SUPPLEMENTARY MATERIAL

FOR THE MANUSCRIPT

Abnormal Hippocampal Morphology in Dissociative Identity Disorder and Posttraumatic Stress Disorder Correlates with Childhood Trauma and Dissociative Symptoms

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Supplementary Material 1

Detailed image acquisition and analysis methods

Image acquisition

Identifying individuals with posttraumatic stress disorder (PTSD), with or without dissociative identity disorder (DID), willing and able to participate in a neuroimaging study is known to provide a great challenge. As minimizing travel time increases the likelihood of participation, participants were scanned in the closest of two (University Medical Center Groningen (UMCG) and of the Amsterdam Medical Center (AMC)) 3T MR scanners (Philips Medical Systems, Best, NL) in The Netherlands. Prior to starting, a reproducibility study was conducted that resulted in an optimized structural MRI protocol with a high contrast-to-noise ratio (important for manual and automated segmentation procedures) and a high reproducibility between the two centers [Chalavi et al., 2012]. At both centers T1-weighted anatomical MR scans were acquired (MPRAGE, TR=9.95ms, TE=5.6ms, flip-angle=8º, 1x1x1mm voxels, number of slices=160, total scan-time=10m14s). All-PTSD patients and their matched HC were scanned interleaved within a short time interval to avoid an interaction between group and time dependent scanner fluctuations. The samples were balanced over the two centers: twenty All-PTSD patients (ten PTSD-DID, ten PTSD-only) and nineteen HC were scanned at UMCG. Two structural MRI scans were collected from each subject whenever possible (fifteen PTSD-DID patients and fourteen HC). Where both scans were artifact-free, the first scan was used. Four HC subjects were excluded due to the presence of (motion) artifacts in the MRI scan. A total of 17 PTSD-DID, 16 PTSD-only and 28 HC subjects were included in the demographic and morphological analyses.

Image analysis

Manual measures of global volume and shape analysis of the hippocampus

Non-cortical tissue was removed from the MR images using the Brain Extraction Tool (BET)[Smith, 2002] and head alignment was standardized by rigidly aligning the individual MR images with the average brain template (ICBM452) using FSL-FLIRT. The hippocampi were manually traced using MultiTracer [Woods, 2003] by a single rater (SC), who was blind to all demographical and clinical variables and was trained by an expert (JHC) in this field and obtained good inter- and intra-rater reliabilities according to the established protocol [Thompson et al., 2004]. The 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 [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 [Thompson et al., 2004]. Hippocampal global volumes obtained from these tracings were statistically analyzed.

To assess the shape deformations of different hippocampal subfields an anatomical surface mesh modeling method [Thompson et al., 2004] matched equivalent hippocampal surface points across subjects following manual tracing of the hippocampal boundaries. In brief: localized gray matter contractions and expansions of the hippocampal surface were established corresponding to the CA1, CA2-3 and subiculum (Figure 1.a). In each individual the medial core, a central 3D curve threading down the long axis of the structure, was computed. From each point on the hippocampal surface a radial distance measure was derived to the medial core. As the same surface grid was imposed on all subjects' hippocampi in the same coordinate space, statistical comparisons were made at each hippocampal surface point between the groups to index contrasts on a local scale. Probability values from these statistical comparisons were mapped onto an average hippocampal shape for the entire

sample to generate a 3D representation of the structural differences between the groups. The approximate overlay of the hippocampal subfields was defined based on the Duvernoy atlas [Duvernoy, 1988].

Supplementary Material 2

Effect of medication

It has been reported that some psychiatric medications including typical antipsychotics [Chakos et al., 2005], anti-epileptics [Watanabe et al., 1992] and antidepressants [Vermetten et al., 2003] change the hippocampal morphology. In this study in order to ascertain genuine findings we repeated the analyses after excluding posttraumatic stress disorder patients with (PTSD-DID) or without (PTSD-only) dissociative identity disorder (DID) and a history of different types of psychiatric medications.

The volumetric analyses were repeated three times while excluding the patients with a history of using (i) typical antipsychotics (3 PTSD-DID), (ii) typical and atypical antipsychotics (9 PTSD-DID), (iii) anti-epileptics (4 PTSD-DID), and (iv) antidepressants (10 PTSD-DID and 2 PTSD-only). To test the effect of PTSD diagnosis, first volumetric measurements were compared between All-PTSD vs. HC (HC: healthy controls) using analysis of covariance (ANCOVA), with group and MRI center as independent factors 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) PTSD-DID vs. HC, 2) PTSD-DID vs. PTSD-only, and 3) PTSD-only vs. HC.

