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The traumatized brain

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**Hippocampal Morphology and Childhood
Traumatization in Dissociative Identity
Disorder and Posttraumatic Stress Disorder**

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Chapter

5

In preparation

Abstract

Background: Experiencing severe and chronic childhood abuse and neglect is associated with a high risk of mental disorders. Whereas the view that dissociative identity disorder (DID) is related to childhood traumatization is disputed, virtually all patients with DID report these types of potentially traumatizing events. Animal research and studies on posttraumatic stress disorder (PTSD) have documented a link between traumatization and small hippocampal volume. Several studies reported small hippocampal volume in DID, but involved limited sample sizes and lacked examination of hippocampal subfields. The current study aims to investigate morphology of the hippocampus as a whole as well as its different subfields in DID and PTSD patients, and to test whether there is a link between hippocampal abnormalities and retrospectively reported childhood traumatization. Such findings can help in better understanding the etiological processes involved in DID.

Methods: Brain structural magnetic resonance images were acquired from seventeen DID patients, sixteen PTSD patients and twenty-eight healthy controls (HC), who were matched for gender, age and education. Hippocampal volume and shape measures were generated and compared using an approach based on manual tracing and surface mesh modeling. Correlations were conducted to investigate associations of reported childhood traumatization with hippocampal volume and shape in patient groups.

Results: Left and right hippocampal volumes were significantly smaller in DID compared to PTSD and HC. Right hippocampal volume showed a trend to be smaller in PTSD relative to HC. Shape analysis results revealed deformations in the CA1, CA2-3 and subiculum subfields in DID and PTSD compared to HC. Furthermore, negative correlations were found between the severity of reported childhood traumatization and hippocampal volume and shape in the patient groups.

Conclusions: Our findings support the view that DID is related to childhood traumatization, and that the severity of hippocampal morphological abnormalities is related to the severity of childhood traumatization.

Introduction

Childhood abuse and neglect are risk factors for many psychiatric disorders such as anxiety disorders (Gibb et al., 2007), posttraumatic stress disorder (PTSD) (Bremner et al., 2003b, Wolfe et al., 2006), mood-related disorders (Wolfe et al., 2006), borderline personality disorder (Ball and Links, 2009), first episode psychosis (Aas et al., 2012, Hoy et al., 2012) and schizophrenia (Sideli et al., 2012). In adults with a history of childhood traumatization, regardless of the kind of psychiatric disorder, small hippocampal volume is the most consistently observed structural brain abnormality (Andersen et al., 2008, Bremner et al., 2003b, Stein et al., 1997, Teicher et al., 2012, Thomaes et al., 2010, Vythilingam et al., 2002). Preclinical research has shown that increased activity of the Hypothalamic-Pituitary-Adrenal Axis (HPA) and subsequently elevated level of glucocorticoid (GC) secretion during exposure to stress are key factors associated with hippocampal volume loss, due to its high density of GC receptors (McEwen et al., 1995, Sapolsky, 1996).

Clinical observations and retrospective correlational research suggest that dissociative identity disorder (DID) is related to a history of severe and chronic childhood traumatization (Boon and Draijer, 1993, Chu and Dill, 1990, Draijer and Langeland, 1999, Mulder et al., 1998, Nijenhuis and Den Boer, 2009, Spiegel, 2006, Van der Hart et al., 2006). The involved reported or documented adverse event include physical and sexual abuse as well as emotional neglect and abuse, often combined with a lack of affect-regulation by or emotional unavailability of caregivers (Boon and Draijer, 1993, Draijer and Langeland, 1999, Van der Hart et al., 2005). In line with these findings, longitudinal research has documented a relationship between disorganized attachment in early childhood, childhood traumatization, and the severity of dissociative symptoms in early adulthood (Lyons-Ruth et al., 2006, Ogawa et al., 1997). Furthermore, it has been reported that childhood neglect may result in limited self-soothing capacities (Draijer and Langeland, 1999) or inefficient affect regulation (Schore, 2002).

According to the DSM-IV, DID involves the presence of two or more different identity states that recurrently take control of a person's behavior and consciousness. These identity states have been found to be related to different patterns of brain activation (Reinders et al., 2003, 2006, 2012). Episodes of dissociative amnesia, depersonalization, derealization, and sensori-motor dissociation are other characteristic features of DID. DID has been indicated to be at the far end of the spectrum of trauma-related disorders (Spiegel, 1984) and thereby to be a severe form of (dissociative) PTSD (Lanius et al., 2006).

Few studies have investigated hippocampal volume in DID patients. While one study reported preserved hippocampal volume in a sample of combined individuals with DID (n=4) and dissociative amnesia (n=9) (Weniger et al., 2008), the other single case (Tsai et al., 1999) and multi-participant (Ehling et al., 2008, Irle et al., 2009, Vermetten et al., 2006) studies reported smaller hippocampal volume in DID patients compared to healthy controls. However, the finding of smaller hippocampal volume in DID reported by Vermetten et al. (2006) has been criticized as being due to differences in age rather than clinical status (Smeets et al., 2006). A recent volumetric study (Chalavi et al., submitted) investigated brain morphology in DID by comparing gray matter measures between DID patients, PTSD patients and healthy controls (HC). Findings of the latter study indicated smaller hippocampal volume along with several other cortical and subcortical gray matter differences in DID relative to HC. The observed similarities in morphological abnormalities in DID and PTSD patients suggest a trauma-related nature for DID. Neuroimaging studies can also help in providing evidence for the clinically observed etiological relationship between DID and reported childhood traumatization.

To date, most neuroimaging studies, including those on DID, have examined differences in overall hippocampal volume. However, the hippocampus consists of several histologically distinct subfields including the subiculum, CA1 (CA: cornu ammonis), CA2, CA3, CA4 and dentate gyrus. Each of these subfields has distinct structural and functional connections with the cortex and specialized functional properties (Wang et al., 2010). A recent neuroimaging study by Teicher et al. (2012)

reported that retrospectively assessed childhood traumatization (using the Childhood Trauma Questionnaire (CTQ)) was associated with smaller volume of the CA1, CA2-3, CA4-DG, and subiculum in un-medicated individuals from the general community. The strongest associations were observed in the CA3, and CA4-DG and subiculum subfields. This proven link between morphological abnormalities of hippocampal subfields and reported childhood traumatization opens an avenue to gain knowledge about the etiology of DID and may aid to provide evidence for the childhood traumatization-related nature of this disorder.

Novel methodologies are available to study possible abnormalities of hippocampal subfields. These include automatic procedures (Teicher et al., 2012) and manual tracing methods (Thompson et al., 2004, Wang et al., 2010). In neuroimaging studies with large sample sizes, automated techniques using fast processing methods are advantageous over (time-consuming) manual tracing methods. On the other hand, automatic procedures can be prone to segmentation errors especially in delineating hippocampal boundaries in small regions (Teicher et al., 2012) for which manual tracing methods may be more precise. An example of the latter methods is a relatively new method developed by Thompson et al. (2004), which is based on manual tracing and hippocampal surface mesh modeling. This method allows for the investigation of both hippocampal global volume and shape regional deformations and has been applied in several psychiatric disorders including major depressive disorder (Cole et al., 2010), bipolar disorder (Bearden et al., 2008b) and first episode schizophrenia (Narr et al., 2004).

In the current study we utilized the manual tracing-based technique to compare total and subfield hippocampal morphology between individuals with DID and gender-, age- and education-matched individuals with PTSD and healthy controls (HC). We also investigated a possible negative correlation between the severity of subjectively reported exposure to potentially traumatizing events and hippocampal morphology. We hypothesized that DID is associated with smaller hippocampal volume and regional shape deformations in various hippocampal subfields

compared to HC. Furthermore, we expected to observe associations between reported childhood traumatization, as assessed by the CTQ, and hippocampal volume and shape.

