

Association Among Clinical Response, Hippocampal Volume, and *FKBP5* Gene Expression in Individuals with Posttraumatic Stress Disorder Receiving Cognitive Behavioral Therapy

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Background: Posttraumatic stress disorder (PTSD) is characterized by a reduced expression of *FKBP5*, a key modulator of the glucocorticoid receptor. Smaller hippocampal volume has also been documented in PTSD. We explored possible changes in *FKBP5* gene expression and brain structure in patients with PTSD after cognitive behavioral therapy (CBT).

Methods: We measured peripheral *FKBP5* RNA and volumes of the hippocampus, amygdala, and medial orbitofrontal cortex in 39 patients with PTSD before and after CBT. The control subjects were 31 trauma-exposed individuals without PTSD who were also assessed twice. Gene expression changes were screened with a microarray toolkit, which was followed by quantitative polymerase chain reaction for *FKBP5* RNA. Brain volumes were measured using FreeSurfer.

Results: At baseline, patients with PTSD showed lower *FKBP5* gene expression and smaller hippocampal and medial orbitofrontal cortex, but not amygdala, volumes relative to control subjects. At follow-up, we found significantly increased *FKBP5* expression and increased hippocampal volume in patients with PTSD. At follow-up, patients did not differ from control subjects in hippocampal volume. Improvement in PTSD symptoms was predicted by increased *FKBP5* expression and increased hippocampal volume, but the primary predictor was *FKBP5* expression. The most significantly altered gene expression in patients with PTSD relative to control subjects was found for ribosomal protein S6 kinase, which did not change after CBT and did not correlate with hippocampal volume.

Conclusions: Clinical improvement in individuals with PTSD was associated with increased expression of *FKBP5* and increased hippocampal volume, which were positively correlated.

Key Words: Cognitive behavioral therapy, *FKBP5*, gene expression, hippocampus, posttraumatic stress disorder, structural brain imaging

In spite of extensive research focusing on the mechanism and treatment of posttraumatic stress disorder (PTSD), the neurobiological correlates of symptom improvement during psychotherapy remain elusive. Results from brain imaging studies and animal models suggest that the medial prefrontal cortex, amygdala, and hippocampus play a role in the development of PTSD. These brain areas are involved in fear conditioning, extinction, emotion regulation, and spatiotemporal context representation (i.e., where and when the traumatic event occurred) (1–4). Pioneering studies found smaller hippocampal volumes in PTSD associated with combat-related trauma and childhood abuse [(5,6); for meta-analytic evidence, see (3,7–9)]. Additional gray matter reductions have been identified in anterior cingulate cortex, ventromedial prefrontal cortex, and left temporal pole/middle temporal gyrus (3,7).

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Functional brain imaging studies, using different tasks or a resting state paradigm during scanning, revealed increased activation in widespread prefrontal and cingulate areas as a potential impact of cognitive behavioral therapy (CBT) in PTSD, although the results are inconsistent [for a review, see (10)]. In contrast to the results of functional brain imaging studies, morphological changes associated with psychotherapy were confined to the hippocampal formation and cingulate cortex [(11–13)] but see (14); reviewed in (10)].

The molecular correlates of these changes are unknown. The role of the overactive hypothalamic-pituitary stress axis has been implicated in PTSD (15), and alteration of the homeostatic regulation of cortisol may lead to structural changes, such as hippocampal atrophy (16,17). There is an abundance of potential target molecules, but *FKBP5* (FK506 binding protein) may be a special hub in complex molecular networks, and multifaceted evidence suggests its role in the pathophysiology of mood disorders and PTSD (18). *FKBP5* binds to the glucocorticoid receptor complex, reducing the affinity of cortisol and the nuclear translocation of the receptor (18). In mice, deletion of the *FKBP5* gene increased the sensitivity of the hypothalamic-pituitary axis for negative feedback by glucocorticoids, leading to a lower cortisol level after acute stress, which is similar to PTSD (19). Genetic polymorphisms of the *FKBP5* gene have been shown to be associated with anxiety and mood disorders, including PTSD, interacting with environmental adverse events [e.g., (20–23)]. These genetic factors and related molecular mechanisms, as well as pre-existing structural brain alterations, might predict the development of future PTSD (24–27).

