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ORIGINAL ARTICLE

# Smaller hippocampal volume as a vulnerability factor for the persistence of post-traumatic stress disorder

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**Background.** Smaller hippocampal volume has often been observed in patients with post-traumatic stress disorder (PTSD). However, there is no consensus whether this is a result of stress/trauma exposure, or constitutes a vulnerability factor for the development of PTSD. Second, it is unclear whether hippocampal volume normalizes with successful treatment of PTSD, or whether a smaller hippocampus is a risk factor for the persistence of PTSD.

**Method.** Magnetic resonance imaging (MRI) scans and clinical interviews were collected from 47 war veterans with PTSD, 25 healthy war veterans (combat controls) and 25 healthy non-military controls. All veterans were scanned a second time with a 6- to 8-month interval, during which PTSD patients received trauma-focused therapy. Based on post-treatment PTSD symptoms, patients were divided into a PTSD group who was in remission ($n = 22$) and a group in whom PTSD symptoms persisted ($n = 22$). MRI data were analysed with Freesurfer.

**Results.** Smaller left hippocampal volume was observed in PTSD patients compared with both control groups. Hippocampal volume of the combat controls did not differ from healthy controls. Second, pre- and post-treatment analyses of the PTSD patients and combat controls revealed reduced (left) hippocampal volume only in the persistent patients at both time points. Importantly, hippocampal volume did not change with treatment.

**Conclusions.** Our findings suggest that a smaller (left) hippocampus is not the result of stress/trauma exposure. Furthermore, hippocampal volume does not increase with successful treatment. Instead, we demonstrate for the first time that a smaller (left) hippocampus constitutes a risk factor for the persistence of PTSD.

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**Key words:** Hippocampal volume, magnetic resonance imaging, post-traumatic stress disorder, trauma-focused therapy, war veterans.

## Introduction

Reduced (left) hippocampal volume has consistently been observed in patients with post-traumatic stress disorder (PTSD) compared with trauma-exposed controls and trauma-unexposed healthy controls (Kitayama *et al.* 2005; Smith, 2005; Karl *et al.* 2006; Kuhn & Gallinat, 2013). However, it is unclear whether a smaller hippocampus is a vulnerability factor for the development of PTSD or whether it is a consequence of stress/trauma exposure. The most compelling evidence for the former is provided by a twin study showing reduced hippocampal volume in the trauma-unexposed twin without PTSD (Gilbertson *et al.* 2002). The latter is, in turn, suggested by primate studies (Sapolsky *et al.* 1990; Gould *et al.* 1998), as well as a human study reporting reduced hippocampal volume in trauma-exposed controls compared with trauma-unexposed healthy controls (Winter & Irle, 2004). However, this was not found by another study comparing trauma-exposed and healthy controls (Bremner *et al.* 2003).

Were stress/trauma exposure to be the cause of a smaller hippocampus in PTSD, it would be possible that successful treatment leads to hippocampal volume increase. Indeed, the hippocampus is known to be a highly plastic brain region that is both positively and negatively affected by experiences and hormones (Gould *et al.* 2000). It is well known that hippocampal growth can be induced by treatment, such as electroconvulsive therapy in depressed patients (Nordanskog *et al.* 2010; Tendolkar *et al.* 2013), and

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by lithium use in bipolar disorder (Yucel *et al.* 2007, 2008). A few longitudinal studies have investigated change in hippocampal volume following treatment in PTSD patients, but results are inconsistent. Three studies showed hippocampal growth after treatment with selective serotonin reuptake inhibitors (SSRIs) (Vermetten *et al.* 2003; Bossini *et al.* 2007) or cognitive–behavioral therapy (CBT) (Levy-Gigi *et al.* 2013), whereas others did not observe any change in hippocampal volume after treatment with phenytoin (Bremner *et al.* 2005) or CBT (Lindauer *et al.* 2005). Of these studies, three did not include a control group (Vermetten *et al.* 2003; Bremner *et al.* 2005; Bossini *et al.* 2007), and could therefore not disentangle the effect of treatment from the effect of time. Moreover, all studies examined the PTSD group as a whole, whereas it is known that about 30 to 50% of patients do not respond to treatment (Bradley *et al.* 2005). It is important to make this distinction and to compare patients who are in remission after treatment (remitted patients) with patients in whom symptoms persist (persistent patients), and with a control group. One study demonstrated reduced hippocampal volume in veterans with current PTSD, but not in (remitted) veterans with lifetime PTSD (Apfel *et al.* 2011). This finding suggests that either hippocampal volume was increased in the remitted group, or raises a third hypothesis, i.e. that a smaller hippocampus is only observed in persistent patients and therefore constitutes a risk for the persistence of PTSD. The essential role of the hippocampus in learning and memory processes (Malenka & Nicoll, 1999) could potentially explain its importance in treatment response. However, as veterans in that study were not scanned pre-treatment, this differentiation could not be made. Answering this question is highly relevant for understanding vulnerability factors and treatment effects for hippocampal volume in PTSD.