Methods

Subjects

Sixty-five subjects underwent magnetic resonance imaging (MRI): seventeen individuals with DID, sixteen individuals with PTSD and thirty-two HC. The sample used in this chapter is similar to the sample used in chapter 4. All participants were given a complete description of the study and gave written informed consent according to procedures approved by the Medical Ethical Committee (METc) of the University Medical Center Groningen (UMCG) and of the Amsterdam Medical Center (AMC).

All participants were female as only female DID patients volunteered to take part in this study. In addition, all subjects were matched for age (DID: 43.82 ± 9.85 ; PTSD: 40.75 ± 12.05 ; HC: 41.75 ± 12.29), number of years of education (DID: 14.88 ± 0.99 ; PTSD: 14.94 ± 0.85 ; HC: 15.04 ± 1.20) and Western European ancestry. DID and PTSD patients were recruited via mental health care institutions and internet advertisements. The diagnosis of DID was assessed by one of the two DID experts (E.N. or N.D.) using the Structural Clinical Interview for DSM-IV Dissociative Disorders (SCID-D) (Boon and Draijer, 1993, Steinberg, 1993) and PTSD comorbidity was determined using the PTSD section of the SCID-D (Steinberg, 1993). All DID patients met the criteria for either comorbid PTSD (82.35%) or PTSD in remission (17.65%). PTSD patients were diagnosed by researchers E.V. and M.G. using the Clinician Administered PTSD Scale (CAPS) interview (Blake et al., 1995). Only PTSD patients with inter-personal trauma were included in the current study. Psychoform and somatoform dissociative symptoms were evaluated using the Dissociative Experiences Scale (DES) (Bernstein and Putnam, 1986) (DID: 54.41 ± 16.18 ; PTSD: 22.18 ± 13.83 ; HC: 7.12 ± 11.54) and Somatoform Dissociation Questionnaire (SDQ-20) (Nijenhuis et al., 1996) (DID:

57.06±17.26; PTSD: 32.69±13.43; HC: 22.71±4.19). DID patients filled in the questionnaires in their most predominant identity state. Participants' psychotropic medication was not discontinued in this study (for details of usage and effect on hippocampal morphology see Supplementary material).

Exclusion criteria for DID and PTSD patients were: age outside the range of 18-65, pregnancy, systemic or neurological illness, claustrophobia, presence of metal implants in the body and alcohol/drug abuse. HC were recruited through advertisements in local newspapers. Additional exclusion criteria for HC were: the presence of dissociative symptoms, as determined with the DES (Bernstein and Putnam, 1986) and SDQ-20 (Nijenhuis et al., 1996), mental illness in the past or at present, or a high score of lifetime potentially traumatizing experiences as measured using the Traumatic Experience Checklist (Nijenhuis et al., 2002) (total lifetime traumatizing experiences of the HC group: 2.25±2.43). All HC were free of present and past psychiatric medication.

Image acquisition

Participants were scanned on a 3T MR scanner (Philips Medical Systems, Best, NL) in one of the participating centers in The Netherlands (UMCG and AMC). Subjects were balanced over the two centers (ten DID patients, ten PTSD patients and nineteen healthy controls were scanned at the UMCG) and an optimized structural MRI protocol with a high reproducibility between the two centers (Chalavi et al., 2012) was used. The details of this optimized protocol were: MPRAGE sequence, TR=9.95 ms, TE=5.6 ms, flip angle=8°, slice thickness=1mm isotropic voxel, number of slices=160, total scan time=10m14s.

Two structural MRI scans were collected from each subject whenever possible (fifteen DID patients and fourteen HC). Where both scans were artifact-free, the first scan was used, resulting in the ultimate exclusion of four scans from HC. This left seventeen DID, sixteen PTSD and twenty-eight HC scans for morphological analyses.

Image preprocessing

Non-cortical tissue was removed from the MR images using the Brain extraction Tool (BET) (Smith, 2002) and head alignment was standardized by linearly aligning the individual MR images with the International Consortium for Brain Mapping (ICBM452) average brain template using FLIRT's (FMRIB's Linear Image Registration Tool). In FreeSurfer (Fischl et al., 2002) (<http://surfer.nmr.mgh.harvard.edu>) all images were segmented into different tissue classes and an estimate of parenchymal volume was calculated as the sum of the total gray matter (GM) and the total white matter (WM) volumes. This measurement was used in subsequent statistical analyses to correct for whole-brain size.

Manual delineation of the hippocampus

The hippocampi were manually traced using MultiTracer (Woods, 2003) (<http://www.loni.ucla.edu/Software/MultiTracer>) by a single trained rater (SC), who was blind to all demographical variables. Good inter- and intra-rater reliability with the established protocol (Narr et al., 2004, Thompson et al., 2004) was obtained, intraclass correlation coefficients for this rater were 0.94 and 0.97 for the left and right hippocampus, respectively, which are comparable to those in previously published studies (Bearden et al., 2008a, Cole et al., 2010). The outline of each hippocampus was traced in contiguous coronal brain sections while the digitized surface contours were displayed simultaneously in all three viewing planes to facilitate the accurate identification of boundaries (Narr et al., 2004, Thompson et al., 2004). Hippocampal volumes obtained from these tracings were retained for statistical analyses.

Hippocampal surface mesh modeling and distance mapping

Anatomical surface mesh modeling methods matched equivalent hippocampal surface points across subjects (Thompson et al., 2004). These methods reveal localized gray matter contractions and expansions of the hippocampal surface corresponding to the CA1, CA2-3 and subiculum (Figure 5.1.a and 5.3.a). These

techniques form a grid over each hippocampal surface so that homologous grid-points can be matched between subjects. The matching procedures allow measurements to be made at corresponding surface locations in each subject that can be compared statistically in 3D. Further, for each hippocampal surface model, a 3D medial curve was derived and the radial distance from each surface point to this central curve was measured. Since radial distances are measured at thousands of points along the surface, the resulting radial distance maps detect non-uniform changes on a local scale which could indicate local expansions or contractions in hippocampal surface morphology. The approximate overlay of the hippocampal subfields was defined based on the Duvernoy atlas (Duvernoy, 1988).

Statistical analysis

Demographical and clinical data were compared between the three groups using analysis of variance (ANOVA) followed by two-sample *t*-tests. To test group differences in hippocampal volume, a repeated-measures analysis of covariance (ANCOVA) was used, with side (left or right) as the repeated measure and age and parenchymal volume as covariates. The analysis was followed by two-sample *t*-tests to compare left and right hippocampal volumes separately between: 1) DID vs. HC, 2) DID vs. PTSD, and 3) PTSD vs. HC.

To assess regional hippocampal shape deformations, statistical regression analyses were performed at each hippocampal surface point to map linkage between radial distance and group and the resulting statistical maps (*P*-map) of group differences (uncorrected) were displayed onto the averaged hippocampal surface template of the entire sample. In these analyses age and parenchymal volume were used as covariates. Furthermore, permutation tests with 10,000 iterations and a threshold of $p < 0.05$, were ran (Thompson et al., 2004) to obtain an empirically corrected *p*-value for each *P*-map.