Segman *et al.* (28) found that the degree of *FKBP5* gene expression within a few hours after a traumatic event correlated significantly with the severity of acute PTSD-like symptoms.

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Yehuda *et al.* (29) reported lower FKBP5 RNA levels in PTSD patients surviving the World Trade Center attacks compared with individuals without PTSD. Other research groups confirmed and extended these findings (30,31). Animal models suggest that the *FKBP5* gene is expressed where glucocorticoid receptors are also abundant, including the hippocampal formation and the central amygdala (32).

In the present study, we aimed to explore morphological changes of brain areas linked to the symptoms of PTSD before and after CBT. In addition, we compared the clinical and morphological changes with peripheral gene expression patterns. We measured the volume of the medial orbitofrontal cortex (mOFC), hippocampus, and amygdala before and after CBT and compared brain volume and symptom changes. Although there is no parsimonious meta-analytic evidence for altered amygdalar volume in PTSD (33), recent large-scale studies reported contradictory but significant findings (smaller versus enlarged amygdala in PTSD) (34,35). Therefore, we included amygdala in the analysis. Regarding the gene expression pattern, we first screened PTSD patients using a microarray platform, which was followed by quantitative polymerase chain reaction (qPCR). Specifically, we intended to replicate the altered expression of the *FKBP5* gene.

We had two main hypotheses. In PTSD patients who underwent CBT, there will be increased levels of FKBP5 messenger RNA relative to the pre-CBT baseline condition. This hypothesis was based on the finding that, in contrast to patients with active symptoms, remitted patients with PTSD did not exhibit significantly diminished *FKBP5* gene expression (30). Given that FKBP5 may be related to glucocorticoid and stress response in the hippocampus (32,36) and the expression of glucocorticoid-related genes (*GLIZ* [glucocorticoid-induced leucine zipper], *SGK-1* [serum- and glucocorticoid-induced protein kinase-1]) are associated with hippocampal atrophy in depression (37), we hypothesized a relationship between changes in hippocampal volume and *FKBP5* expression (increased hippocampal volume associated with increased *FKBP5* expression after CBT relative to pre-CBT baseline).

Methods and Materials

Participants and Psychological Assessment

We enrolled 47 previously untreated patients with PTSD and 31 trauma-exposed non-PTSD volunteers of Caucasian origin at the early trauma intervention center (National Psychiatry Center, Budapest, Hungary). Thirty-nine patients with PTSD completed the study (Table 1). Volunteers were referred by general practitioners, clinical psychologists, psychiatrists, and social workers who were trained to recognize probable PTSD using the Primary Care PTSD Screen (38). Additionally, volunteers could contact the center directly via telephone or e-mail. After psychoeducation and description of research, we offered the following opportunities: 1) CBT only (supplemented by pharmacologic interventions if needed and agreed); 2) CBT plus participation in research; 3) regular psychiatric treatment; and 4) none. The 47 patients enrolled in this study chose the second option. Twenty-seven patients not reported here chose another option. For psychological assessment, we administered the Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (39), the Trauma and Life Events Self-Report Inventory (40), the Clinician-Administered PTSD Scale (CAPS) (41), the Hamilton Depression Rating Scale (42), and the Wechsler Abbreviated Scale of Intelligence (43). All scales were administered by trained and regularly supervised clinical psychologists who were blind to the aim of the study. The

diagnosis was confirmed by two independent experts. Exclusion criteria included history of psychiatric or neurological disorders and current comorbid DSM-IV mood disorders, psychotic disorders, and substance misuse. Four patients with PTSD had comorbid panic disorder and specific phobia. The demographic and clinical data, including trauma type and duration of symptoms, are presented in Table 1. The study was carried out in accordance with the Declaration of Helsinki and it was approved by the local ethics board (TUKÉB 65/2010). All participants gave written informed consent.