In the current study, we collected magnetic resonance imaging (MRI) scans from 47 male war veterans with PTSD, 25 healthy male war veterans (combat controls) and 25 healthy non-military men. We measured hippocampal volumes to investigate whether trauma/stress exposure and PTSD status is related to reduced hippocampal volume. Since reduced volume of the left hippocampus was predominantly associated with PTSD, we analysed the left and right hippocampus separately. Our first aim was to test the hypotheses that hippocampal volumes of PTSD patients are smaller than hippocampal volumes of both control groups, and that combat controls have smaller hippocampal volumes than healthy controls. Furthermore, all veterans were scanned twice with a 6- to 8-month interval in which PTSD patients received trauma-focused therapy, the treatment for PTSD as recommended by international guidelines (Foa *et al.* 2009). Trauma-focused therapy includes trauma-focused CBT or eye-movement desensitization and reprocessing (EMDR), which have been found to be equally effective in treating PTSD symptoms (Bisson *et al.* 2007). Based on post-treatment PTSD symptoms, patients were divided into a PTSD group who was in remission and a group in whom PTSD symptoms persisted. Pre- and post-treatment volume of the hippocampus was compared for the combat controls, remitted and persistent PTSD patients. With this longitudinal design our second aim was to investigate whether reduced hippocampal volume in PTSD patients normalizes after successful treatment.

## Method

### *Participants*

War veterans who were diagnosed with combat-related PTSD by a psychologist or psychiatrist at one of the four Military Mental Healthcare out-patient clinics were invited to participate in the current study. A total of 47 male war veterans with PTSD were included and 25 male veterans without a current psychiatric disorder were included as combat controls. Additionally, 25 non-military men without a current psychiatric disorder and who had not been exposed to enduring high levels of stress in their lives were included as healthy controls. All participants underwent 3-T MRI scanning. A second MRI scan was collected from all veterans with a 6- to 8-month interval in which PTSD patients received trauma-focused therapy (treatment as usual). To confirm and quantify the severity (or absence) of PTSD symptoms at both time points, the Clinician-Administered PTSD Scale (CAPS; Blake *et al.* 1990) was applied by a trained researcher. PTSD patients were divided into remitted and persistent PTSD groups to investigate (the lack of) PTSD symptoms post-treatment. PTSD in remission was defined as a post-treatment CAPS score below 45 as this has previously been found to indicate the absence of clinically significant PTSD symptoms (Weathers *et al.* 1999). A post-treatment CAPS score of 45 or above was taken as a measure for persistence of PTSD. To examine (co-morbid) psychiatric disorders at both time points the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I; First *et al.* 2002) was administered. Subjects with current alcohol abuse and/or dependence or with a history of neurological illness were excluded. All participants gave written informed consent after having received complete written and verbal explanation of the study, in accordance with procedures approved by the University Medical Center Utrecht ethics committee and the declaration of Helsinki of 2008.

### Image acquisition

A 3.0-T MRI scanner (Philips Medical System, The Netherlands) at the University Medical Center Utrecht was used to acquire a T1-weighed image (200 slices, repetition time = 10 ms, echo time = 3.8 ms, flip angle 8°, field of view = 240 × 240 × 160 mm, matrix of 304 × 299).

### Image processing

The freely available and extensively validated Freesurfer software (version 5.1.0; http://surfer.nmr.mgh.harvard.edu) was used to estimate the volumes of the left and right hippocampus. Technical details on this analysis are described elsewhere (e.g. Dale *et al.* 1999; Fischl *et al.* 1999). For these volumes, neuroanatomical labels were automatically assigned. This automatic labeling was based on probabilistic information from a manually labeled set, and was previously shown to give similar results as when manually labeled (Fischl *et al.* 2002).

Before the group analyses on our regions of interest could be performed, all output was visually inspected to confirm that the hippocampus was properly segmented. Subcortical labeling was inspected following the standardized ENIGMA protocol (http://enigma.ini.usc.edu/protocols/imaging-protocols/quality-checking-subcortical-structures/).

### Statistical analyses

Group differences for participant characteristics were analysed with analyses of variance for means and $\chi^2$ tests for proportions.

For the MRI data, univariate analyses were performed to compare left and right hippocampal volume (pre-treatment) for PTSD patients, combat controls and healthy controls. Second, repeated-measures analyses with pre- and post-treatment as a within-subject factor and group (remitted patients, persistent patients, and combat controls) as the between-subjects factor were performed for the left and right hippocampus. Age and intracranial volume (determined from a T1-weighted image using Freesurfer) at the first scan were included as covariates in all MRI analyses.

Several *post-hoc* analyses were performed. First, the pre-treatment analyses were repeated with four groups, i.e. the two control groups and the remitted and persistent patients, to investigate if the findings could be explained by one of the PTSD groups in particular. Second, the pre- and post-treatment measurements were re-analysed with only medication-naive patients to investigate the effect of medication use, because medication is thought to influence hippocampal volume (Duman *et al.* 2001). Furthermore, pre- and post-treatment hippocampal volumes were compared between medication-naive PTSD patients and patients using SSRIs pre-treatment. Third, as alcoholism was found to contribute to hippocampal volume deficits associated with PTSD (Hedges & Woon, 2010), analyses were repeated after excluding patients with lifetime (but not current) alcohol abuse and/or dependence. Finally, it was investigated whether there was a correlation between hippocampal volume and PTSD severity, and change in hippocampal volume and clinical improvement in the PTSD group.

### Ethical Standards

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

## Results

### Participants

Participant characteristics are presented in Tables 1 and 2. Freesurfer analyses were not performed on MRI scans of two combat controls and one PTSD patient, due to poor scan quality (movement). One PTSD patient had severe temporal lobe atrophy and was therefore excluded from further analyses. Furthermore, one patient had not received treatment in between the two MRI scans and was also excluded. This yielded 44 PTSD patients, 23 combat controls and 25 healthy controls for the analyses. The three groups did not differ in age. The healthy control group had a higher education level compared with the veteran groups, but parental education level did not differ between the groups (Table 1).