Correlation between reported childhood traumatization and hippocampal morphology

Childhood traumatization-related experiences were retrospectively assessed using Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1994) in two different types of dissociative identity states. Following the terminology of Reinders et al., 2006 and conceptualization of Van der Hart et al., 2006, we obtained the CTQ scores in 1) a Traumatic Identity State (TIS), a DID identity state in which traumatic memories are recognized as first-hand autobiographical memories, showing an emotional and physiological response to trauma cues; and 2) a Neutral Identity State (NIS) which reports either partial or complete dissociative amnesia for traumatic memories, experiencing them without a first-hand sense of personal autobiographical experience. The indicators NIS and TIS are derived from the terms “apparently normal part of the personality (ANP)” and “emotional part of the personality (EP)”, respectively, which are conceptualized in the theory of ‘structural dissociation’ (Van der Hart et al., 2006). This theory defines dissociation as a division of personality into different types of subsystems (identity states), each with their own first-person perspective, that is, their own subjective point of view as to who they are, what the world is like, and how they relate to that world. When functioning in a NIS or in the most prominent identity state the CTQ scores are likely to represent an underestimation due to partial or complete functional amnesia for, and denial of, trauma-related memories. However, the CTQ scores given in the TIS may be more representative for the actual childhood traumatization that DID patients experienced as in this state they are not mentally avoiding the trauma-related memories. The CTQ data in both the NIS and the TIS from one DID patient were missing and another DID patient filled in the CTQ only in the NIS. The PTSD patients filled in the CTQ only once. To avoid spurious correlations, the HC group was not included in the correlation analyses since due to the inclusion criteria they all reported low lifetime traumatizing experiences.

Paired *t*-tests were used to compare the CTQ sub-scores between the two identity states within the DID patients and two-sample *t*-tests were conducted to

compare the CTQ sub-scores between DID (NIS or TIS) and PTSD. Associations of reported childhood traumatization with hippocampal volume (normalized by the parenchymal volume) and radial distance measures (i.e. shape) were investigated using Pearson's correlations on the NIS and TIS scores separately in combination with the PTSD data.

Results

Demographic and clinical assessments

The three groups did not differ significantly with respect to age ($F(2,58)=0.31$, $p=0.74$) and number of years of education ($F(2,58)=0.11$, $p=0.89$). Dissociative symptom scores (both psychoform and somatoform dissociation) were significantly different between the three groups (DES: $F(2,58)=64.63$, $p<0.001$; SDQ-20: $F(2,58)=45.94$, $p<0.001$). DID reported higher dissociative symptom scores compared to HC (DES: $p<0.001$; SDQ-20: $p<0.001$) and to PTSD (DES: $p<0.001$; SDQ-20: $p<0.001$). PTSD patients also reported significantly higher psychoform (DES: $p<0.001$) and somatoform (SDQ-20: $p<0.001$) dissociative symptom scores compared to HC.

Hippocampal volume

A significant effect of group on hippocampal volume ($F(2,56)=8.02$, $p=0.001$) was found (Table 5.1), independent of lateralization (group x side) ($F(2,56)=0.84$, $p=0.44$). The groups did not differ significantly with respect to parenchymal volume. *Post hoc* pairwise *t*-tests on the left and right hippocampal volumes (Table 5.1) revealed that both left ($p=0.001$) and right ($p<0.001$) hippocampal volumes were significantly smaller in DID relative to HC by 10.19% and 11.37%, respectively. Furthermore, DID patients showed significantly smaller left ($p=0.046$, 7.24%) and right ($p=0.047$, 6.58%) hippocampal volumes relative to PTSD. Comparing PTSD to HC only a trend for a smaller right hippocampal volume ($p=0.067$, 5.13%) was found in individuals with PTSD.

Table 5.1. Statistical analyses of parenchymal (cm³) and hippocampal (mm³) volumes

	Mean(SD)			pairwise comparisons: Effect size [^] , change (%), P-Value								
	DID	PTSD	HC	DID vs. HC			DID vs. PTSD			PTSD vs. HC		
				Cohen's d	Change %	P-Value	Cohen's d	Change %	P-Value	Cohen's d	Change %	P-Value
Parenchymal	1068(819)	1075(759)	1084(599)	-0.02	-1.48	0.479	-0.01	-0.65	0.777	-0.01	-0.83	0.703
Hippocampus												
Left	2009(208)	2166(228)	2237(196)	-1.13	-10.19	0.001*	-0.72	-7.25	0.046*	-0.33	-3.17	0.298
Right	2074(185)	2220(202)	2340(205)	-1.36	-11.37	<0.001*	-0.75	-6.58	0.047*	-0.59	-5.13	0.067

DID= dissociative identity disorder; PTSD= posttraumatic stress disorder; HC= healthy controls.

* P-value<=0.05

[^] = Cohen's d

Hippocampal shape

Statistical 3D maps of hippocampal shape differences between the groups are shown in Figure 5.1. Results of the permutation tests revealed that these maps did not survive multiple comparison correction; therefore uncorrected shape deformation results are presented for exploratory purposes only. Compared to HC, both DID and PTSD showed contractions in the CA1, CA2-3 and subiculum subfields. Direct comparison of shape measures between DID and PTSD showed contractions in all three hippocampal subfields (i.e. CA1, CA2-3 and subiculum), however, the pattern of contraction was less widespread than DID vs. HC and PTSD vs. HC comparisons.

Relationship with childhood traumatization

Within the DID group, in the TIS subjects reported significantly higher childhood physical neglect ($p=0.029$), emotional abuse ($p=0.001$) and total trauma ($p=0.022$) than in the NIS (Table 5.2). Furthermore, all CTQ sub-scores were significantly higher in both identity states of DID than in PTSD (Table 5.2). Correlation analyses on the combined data of the PTSD patients and the DID patients in the NIS revealed significant negative correlations between the left hippocampal volume and childhood emotional neglect, sexual abuse and total childhood traumatization (Table 5.3 and Figure 5.2). Furthermore, when correlation analyses were conducted on the combined data of the PTSD patients and the DID patients in the TIS, significant negative correlations were found between the left hippocampal volume and childhood emotional neglect, physical neglect, emotional abuse, sexual abuse and total childhood traumatization (Table 5.3 and Figure 5.2). The latter analysis also showed significant negative correlations between the right hippocampal volume and childhood emotional neglect, physical neglect, sexual abuse and total childhood traumatization (Table 5.3 and Figure 5.2).

All the DID patients in the current study reported severe (Heim et al., 2009) childhood traumatization in their NIS and TIS (CTQ total score \geq 63) and the PTSD patients scored over a broader range (low scores of 30 to severe scores of 113).

Chapter 5

Therefore, *post hoc* we categorized the PTSD patients into two subgroups including PTSD with low childhood traumatization (CTQ total score \leq 53, n=7) and PTSD with moderate-to-severe childhood traumatization (CTQ total score \geq 56, n=9) (Heim et al., 2009) and compared the hippocampal volume between these subgroups using two-sample *t*-test. Results of this analysis revealed that the left hippocampal volume tended to be smaller in the PTSD subgroup with moderate-to-severe childhood traumatization as compared to the PTSD subgroup with low childhood traumatization ($p=0.058$), but right hippocampal volume did not show a significant effect ($p=0.34$).

Table 5.2. Statistical analysis of childhood traumatization data reported by the DID and PTSD groups.

