2.75	1.57	1.32	1.56	1.46	2.19	1.07	2.10
JHUTD4	2.80	3.19	3.48	4.31	3.39	3.08	1.43	4.54
JHUTD6	3.38	3.57	3.69	4.48	3.65	3.16	1.32	4.94
JHUTD14	2.47	3.24	3.15	3.73	3.44	2.94	1.41	4.09
JHUTD105	3.73	.	2.86	.	.	3.30	2.11	3.73
JHUTD1001	4.59	.	3.64	.	.	4.29	2.57	4.86
JHUTD1002	3.83	.	2.77	.	.	3.66	2.21	3.73
JHUTD1005	3.58	.	2.77	.	.	3.24	2.02	3.26
JHUASD3	2.17	3.06	3.26	4.05	3.43	3.87	1.27	3.97
JHUASD4	2.62	3.43	3.10	4.17	3.99	3.03	1.45	3.85
JHUASD5	2.79	3.25	3.48	3.84	3.25	2.78	1.46	4.17
JHUASD7	3.13	.	2.03	2.74	.	2.84	1.67	2.72
JHUASD8	3.12	3.54	3.42	4.18	3.74	3.36	1.55	4.21
JHUASD9	3.06	3.35	3.43	4.23	3.64	3.36	1.32	4.58
JHUASD12	3.25	2.46	3.41	4.11	3.34	2.99	1.75	4.58
INDFXS34	1.96	1.00	1.06	1.42	1.37	2.01	0.83	1.40
INDFXS38	1.58	0.69	0.59	0.82	0.79	1.08	0.61	0.92
INDFXS40	1.65	0.81	0.82	0.96	1.40	1.13	0.39	1.14
INDFXS41	2.14	1.25	1.08	1.36	1.56	1.65	0.56	1.59
INDFXS42	3.45	2.24	1.78	2.09	2.18	2.55	1.25	2.68
INDFXS44	1.76	1.01	1.03	1.23	1.23	1.69	0.73	1.37
INDFXS45	2.89	1.74	1.47	1.73	1.91	2.16	1.32	2.13
INDFXS-M50	2.99	2.24	1.73	1.77	1.82	2.50	1.19	2.53
JHUFXS2	2.05	2.71	.	.	3.01	2.01	0.97	.
JHUFXS4	2.00	2.21	.	2.7	2.25	1.98	0.89	.

CN: Caudate nucleus; mTp: Medial temporal cortex; Oc: Occipital cortex; Pa: Parietal cortex; pCg: Posterior cingulate cortex; Pu: Putamen; Th: Thalamus; Tp: Temporal cortex.

The ages of participants with IASD were lower than those with FXS and TD (Table 1) [17,26,29]. BMIs were ordered IASD < TD < FXS (Table 1) [17,26,29].

$mGluR_5$ uptake was ordered FXS < TD < IASD in cortical (Oc, Pa, Tp, and pCg) structures (Figure 2) [26,30].