Based on post-treatment PTSD severity, 22 PTSD patients were classified as remitted and 22 as persistent patients. The three veteran groups did not differ in age, education level, months since deployment, number of missions, and early traumatic experiences (investigated with the Early Trauma Inventory – Self Report; Bremner *et al.* 2007) (Table 2). Pre-treatment total CAPS score was larger in patients than in controls, and larger in persistent patients than remitted patients. This variance between persistent and remitted patients was explained by a group difference in hyperarousal symptoms. Re-experiencing and avoiding and numbing symptoms did not significantly differ between remitted and persistent patients. All patients included in the analyses had received trauma-focused therapy, and number of treatment sessions did not differ between the two patient groups (Table 3). Remitted and persistent patients were comparable on pre-treatment

**Table 1.** *Age, education level and PTSD symptoms pre-treatment of healthy controls, combat controls and PTSD patients*

| | Healthy controls ($n = 25$) | Combat controls ($n = 23$) | PTSD patients ($n = 44$) | $F$ | $p$ |
|---|---|---|---|---|---|
| Age, years | 35.4 (10.1) | 36.7 (10.5) | 36.5 (9.2) | 0.13 | 0.876 |
| Education level: ISCED | | | | | |
| Own | 5.2 (1.2) | 3.5 (1.9) | 3.5 (1.2) | 13.2 | <0.001 |
| Father | 2.8 (1.7) | 4.0 (1.8) | 3.5 (1.9) | 2.27 | 0.110 |
| Mother | 3.0 (1.9) | 2.8 (1.5) | 2.4 (1.5) | 1.15 | 0.319 |
| PTSD symptoms pre-treatment | | | | | |
| Re-experiencing: CAPS B | 0.7 (1.7) | 0.7 (1.3) | 23.2 (5.4) | 377.7 | <0.001 |
| Avoiding: CAPS C | 1.2 (2.6) | 0.7 (2.0) | 23.7 (9.2) | 134.3 | <0.001 |
| Hyperarousal: CAPS D | 3.1 (3.6) | 3.1 (2.9) | 24.5 (4.9) | 203.3 | <0.001 |
| Total: CAPS total | 5.0 (4.4) | 4.5 (4.1) | 71.4 (13.2) | 543.6 | <0.001 |

Data are given as mean (standard deviation).

PTSD, Post-traumatic stress disorder; ISCED, International Standard Classification of Education (Schneider, 2013); CAPS, Clinician Administered PTSD Scale (Blake *et al.* 1990).

medication use; however, persistent patients had more SSRI use post-treatment. Most patients had the same medication status or used the same type of medication throughout their treatment, and some patients changed medication. However, there were no group differences in the number of patients that changed medication status (remitted, $n = 5$; persistent, $n = 6$; $p = 0.728$) or type of medication (remitted, $n = 3$; persistent, $n = 5$; $p = 0.434$). Persistent patients had more co-morbid anxiety disorders pre-treatment and co-morbid depression post-treatment. The two patient groups did not significantly differ with respect to lifetime alcohol abuse and/or dependence (Table 3).

### *Relation to stress/trauma exposure*

Results of the comparison of PTSD patients, combat controls and healthy controls are displayed in Fig. 1. The univariate analyses revealed a group difference in the left hippocampus ($F_{2,87} = 3.98$, $p = 0.022$). *Post-hoc t* tests showed that PTSD patients differed significantly from the healthy controls ($p = 0.019$) and the combat controls ($p = 0.027$). The combat control and healthy control groups did not differ from each other ($p = 0.952$). No significant group difference was observed in the right hippocampus ($F_{2,87} = 2.27$, $p = 0.109$).

### *Effect of treatment*

Results of the pre- and post-treatment analyses for the remitted and persistent patients and combat controls are displayed in Fig. 2. No significant interaction between group and time was observed. Instead, in the left hippocampus a significant main effect of group was found ($F_{2,62} = 4.72$, $p = 0.012$). Persistent PTSD patients had a significantly smaller left hippocampus across both time points than both combat controls ($p = 0.005$) and remitted PTSD patients ($p = 0.027$). In the right hippocampus only a marginally significant group difference was found ($F_{2,62} = 2.86$, $p = 0.069$). *Post-hoc* tests revealed a smaller right hippocampus in the persistent PTSD patients compared with combat controls ($p = 0.037$), whereas remitted patients did not differ from combat controls or persistent patients.

### Post-hoc *analyses*

As the persistent patients probably explain the group difference for the comparison of PTSD patients, combat controls and healthy controls, the data were re-analysed with four groups. Again, a group difference was observed in the left hippocampus ($F_{3,86} = 4.08$, $p = 0.009$). Indeed, only the persistent PTSD group differed from the healthy controls ($p = 0.003$), combat controls ($p = 0.004$) and from the remitted PTSD group ($p = 0.049$). There was no significant group difference in the right hippocampus.

Second, to investigate the potential effect of medication on our results, pre- and post-treatment measures of hippocampal volume were compared for medication-naive remitted patients ($n = 10$), medication-naive persistent patients ($n = 8$) and combat controls ($n = 23$). Again a group difference in the left hippocampus ($F_{2,36} = 3.74$, $p = 0.036$) was observed, showing that this finding cannot be explained by medication use. In the right hippocampus, again no significant difference was observed ($F_{2,36} = 1.27$, $p = 0.289$). The potential effect of SSRIs was analysed by comparing hippocampal volume, pre- and post-treatment, between patients using SSRIs pre-treatment and medication-naive patients in the whole PTSD sample (SSRI, $n = 11$;