	Mean(SD)		P-value	
	DID (n=17)	PTSD (n=16)	Paired <i>t</i> -test: NIS vs. TIS	Two sample <i>t</i> -test: DID vs. PTSD
Childhood trauma Questionnaire (CTQ) ^a				
<i>emotional neglect</i>	NIS:21.81(2.90) TIS:23.40(2.26)	16.63(6.02)	0.074	NIS: 0.004* TIS: <0.001*
<i>physical neglect</i>	NIS:16.56(4.37) TIS:17.47(3.87)	10.50(3.93)	0.029*	NIS: <0.001* TIS: <0.001*
<i>emotional abuse</i>	NIS:19.68(4.61) TIS:22.80(3.30)	14.44(6.31)	0.001*	NIS: 0.012* TIS: <0.001*
<i>physical abuse</i>	NIS:14.00(6.02) TIS:15.60(5.37)	9.31(4.84)	0.180	NIS: 0.021* TIS: 0.002*
<i>sexual abuse</i>	NIS:16.19(7.26) TIS:17.87(7.32)	10.06(6.06)	0.375	NIS: 0.015* TIS: 0.003*
<i>total trauma score</i>	NIS:88.25(18.62) TIS:97.13(16.63)	60.94(22.70)	0.022*	NIS: 0.001* TIS: <0.001*

DID= dissociative identity disorder; PTSD= posttraumatic stress disorder.

* P-value<0.05

^a The CTQ data from both identity states of one DID patient was missing and another DID patient only filled in the CTQ in her NIS.

Table 5.3. Correlation analyses between hippocampal volume ^a and CTQ sub-scores of the patient groups

	Pearson's correlation r(P-Value)					
	emotional neglect	physical neglect	emotional abuse	physical abuse	sexual abuse	Total score
Left hippocampus	NIS:-0.466(0.007*)	NIS:-0.316(0.078)	NIS:-0.224(0.218)	NIS:-0.186(0.307)	NIS:-0.349(0.050*)	NIS:-0.369(0.038*)
	TIS:-0.527(0.002*)	TIS:-0.412(0.021*)	TIS:-0.363(0.045*)	TIS:-0.271(0.140)	TIS:-0.363(0.045*)	TIS:-0.443(0.013*)
Right hippocampus	NIS:-0.271(0.134)	NIS:-0.206(0.258)	NIS:-0.111(0.544)	NIS:-0.112(0.543)	NIS:-0.225(0.215)	NIS:-0.221(0.223)
	TIS:-0.373(0.039*)	TIS:-0.358(0.048*)	TIS:-0.322(0.077)	TIS:-0.263(0.152)	TIS:-0.365(0.043*)	TIS:-0.389(0.030*)

CTQ= childhood trauma questionnaire.

* P-value<=0.05

^a hippocampal volumes were normalized by the parenchymal volumes.

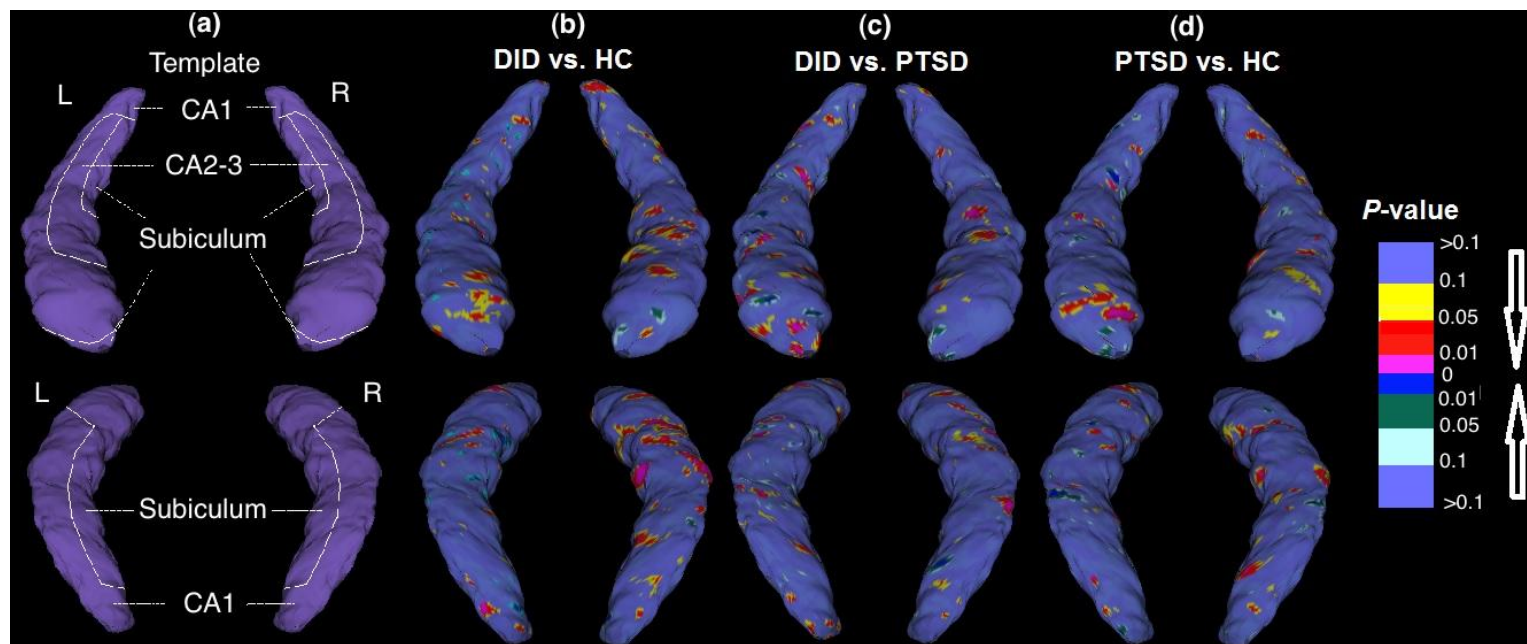


Figure 5.1. (a) A schematic representation of the hippocampal subfields mapped onto a representative hippocampal surface obtained by averaging the surface from all the participants. In addition, 3D maps of hippocampal regional shape differences are shown comparing (b) DID vs. HC, (c) DID vs. PTSD and (d) PTSD vs. HC. The maps are uncorrected. Upper maps represent anterior view and lower maps represent posterior view. Abbreviations: DID, dissociative identity disorder; PTSD, posttraumatic stress disorder; HC, healthy controls.

