Correction

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Correction for "Altered metabotropic glutamate receptor 5 markers in PTSD: In vivo and postmortem evidence," by Sophie E. Holmes, Matthew J. Girgenti, Margaret T. Davis, Robert H. Pietrzak, Nicole DellaGioia, Nabeel Nabulsi, David Matuskey, Steven Southwick, Ronald S. Duman, Richard E. Carson, John H. Krystal, Irina Esterlis, and the Traumatic Stress Brain Study Group, which was first published July 17, 2017; 10.1073/pnas.1701749114 (*Proc Natl Acad Sci USA* 114:8390–8395).

The authors note that the affiliation for [c] should instead appear as National Center for PTSD, Clinical Neurosciences Division, West Haven, CT 06516. The authors also note that this affiliation should be applied to Steven Southwick, in addition to affiliations [a] and [b].

The corrected author and affiliation lines appear below. The online version has been corrected.

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Altered metabotropic glutamate receptor 5 markers in PTSD: In vivo and postmortem evidence

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Posttraumatic stress disorder (PTSD) is a prevalent and highly disabling disorder, but there is currently no targeted pharmacological treatment for it. Dysfunction of the glutamate system has been implicated in trauma and stress psychopathology, resulting in a growing interest in modulation of the glutamate system for the treatment of PTSD. Specifically, the metabotropic glutamate receptor 5 (mGluR5) represents a promising treatment target. We used [18F]FPEB, a radioligand that binds to the mGluR5, and positron emission tomography (PET) to quantify in vivo mGluR5 availability in human PTSD vs. healthy control (HCs) subjects. In an independent sample of human postmortem tissue, we investigated expression of proteins that have a functional relationship with mGluR5 and glucocorticoids in PTSD. We observed significantly higher cortical mGluR5 availability in PTSD in vivo and positive correlations between mGluR5 availability and avoidance symptoms. In the postmortem sample, we observed up-regulation of SHANK1, a protein that anchors mGluR5 to the cell surface, as well as decreased expression of FKBP5, implicating aberrant glucocorticoid functioning in PTSD. Results of this study provide insight into molecular mechanisms underlying PTSD and suggest that mGluR5 may be a promising target for mechanism-based treatments aimed at mitigating this disorder.

PTSD | PET | glutamate | glucocorticoid | RNA

The lifetime prevalence of posttraumatic stress disorder (PTSD) among adults in the United States is ~6% (1). PTSD is triggered by a traumatic event and associated with reexperiencing, avoidance, negative changes in cognition and mood, and symptoms of arousal (2). Despite the clinical, social, and economic burden of PTSD (3), current pharmacological treatments are ineffective in about 40% of patients (4), highlighting the need to identify novel molecular mechanisms underlying PTSD to develop more targeted and efficacious pharmacotherapies.

Glutamate is thought to play a key role in the pathogenesis of PTSD (5, 6) for several reasons: glutamate underlies synaptic plasticity and memory formation (7), stress significantly impacts glutamate transmission (8), and the glutamate receptor antagonist ketamine may have efficacy in treating PTSD (9). Metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors that mediate neuromodulatory effects of glutamate, making them a promising target for drug development (10). Considerable work has focused on the mGluR5 subtype, with multiple studies showing that administration of mGluR5 antagonists, such as 2-methyl-6-(phenylethynyl)pyridine (MPEP), leads to anxiolytic effects in animal models (11–16). Of particular relevance to PTSD, animal studies indicate that mGluR5 activity underlies stress-induced fear conditioning (17) and that antagonism of mGluR5 blocks the acquisition and expression of conditioned fear (18), suggesting that mGluR5 may play a crucial role in the storage and retrieval of trauma-related memories in PTSD. However, studies examining in vivo mGluR5 availability in humans with PTSD are lacking.