**Table 2.** *Age, education level, time since deployment, number of missions, number of early traumatic experiences and PTSD symptoms pre- and post-treatment of combat controls, remitted PTSD patients and persistent PTSD patients*

| | Combat controls ($n = 23$) | Remitted patients ($n = 22$) | Persistent patients ($n = 22$) | $F$ | $p$ |
|---|---|---|---|---|---|
| Age, years | 36.7 (10.5) | 34.7 (9.5) | 38.3 (8.9) | 0.79 | 0.457 |
| Education level: ISCED | | | | | |
| Own | 3.5 (1.9) | 3.7 (1.4) | 3.2 (0.9) | 0.54 | 0.585 |
| Father | 4.0 (1.8) | 3.6 (1.7) | 3.3 (2.1) | 0.65 | 0.527 |
| Mother | 2.8 (1.5) | 2.6 (1.5) | 2.3 (1.5) | 0.80 | 0.456 |
| Time since deployment, months | 68.1 (68.0) | 86.3 (108.0) | 106.0 (95.2) | 0.94 | 0.395 |
| Number of missions | 2.6 (1.5) | 3.1 (4.3) | 2.4 (1.7) | 0.38 | 0.688 |
| Missions, *n* | | | | | |
| 1 | 7 | 8 | 10 | | |
| 2 | 6 | 6 | 3 | | |
| 3 | 4 | 4 | 4 | | |
| >3 | 6 | 4 | 5 | | |
| Early traumatic experiences[a] | 2.9 (2.8) | 4.1 (3.7) | 5.2 (4.6) | 1.94 | 0.153 |
| PTSD symptoms pre-treatment | | | | | |
| Re-experiencing: CAPS B | 0.7 (1.3) | 22.1 (4.7) | 24.4 (5.9) | 198.5 | <0.001 |
| Avoiding: CAPS C | 0.7 (2.0) | 21.6 (9.7) | 25.7 (8.5) | 72.6 | <0.001 |
| Hyperarousal: CAPS D | 3.1 (2.9) | 22.8 (5.3) | 26.2 (3.9) | 204.3 | <0.001* |
| Total: CAPS total | 4.5 (4.1) | 66.5 (12.3) | 76.3 (12.5) | 321.0 | <0.001* |
| PTSD symptoms post-treatment | | | | | |
| Re-experiencing: CAPS B | 1.4 (2.3) | 7.1 (6.6) | 21.9 (6.3) | 86.8 | <0.001* |
| Avoiding: CAPS C | 0.7 (1.6) | 6.4 (5.3) | 21.0 (8.1) | 76.8 | <0.001* |
| Hyperarousal: CAPS D | 3.3 (2.8) | 11.5 (5.9) | 23.5 (5.9) | 91.0 | <0.001* |
| Total: CAPS total | 5.4 (4.4) | 25.0 (14.2) | 66.4 (15.2) | 145.8 | <0.001* |

Data are given as mean (standard deviation) unless otherwise indicated.

PTSD, Post-traumatic stress disorder; ISCED, International Standard Classification of Education (Schneider, 2013); CAPS, Clinician Administered PTSD Scale (Blake *et al.* 1990).

[a] Early traumatic experiences were investigated with the Early Trauma Inventory – Self Report (Bremner *et al.* 2007).

* Mean values for remitted and persistent patients were significantly different.

medication naive, $n = 25$), and in the persistent PTSD group only (SSRI, $n = 7$; medication naive, $n = 14$). These analyses revealed no significant hippocampal volume differences in the whole PTSD sample (group × time interaction, $F_{1,34} = 1.363$, $p = 0.251$; main effect group $F_{1,34} = 0.002$, $p = 0.996$) or in the persistent patients alone (group × time interaction, $F_{1,19} = 0.151$, $p = 0.702$; main effect group $F_{1,19} = 0.138$, $p = 0.715$).

Third, although the $\chi^2$ test did not reveal a significant group difference for lifetime alcohol abuse/dependence, only a few patients ($n = 3$) in the persistent group had a history of alcohol dependence and/or abuse. Therefore, the original group analyses were repeated after excluding these three patients. Again, a significant main effect of group was found in the left hippocampus ($F_{2,58} = 6.109$, $p = 0.004$). Furthermore, the marginally significant effect of group in the right hippocampus reached significance after excluding patients with a history of alcoholism ($F_{2,58} = 3.621$, $p = 0.033$).

Finally, no significant correlations were observed in the PTSD group between PTSD severity (total CAPS score) and hippocampal volume, either pre-treatment (left, $r = -0.089$, $p = 0.566$; right, $r = -0.044$, $p = 0.777$) or post-treatment (left, $r = -0.062$, $p = 0.689$; right, $r = -0.052$, $p = 0.736$). Also, no correlations were observed in the PTSD group between clinical improvement (Δ total CAPS score) and change in hippocampal volume in the PTSD group as a whole (left, $r = 0.011$, $p = 0.942$; right, $r = -0.175$, $p = 0.256$) or when analysed separately for the remitted (left, $r = 0.236$, $p = 0.290$; right, $r = 0.091$, $p = 0.688$) and persistent patients (left, $r = -0.117$, $p = 0.603$; right, $r = -0.125$, $p = 0.578$).