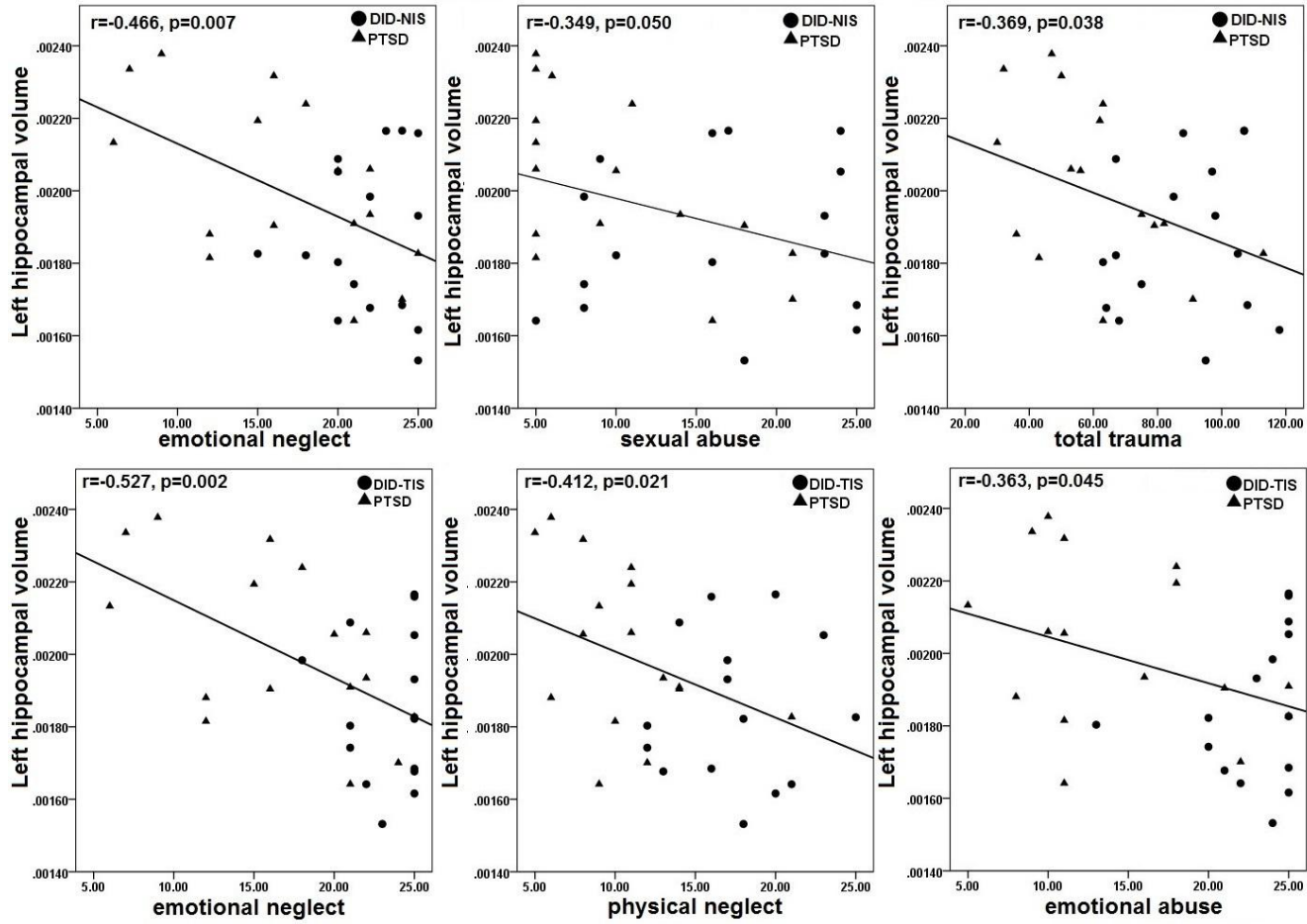


Figure 5.2 (part1).

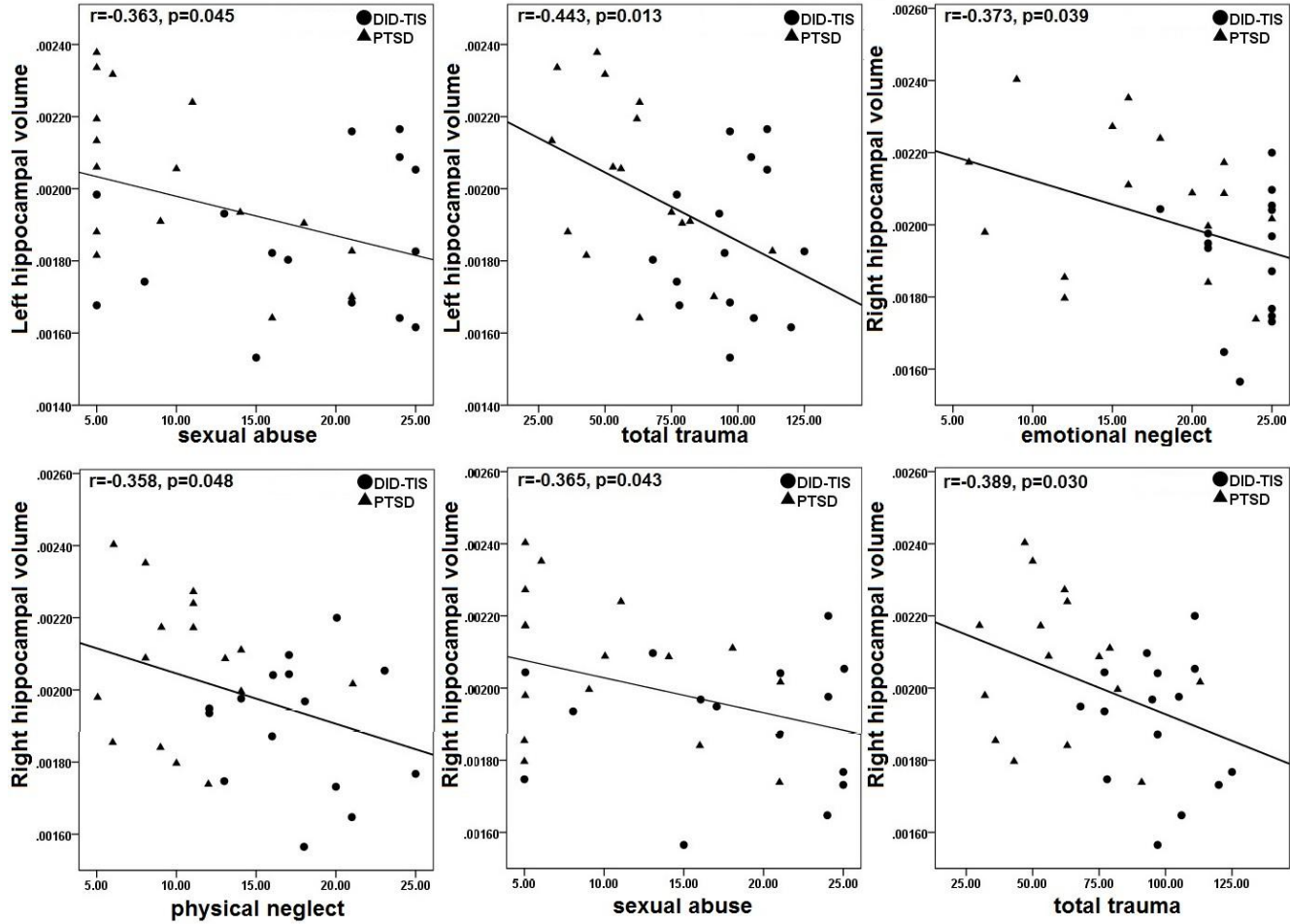


Figure 5.2 (part2).

Figure 5.2. (part 1 and 2). *Linear regression scatter plots performed on the combined data from the DID patients in the NIS (DID-NIS) or in TIS (DID-TIS) and PTSD patients showing significant negative correlations between hippocampal volumes and reported childhood traumatization scores obtained using the CTQ. The hippocampal volumes were normalized by parenchymal volume, which is representative for whole brain volume. Abbreviations: DID, dissociative identity disorder; NIS: neutral identity state; TIS; traumatic identity state; PTSD, posttraumatic stress disorder; CTQ, childhood trauma questionnaire.*

Correlation analyses between the hippocampal radial distance measures and the CTQ total scores of the combined data from the patient groups revealed widespread effects across the hippocampal surface, encompassing the CA1, CA2-3 and subiculum subfields (Figure 5.3). These negative correlations were more pronounced (more dark blue regions) when using the CTQ scores of the DID patients in the TIS than in the NIS. Furthermore, comparing the results of DID vs. HC contrast (Figure 5.1) with the results of correlation analyses (Figure 5.3), we found that the contractions in the CA2-3 of the right hippocampus (in the anterior view) and in the anterior subiculum of both left and right hippocampi (in the posterior view) overlapped with the negative correlations observed in the correlation analyses (Figure 5.3).