Procedure

At the first assessment, patients with PTSD and trauma-exposed control subjects underwent psychological assessment, brain magnetic resonance imaging, and blood sample drawing. After this assessment, patients with PTSD received trauma-focused CBT according to the protocol of Marks *et al.* (44) [for Hungarian applications, see (45)]. Cognitive behavioral therapy helps identify, challenge, and modify negative automatic thoughts and maladaptive cognitive schemas. Participants learned how to identify dysfunctional automatic thoughts and cognitive distortions, find evidences for and against thoughts, create alternatives, and finally reappraise their beliefs about themselves and the trauma by creating a new narrative of the traumatic event. Following a psychoeducation section on PTSD, patients learned to detect their automatic thoughts using daily monitoring and diaries. During homework sections, patients prepared regular thought records and audiotaped their sessions (44,45). Clinical psychologists or psychiatrists with adequate training, regular supervision, and certification of Good Clinical Practice administered 12 weekly 1.5-hour sessions. We measured the clinicians' adherence to treatment protocol by videotaping and independent ratings. The control subjects did not participate in any psychological intervention. We preferred CBT instead of prolonged exposure therapy because most of the patients expressed concerns, anxiety, and discomfort after the description of exposure-based intervention. After the 12-week period, patients with PTSD and control subjects were re-assessed, including magnetic resonance imaging and blood sample drawing. Investigators analyzing imaging and molecular data were blind to diagnosis and time point of investigation. Clinical scales, interviews, and CBT sessions were administered by experts who were naïve to the aim of the study and did not have information on brain imaging and molecular data.

From the 47 patients with PTSD, 39 completed the study. The remaining eight patients decided to stop the CBT sessions and continued treatment according to the official protocol used in psychiatric practice, including antidepressant medications. The 39 patients who completed CBT did not receive antidepressant medications. Sixteen patients received benzodiazepines for less than 4 weeks to ameliorate sleep disorder and daytime anxiety, as well as beta blockers to reduce autonomic symptoms of anxiety and arousal. The changes in clinical symptoms across the two assessment points are shown in Table 1.

Structural Brain Imaging

We used the standard FreeSurfer protocol for structural neuroimaging (Martinos Center for Biomedical Imaging, Boston, Massachusetts; <http://surfer.nmr.mgh.harvard.edu>; version: v5.1.0, Dell XPS workstation; Dell Inc., Austin, Texas). We applied a multiecho FLASH sequence with a 1 mm³ isotropic resolution (Siemens Trio 3T scanner [Munich and Berlin, Germany]) (256 × 256 matrix, 176 sagittal slices with a thickness of 1 mm, repetition time 2530 msec, inversion time 1100 msec, echo time 1.64/3.5/5.36/7.22 msec,

Table 1. Clinical and Demographic Characteristics

	PTSD (N = 39; 30 Female Subjects, 9 Male Subjects)		Trauma-Exposed Control Subjects (N = 31; 20 Female Subjects; 11 Male Subjects)	
	Mean	SD	Mean	SD
Type of Trauma	Environmental disaster (red sludge and flood): n = 12 Violent crime: n = 8 Traffic accident: n = 13 Combat: n = 3 Emergency service workers: n = 3		Environmental disaster (red sludge and flood): n = 10 Violent crime: n = 5 Traffic accident: n = 10 Combat: n = 3 Emergency service workers: n = 3	
Smoking	n = 14		n = 12	
Occasional Alcohol Consumption	n = 25		n = 23	
Age (Years)	35.9	12.0	37.0	10.4
Education (Years)	11.6	2.9	11.4	2.7
IQ	110.6	12.2	108.8	8.9
Trauma and Life Events Self-Report Inventory	6.3	2.5	5.4	1.7
Duration of Symptoms (Months)	3.8	2.5		
Time Since Trauma (Months)	8.9	2.4		
	Before Cognitive Behavioral Therapy		After Cognitive Behavioral Therapy	
	Mean	SD	Mean	SD
Clinician-Administered PTSD Scale				
Re-experience ^a	15.6	6.9	10.3	6.9
Avoidance ^a	22.2	6.6	14.3	6.6
Arousal ^a	24.6	5.2	15.8	7.0
Total ^a	62.4	12.8	40.4	20.3
Hamilton Depression Rating Scale ^b	15.3	8.2	11.2	8.3

Patients with posttraumatic stress disorder (PTSD) and trauma-exposed control subjects did not differ in gender distribution, smoking, occasional alcohol use (chi-square tests, $p > .1$), age, education, and IQ (t tests, $p > .1$). Cognitive behavioral therapy was applied only in PTSD patients. Trauma-exposed control subjects did not receive psychological treatment.