## Discussion

In addition to the replication of earlier findings that PTSD patients have smaller left hippocampal volumes than controls, we report that hippocampal volumes of combat controls and healthy non-military controls did

**Table 3.** *Number of treatment sessions, use of medication and presence of co-morbid disorders in remitted and persistent PTSD patients*

| | Remitted patients ($n = 22$) | | Persistent patients ($n = 22$) | | $P^a$ | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post |
| Treatment: mean total sessions, number (S.D.) | 9.2 (6.5) | | 10.0 (4.6) | | $t = -0.48$, | $p = 0.632$ |
| Medication, *n* | 11 | 8 | 9 | 13 | 0.545 | 0.131 |
| SSRI | 4 | 3 | 7 | 12 | 0.296 | 0.004 |
| Benzodiazepine | 6 | 6 | 4 | 2 | 0.472 | 0.118 |
| SARI | 0 | 1 | 2 | 1 | 1.000 | 1.000 |
| Antipsychotics | 1 | 1 | 1 | 3 | 1.000 | 0.294 |
| Nicotine antagonist | 1 | 0 | 0 | 0 | 0.312 | 1.000 |
| Beta blocker | 1 | 0 | 1 | 0 | 1.000 | 1.000 |
| Co-morbid disorders, *n* | 13 | 3 | 18 | 10 | 0.099 | 0.021 |
| Mood | 11 | 1 | 13 | 6 | 0.545 | 0.039 |
| Anxiety | 3 | 2 | 11 | 5 | 0.010 | 0.216 |
| Somatic | 1 | 0 | 1 | 1 | 1.000 | 0.312 |
| Lifetime (not current) alcohol abuse and/or dependence | 0 | | 3 | | 0.073 | |

PTSD, Post-traumatic stress disorder; pre, pre-treatment; post, post-treatment; S.D., standard deviation; SSRI, selective serotonin reuptake inhibitor; SARI, serotonin antagonist and reuptake inhibitor.

[a] *p* Values of medication and co-morbid disorder analyses are based on $\chi^2$ analyses.

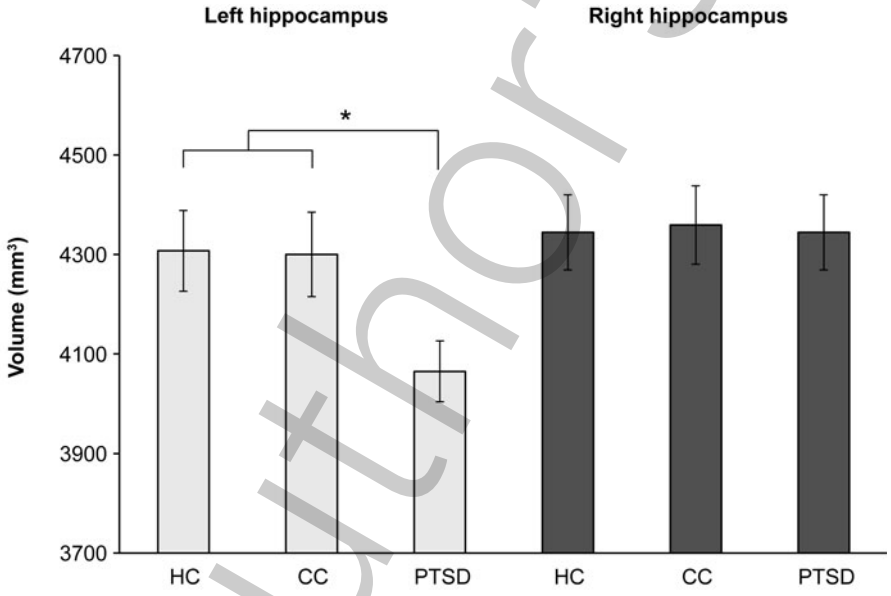


**Fig. 1.** Left and right hippocampal volumes of healthy non-military controls (HC), healthy war veterans (combat controls; CC) and post-traumatic stress disorder (PTSD) patients. Values are means, with standard errors represented by vertical bars. *Significant group difference ($p < 0.05$).

not differ. This suggests that trauma or stress exposure during deployment does not result in decreased hippocampal volumes. Second, we found that hippocampal volume of PTSD patients did not normalize with successful treatment. Instead, we showed that (left) hippocampal volumes of PTSD patients in whom PTSD symptoms persisted after trauma-focused therapy were smaller than hippocampal volumes of combat controls and remitted patients both prior to treatment and after treatment. Previous studies on hippocampal volume and treatment in PTSD did not collect pre- and post-treatment MRI scans or did not differentiate

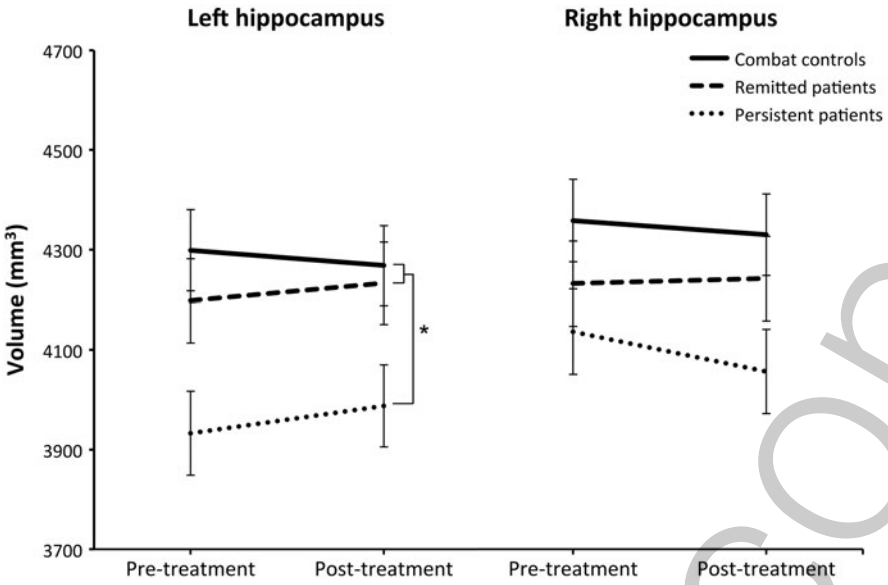


**Fig. 2.** Effect of treatment on hippocampal volumes. Pre- and post-treatment hippocampal volumes (in $mm^3$) of healthy war veterans (combat controls), post-traumatic stress disorder (PTSD) patients in remission (remitted patients) and PTSD patients in whom symptoms persisted after treatment (persistent patients) are displayed separately for the left and right hippocampus. Values are means, with standard errors represented by vertical bars. * Significant group difference ($p < 0.05$).

between remitted and persistent patients. Therefore, we are the first to conclude that a smaller hippocampus represents a pre-treatment vulnerability factor for the persistence of PTSD.