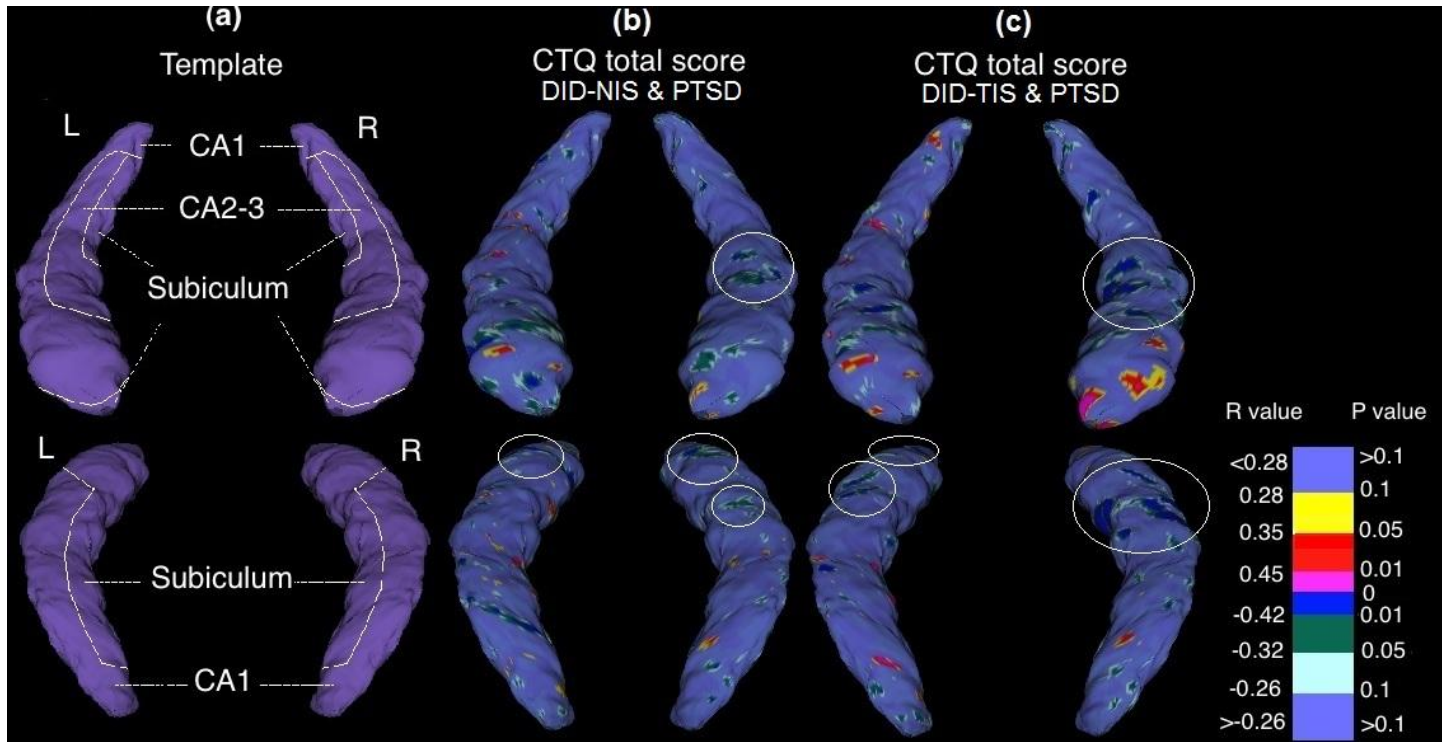


Figure 5.3. (a) A schematic representation of the hippocampal subfields mapped onto a representative hippocampal surface obtained by averaging the surface from all the participants. In addition, statistical 3D maps showing correlations between each hippocampal surface point and childhood traumatization obtained using the CTQ scores reported by the PTSD patients and the DID patients in the (b) NIS or (c) TIS. Areas which are depicted with circle are overlapping areas with DID vs. HC comparison. Abbreviations: DID, dissociative identity disorder; NIS, neutral identity state; TIS, traumatic identity state; PTSD, posttraumatic stress disorder; CTQ, childhood trauma questionnaire.

Discussion

This study is the first to report abnormalities of hippocampal volume and shape in DID patients in comparison with PTSD patients and HC. Compared to HC, we found significantly smaller left (10.19%) and right (11.37%) hippocampal volumes in DID patients and a trend for smaller right (5.13%) hippocampal volume in PTSD patients. DID patients were found to have significantly smaller left (7.25%) and right (6.58%) hippocampal volume compared to PTSD patients. Shape analyses indicated surface contractions in the subfields CA1, CA2-3 and subiculum in DID compared to HC. For PTSD vs. HC also regional surface contractions were observed in the CA1, CA2-3 and subiculum, although these deformations were less widespread than those observed when comparing DID to HC. Furthermore, compared to PTSD, DID showed contractions in several hippocampal subfields. Correlation analyses revealed significant negative correlations between bilateral hippocampal volume and reported childhood traumatization. The effects were more pronounced when the childhood traumatization scores of the DID patients in the TIS were used instead of those in the NIS. With regard to the smaller hippocampal volume in DID compared to HC, our results corroborate results of our previous study in which we used automatic segmentation methods (Chalavi et al., submitted). Furthermore, our findings of negative correlations between reported childhood traumatization and hippocampal volume and shape deformation provide additional support for previous studies reporting that DID is etiologically related to childhood traumatization (Boon and Draijer, 1993, Chu and Dill, 1990, Draijer and Langeland, 1999, Mulder et al., 1998, Nijenhuis and Den Boer, 2009, Spiegel, 2006, Van der Hart et al., 2006).

Volumetric differences

Our finding of smaller hippocampal volume in DID and PTSD concur with prior neuroimaging studies in DID (Chalavi et al., submitted, Ehling et al., 2008, Irle et al., 2009, Tsai et al., 1999, Vermetten et al., 2006) and in adult victims of childhood maltreatment, with or without PTSD (Andersen et al., 2008, Bremner et al., 2003b, Stein et al., 1997, Teicher et al., 2012, Vythilingam et al., 2002).

It has been shown that acute elevation of stress hormones, such as GCs, in a short period of time may facilitate the formation of memories of events associated with strong emotions (McGaugh, 2000). However, prolonged and excessive exposure to stress hormones may result in damage to brain structures such as the hippocampus (McEwen, 2000). In line with this, in patients with Cushing's syndrome, which is characterized by elevated level of cortisol (GCs in human), a neuroimaging study revealed that hippocampal volume was negatively associated with plasma cortisol levels and was positively correlated with memory performance (Starkman et al., 1992). This indicates a mechanism in which an elevated stress response can negatively impact hippocampal volume. The current results of smaller hippocampal volume in DID and PTSD may be (Bremner et al., 2003a, Santa Ana et al., 2006) induced by trauma-related stress and the resultant chronic exposure of the hippocampus to excessive GCs and detrimental effects of these hormones on the hippocampus.

Regional shape differences

While volumetric studies provide information about the hippocampal volume as a single unity, using shape analysis tools regional hippocampal deformations can be assessed which can provide information regarding morphological alterations in different hippocampal subfields. In the current study, regional hippocampal contractions were observed in the CA1, CA2-3 and subiculum of DID and PTSD patients as compared to HC. Although these results are of an exploratory nature only, they are in agreement with several studies which reported associations between stress or elevated level of GCs and volumetric morphological changes in the hippocampal subfields CA1 and CA2-3 (Andersen and Teicher, 2004, Gould et al., 1997, Kadar et al., 1998, McEwen, 1999, Teicher et al., 2012, Wang et al., 2010).