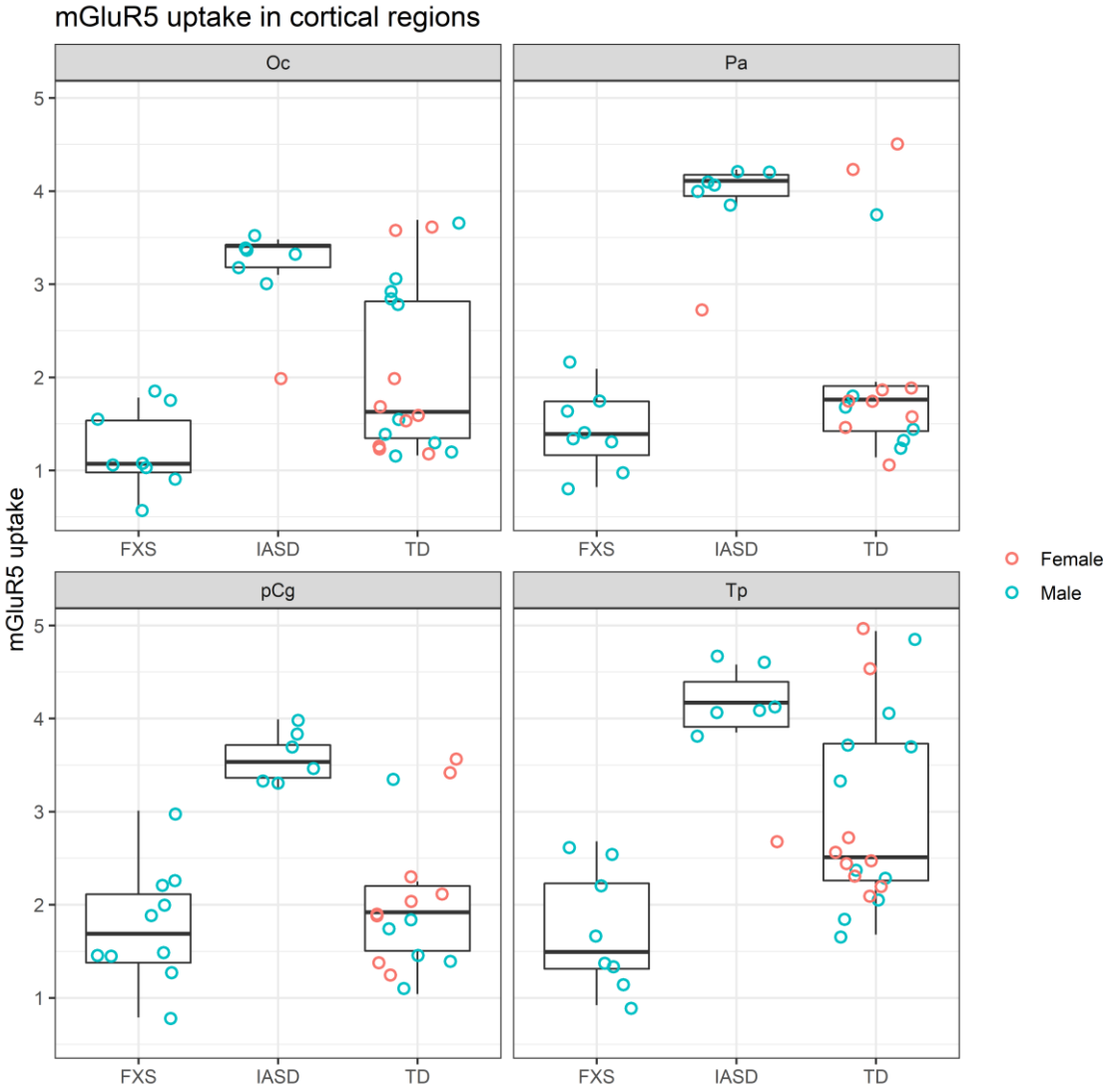


Figure 2. mGluR$_5$ uptake in cortical regions of participants grouped by cohort [26,30]. FXS: Fragile X syndrome; IASD: Idiopathic autism spectrum disorder; mGluR$_5$: Metabotropic glutamate receptor subtype 5; Oc: Occipital cortex; Pa: Parietal cortex; pCg: Posterior cingulate cortex; TD: Typical development; Tp: Temporal cortex.

By contrast mGluR$_5$ uptake was lower in participants with FXS than in participants with TD and IASD in subcortical (CN, Pu, and Th) structures (Figure 3) [26,30].

The initial visual analysis of the data indicated that the mGluR$_5$ uptake differed across the groups of FXS, IASD, and TD in multiple regions. Analysis of variance (ANOVA) confirmed that group had a significant effect across all regions (d.f. = 2, F = 51.6, $p < 0.001$) (Table 3) [30]. *Post hoc* pair-wise comparisons using Tukey's Honest Standard Differences (HSD) method further confirmed specific differences (Table 4) [30,31]. The pairwise comparisons highlighted the largest group differences in the temporal cortex (adjusted mean difference, FXS versus IASD = -2.19 ± 0.49 ($p < 0.001$) the parietal cortex (FXS versus IASD = -2.31 ± 0.48, $p < 0.001$), and the occipital cortex (FXS versus IASD = -1.88 ± 0.41, $p < 0.001$) [30,31].