^a $p < .0005$.

^b $p < .05$.

bandwidth 651 Hz, nonselective excitation at 7°). Automated image processing consisted of the following steps: removal of nonbrain tissue with a hybrid watershed/surface deformation procedure, automated Talairach transformation, and segmentation of white and gray matter (46). We also used laser alignment to optimize the standardization of head position across participants and assessments. The dependent measures were as follows: right and left hippocampus, amygdala, mOFC, intracranial volume, and total brain volume. These brain regions have been defined previously in the FreeSurfer protocol, and reliable and replicable volume measurements have been established (47,48) (<http://surfer.nmr.mgh.harvard.edu>). The accuracy of each FreeSurfer region of interest (ROI) was visually inspected. In a pilot study, we also performed the manual parcellation of these ROIs in 20 healthy control subjects. The intraclass correlation coefficient describing the correlation of FreeSurfer and manual parcellation methods were high ($r > .8$ for each ROI). We used the same computer (Dell XPS) and operation system (Linux; GNU/Linux, Free Software Foundation) for all measurements to avoid any bias stemming from different systems (49). Region of interest volumes were corrected for total intracranial volume (50).

Gene Expression and Plasma Cortisol

We adapted the method of Yehuda *et al.* (29). For the sake of comparability between the studies, we followed their procedure exactly, and therefore the technical details will not be described herein. We drew fasting blood samples between 08:00 and 09:00 hours. For screening, we used Human Genome U133 Plus 2.0

Array (Affymetrix, Santa Clara, California), which was followed by qPCR for *FKBP5* expression. At the second assessment, we performed *FKBP5* qPCR to study its potential changes after CBT. For a control analysis, we examined the factor that showed the most significantly altered expression in PTSD. This control analysis was hypothesis free. Serum cortisol concentration was measured at both assessments with a commercial radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, Texas).

Data Analysis

We used STATISTICA 11 software for data analysis (StatSoft, Inc., Tulsa, Oklahoma). First, data were entered into Kolmogorov-Smirnov and Levene's tests to verify normal distribution and homogeneity of variance, respectively. Second, we used repeated measures analyses of variance (ANOVAs) on ROI volumes. These ANOVAs investigated the main effects of group (PTSD vs. control subjects), assessment session (before vs. after CBT in patients, baseline vs. follow-up in control subjects), laterality (left vs. right), and their interactions. All ROI volumes were corrected for intracranial volume. Whole brain volume was included as a covariate in the ANOVAs. A similar ANOVA design was used for the analysis of *FKBP5* expression without laterality and volume corrections. Tukey's honestly significant difference (HSD) tests were used for post hoc comparisons. We calculated Pearson's product moment correlation coefficients and performed multiple regression analyses to explore the relationship between changes in PTSD symptoms, *FKBP5* expression, and hippocampal volume.

All analyses were corrected for gender, age, education, and IQ. Clinical and demographic data were compared with chi-square tests and two-tailed Student *t* tests. The level of statistical significance was set at $\alpha < .05$.

Results

Structural Brain Imaging

The ANOVA conducted on the hippocampal volume revealed a significant main effect of group (PTSD vs. control subjects, $F_{1,68} = 8.18, p < .05, \eta^2 = .11$). There was a significant interaction between group and assessment session (before and after CBT in patients) ($F_{1,68} = 13.29, p < .005, \eta^2 = .14$). The effect of laterality (left vs. right hippocampus) and its interactions with group and assessment session were not significant (all *p* values $> .3$).