Each of the hippocampal subfields has a distinct pattern of structural cortical connections and accordingly has a distinct functional role. Functional MRI studies have revealed that the subiculum is involved in memory retrieval while regions corresponding to CA2-3 are associated with the encoding of information (Eldridge

et al., 2005, Gabrieli et al., 1997, Zeineh et al., 2003). The role of CA1 in encoding and retrieval of contextual memory has been reported in animal studies (Daumas et al., 2005). Therefore, abnormalities of any of these subfields could result in memory abnormalities (Squire, 2004) and hence the localized deformations in different hippocampal subfields observed in DID and PTSD patients could underlie some of the memory alterations reported in DID patients (Dorahy, 2001, Elzinga et al., 2003, Van der Hart et al., 2005) and the impaired declarative and non-declarative memory often reported in PTSD (for review see Samuelson (2011)). However, future research linking focal hippocampal abnormalities to performance on standardized memory tasks in DID is warranted.

Childhood traumatization

In the TIS, DID patients reported more severe childhood traumatization than in the NIS which is in line with identity state dependent features of the different types of identity states (Van der Hart et al., 2006). Correlation analyses on the combined data from the DID (NIS/TIS) and PTSD patients revealed that reported childhood traumatization was associated with smaller left hippocampal volume as well as contractions of the hippocampal surface in the CA1, CA2-3 and subiculum. The effects were stronger when the CTQ scores in the TIS were used than the ones in the NIS. Furthermore, overlapping areas in the CA2-3 and subiculum were observed between the correlation findings and the results of comparing DID to HC. These results are in agreement with negative correlations previously reported between the CTQ and hippocampal global volume (Andersen et al., 2008, Dannlowski et al., 2012) and hippocampal subfield volumes (Teicher et al., 2012) in adults with a history of early-life adversity. Teicher et al. (2012) found that the strongest associations were related to the CA2-3 and subiculum subfields. Furthermore, *post-hoc* analyses revealed a trend for smaller left hippocampal volume in PTSD patients who reported moderate-to-severe childhood traumatization as compared to those who reported low childhood traumatization. This finding may indicate that reduced left hippocampal volume, independent of PTSD diagnosis, is related to the severity of reported childhood traumatization.

An alternative explanation of our findings of smaller hippocampal volume in DID and PTSD is that smaller hippocampal volume can be a predisposing factor putting an individual at risk to develop trauma-related disorders after subsequent trauma in their life (Gilbertson et al., 2002). While a prospective longitudinal study would be necessary to establish direct causality, we suggest that besides an environmental factor, such as childhood stress, genetic factors as well as gene-environment interactions (Auxemery, 2012, van Zuiden et al., 2012) may be involved in the hippocampal volume and shape differences as observed in DID and PTSD. However, more research is needed to investigate the relationship between genetic predispositions, stress hormone levels and hippocampal structure.

It has been reported that some medications including typical antipsychotics (Chakos et al., 2005), anti-epileptics (Watanabe et al., 1992) and antidepressants (Vermetten et al., 2003) could change hippocampal morphology. However, when we repeated the volumetric analyses after excluding the patients with a history of using these medications we obtained similar findings, i.e., smaller hippocampal volume in DID compared to HC and a negative correlation with reported childhood traumatization (See Supplementary material 1).

Strengths and limitations

Some strengths and limitations of the present study should be noted. One important strength of this study is that the DID diagnosis assessment was established by one of two independent experts in this field limiting the chance of including false positive cases. Furthermore, in the present study only PTSD patients with interpersonal trauma were recruited in order to match the trauma-related background of the DID patients. The sample size of 17 DID and 16 PTSD patients could be considered as modest. However, this sample size is the largest among volumetric studies in DID. While we did observe significant hippocampal volume differences between the groups, the results of shape analysis did not survive multiple comparison correction. This is likely to be due to insufficient statistical power necessary for conducting multiple tests across the hippocampal surface. However, it should be noted that permutation testing can be considered

rather conservative in this context as all the tests are treated independently and it does not account for local correlations between surface points. Only female patients and controls were included, so that our findings cannot be generalized to the whole DID and PTSD populations. We did not match the PTSD and DID groups with regard to childhood traumatization. While all the DID patients reported severe childhood traumatization, some (nine out of sixteen) of the PTSD patients reported moderate-to-severe childhood traumatization and the other PTSD patients reported less childhood traumatization. Therefore it is possible that part of the effects observed in the PTSD patients were related to the differences in childhood traumatization. Another limitation is that the history of childhood traumatization was assessed by means of a retrospective self-report questionnaire (i.e. CTQ) which could be subject to recollection bias, but on the other hand the CTQ has been shown to be of reliable tool in previous research (Teicher et al., 2012).

In this study we chose to use manual tracing and shape-based analyses techniques since they might be more sensitive in detecting deformations compared to automated atlas-based segmentation approaches. Although the intra-rater's reliability was very high and comparable to the previous studies, manual tracing techniques are prone to measurement error, which could overcome by repeated tracing using multiple raters, if available, or using automated techniques in combination with a careful segmentation quality check.

Conclusions

The current study found smaller hippocampal volume in DID as compared to PTSD and HC. Importantly, these volumetric abnormalities were correlated with the severity of reported childhood traumatization, particularly when the childhood traumatization scores of the DID patients in the TIS were used. Interestingly, as was expected, all DID patients in the study met criteria for PTSD as well, which provides another indication that DID is a more severe trauma-related condition compared to PTSD. DID and PTSD were associated with contractions in several subfields of the hippocampus which were linked to childhood traumatization. Therefore, our findings confirm clinical observations and previous empirical

research that DID is related to chronic childhood abuse and neglect and are therefore indicative of an etiology related to chronic childhood adversity for this disorder. These findings are important for translation into clinical treatment of this disorder and for the investigation of the neurobiological mechanisms involved in DID.

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Supplemental Material for:

**Hippocampal Morphology and Childhood
Traumatization in Dissociative Identity
Disorder and Posttraumatic Stress Disorder**

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Chapter

5

Effect of medication

Medication history in the DID group included antipsychotics (past: n=2; current: n=8), antidepressants (past: n=2; current: n=10), anti-epileptics (past: n=1; current: n=3). Two PTSD patients reported using antidepressants at the time of scan. All HC were free of present and past psychiatric medication. It has been reported that some medications including typical antipsychotics (Chakos et al., 2005), anti-epileptics (Watanabe et al., 1992) and antidepressants (Vermetten et al., 2003) have been shown to change the hippocampal morphology.

The results listed in Table 5.1 of the main text indicated that DID is associated with smaller bilateral hippocampal volume as compared to HC and PTSD. To investigate whether history of medication has any effect on these findings we repeated the volumetric analyses three times while excluding the patients with a history of using (i) typical antipsychotics (3 DID), (ii) anti-epileptics (4 DID) and (iii) antidepressants (10 DID and 2 PTSD). To test group differences in hippocampal volume, a repeated-measures analysis of covariance (ANCOVA) was used, with side (left or right) as the repeated measure and age and parenchymal volume as covariates. The analysis was followed by two-sample t-tests to compare left and right hippocampal volumes separately between: 1) DID vs. HC, 2) DID vs. PTSD, and 3) PTSD vs. HC. Furthermore, correlation analyses were repeated to investigate if excluding patients with a history of using medication has any effect on the findings presented in Table 5.3.