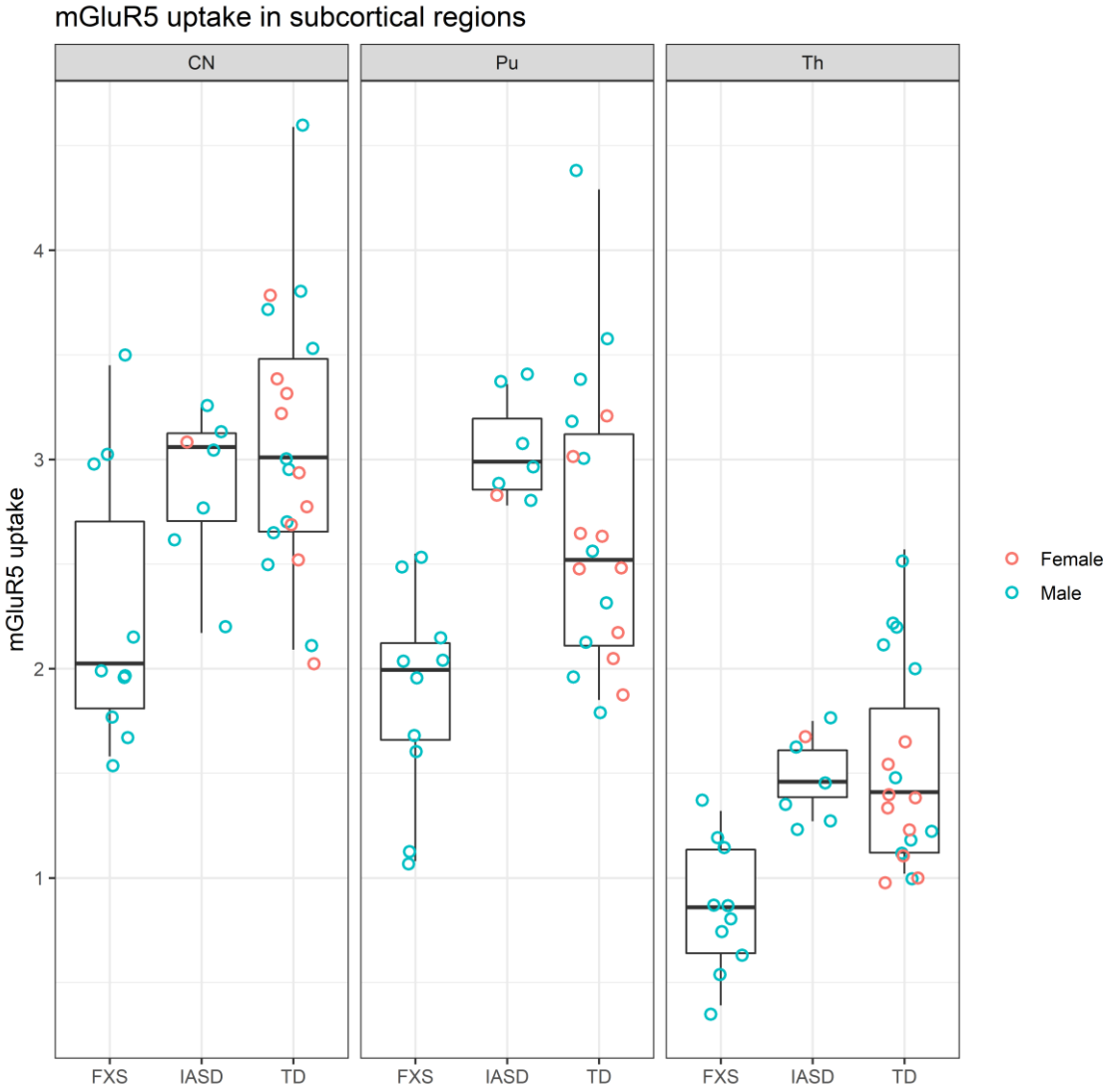


Figure 3. $mGluR_5$ uptake in subcortical regions of participants grouped by cohort [26,30]. CN: Caudate nucleus; FXS: Fragile X syndrome; IASD: Idiopathic autism spectrum disorder; $mGluR_5$: Metabotropic glutamate receptor subtype 5; Pu: Putamen; TD: Typical development; Th: Thalamus.