Hippocampal volumes did not differ between trauma-exposed controls and healthy controls, suggesting that reduced hippocampal volume is not the consequence of stress or trauma exposure during deployment. This conclusion is supported by the twin study of Gilbertson *et al.* (2002) who showed reduced hippocampal volume in the non-deployed twin without PTSD (Gilbertson *et al.* 2002), and by a study that found smaller hippocampal volumes in war veterans with PTSD, and war veterans without PTSD, but also in non-deployed reservists compared with healthy controls (Vythilingam *et al.* 2005). In two non-military studies opposing results were found. A study on childhood abuse observed comparable hippocampal volumes of women with and without early childhood abuse (Bremner *et al.* 2003), whereas a study that compared burns victims with and without PTSD and healthy controls observed reduced hippocampal volume in all burns victims (Winter & Irle, 2004). Two meta-analyses (Karl *et al.* 2006; Woon *et al.* 2010) concluded that trauma exposure regardless of PTSD was associated with reduced (left) hippocampal volume. This conclusion is not supported by our data; however, the discrepancy in findings can potentially be explained by differences in type of trauma and, in particular, the timing of traumatic events. Early traumatic experiences have often been associated with reduced hippocampal volume (Vythilingam *et al.* 2002; Teicher *et al.* 2003). It can therefore be postulated that negative environmental factors may influence hippocampal growth (in particular) during sensitive periods, putting these individuals at risk for PTSD. Building upon this hypothesis, trauma exposure in adulthood does not necessarily result in hippocampal volume reduction, which was indeed observed in the current study.

With this study we also investigated whether reduced hippocampal volume in PTSD patients normalizes after successful treatment, or if reduced hippocampal volume constitutes a vulnerability factor for the persistence of PTSD. We observed a smaller left hippocampus before and after treatment in persistent patients compared with remitted patients and combat controls. With this finding we support and extend the conclusion of Gilbertson *et al.* (2002) that a smaller hippocampus is a vulnerability factor for the persistence of PTSD. The present study also showed that a smaller hippocampal volume is not a necessary condition for developing PTSD, because mean hippocampal volume of remitted patients was comparable with that of combat controls and healthy controls, both pre- and post-treatment. Moreover, the current finding clarifies why not all previous MRI studies in PTSD have observed reduced hippocampal volume in patients, e.g. in a study with recently traumatized patients, PTSD was not associated with smaller hippocampal volumes (Bonne *et al.* 2001). In line with earlier meta-analyses (e.g. Smith, 2005; Karl *et al.* 2006), we

also showed that reduced hippocampal volume in persistent patients was most pronounced in the left hippocampus. Group differences in the right hippocampus were marginally significant, though this difference became significant when excluding patients with a lifetime history of alcohol abuse and/or dependence.

The finding that reduced hippocampal volume constitutes a risk factor for the persistence of PTSD has potential implications for the prognosis and prediction of treatment success. PTSD is effectively treated with trauma-focused therapy (Bisson *et al.* 2007), which is based on extinction learning and aims at updating the traumatic memory by focusing on contextual (safety) information (Izquierdo *et al.* 2004). The hippocampus is involved in learning, formation of contextual memories and consolidation of new memories (Fendt & Fanselow, 1999). Furthermore, hippocampal volume has been correlated with memory performance (Tischler *et al.* 2006; Pohlack *et al.* 2014) and improvement after memory training (Engvig *et al.* 2012). Therefore, it seems plausible to conclude that a larger hippocampus is associated with an enhanced ability to learn and store newly formed contextual memories, resulting in a better prospect to recover from PTSD. On the other hand, individuals with a smaller hippocampus might benefit from a different treatment approach. This is relevant because trauma-focused therapy is often experienced as strenuous and might initially worsen symptoms (Bisson *et al.* 2007). Therefore, more research on alternative treatment for this group is required.

Treatment response in our sample was not associated with change in hippocampal volume. Previous findings of treatment effects on hippocampal volume in PTSD patients were conflicting. Two studies observed hippocampal growth after treatment with SSRIs (Vermetten *et al.* 2003; Bossini *et al.* 2007), whereas another study did not observe increase in hippocampal volume (Bremner *et al.* 2005). These studies did not include a control group, which hampers the interpretation of the findings, because the effect of treatment cannot be separated from the potential effect of time. A study on the effect of CBT on hippocampal volume demonstrated a mean increase of 100 $mm^3$ in PTSD patients after 12 weeks of CBT, while controls showed a hippocampal decrease of about 70 $mm^3$ (Levy-Gigi *et al.* 2013). Furthermore, a correlation between change in hippocampal volume and change in symptoms in the PTSD group was observed. We could neither replicate this pre- to post-treatment difference, nor this correlation. Remarkably, in that study the change in hippocampal volume in some patients was about 25%, which is very large and might be caused by movement or measurement error at one of two time points, thereby influencing hippocampal volume (change). Our findings support results from another CBT treatment study (Lindauer *et al.* 2005), in which patients who received CBT were compared with patients on a waiting list and a group of controls. In that study also no change in hippocampal volume was observed.