When PTSD-DID patients with a history of using typical antipsychotics were excluded, the majority of the findings remained significant and only the difference in right hippocampal global volume in PTSD-DID vs. PTSD-only comparison and right CA1 in the PTSD-DID vs. HC comparison changed from significant to trend ($p=0.073$ and $p=0.059$, respectively). However, when all the PTSD-DID patients with a history of using either typical or atypical antipsychotics were excluded only left CA4-DG showed a trend to be smaller in PTSD-DID than HC and the rest of statistical analyses results did not reach a

significant level which is probably due to the lack of statistical power.

After excluding PTSD-DID patients with a history of using anti-epileptics, we found that the effect size of group differences for PTSD-DID vs. HC as well as PTSD-DID vs. PTSD-only became larger for right and especially left hippocampal global volumes and the differences between the PTSD-DID and PTSD-only became significant in the bilateral hippocampal global volume and volume of the left CA1, right CA2-3, bilateral CA4-DG, bilateral subiculum. This may indicate that PTSD-DID patients with a history of using anti-epileptic drugs had *larger* hippocampal volumes compared to those of PTSD-DID patients without a history of using anti-epileptic drugs and therefore resulted in an underestimation of the hippocampal reductions in the PTSD-DID group.

When PTSD-DID and PTSD-only patients with a history of using antidepressants were excluded from the volumetric analyses the results of smaller left and right hippocampal volume in PTSD-DID relative to HC became less significant, which is most likely caused by insufficient statistical power. However, the pattern of differences remained the same.

In sum: The results of these *post hoc* tests show that smaller hippocampal volume in PTSD-DID compared to HC (Table II main manuscript) was a robust finding and was not due to the history of medication.

Supplementary Material 3

PTSD with childhood onset traumatization

Experienced inter-personal trauma as reported by PTSD-only patients during the Clinician Administered PTSD Scale (CAPS) interview included: emotional neglect (n=4), physical and sexual abuse/violence (n=13), attack with a weapon (n=3) and witnessing drowning of a family member (n=1). Eleven of the PTSD-only patients reported experiencing multiple types of interpersonal traumas during their childhood (n=6) or trauma starting from childhood and continuing into their adult life (n=5). The remaining 5 PTSD-only patients reported experiencing trauma only in their adult life. This is while all the PTSD-DID

patients reported childhood traumatic experiences on the Trauma Experience Checklist (TEC)[Nijenhuis et al., 2002]. To investigate the childhood trauma-related nature of smaller hippocampal (global and subfield) volumes in PTSD-only and PTSD-DID patients, we repeated the pairwise comparisons between groups by removing those five PTSD-only patients with only adult trauma. Results of this *post hoc* test revealed that after excluding PTSD-only patients with only adult trauma, bilateral hippocampal global volumes in the PTSD-only became significantly smaller compared to HC. Left and right global hippocampal volumes of the PTSD-only group became significantly smaller and the volume differences changed from 3.17 % and 5.13% in the original analysis to 7.11% and 7.31% in the *post hoc* analysis. Interestingly, bilateral hippocampal global volumes did not differ significantly between the PTSD-DID and PTSD-only groups anymore. With regard to the hippocampal subfield volumes, the *post hoc* analysis revealed that in addition to the findings of the main analysis, as compared to HC, All-PTSD patients had smaller volumes of the left CA4-DG and bilateral subiculum. PTSD-only group had smaller right presubiculum and trends for smaller right CA4-DG ($p=0.059$) and left presubiculum ($p=0.074$) as compared to HC. Furthermore, when the shape analysis was repeated for the second *pos hoc* test, we found a trend ($p=0.090$) for a significant pattern of deformations for All-PTSD vs. HC comparison. The results revealed that after excluding PTSD-only patients with only adult trauma, the results became more significant for both hippocampal volume and shape.

This shows that hippocampal size differs between PTSD-only with childhood onset and PTSD-only with adult onset trauma and suggests that future research in PTSD needs to carefully assess the presence of childhood trauma to limit heterogeneity.

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