Table 3. Analysis of variance of $mGluR_5$ uptake by group (FXS, IASD, and TD) and region [30].

Analysis of Variance by Group Status and Region			
Region	Degrees of freedom	Test statistic	Probability
Caudate nucleus	2	6.77	0.00364
Occipital cortex	2	12.8	0.00010
Parietal cortex	2	16.2	0.00003
Posterior cingulate cortex	2	14.6	0.00006
Putamen	2	10.1	0.00043
Thalamus	2	10.3	0.00038
Temporal cortex	2	12.3	0.00014

FXS: Fragile X syndrome; IASD: Idiopathic autism spectrum disorder; $mGluR_5$: Metabotropic glutamate receptor subtype 5; TD: Typical development.

Table 4. *Post hoc* pairwise comparisons of $mGluR_5$ uptake by group (FXS, IASD, and TD) and region [30,31].

Post hoc Pairwise Comparisons by Tukey's Honest Standard Differences [30,31]				
Region	Pairwise Comparison	Adjusted Mean Difference	Standard Error	Probability
Caudate nucleus	FXS-IASD	−0.81837	0.301489	0.028089
	TD-IASD	−0.02072	0.298712	0.997341
	TD-FXS	0.797653	0.259542	0.011671
Occipital cortex	FXS-IASD	−1.88121	0.409271	0.000218
	TD-IASD	−0.74904	0.384949	0.143727
	TD-FXS	1.132169	0.353062	0.008855
Parietal cortex	FXS-IASD	−2.31154	0.476181	0.000173
	TD-IASD	−1.56255	0.493592	0.010802
	TD-FXS	0.748987	0.462568	0.25626
Posterior cingulate cortex	FXS-IASD	−1.6965	0.362152	0.000203
	TD-IASD	−1.5125	0.425317	0.00414
	TD-FXS	0.184006	0.356537	0.863175
Putamen	FXS-IASD	−1.23401	0.29236	0.000529
	TD-IASD	−0.31288	0.289668	0.532202
	TD-FXS	0.921134	0.251683	0.002488
Thalamus	FXS-IASD	−0.69753	0.197012	0.003572
	TD-IASD	0.084401	0.195197	0.902066
	TD-FXS	0.781931	0.169601	0.000214
Temporal cortex	FXS-IASD	−2.18986	0.490827	0.000294
	TD-IASD	−0.75798	0.461658	0.243837
	TD-FXS	1.431881	0.423417	0.005678

FXS: Fragile X syndrome; IASD: Idiopathic autism spectrum disorder; $mGluR^5$: Metabotropic glutamate receptor subtype 5; TD: Typical development.

3. Discussion

We confirmed our earlier finding that $mGluR_5$ expression is reduced in all brain regions in men with FXS [17] on a sample of men with FXS compared to participants of both sexes with IASD [14,23] and TD [14,17,25,26]. In men with FXS, reduced $mGluR_5$ expression in (A) cortical regions provides a basis for ID and (B) limbic regions provides a basis for the neurobehavioral symptoms [10,17].

We expanded our finding of increased $mGluR_5$ expression in the postcenteral gyrus and cerebellum of men with IASD [14] to show increased $mGluR_5$ expression in cortical regions of a sample from two separate institutions (IND and JHU) that includes participants with IASD and TD of both sexes. There are several possible explanations for the opposite results in IASD versus FXS. First, there may be different characteristic mechanisms for the development of $mGluR_5$ expression in IASD and FXS. Second, there may be other characteristics of these cohorts, specifically age and ID, that caused the differences in $mGluR_5$ expression in the cohorts with FXS and IASD. The participants with IASD were all younger than the participants with FXS. There may be reductions in $mGluR_5$ expression correlated with age as for dopamine D2 and serotonin S2 receptors [32]. Additionally all participants with IASD were recruited from samples with high-functioning autism; all participants with IASD had normal or superior intelligence. By contrast all participants with FXS had ID. Therefore, the opposite results of $mGluR_5$ expression in IASD versus FXS may simply reflect the differences in age and ID between the cohorts. The opposite results

of $mGluR_5$ expression in IASD versus FXS may therefore be unrelated to the diagnosis of FXS and IASD.