### *Limitations and future directions*

The distinction between remitted and persistent patients resulted in some group differences probably related to post-treatment symptom severity, such as pre-treatment severity, co-morbidity and post-treatment medication use. As symptom severity did not correlate with left hippocampal volume within the groups, it is unlikely that group differences can be attributed to pre-treatment PTSD severity. Second, analyses were repeated with medication-naive patients and similar results were obtained. Third, it is also not likely that co-morbidity explains the findings, because the same pattern of results was observed when patients without co-morbidity were compared. Yet, based on these findings we cannot ascertain that medication and co-morbidity have not affected hippocampal volume, and future studies should confirm our results.

Education level differed between the veterans and non-military healthy controls. This is probably due to the fact that most military men joined the armed forces after high school, whereas the non-military men continued education. Parental education did not differ between groups.

We conclude that a smaller hippocampal volume is a vulnerability factor for treatment non-response in PTSD. This finding is of potential clinical significance in predicting persistence of PTSD; however, its exact predictive value should be further established. Furthermore, investigating (contextual) memory consolidation and retrieval in a group of remitted and persistent PTSD patients would inform us whether structural brain characteristics observed in this study indeed translate to behavioral differences.

## Conclusion

Our findings suggest that a smaller (left) hippocampus is not the result of stress/trauma exposure. Furthermore, hippocampal volume does not increase with successful treatment. Instead, we demonstrate for the first time that a smaller (left) hippocampus constitutes a risk factor for the persistence of PTSD.

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## Declaration of Interest

None.

## References


**Apfel BA, Ross J, Hlavin J, Meyerhoff DJ, Metzler TJ, Marmar CR, Weiner MW, Schuff N, Neylan TC** (2011). Hippocampal volume differences in gulf war veterans with current *versus* lifetime posttraumatic stress disorder symptoms. *Biological Psychiatry* **69**, 541–548.

**Bisson JI, Ehlers A, Matthews R, Pilling S, Richards D, Turner S** (2007). Psychological treatments for chronic post-traumatic stress disorder: systematic review and meta-analysis. *British Journal of Psychiatry* **190**, 97–104.

**Blake D, Weathers F, Nagy L, Kaloupek D, Klauminzer G, Charney DA** (1990). Clinician rating scale for assessing current and lifetime PTSD: the CAPS-1. *Behavioral Therapy* **13**, 187–188.

**Bonne O, Brandes D, Gilboa A, Gomori JM, Shenton ME, Pitman RK, Shalev AY** (2001). Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. *American Journal of Psychiatry* **158**, 1248–1251.

**Bossini L, Tavanti M, Lombardelli A, Calossi S, Polizzotto NR, Galli R, Vatti G, Pieraccini F, Castrogiovanni P** (2007). Changes in hippocampal volume in patients with post-traumatic stress disorder after sertraline treatment. *Journal of Clinical Psychopharmacology* **27**, 233–235.

**Bradley R, Greene J, Russ E, Dutra L, Westen D** (2005). A multidimensional meta-analysis of psychotherapy for PTSD. *American Journal of Psychiatry* **162**, 214–227.

**Bremner JD, Bolus R, Mayer EA** (2007). Psychometric properties of the early trauma inventory – self report. *Journal of Nervous and Mental Disease* **195**, 211–218.

**Bremner JD, Mletzko T, Welter S, Quinn S, Williams C, Brummer M, Siddiq S, Reed L, Heim CM, Nemeroff CB** (2005). Effects of phenytoin on memory, cognition and brain structure in post-traumatic stress disorder: a pilot study. *Journal of Psychopharmacology* **19**, 159–165.

**Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashan T, Nazeer A, Khan S, Vaccarino LV, Soufer R, Garg PK, Ng CK, Staib LH, Duncan JS, Charney DS** (2003). MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. *American Journal of Psychiatry* **160**, 924–932.

**Dale AM, Fischl B, Sereno MI** (1999). Cortical surface-based analysis: I. Segmentation and surface reconstruction. *NeuroImage* **9**, 179–194.

**Duman RS, Nakagawa S, Malberg J** (2001). Regulation of adult neurogenesis by antidepressant treatment. *Neuropsychopharmacology* **25**, 836–844.

**Engvig A, Fjell AM, Westlye LT, Skaane NV, Sundseth T, Walhovd KB** (2012). Hippocampal subfield volumes correlate with memory training benefit in subjective memory impairment. *NeuroImage* **61**, 188–194.

**Fendt M, Fanselow MS** (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews* **23**, 743–760.

**First MB, Spitzer RL, Gibbon M, Williams JBW** (2002). *Structured Clinical Interview for DSM-IV Axis I Disorders, Research Version, Patient Edition (SCID-I/P)*. Biometrics Research, New York State Psychiatric Institute: New York.

**Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, Van Der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM** (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* **33**, 341–355.

**Fischl B, Sereno MI, Dale AM** (1999). Cortical surface-based analysis: II. Inflation, flattening, and a surface-based coordinate system. *NeuroImage* **9**, 195–207.

**Foa EB, Keane TM, Friedman MJ, Cohen JA** (2009). *Effective Treatments for PTSD: Practice Guidelines from the International Society of Traumatic Stress Studies*. Guilford Press: New York.