In this study, we used in vivo positron emission tomography (PET) and RNA sequencing to advance our understanding of the role of the glutamatergic system in the pathophysiology of PTSD. In study 1, we used PET and [18F]FPEB, a radioligand that binds to the negative allosteric modulator site on mGluR5 with high selectivity and specificity (19, 20), to quantify mGluR5 availability in individuals with PTSD. We hypothesized that cortical mGluR5 availability would be altered in PTSD, with the largest alterations in individuals with the most severe PTSD symptoms. We also hypothesized that PTSD-related alterations in mGluR5 availability in the PET study might reflect stress effects on receptor trafficking, which could be mediated by alterations in the levels of scaffolding proteins for mGluR5s, Homer, and SHANK-1. To evaluate this hypothesis, in a second study, we measured the gene expression levels for Homer and SHANK-1 in ventral prefrontal cortex (PFC) postmortem tissue from an independent sample of individuals with and without PTSD. We also examined gene expression for FKBP5, a cochaperone for the glucocorticoid receptor (GR) that has been implicated in PTSD (21–25).

Results

Elevated mGluR5 Availability in PTSD—PET Findings. Cortical mGluR5 availability was significantly higher in the PTSD group relative to the healthy control (HC) group by an average of 19% ($F_{1,28} = 4.24$, $P = 0.049$) (Fig. 1 A and B and Table 1). Regional exploratory analyses revealed significantly higher mGluR5 availability and large effect sizes in the PTSD group compared with the HC group in dorsolateral prefrontal cortex (dlPFC; $P = 0.034$; Cohen's $d = 0.77$) and orbitofrontal cortex (OFC; $P = 0.044$; $d = 0.74$), with trend significance in ventromedial prefrontal cortex (vmPFC; $P = 0.052$; $d = 0.70$) and parietal ($P = 0.050$; $d = 0.71$), temporal ($P = 0.072$; $d = 0.65$), and occipital cortices ($P = 0.066$; $d = 0.66$). Variances

Significance

Posttraumatic stress disorder (PTSD) is a highly prevalent and disabling disorder, but there are currently no targeted medications for its treatment. Glutamate dysfunction is thought to be involved in the pathophysiology of PTSD, and the metabotropic glutamate receptor 5 (mGluR5) may represent a treatment target. We show alterations in mGluR5 availability in vivo and mGluR5- and glucocorticoid-related gene expression in postmortem tissue in PTSD, providing insight into the molecular mechanisms underlying this disorder. Our findings could, therefore, help inform the development of critically needed targeted and effective treatments for those suffering from PTSD.
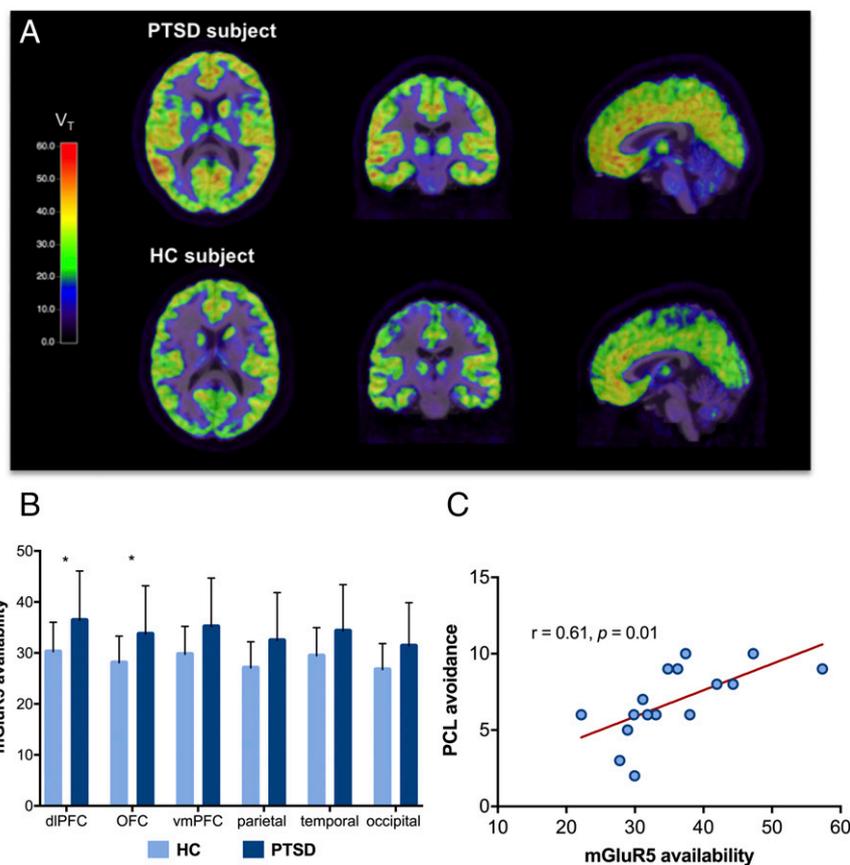
Fig. 1. mGluR5 availability in PTSD: PET findings. (*A*) Parametric [^{18}F]FPEB V_T images overlaid onto T1-weighted MRI in template space for a representative PTSD subject (*Upper*: 29-y-old male) and HC subject (*Lower*: 27-y-old male) in transaxial, coronal, and sagittal planes. (*B*) mGluR5 availability in PTSD (*n* = 16) compared with HC (*n* = 16). (*C*) Correlation between mGluR5 availability in the dlPFC and scores on the avoidance subscale of the PCL. Error bars represent SD. *P* values obtained from exploratory MANCOVAs. Pearson's *r* was used for correlation analysis. *Significant at *P* < 0.05.