Tukey's HSD conducted on the group by assessment session interaction indicated significantly smaller hippocampal volumes in patients with PTSD relative to control subjects at the first assessment (before CBT) ($p < .005$). This difference was not observed at the second assessment (after CBT) ($p > .3$). There were greater hippocampal volumes in patients with PTSD at the second assessment relative to the first assessment ($p < .05$). In control subjects, hippocampal volumes did not differ across assessments ($p > .2$) (Figure 1).

For mOFC volume, we also found a main effect of group ($F_{1,68} = 5.64, p < .05, \eta^2 = .08$), indicating a reduced volume in patients relative to control subjects, but the remaining main effects and interactions did not reach the level of statistical significance (all *p* values $> .1$). For amygdala and total brain volume, none of the main effects or interactions appeared significant (all *p* values $> .2$) (Table 2).

Gene Expression

At the first assessment, we replicated several differentially expressed transcripts in PTSD patients compared with control subjects, as reported by Yehuda *et al.* (29) (data not shown because this is beyond the scope of the study). The ANOVA conducted on the level of *FKBP5* expression measured by qPCR revealed significant main effects of group ($F_{1,68} = 18.0, p < .001, \eta^2 = .21$) and assessment session ($F_{1,68} = 12.2, p < .005, \eta^2 = .14$). The two-way interaction between group and assessment session was also significant ($F_{1,68} = 6.26, p < .05, \eta^2 = .08$).

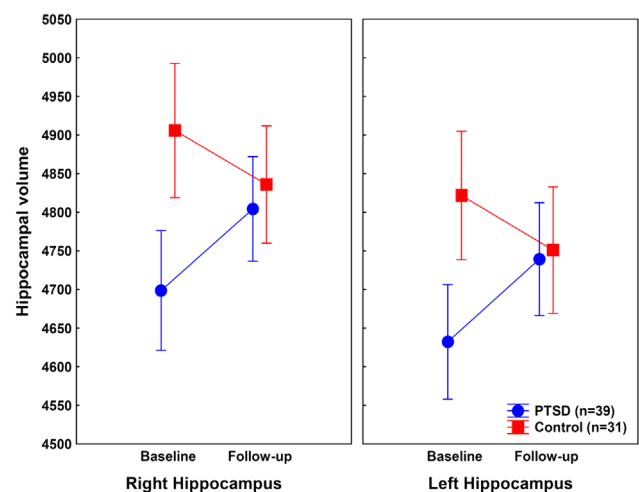


Figure 1. Hippocampal volume (mm^3) in patients with posttraumatic stress disorder (PTSD) and control subjects. Error bars indicate 95% confidence intervals. There were significant bilateral differences between patients and control subjects only before cognitive behavioral therapy ($p < .005$, Tukey's honestly significant difference test).

Tukey's HSD tests indicated lower *FKBP5* expression in individuals with PTSD relative to control subjects at the first assessment ($p < .001$), but this difference did not retain statistical significance at the second assessment ($p = .06$). In patients with PTSD, *FKBP5* expression increased across the first and second assessment ($p < .001$), whereas in control subjects these values did not change ($p = .9$) (Figure 2).

Serum Cortisol

There were statistically similar values in individuals with PTSD and control subjects at both assessments (first assessment PTSD: $14.6 \mu\text{g/dL}$, $\text{SD} = 5.0$; control subjects: $14.8 \mu\text{g/dL}$, $\text{SD} = 5.8$; second assessment PTSD: $15.0 \mu\text{g/dL}$, $\text{SD} = 5.2$; control subjects: $14.5 \mu\text{g/dL}$, $\text{SD} = 4.9$; $p > .5$).

Correlations between Symptoms, Hippocampal Volume, and *FKBP5* Expression

First, we examined the correlation among total CAPS scores, hippocampal volume, and *FKBP5* expression before and after CBT. We restricted this analysis to hippocampal volume because only