These findings confirm the hypothesis that $mGluR_5$ expression plays a role in the pathogenesis of FXS and other subtypes of IASD. The protocol for this investigation provides a feasibility tool that may facilitate the measurement of a biomarker of $mGluR_5$ expression to conduct rigorously designed clinical trials of FXS [9] and perhaps other subtypes of IASD. That said, the findings of this study merit replication in a larger sample of the groups studied here and other neurodevelopmental disorders [33]. Indeed the current protocol may be expanded to promote knowledge about multiple neuromodulators in FXS, Rett syndrome [34,35] and other subtypes of IASD.

Limitations. Estimation of binding potential for participants from IND as [standard uptake value ratio (SUVR)-1] [36] introduced uncertainty in the analysis [17]. Additionally the comparison of results from IND and JHU was confounded by the use of differences in scanners, scanning times, and analysis [17,26]. The similarity of results from both IND and JHU suggests the presence of convergent validity that the findings represent the characteristics of the status (IASD, FXS, and TD) of the participants. Future investigations will be enhanced by contemporaneous conduct of all investigations at all participating institutions with identical protocols and analyses [17].

Additionally, since some participants with TD are much older than other participants, the age difference may represent a confounding influence. $mGluR_5$ density may be reduced with age just as the density of dopamine D2 and serotonin S2 receptors is reduced with age [32]. The variability of BMIs may represent another confounding influence. Since all participants with IASD were high-functioning [14,23,26], both samples of participants with IASD and TD lacked the intellectual disability (ID) that characterized the sample of males with FXS [17,26].

Future directions. Our finding of increased $mGluR_5$ in the post central gyrus and cerebellum of men with IASD [14] was expanded in the current report with a sample of participants with IASD and TD of both sexes and men with FXS. We confirmed the reductions in $mGluR_5$ in all regions in men with FXS [17]. We showed increased $mGluR_5$ in cortical regions of participants with IASD. A study of the left striatum of a different cohort of participants with ID and TD demonstrated a trend of increased $mGluR_5$ in participants with IASD by PET with [^{18}F]FPEB, no change in glutamate by magnetic resonance spectroscopy (MRS), a trend of decreased gamma amino butyric acid (GABA) by MRS, and a strong negative correlation between $mGluR_5$ and GABA [37]. This finding supports the hypothesis of abnormal excitatory/inhibitory ratio in participants with IASD [2] and merits expansion and confirmation in other cohorts using both PET and MRS to assess both $mGluR_5$ and GABA.

Future investigations utilizing the protocol of this study may provide the tools for successful clinical trials of negative allosteric modulators (NAMs) for FXS and IASD. Despite the evidence that NAMs ameliorate behavioral symptoms in animal models of FXS, there have not been beneficial effects demonstrated in multiple clinical trials of NAMs in FXS. Flaws in the design of the clinical trials including the absence of a tool to measure $mGluR_5$ expression in the living human brains of participants with FXS have been identified as likely explanations for the unsuccessful clinical trial of NAMs in FXS [9]. Therefore, utilization of the procedure in this study may provide the crucial tool to generate rigorous measurements to demonstrate beneficial neurobehavioral effects of NAMs in clinical trials of FXS and IASD and related conditions.

Additional investigations will be enhanced with multiple imaging techniques including PET, MRS, PET/MRI [38], electroencephalography (EEG) [39,40], event-related brain potential (ERP) [39–41], resting state functional magnetic resonance imaging (rs-fMRI), diffusion tensor imaging (DTI), movement measurements [42], and quantitative measurements of FMRP and the *FMR1* gene [43]. Further prospective studies of ASD [44] may be enhanced by including these measurement tools, neuropsychological assessments, and whole exome sequencing (WES) [45]. The evidence for decreased expression of FMRP in

IASD [46] and FXS [43] indicates that correlation of FMRP with $mGluR_5$ [43,46] and GABA in ASD [37] is appropriate for future studies.