were equal across groups as determined by Levene's test (*P* values: dlPFC, 0.132; OFC, 0.078; vmPFC, 0.075; parietal, 0.054; occipital, 0.114; temporal, 0.155). mGluR5 availability was assessed in subcortical regions (caudate, putamen, amygdala, hippocampus, and ventral striatum) as an exploratory analysis. Volume of distribution (V_T) values were consistently higher in the PTSD vs. HC group, but only ventral striatum reached significance (Table S1).

In the PTSD group, we found significant positive correlations between mGluR5 availability and scores on the avoidance subscale of the PTSD checklist (PCL) across all regions [dlPFC (*r* = 0.61, *P* = 0.012), OFC (*r* = 0.58, *P* = 0.020), vmPFC (*r* = 0.59, *P* = 0.016), parietal cortex (*r* = 0.56, *P* = 0.025), temporal cortex (*r* = 0.60, *P* = 0.014), and occipital cortex (*r* = 0.58, *P* = 0.018) (Fig. 1*C*)] and with the avoidance subscale of the Clinician-

Administered PTSD Scale (CAPS) in all PFC regions [dlPFC (*r* = 0.56, *P* = 0.037), OFC (*r* = 0.56, *P* = 0.039), vmPFC (*r* = 0.56, *P* = 0.039), and temporal cortex (*r* = 0.58, *P* = 0.031)]. No significant correlations between total CAPS or PCL scores or with the other subscales (reexperiencing, numbness, hyperarousal) were observed. There were no significant correlations between mGluR5 availability and age, body mass index, or duration of illness in either PTSD or HC group.

To assess the reliability of these analyses, we conducted bootstrapped analyses with 10,000 replicates; results of these analyses revealed *P* values of <0.001 for correlations between avoidance symptoms and mGluR5 in dlPFC, vmPFC, OFC, and temporal cortex and 0.001 for mGluR5 in parietal and occipital cortices. *P* values for all other correlations were >0.046.

Table 1. Regional [^{18}F]FPEB V_T (mGluR5 availability) for PTSD vs. HC

Region	HC (*n* = 16)	PTSD (*n* = 16)	Difference, %	Cohen's *d*	*P* value*
dlPFC	30.25 (5.78)	36.40 (9.67)	20.4	0.77	0.034[†]
OFC	28.07 (5.23)	33.71 (9.45)	20.1	0.74	0.044[†]
vmPFC	29.73 (5.48)	35.16 (9.54)	18.2	0.70	0.052
Parietal cortex	27.06 (5.12)	32.44 (9.40)	19.9	0.71	0.050
Temporal cortex	29.44 (5.52)	34.30 (9.09)	16.5	0.65	0.072
Occipital cortex	26.73 (5.11)	31.38 (8.47)	17.4	0.66	0.066

Values are given as mean (SD).
P values obtained from MANCOVAs, with age and sex as covariates.
[†]Significant at *P* < 0.05.