Results of the group comparisons are listed in Supplementary Table ST5.1. When DID patients with a history of using typical antipsychotics were excluded, the results of smaller left and right hippocampal volume in DID relative to HC and smaller left hippocampal volume in DID compared to PTSD remained significant. However, comparing DID to PTSD, right hippocampal volume showed only a trend to be smaller in DID. Furthermore, we found that after excluding DID patients with a history of using anti-epileptics, the effect size of group differences for DID vs. HC as well as DID vs. PTSD became larger for right and (particularly) left hippocampal volumes. This may indicate that DID patients with a history of using anti-epileptic

drugs had larger hippocampal volumes compared to those of DID patients without a history of using anti-epileptic drugs. It has been proposed that administration of anti-epileptic drugs, via interfering with excitatory amino acid release and action, may prevent stress and GC-induced atrophy in the hippocampal subfield CA3 (Watanabe et al., 1992). Unfortunately, the effect of anti-epileptics on the CA1 region (which we observed) is not clear yet as little research has been done. Although the current finding could have important clinical implications, our results are limited by the number of patients with and without a history of anti-epileptic medications. Therefore more research is needed to further investigate the effect of anti-epileptic drugs on the hippocampus in the DID patients.

When DID and PTSD patients with a history of using antidepressants were excluded from the volumetric analyses the results of smaller left and right hippocampal volume in DID relative to HC remained significant although the effects sizes were smaller than those of the original analyses. However, comparing DID to PTSD, left hippocampal volume showed only a trend to be smaller in DID ($p=0.077$) and comparing PTSD to HC no significant difference in left and right hippocampal volumes were observed which could be because of insufficient statistical power due to excluding ten DID and two PTSD patients. The results of these *post hoc* tests could indicate that reduced hippocampal volume in DID compared to HC (See Table 5.1) was a robust finding and was not due to the history of medication.

Results of the correlation analyses after excluding patients with a history of using psychiatric medications are presented in Supplementary Table ST5.2. These results indicate that the correlation results presented in Table ST5.3 were not affected by medication.

References

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Supplementary Table ST5.1. Statistical analyses of parenchymal (cm³) and hippocampal (mm³) volumes

	Mean(SD)			pairwise comparisons: Effect size [^] , change (%), P Value									
	DID	PTSD	HC	DID vs. HC			DID vs. PTSD			PTSD vs. HC			
				Cohen's d	Change %	P-Value	Cohen's d	Change %	P-Value	Cohen's d	Change %	P-Value	
Exclusion of typical antipsychotic users ^a													
Left hippocampus	1980(216)	2166(228)	2237(196)	-1.25	-11.49	0.001*	-0.84	-8.59	0.036*	-0.33	-3.17	0.310	
Right hippocampus	2067(204)	2220(202)	2340(205)	-1.33	-11.67	<0.001*	-0.75	-6.89	0.073	-0.59	-5.13	0.076	
Exclusion of anti-epileptic users ^b													
Left hippocampus	1932(160)	2166(228)	2237(196)	-1.71	-13.63	<0.001*	-1.21	-10.80	0.003*	-0.33	-3.17	0.291	
Right hippocampus	2027(160)	2220(202)	2340(205)	-1.72	-13.38	<0.001*	-1.07	-8.60	0.011*	-0.59	-5.13	0.066	
Exclusion of antidepressant users ^c													
Left hippocampus	2024(231)	2179(230)	2237(196)	-1.00	-9.52	0.014*	-0.67	-7.11	0.077	-0.27	-2.59	0.485	
Right hippocampus	2114(252)	2215(209)	2340(205)	-0.99	-9.66	0.012*	-0.44	-4.56	0.229	-0.60	-5.34	0.107	

DID= dissociative identity disorder; PTSD= posttraumatic stress disorder; HC= healthy controls.

* P-value<0.05

[^] = Cohens'd

^a Three DID patients with a history of using typical antipsychotic medications were excluded, leaving 14 DID, 16 PTSD and 28 HC.

^b Four DID patients with a history of using anti-epileptic medications were excluded, leaving 13 DID, 16 PTSD and 28 HC.

^c Ten DID and two PTSD patients with histories of using antidepressant medications were excluded, leaving 7 DID, 14 PTSD and 28 HC.

Supplementary Table ST5.2. Correlation analyse between hippocampal volume ^d and CTQ sub-scores of the patient groups

	Pearson's correlation r (P Value)					
	emotional neglect	physical neglect	emotional abuse	physical abuse	sexual abuse	Total score
Exclusion of typical antipsychotic users ^a						
Left hippocampus	NIS:-0.529(0.003*) TIS:-0.602(0.001*)	NIS:-0.347(0.065) TIS:-0.461(0.013*)	NIS:-0.277(0.146) TIS:-0.427(0.024*)	NIS:-0.226(0.238) TIS:-0.241(0.216)	NIS:-0.481(0.008*) TIS:-0.418(0.027*)	NIS:-0.439(0.017*) TIS:-0.492(0.008*)
Right hippocampus	NIS:-0.290(0.127) TIS:-0.399(0.035*)	NIS:-0.186(0.333) TIS:-0.347(0.070)	NIS:-0.130(0.501) TIS:-0.338(0.079)	NIS:-0.163(0.397) TIS:-0.232(0.236)	NIS:-0.338(0.073) TIS:-0.358(0.061)	NIS:-0.265(0.165) TIS:-0.387(0.042*)
Exclusion of anti-epileptic users ^b						
Left hippocampus	NIS:-0.531(0.003*) TIS:-0.616(<0.001*)	NIS:-0.379(0.043*) TIS:-0.503(0.006*)	NIS:-0.260(0.173) TIS:-0.481(0.010*)	NIS:-0.189(0.326) TIS:-0.316(0.101)	NIS:-0.404(0.030*) TIS:-0.516(0.005*)	NIS:-0.414(0.025*) TIS:-0.560(0.002*)
Right hippocampus	NIS:-0.286(0.133) TIS:-0.404(0.033*)	NIS:-0.243(0.204) TIS:-0.405(0.033*)	NIS:-0.129(0.506) TIS:-0.361(0.059)	NIS:-0.107(0.582) TIS:-0.270(0.165)	NIS:-0.255(0.181) TIS:-0.427(0.024*)	NIS:-0.240(0.210) TIS:-0.431(0.022*)
Exclusion of antidepressant users ^c						
Left hippocampus	NIS:-0.567(0.009*) TIS:-0.663(0.002*)	NIS:-0.470(0.037*) TIS:-0.696(0.001*)	NIS:-0.339(0.144) TIS:-0.617(0.005*)	NIS:-0.049(0.838) TIS:-0.404(0.086)	NIS:-0.374(0.104) TIS:-0.583(0.009*)	NIS:-0.431(0.058) TIS:-0.677(0.001*)
Right hippocampus	NIS:-0.320(0.168) TIS:-0.406(0.085)	NIS:-0.222(0.347) TIS:-0.439(0.060)	NIS:-0.096(0.688) TIS:-0.384(0.105)	NIS:-0.160(0.500) TIS:-0.187(0.442)	NIS:-0.130(0.585) TIS:-0.400(0.090)	NIS:-0.154(0.517) TIS:-0.418(0.075)

CTQ= childhood trauma questionnaire.

* *P*-value<=0.05

^a Three DID patients with a history of using typical antipsychotic medications were excluded, leaving 14 DID, 16 PTSD and 28 HC.

^b Four DID patients with a history of using anti-epileptic medications were excluded, leaving 13 DID, 16 PTSD and 28 HC.

^c Ten DID and two PTSD patients with histories of using antidepressant medications were excluded, leaving 7 DID, 14 PTSD and 28 HC.

^d hippocampal volumes were normalized by the parenchymal volumes.