4. Materials and Methods

4.1. Participants

4.1.1. Recruiting Sites

The study is approved by Johns Hopkins Medicine Institutional Review Board IRB 169,249 [17]. The protocols for the study of humans were approved by the Institutional Review Boards of the Institute for Neurodegenerative Disorders (IND) in New Haven, Connecticut [47] and the Johns Hopkins University (JHU) in Baltimore, Maryland [48,49]. Since exposure to radioactivity in PET constitutes greater than minimal risk, this pilot study was restricted to adults [17]. Written informed consent was obtained from each participant at both locations.

We report the findings of cohorts of independent investigations conducted at the IND on seven men with FXS (mean age 27 ± 4.76, range 12 years) [17,26], one man with fragile X syndrome allele size mosaicism (FXS-M) aged 56.6 years [17,26], and five men and six women with TD (mean age 38.27 ± 15.68, range 42 years) [17,26], and at the JHU on two men with FXS (mean age 25.5 ± 2.12, range 3 years), six men and one woman with IASD (mean age 19.71 ± 2.06, range 5 years), and five men and two women with TD (mean age 26.57 ± 7.14 range 20 years) [14,17,23,25,26]. In contrast to the participants with FXS and FXS-M, all participants with IASD and TD had no evidence of intellectual disability (ID) [26]. In order to maximize the size effect, this report with focus on the combined sample of participants with IASD (N = 7, age 19.71 ± 2.06), FXS (N = 10, age 29.7 ± 10.39), and TD (N = 19, age 34.89 ± 14.57) [26,29].

4.1.2. Inclusion Criteria

Inclusion criteria for all participants included age between 18–60 years. Participants with IASD had a diagnosis of autism based on the Autism Diagnostic Interview-Revised [14,23,50], the Autism Diagnostic Observation Schedule [14,23,51], the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (*DSM-5*) [1,14], and other diagnostic tools documented in our prior publication [14,17,26]. Participants with FXS had a diagnosis of FXS based on *FMR1* DNA gene testing by polymerase chain reaction (PCR)/Southern Blot, supplemented by clinical neurobehavioral profiling [17,26,43].

4.1.3. Exclusion Criteria

Exclusion criteria were clinically significant abnormal laboratory values and/or clinically significant unstable serious medical, neurological, or psychiatric illnesses [14,17,47].

4.2. Procedures

4.2.1. Positron Emission Tomography (PET)

All participants underwent scans conducted by an experienced research staff of Certified Nuclear Medicine Technologists (CNMT) who had attained certification by the Nuclear Medicine Technology Certification Board (NMTCB). The technologists had conducted many PET scans before this study. The technologists maintained the physical conditions of each scan optimally for the completion of the scans. Participants were positioned by the technologists in the most comfortable manner for scans. Heads were stabilized in the scanner by gauge strips at IND and by face masks at JHU [17,34]. In order to maintain a comfortable environment during the scans, technologists utilized blankets and pads to raise legs. The physical conditions of the scans were maintained in optimal manners for participants by outstanding technologists.

Positron emission tomography (PET) after the intravenous bolus injection 185 MBq (5 mCi) of [^{18}F]FPEB [14,17,23,26] was conducted at IND on an ECAT EXACT HR+ PET manufactured by Siemens/CTI (Knoxville, TN) [52] for 90–120 min after injection and at JHU on an ECAT high resolution research tomograph (HRRT) manufactured by

Siemens/CTI (Knoxville, TN) [53] for 0–90 min after injection. Injectors obtained measured doses of [^{18}F]FPEB synthesized by radiochemists in the adjacent radiochemistry laboratory following the published methods [25] to be administered to participants in the scanning chambers.

4.2.2. Statistical Analyses

Data for participants from IND were expressed as the standard uptake value ratio (SUVR) with the cerebellum as reference region because there is minimal radio tracer uptake in the cerebellum [26,54]. Assuming that there is no difference in nonspecific tracer binding between regions and between participant cohorts, we approximated nondisplaceable binding potentials (BP_{ND}) [17,26] as the (SUVR-1) [36] for participants from IND (Table 2).

Data for participants from JHU were represented as regional nondisplaceable binding potentials (BP_{ND}s) [14,17,23,26] by reference tissue graphical analysis (RTGA) [55] with the cerebellar white matter as the reference region [14,17,25,26,54].