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Up-Regulation of SHANK-1 and Down-Regulation of FKBP5 in PTSD—Postmortem Findings. Our analysis revealed a statistically significant 3.5-fold reduction in FKBP5 expression (±1.4; Bonferroni-corrected $P < 0.05$; $n = 19$) and a 3.8-fold up-regulation of SHANK-1 (±0.45; Bonferroni-corrected $P < 0.05$; $n = 19$) in postmortem tissue of individuals with PTSD (Fig. 2 and Table S2). mGluR5, Homer, FKBP1a, and FKBP8 did not differ between groups (Table S2). There were no significant differences in transcript expression between the individuals with PTSD who were medicated and nonmedicated at time of death.

Discussion

We report significantly higher cortical mGluR5 availability in medication-free individuals with PTSD compared with HCs. We also found an association between higher mGluR5 availability and an increase in avoidance symptoms, with the strongest associations occurring in the PFC. With [^{18}F]FPEB PET, we are likely quantifying mGluR5 at the cell surface but not internalized receptors. Given that the mGluR5 gene expression was not increased in the postmortem tissue, higher ligand binding to mGluR5 receptors in vivo might reflect increased trafficking of receptors or stabilization of receptors at the neuronal membrane surface. Consistent with this hypothesis, SHANK-1 gene expression was up-regulated.

The mechanism underlying the up-regulation of mGluR5 is not yet clear. A growing range of glutamate-related alterations are associated with PTSD (26). The association between higher mGluR5 availability and increased SHANK-1 gene expression raises the possibility of a coordinated pattern of synaptic adaptations in PTSD that serves to enhance the stabilization of mGluR5s at the synapse. The processes driving these adaptations need to be examined. Preclinical studies indicate that acute administration of corticosterone (27) and acute stress-induced increases in corticosterone (28) reduce the expression of mGluR5. However, PTSD seems to be associated with down-regulation of the glucocorticoid-related gene serum- and glucocorticoid-regulated kinase 1 (SGK1) (29) and now, FKBP5. Both SGK1 and FKBP5 gene expressions are enhanced by cortisol exposure in animals (30, 31). These findings raise the possibility that up-regulated mGluR5 availability and SHANK-1 gene expression could be related to the observed deficits in glucocorticoid signaling in PTSD (32–35).

The 3.5-fold decrease in FKBP5 expression that we found in postmortem PTSD compared with comparison samples is consistent with previous work showing decreased FKBP5 expression in WBCs and brain after trauma (21, 22, 36). FKBP5 regulates
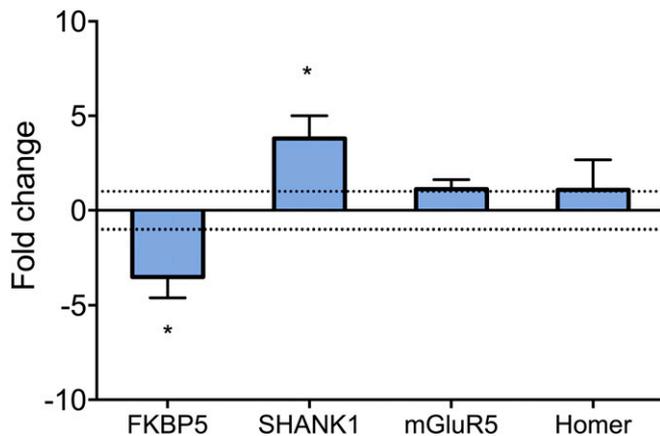
the cortisol binding affinity and nuclear translocation of the GR (23). It is thought that changes in FKBP5 may facilitate enhanced GR responsiveness and lower cortisol levels in PTSD (23). Indeed, deletion of the FKBP5 gene in mice increases the sensitivity of the hypothalamic–pituitary–adrenal (HPA) axis to negative feedback by glucocorticoids, leading to lower cortisol levels after acute stress (24), similar to what is seen in PTSD (32–35). Decreased FKBP5 adds to the evidence implicating dysfunction of glucocorticoid function in PTSD and is consistent with our previous finding of decreased SGK1 (29). The fact that we found no differences in FKBP1a or FKBP8 suggests that our findings are specific to FKBP5.