Table 2. Structural MRI Results

	First Assessment				Second Assessment			
	PTSD (<i>n</i> = 39)		Control Subjects (<i>n</i> = 31)		PTSD (<i>n</i> = 39)		Control Subjects (<i>n</i> = 31)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Right Hippocampus ^a	4698.7	253.3	4905.8	228.5	4804.4	217.0	4836.0	205.6
Left Hippocampus ^a	4632.1	247.7	4821.8	211.7	4739.4	243.9	4751.0	207.7
Right Amygdala	1728.9	103.9	1703.9	113.5	1727.4	111.9	1710.0	109.7
Left Amygdala	1683.7	107.2	1665.6	116.7	1705.3	117.2	1674.0	122.2
Right Medial Orbitofrontal Cortex ^b	5338.2	484.9	5642.4	487.3	5362.6	477.6	5632.1	498.4
Left Medial Orbitofrontal Cortex ^b	5311.5	518.4	5548.2	486.9	5330.0	509.6	5531.9	461.7
Total Brain Volume	1.5	.2	1.4	.2	1.4	.2	1.4	.2
Intracranial Volume	1.7	.2	1.7	.1	1.7	.2	1.7	.1

CBT, cognitive behavioral therapy; MRI, magnetic resonance imaging; PTSD, posttraumatic stress disorder.

Data are in mm^3 ($\times 10^6$ for total brain volume and intracranial volume).

^aSmaller volume in PTSD relative to control subjects, which was not observed after CBT.

^bSmaller volume in PTSD relative to control subjects, which was similar before and after CBT (for statistical details, see the text).

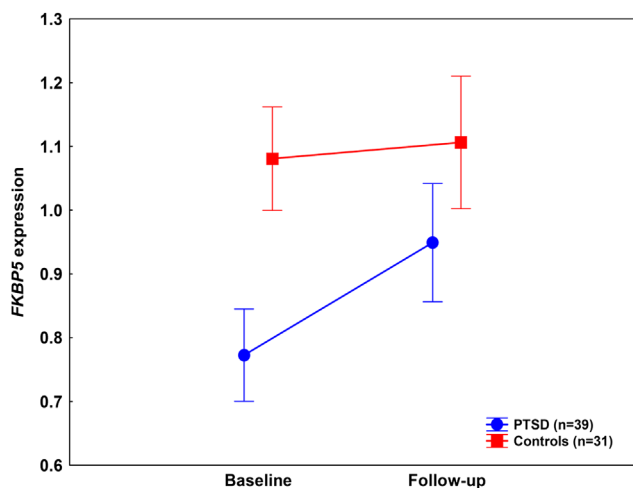


Figure 2. Peripheral *FKBP5* expression from quantitative polymerase chain reaction measurements. Error bars indicate 95% confidence intervals. There was a significant difference between patients and control subjects only before cognitive behavioral therapy ($p < .001$, Tukey's honestly significant difference test). PTSD, posttraumatic stress disorder.

this brain structure showed volume changes across assessments. These correlation analyses revealed no significant results ($-.2 < r < .2$, $p > .1$). Second, we examined the correlation between changes in total CAPS scores, changes in total hippocampal volume, and changes in *FKBP5* expression (change: values after CBT minus values before CBT). We found significant correlations between changes in CAPS scores and changes in *FKBP5* expression ($r = -.63$, $p < .001$), changes in hippocampal volume and changes in *FKBP5* expression ($r = .56$, $p < .001$), and changes in CAPS scores and changes in hippocampal volume ($r = -.52$, $p < .005$) (Bonferroni-corrected level of significance: $.05/9 = .006$) (Figure 3).

Regression analyses revealed that both changes in total hippocampal volume and changes in *FKBP5* expression significantly predicted changes in total CAPS scores (hippocampal volume: $b^* = -.52$, $t_{37} = -3.69$, $p < .005$, $R^2 = .25$; *FKBP5*: $b^* = -.63$, $t_{37} = -4.92$, $p < .001$, $R^2 = .38$). However, when both changes in *FKBP5* expression and changes in total hippocampal volume were entered into the analysis, only changes in *FKBP5* expression remained significant as a primary predictor ($b^* = .49$, $t_{37} = -3.26$, $p < .005$, $R^2 = .40$).

Control Analyses

We examined the specificity of our findings for *FKBP5*. Instead of a hypothesis-driven analysis, we investigated the factor exhibiting the most significantly altered expression in untreated patients with PTSD relative to control subjects. This factor was ribosomal protein S6 kinase (RPS6K) (90 kDa; Affy ID: 204633_s_at; $p < .00001$), which is in accordance with the results of Yehuda *et al.* (29). Expression of RPS6K did not change after CBT and did not correlate with hippocampal volumes ($-.1 > r > .1$, $p > .5$).