Due to the small sample size we expressed the results for the combined cohorts from IND and JHU as dot plots with box plots representing descriptive statistics utilizing R (R Foundation, Vienna, Austria) [30].

After constructing the plots of our data, several group differences were observed across the regions tested. To confirm the effect of group status (e.g., FXS versus TD versus IASD), we used analysis of variance (ANOVA) utilizing R (R Foundation, Vienna, Austria) [30], using group and region as the main factors, with age and sex as covariates. As the ANOVA showed evidence of a significant effect of group on $mGluR_5$ uptake, we then conducted post hoc pairwise comparisons with Tukey's Honest Standard Differences (HSD) utilizing R (R Foundation, Vienna, Austria) [30,31]. HSD was chosen as the more traditional Bonferroni correction lacked statistical power given our smaller sample size.

5. Conclusions

We confirmed our earlier finding of reduced cerebral $mGluR_5$ expression [17] in a sample of men with FXS in contrast to participants with IASD and TD of both sexes. The significantly reduced $mGluR_5$ expression in all brain regions of men with FXS provides a possible molecular basis for the neurobehavioral phenotype of individuals with FXS [10]. Reduced cortical $mGluR_5$ expression may provide a basis for the cognitive deficits (delayed socialization) of individuals with FXS [56]. Reduced limbic $mGluR_5$ expression may provide a basis for the avoidance behaviors of individuals with FXS [56].

We showed increased cortical cerebral $mGluR_5$ expression in participants of both sexes with IASD in contrast to participants with TD and men with FXS. Since all participants with IASD were recruited initially for studies of children with high-functioning autism, the increased cortical cerebral $mGluR_5$ expression may represent a molecular feature of IASD or of superior intelligence.

The proposed protocol may provide a biomarker for measurement of $mGluR_5$ expression for clinical trials of FXS and other subtypes of ASD. The proposed protocol may provide a tool to utilize precision medicine for diagnostic and therapeutic interventions for ASD and related conditions.

Author Contributions: Conceptualization, J.R.B., D.S.R., D.F.W. and D.B.B.; data curation, J.R.B., A.N., D.S.R., D.J., O.B., S.D.M., D.F.W. and D.B.B.; formal analysis, J.R.B., A.N., O.B., D.F.W. and D.B.B.; funding acquisition, J.R.B., D.S.R., D.F.W. and D.B.B.; investigation, J.R.B., A.N., D.S.R., D.J., S.D.M., K.S., T.S., J.P.S., D.F.W. and D.B.B.; methodology, J.R.B., A.N., D.S.R., D.J., O.B., S.D.M., K.S., T.S., J.P.S., D.F.W. and D.B.B.; project administration, J.R.B., A.N., D.S.R., D.J., J.P.S., D.F.W. and D.B.B.; resources, J.R.B., A.N., D.S.R., O.B., K.S., T.S., J.P.S., D.F.W. and D.B.B.; software, J.R.B., A.N., O.B. and S.D.M.; supervision, J.R.B., D.S.R., K.S., T.S., J.P.S., D.F.W. and D.B.B.; validation, J.R.B., A.N., D.S.R., D.J., O.B., S.D.M., K.S., T.S., J.P.S., D.F.W. and D.B.B.; visualization, J.R.B., A.N., D.S.R., D.J., O.B., S.D.M., J.P.S., D.F.W. and D.B.B.; and editing, J.R.B., A.N., D.S.R., S.D.M. and D.B.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was made possible by a Radiology BRidge/Development Funding Initiative to STimulate and Advance Research (RAD BriteStar Bridge) Award from the Johns Hopkins University School of Medicine, Baltimore, Maryland to J.R.B. with the assistance of D.F.W.; the Intellectual & Developmental Disabilities Research Center (U54 HD079123), Kennedy Krieger Institute, and Johns Hopkins Medical Institutions, Baltimore, Maryland, vto J.R.B.; and the Johns Hopkins Institute for Clinical and Translational Research (ICTR), Johns Hopkins University School of Medicine, Baltimore, Maryland, to J.R.B., which is funded in part by Grant Number UL1 TR003098 from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the Johns Hopkins ICTR, NCATS, or NIH.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki [49], and approved by the Institutional Review Board of the Johns Hopkins School of Medicine in Baltimore, Maryland (Protocol Number: IRB00169249 and Initial Approval Date: 11 July 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are openly available in [Zenodo]. Available online: https://doi.org/10.5281/zenodo.4395102 (accessed on 6 March 2021) [26].