Alterations in mGluR5 could play a role in the etiology of PTSD. Preclinical research indicates that mGluR5 plays a crucial role in fear-conditioning models, which are used to mimic the traumatic events that induce the symptoms of PTSD. For example, mGluR5 activity leads to enhancement of contextual fear conditioning after stress, and antagonism of mGluR5 abolishes this effect (17). Several other studies have shown that antagonism of mGluR5 (18, 37, 38) and genetic deletion of mGluR5 (39) block or reduce fear conditioning and that fear conditioning is associated with increased expression of mGluR5 (18). Furthermore, a wealth of research shows that mGluR5 antagonists have anxiolytic effects in animal models (10–15), and we recently showed that down-regulating mGluR5 is associated with a decrease in anxiety symptoms in individuals with major depressive disorder (MDD) (40). The correlation between mGluR5 availability and avoidance symptoms suggests that mGluR5 could be a symptom-specific target. Indeed, PTSD symptom clusters may have distinct neurobiological underpinnings (41–43). The fact that we found significant correlations between mGluR5 availability and avoidance symptoms as measured by both the clinician-administered CAPS and the self-rated PCL limits the likelihood of a false positive. Avoidance is hypothesized to be the driving force that perpetuates PTSD (44) and significantly impairs quality of life (45, 46), highlighting the importance of elucidating its molecular underpinnings. The fact that we found a correlation with PTSD symptoms in the PFC specifically is consistent with the known role of the PFC in regulating threat-related processing (47) and data from neuroimaging studies showing impaired function in PFC in individuals with PTSD (48–50). Furthermore, although we show that mGluR5 is globally higher in PTSD, the dlPFC and OFC showed the greatest difference. Dysfunction of the PFC in PTSD is thought to play a key role in fear conditioning (47) as well as cognitive–emotional interactions and social–emotional processing (51). Dysregulation of mGluR5 in the dlPFC might, therefore, underlie PTSD symptoms. Impairments in OFC functioning have been associated with impulsivity (52). Dysregulation of mGluR5 in the OFC might, therefore, play a role in the association between PTSD and impulsive behaviors, including substance abuse (53), self-harm (54), and aggression (55). Interestingly, Akkus et al. (56) showed a positive correlation between mGluR5 and anxiety-related symptoms in Obsessive Compulsive Disorder (OCD) using PET. There is substantial symptomatic overlap between OCD and PTSD, with fear, anxiety, and avoidance symptoms being key to both disorders. Elevated mGluR5 availability in both PTSD and OCD could, therefore, represent a shared neurobiological substrate.

Methodological limitations of this study must be noted. First, the comparison groups in the PET and postmortem studies were not matched for trauma exposure. A comparison of individuals who developed PTSD after trauma vs. trauma-exposed individuals without PTSD would be most informative in identifying genetic and molecular mechanisms underlying PTSD. However, the use of trauma-expose comparison subjects is not always practically feasible, especially with postmortem samples. Second, in studies using postmortem human brain tissue, it is not always possible to rule out perimortem or postmortem confounds, and

Fig. 2. RT-PCR analysis of selected genes in control and PTSD subgenual PFC. Dotted lines indicate control (no change). Error bars represent SEM. *Significant at $P < 0.05$ (independent samples t tests).