**Gilbertson MW, Shenton ME, Ciszewski A, Kasai K, Lasko NB, Orr SP, Pitman RK** (2002). Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nature Neuroscience* **5**, 1242–1247.

**Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E** (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proceedings of the National Academy of Sciences* **95**, 3168–3171.

**Gould E, Tanapat P, Rydel T, Hastings N** (2000). Regulation of hippocampal neurogenesis in adulthood. *Biological Psychiatry* **48**, 715–720.

**Hedges DW, Woon FL** (2010). Alcohol use and hippocampal volume deficits in adults with posttraumatic stress disorder: a meta-analysis. *Biological Psychology* **84**, 163–168.

**Izquierdo I, Cammarota M, Vianna MRM, Bevilaqua LRM** (2004). The inhibition of acquired fear. *Neurotoxicity Research* **6**, 175–188.

**Karl A, Schaefer M, Malta LS, Dorfel D, Rohleder N, Werner A** (2006). A meta-analysis of structural brain abnormalities in PTSD. *Neuroscience and Biobehavioral Reviews* **30**, 1004–1031.

**Kitayama N, Vaccarino V, Kutner M, Weiss P, Bremner JD** (2005). Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. *Journal of Affective Disorders* **88**, 79–86.

**Kuhn S, Gallinat J** (2013). Gray matter correlates of posttraumatic stress disorder: a quantitative meta-analysis. *Biological Psychiatry* **73**, 70–74.

**Levy-Gigi E, Szabo C, Kelemen O, Keri S** (2013). Association among clinical response, hippocampal volume, and fkbp5 gene expression in individuals with posttraumatic stress disorder receiving cognitive behavioral therapy. *Biological Psychiatry* **74**, 793–800.

**Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, Den Heeten GJ, Gersons BP** (2005). Effects of psychotherapy on hippocampal volume in out-patients with post-traumatic stress disorder: a MRI investigation. *Psychological Medicine* **35**, 1421–1431.

**Malenka RC, Nicoll RA** (1999). Long-term potentiation – a decade of progress? *Science* **285**, 1870–1874.

**Nordanskog P, Dahlstrand U, Larsson MR, Larsson EM, Knutsson L, Johanson A** (2010). Increase in hippocampal volume after electroconvulsive therapy in patients with

depression: a volumetric magnetic resonance imaging study. *Journal of ECT* **26**, 62–67.

**Pohlack ST, Meyer P, Cacciaglia R, Liebscher C, Ridder S, Flor H** (2014). Bigger is better! Hippocampal volume and declarative memory performance in healthy young men. *Brain Structure and Function* **219**, 255–267.

**Sapolsky RM, Uno H, Rebert CS, Finch CE** (1990). Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *Journal of Neuroscience* **10**, 2897–2902.

**Schneider SL** (2013). The International Standard Classification of Education 2011. *Comparative Social Research* **30**, 365–379.

**Smith ME** (2005). Bilateral hippocampal volume reduction in adults with post-traumatic stress disorder: a meta-analysis of structural MRI studies. *Hippocampus* **15**, 798–807.

**Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP, Kim DM** (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience and Biobehavioral Reviews* **27**, 33–44.

**Tendolkar I, Van Beek M, Van Oostrom I, Mulder M, Janzing J, Voshaar RO, Van Eijndhoven P** (2013). Electroconvulsive therapy increases hippocampal and amygdala volume in therapy refractory depression: a longitudinal pilot study. *Psychiatry Research – Neuroimaging* **214**, 197–203.

**Tischler L, Brand SR, Stavitsky K, Labinsky E, Newmark R, Grossman R, Buchsbaum MS, Yehuda R** (2006). The relationship between hippocampal volume and declarative memory in a population of combat veterans with and without PTSD. *Annals of the New York Academy of Sciences* **1071**, 405–409.

**Vermetten E, Vythilingam M, Southwick SM, Charney DS, Bremner JD** (2003). Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. *Biological Psychiatry* **54**, 693–702.

**Vythilingam M, Heim C, Newport J, Miller AH, Anderson E, Bronen R, Brummer M, Staib L, Vermetten E, Charney DS, Nemeroff CB, Bremner JD** (2002). Childhood trauma associated with smaller hippocampal volume in women with major depression. *American Journal of Psychiatry* **159**, 2072–2080.

**Vythilingam M, Luckenbaugh DA, Lam T, Morgan III CA, Lipschitz D, Charney DS, Bremner JD, Southwick SM** (2005). Smaller head of the hippocampus in gulf war-related posttraumatic stress disorder. *Psychiatry Research: Neuroimaging* **139**, 89–99.

**Weathers F, Ruscio AM, Keane TM** (1999). Psychometric properties of nine scoring rules for the Clinician-Administered Posttraumatic Stress Disorder Scale. *Psychological Assessment* **11**, 124–133.

**Winter H, Irle E** (2004). Hippocampal volume in adult burn patients with and without posttraumatic stress disorder. *American Journal of Psychiatry* **161**, 2194–2200.

**Woon FL, Sood S, Hedges DW** (2010). Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: a meta-analysis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **34**, 1181–1188.

**Yucel K, McKinnon MC, Taylor VH, Macdonald K, Alda M, Young LT, MacQueen GM** (2007). Bilateral hippocampal volume increases after long-term lithium treatment in patients with bipolar disorder: a longitudinal MRI study. *Psychopharmacology (Berlin)* **195**, 357–367.

**Yucel K, Taylor VH, McKinnon MC, Macdonald K, Alda M, Young LT, MacQueen GM** (2008). Bilateral hippocampal volume increase in patients with bipolar disorder and short-term lithium treatment. *Neuropsychopharmacology* **33**, 361–367.