Acknowledgments: The authors thank the patients and families for their participation and dedication to these studies; they are the inspiration for our efforts at improving treatments. The authors thank the FORWARD Database and Registry of the National Fragile X Foundation (NFXF) funded by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, for referral of participants. The authors thank the teams of the Institute of Neurodegenerative Disorders, the Positron Emission Tomography (PET) Radiotracer Service Center, and the Research Magnetic Resonance Imaging (MRI) Service Center of the Johns Hopkins University School of Medicine for conducting the scans. The authors thank Hiroto Kuwabara for PET analysis. The authors thank Brian Hwang for posing for the photograph by J.R.B. on the Graphical Abstract/Earlier versions of this article were presented at the 2020 Annual Meeting, Society of Nuclear Medicine and Molecular Imaging, 11–14 July 2020, the World Molecular Imaging Congress 2020, and the Society for Neuroscience Global Connectome, Virtual [43].

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

[^{18}F]FPEB	3-[^{18}F]fluoro-5-(2-pyridinylethynyl)benzonitrile
ADI-R	Autism Diagnostic Interview-Revised [50]
ADOS	Autism Diagnostic Observation Schedule [51]
ANOVA	analysis of variance
ASD	autism spectrum disorder
BMI	basal metabolic index
BP_{ND}	non-displaceable binding potential
CEA	Commissariat à l'Énergie Atomique et aux Énergies Alternatives
CDC	Centers for Disease Control and Prevention
CN	caudate nucleus
CNAMI	CNS Neuropsychopharmacology and Multimodal Imaging
CNMT	Certified Nuclear Medicine Technologist
CNRS	Centre National de la Recherche Scientifique
CNS	central nervous system
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition [1]

DTI	diffusion tensor imaging
EEG	electroencephalography
ERK	extracellular signal-regulated kinase
ERP	event-related brain potential
FORWARD	Fragile X Online Registry With Accessible Research Database of the National Fragile X Foundation (NFXF)
FMR1	fragile X mental retardation 1 gene
fmr1	*fmr1* gene in knockout mouse model of fragile X syndrome
FMRP	Fragile X Mental Retardation Protein
FXS	fragile X syndrome
FXS-M	fragile X syndrome allele size mosaicism
GABA	gamma amino butyric acid
HRRT	high resolution research tomograph [53]
HSD	Honest Standard Differences [31]
IASD	idiopathic autism spectrum disorder
ID	intellectual disability
IND	Institute for Neurodegenerative Disorders
IJMS	International Journal of Molecular Sciences
JHU	Johns Hopkins University
KO	knockout
LTD	long-term depression
MDPI	Multidisciplinary Digital Publishing Institute
MPAK	mitogen-activated protein kinase (MAPK)
MBq	megabecquerel
mCi	millicurie
$mGluR_{1/5}$	metabotropic glutamate receptors subtypes 1 and 5
$mGluR_5$	metabotropic glutamate receptor subtype 5
mGluR-LTD	metabotropic glutamate receptor dependent longterm depression
MIRCen	Molecular Imaging Research Center
MR	magnetic resonance
MRS	magnetic resonance spectroscopy
mTOR	mammalian target of rapamycin
mTp	medial temporal cortex
NAM	negative allosteric modulator
NFXF	National Fragile X Foundation
NMTCB	Nuclear Medicine Technology Certification Board
Oc	occipital cortex
Pa	parietal cortex
pCg	posterior cingulate cortex
PCR	polymerase chain reaction
PET	positron emission tomography
PET/MRI	positron emission tomography/magnetic resonance imaging
Pu	putamen
ROI	region of interest
rs-fMRI	resting state functional magnetic resonance imaging
RTGA	reference tissue graphical analysis
SPM	Statistical Parametric Mapping
TD	typical development
Th	thalamus
Tp	temporal cortex
(period)	missing data

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