results from postmortem samples should be interpreted with this in mind. Furthermore, in postmortem studies, it is not feasible to control for comorbid diagnoses as stringently as in in vivo studies. Table S3 indicates that the majority of PTSD subjects who donated their brains had some form of comorbid drug or alcohol disorder, consistent with PTSD and substance abuse/dependence being highly comorbid (53). Therefore, some caution is required in the interpretation of findings. However, based on existing literature, we attribute our postmortem results to the diagnosis of PTSD rather than comorbidities or peri- or postmortem factors. For example, a recent [18F]FPEB PET study found a reduction in mGluR5 in alcohol addiction (57), and another study using the mGluR5 radioligand [11C]ABP688 showed reduced binding in cocaine-dependent subjects (58). Conversely, we found no evidence of down-regulation of mGluR5, and the up-regulation of SHANK1 is consistent with increased expression of mGluR5 at the cell surface as shown by our PET findings. Third, a comprehensive measure of postmortem protein levels was beyond the scope of our study because of the limited number of samples available in the current PTSD brain bank. The proposed correlation of RNA to protein is normally between 30 and 60% (59). Our previous finding of decreased expression of SGK1 (29) and this finding of decreased FKBP5 expression in postmortem PTSD are consistent with research indicating that PTSD is associated with glucocorticoid dysfunction. Taken together with the research showing that changes in glucocorticoid function are associated with changes in mGluR5 and scaffolding proteins (17, 60, 61), we conclude that our RNA findings are consistent with changes in protein levels. Fourth, 9 of 16 PTSD participants in the PET study had comorbid MDD, reflecting the high comorbidity and symptomatic overlap between PTSD and depressive symptoms (62). Previously, mGluR5 availability was reported to be lower in MDD (63, 64). However, more recent studies have shown no differences between MDD and HC groups (65, 66). Sample size limited examination of the additive effects of MDD in this study; however, future work should examine the potential effects. Fifth, our outcome measure was V_T, which includes both specific and nonspecific binding. It was not possible to measure specific binding directly because of the lack of a region devoid of mGluR5 in the human brain (67, 68). Potential confounds that might affect mGluR5 binding were taken into account. Smoking has been shown to be associated with a marked global reduction in mGluR5 (69). The PTSD and HC groups were, therefore, matched for smoking status. Although the number of smokers was low in each group ($n = 4$), limiting the investigation of smoking effects on mGluR5 availability, we found no significant main or interaction effects of smoking (Table S4). Glutamate contributes to control of the circadian system, and mGluRs are thought to be play a role in circadian rhythms (70), raising the possibility that mGluR5 binding may be affected by the time of scanning. To limit this potential confound, participants in both groups were well-matched for time of scan (all were scanned in the afternoon within 2 h of each other). As an exploratory analysis, we investigated the relationship between sleep disturbance and mGluR5 availability within the PTSD group but found no correlations (SI Text).

This study investigates mGluR5 in vivo in PTSD and aims to examine mGluR5- and glucocorticoid-related protein expression in postmortem tissue of individuals with PTSD. We provide evidence for higher mGluR5 availability in PTSD, which may be related to avoidance symptoms specifically. In the postmortem study, we found increased expression of SHANK-1, which could be responsible for anchoring a greater number of mGluR5s to the cell surface, possibly as a result of aberrant glucocorticoid functioning. Collectively, results of this study provide insight into the molecular mechanisms underlying PTSD and could help inform the development of targeted and effective treatments, which are critically important because of the impairing nature of PTSD, the associated high suicide risk, and the current lack of

targeted pharmacological treatments. Additional research is needed to confirm our findings, clarify the association between mGluR5 and glucocorticoid functioning, and evaluate whether targeting mGluR5 may help mitigate symptoms of PTSD.

Materials and Methods

PET Imaging of mGluR5.