We also conducted some secondary analyses to test other factors and confounders. There were no significant correlations or predictive relationships among CAPS scores and serum cortisol ($p > .5$). Hamilton Depression Rating Scale scores did not correlate and predict brain volumes and *FKBP5* expression ($p > .5$). Demographic parameters, as depicted in Table 1, did not predict symptom reduction ($p > .5$).

Discussion

The most noteworthy finding of this study was that clinical improvement during CBT in PTSD was associated with increased hippocampal size and elevated *FKBP5* gene expression, a cellular regulator of the glucocorticoid receptor. Increased hippocampal volume and elevated *FKBP5* expression were significantly correlated, and the primary predictor of clinical improvement was increased *FKBP5* expression. We found smaller volumes of mOFC, but not amygdala, in PTSD at both assessments (before and after CBT).

The hippocampus may have a multifaceted role in the pathogenesis of PTSD. First, it participates in the formation of memory traces for contextual information of traumatic events (i.e., when and where the trauma happened), providing a representation of safety or danger of the environment (51). Second, the primate anterior hippocampus is essential in emotional stress response, with inhibitory pathways to the hypothalamic nuclei that regulate the secretion of cortisol. Novel evidence from gene expression profiling and anatomical connectivity suggests that the rodent dorsal (posterior in primates) hippocampus is linked to cortical regions participating in learning and spatial-contextual representation, whereas the ventral (anterior in primates) hippocampus is functionally associated with the amygdala and hypothalamus (52). Although pioneering structural imaging studies indicated that stress led to hippocampal atrophy (5,6,16,17), more recent studies raised the possibility that small hippocampal size is a premorbid vulnerability factor for PTSD (26) and larger hippocampal size is associated with clinical recovery (53). Admon *et al.* (54) showed that soldiers with decreased hippocampal volume after military service had more PTSD-related symptoms than those with increased hippocampal volume. Vermetten *et al.* (55) demonstrated that treatment with paroxetine, a selective serotonin reuptake inhibitor antidepressant, was accompanied by increased hippocampal volume in PTSD.

Although a previous study did not detect hippocampal volume changes during CBT (14), we found a significant volume expansion during a 12-week treatment period, which correlated with clinical improvement. The discrepancy between the negative

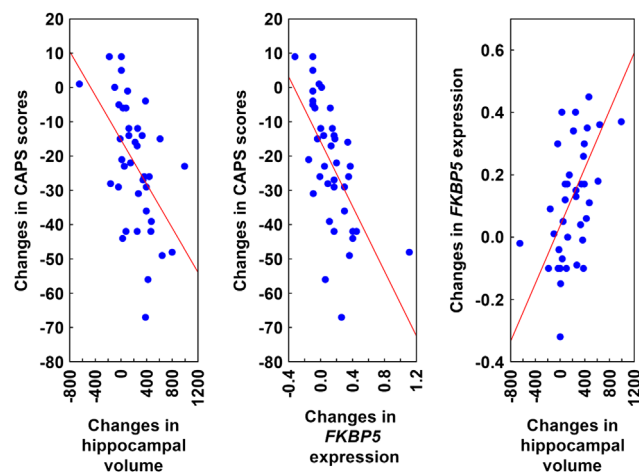


Figure 3. Correlations among Clinician-Administered PTSD Scale (CAPS) total scores, total hippocampal volume (mm^3), and *FKBP5* RNA expression after versus before cognitive behavioral therapy. Increased *FKBP5* expression was associated with greater total hippocampal volume expansion and better clinical improvement. Pearson's correlation coefficients: CAPS – hippocampus: $-.52$, $p < .005$; CAPS – *FKBP5*: $-.63$, $p < .001$; hippocampus – *FKBP5*: $r = .56$, $p < .001$.

findings of Lindauer *et al.* (14) and the present study can be explained by differences in patient populations (e.g., chronic features, presence of depression), sample size, and time passed since trauma; in the present study, we included less severely affected, recent-onset patients without comorbidity and with negative history of psychiatric disorders. This suggests that early intervention is especially important by targeting potentially more viable neuroplastic capacities. However, at the same time, it is dubious whether our findings can be generalized to chronic and more severely affected patients. Less severe depressive symptoms with relatively low variance may also explain a lack of correlation between imaging data and Hamilton Depression Rating Scale scores.