Participants. Sixteen medication-free individuals with PTSD (mean ± SD age = 36.5 ± 8.8 y old; 10 females) and 16 age-, smoking-, and sex-matched HC individuals (mean ± SD age = 36.4 ± 11.0 y old) participated in the PET study; 9 of 16 individuals with PTSD also met criteria for MDD, but there were no other comorbid diagnostic and statistical manual of mental disorders (DSM-IV) diagnoses. All participants ranged in age from 18 to 55 y old. Table S5 shows demographic and clinical characteristics of the sample. Participants underwent physical and neurological examination to rule out any major medical or neurological illness. Screening involved electrocardiography, complete blood counts, serum chemistries, thyroid function test, liver function test, urinalysis and urine toxicology screening, and plasma pregnancy tests (for women). Diagnosis was confirmed using the Structured Clinical Interview for DSM-IV (71). PTSD and mood symptoms were additionally assessed using the DSM-IV CAPS (72), the DSM-IV PCL (71), the Montgomery–Asberg Depression Scale (73), and the Hamilton Depression Rating Scale (74). Scores on the CAPS and the PCL were broken down according to the following symptom clusters: reexperiencing (including distressing recollections, dreams and flashbacks of the traumatic event, and psychological and physiological reactions to cues associated with the event), avoidance (including efforts to avoid thoughts, feelings, places, or people associated with the trauma and an ability to recall important aspects of the trauma), numbing (including anhedonia, feelings of detachment, and restricted range of affect), and hyperarousal (including hypervigilance, exaggerated startle response, difficulties sleeping, and concentrating). The DSM-IV combines avoidance and numbing symptom in one subscale, however, factor analysis (75) indicates that avoidance and numbing are distinct symptom clusters of PTSD, which are reflected in DSM-V criteria. As such, we combined the scores of the avoidance items (efforts to avoid thoughts, feelings, or conversations associated with the trauma and efforts to avoid activities, places, or people that arouse recollections of the trauma) and scores of the numbing items separately. Types of trauma included sexual abuse ($n = 4$), witnessing of shooting ($n = 3$), military combat ($n = 2$), physical abuse ($n = 2$), car accident ($n = 2$), sexual assault ($n = 1$), human trafficking ($n = 1$), and robbery at gunpoint ($n = 1$). Exclusion criteria were lifetime history of bipolar disorder or schizophrenia; diagnosis of alcohol or substance abuse (past 6 mo) or dependence (past 12 mo), except for nicotine dependence; positive urine toxicology or pregnancy tests before any scan; psychotropic medication within the past 2 mo; history of loss of consciousness for more than 5 min; significant medical condition; and contraindications to MRI/PET scans. Exclusion criteria were the same for the HC group, except for the addition of no current or history of any DSM-IV diagnosis, except for nicotine dependence. The Yale University Human Investigation Committee and the Radioactive Drug Research Committee approved the study. All participants provided written informed consent before inclusion in the study.

MRI and PET scanning. T1-weighted MRI scans were acquired on a 3T scanner (Trio; Siemens Medical Systems) to exclude structural abnormality and for coregistration with PET. [18F]FPEB was synthesized onsite as described previously (19). [18F]FPEB was administered i.v. as bolus plus infusion for 120 min, with a K_{bol} of 190 min (19, 76). Based on previous studies showing that equilibrium was reached at 60 min, emission data were acquired 90–120 min after the start of injection on the high-resolution research tomograph (Siemens/CTI), which has intrinsic spatial resolution of ~2.5 mm FWHM. We have previously shown that venous and arterial concentrations are well-matched at equilibrium, allowing venous sampling to be collected for metabolite correction as opposed to the more invasive arterial sampling (19). We also showed good test–retest variability (mean TRV of 12%) of [18F]FPEB binding using this approach (19). Venous samples were acquired at 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min postinjection for calculation of a metabolite-corrected venous input function as validated previously (19). A 6-min transmission scan was obtained for attenuation correction. Head motion was tracked using the Polaris Vicra optical tracking system (Vicra; NDI System). There were no significant differences in the injected dose or mass between PTSD and HC groups ($P = 0.40$ and $P = 0.78$, respectively) (Table S5). Dynamic scan data were reconstructed with corrections for attenuation, normalization, randoms, scatter, dead time, and motion using the ordered subset expectation maximization-based MOLAR algorithm (77). PET acquisition and quantitation were performed under blind conditions: radiotracer synthesis and data analyses

were performed based on anonymized identification numbers and without knowledge of psychiatric status.

PET image analysis. PET images were coregistered to each participant's T1-weighted MRI images using a six-parameter mutual information algorithm (FLIRT; FSL 3.2, Analysis Group, FMRIB), which was then coregistered to the magnetic resonance template by nonlinear transformation using the Bioimagesuite software (version 2.5; www.bioimagesuite.com). Regions of interest (ROIs) from the Anatomical Automatic Labeling for SPM2 template were used. ROIs included subdivisions of the PFC: vmPFC, OFC, dlPFC, parietal cortex, temporal cortex, and occipital cortex. The primary dependent variable, mean cortical mGluR5 availability, represents mean mGluR5 availability across these regions. Gray matter segmentation was conducted using FSL-FAST, and a gray matter mask was applied to the ROIs. Given the lack of a reference region for mGluR5 targets (78), V_T (ratio of radioligand concentration in ROI to the concentration in plasma at equilibrium) was used as the outcome measure. V_T was estimated by the equilibrium analysis method described previously (76) and a venous plasma input function (19).

Postmortem Gene Expression Analysis. Quantitative RT-PCR (qRT-PCR) was performed on Brodmann Area 25 (BA 25; part of vmPFC and anterior cingulate) in 19 individuals with PTSD (mean age $\pm$ SD = 47.6 $\pm$ 10.9 y old; 10 females) and 19 matched comparison subjects without PTSD (mean age = 50.1 $\pm$ 10.4 y old; 10 females) (Table S6). qRT-PCR was performed, and primers for the transcripts of FKBP5, SHANK-1, mGlur5, and Homer were designed to test mGluR5- and glucocorticoid-related genes. At the time of death, 13 of 19 individuals with PTSD were on an antidepressant, and 10 of 19 comparison subjects were using an over the counter medication. Details of cause of death are in Tables S3 and S7. The average postmortem interval (PMI) was 15.6 h ($\pm$5.7) in the PTSD samples and 18.8 h ($\pm$7.1) in comparison samples. There were no significant differences between PTSD and HC samples in age, PMI, pH, or RNA integrity number. RNA was isolated from subgenual anterior cingulate cortex (sgACC) (BA 25) using the RNEasy Plus Mini kit (Qiagen); 1 μg was reverse-transcribed into cDNA using oligo-dT primers and reverse transcriptase. RNA was then hydrolyzed and resuspended in nuclease-free water. Gene-specific primers were designed using Primer 3 freeware (bioinfo.ut.ee/primer3-0.4.0/primer3/)

and tested for efficiency and specificity by serial dilution and melt curve analysis. Sybr Green mix (ABI) was used to amplify cDNA, and fold regulation was calculated using the 2-delta delta threshold cycle (2-DDCt) method. The 2-DDCt analysis calculates relative gene expression levels between different samples by using threshold cycles generated by the fluorescent signal of the amplicon during quantitative PCR (79).

Statistics. For the PET study, statistical analysis was performed in SPSS Statistics v22 (IBM Corp.). Independent samples *t* tests were used to assess differences between demographic and radiotracer characteristics across groups. Group differences in mGluR5 availability ([^{18}F]FPEB V_T) were assessed using a univariate analysis of covariance, with mean cortical V_T as the dependent variable, diagnosis as the independent variable, and age and sex as covariates. Regional exploratory analyses were carried out using multivariate analysis of covariance (MANCOVA) adjusted for age and sex. Sphericity of the data was checked by Mauchly's test and corrected by the Greenhouse–Geisser procedure if violated. Homogeneity of variances was checked by Levene's test. Pairwise comparisons were subsequently carried out to determine regional V_T differences. To evaluate associations between mGluR5 availability and clinical and demographic variables, which were normally distributed based on Shapiro–Wilk tests, we computed Pearson's *r*. Findings were considered significant at the $P < 0.05$ level. For the postmortem study, differences in transcriptional changes in PTSD relative to comparison samples were evaluated using Graphpad Prism v6 (Graphpad Software Inc.), with fold changes calculated using the 2-DDCt method. Independent samples *t* tests followed by Bonferroni correction were used to assess statistical differences in the fold regulations calculated.

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