We also found a correlation between changes in hippocampal volume and *FKBP5* expression, which were both associated with clinical improvement. However, regression analyses indicated that *FKBP5* expression change was the primary predictor. Given that *FKBP5* is a regulator protein of the cortisol receptor (18) and abnormal cortisol secretion is linked to hippocampal atrophy (16,17), it is reasonable to hypothesize that the amelioration of *FKBP5* gene expression had a causal role in the normalization of hippocampal volume. Possible mechanisms may be enhanced neurogenesis, increased neuronal size, and enrichment of dendritic arborization (16–18,56). Intriguingly, it may occur together with an unaltered cortisol level, which is modulated by the polymorphisms of the *FKBP5* gene (57). The other direction of causality, presupposing that increased hippocampal size led to the improvement of *FKBP5* expression via its regulatory role on the hypothalamic-pituitary-adrenal stress axis, is less likely. Possible causal relationships, however, need to be confirmed directly.

This study has limitations. First, we did not use a randomized controlled design, and therefore it remained unclear whether the effects were specifically caused by CBT or spontaneous symptom improvement. Second, brain imaging was confined to predefined brain regions, providing no information about the structure and function of large-scale neuronal networks. We were not able to investigate functional and structural subdivisions of the hippocampal formation.

Hippocampal volume was slightly reduced in control subjects during the follow-up phase, but it was not significant. Given that automatic segmentation methods can lead to errors in the case of small subcortical structures, this is a critical issue. However, we did not observe such systematic changes in the case of amygdala, and there was no significant correlation between hippocampal and amygdala volumes ($-0.1 < r < 0.1$). This is against the possibility of a systematic error. Given that the control group included highly traumatized individuals without PTSD, one can speculate that these individuals showed decreasing hippocampal volume during the follow-up period, which may be an indicator of PTSD vulnerability in the absence of full clinical symptoms.

The third limitation is that the study was hypothesis-driven. These methodological restrictions were necessary to avoid statistical confounds due to multiple comparisons at this sample size. Nevertheless, we conducted a control analysis with the most significantly altered molecular factor in PTSD, RPS6K, which is an abundant signal transduction molecule critical in the regulation of ribosomes and protein translation, cell growth, and survival (58). Despite the statistically robust alteration, this factor was not related to symptom changes and hippocampal structure, and its role in PTSD is unknown. *FKBP5* expression can be considered as a state marker, which tends to be normalized along with symptomatic improvement, and therefore it can be a

marker of actual disease state. Together with other factors, these might be useful in the evaluation of vulnerability, diagnosis, disease progression, and treatment response (25,31).

The final limitation to mention is that a subgroup of patients received benzodiazepines and beta blockers for 4 weeks, which may have confounded the results. However, patients with PTSD and control subjects did not differ in smoking and alcohol use habits, and therefore the confounding effect of these factors was unlikely.

The strengths of the study lie in the recruitment of recent-onset, unmedicated patients with negative psychiatric history and the inclusion of trauma-exposed control subjects who were also assessed twice in a longitudinal design. Our data did not provide information about the effect of trauma without the development of PTSD because trauma nonexposed, supernormal individuals (59) were not included in the study. Nevertheless, the definitive difference in hippocampal volume between trauma-exposed control subjects and patients with PTSD at the first assessment was somewhat unexpected (7), providing evidence that smaller hippocampal volume is specifically associated with fully developed PTSD.

In conclusion, the results of the present study show a definitive link between clinical improvement during psychotherapy, structural changes of the brain, and peripheral expression of genes responsible for stress response. The results highlight the potential relevance of these joined mechanisms, together with the importance of early intervention in PTSD when neuroplastic capacities may be more viable.

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