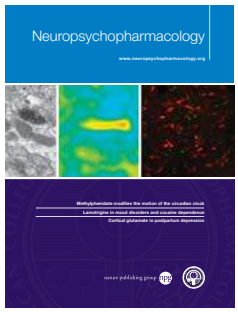

Treatment Outcome Related White Matter Differences in Veterans with Posttraumatic Stress Disorder

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Treatment outcome related white matter differences in veterans with Posttraumatic Stress Disorder

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Running title

Increased dorsal cingulum FA in persistent PTSD

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Abstract

Post-traumatic stress disorder (PTSD) is a debilitating disorder that has been associated with brain abnormalities, including white matter alterations. However, little is known about the effect of treatment on these brain alterations. To investigate the course of white matter alterations in PTSD, we used a longitudinal design investigating treatment effects on white matter integrity using diffusion tensor imaging (DTI).

Diffusion tensor and magnetization transfer images were obtained pre- and post-treatment from veterans with (n=39) and without PTSD (n=22). After treatment, 16 PTSD patients were remitted, and 23 had persistent PTSD based on PTSD diagnosis. The dorsal and hippocampal cingulum bundle, stria terminalis, and fornix were investigated as regions of interest. Exploratory whole brain analyses were also performed. Groups were compared with repeated measures ANOVA for fractional anisotropy (FA), and magnetization transfer ratio.

Persistently symptomatic PTSD patients had increasing FA of the dorsal cingulum over time, and at reassessment these FA values were higher than both combat controls and the remitted PTSD group. Group by time interactions for FA were found in the hippocampal cingulum, fornix, and stria terminalis, posterior corona radiata, and superior longitudinal fasciculus.

Our results indicate that higher FA of the dorsal cingulum bundle may be an acquired feature of persistent PTSD that develops over time. Furthermore, treatment might have differential effects on the hippocampal cingulum, fornix, stria terminalis, posterior corona radiata and superior longitudinal fasciculus in remitted versus persistent PTSD patients. This study contributes to a better understanding of the neural underpinnings of PTSD treatment outcome.

Introduction

Post-traumatic stress disorder (PTSD) is a trauma and stressor-related disorder that is prevalent in about 6-13% of veterans deployed to Iraq or Afghanistan (Hoge*, et al* 2004; Reijnen*, et al* 2014). Understanding PTSD pathophysiology and treatment can contribute to the improvement of interventions and perhaps the prevention of the development of PTSD (Linden 2006). Although trauma-focused therapy is available and effective to treat PTSD, by inducing fear extinction of trauma-related memories (Foa and Kozak 1986; Rothbaum and Davis 2003), not all patients remit from PTSD (Bisson*, et al* 2007). Using a longitudinal design we investigated neurobiological alterations in PTSD patients and combat controls before and after treatment.

PTSD has been associated with a hyperactive limbic system (e.g. amygdala), and a hypoactive emotional regulation system (e.g. anterior cingulate cortex (ACC), prefrontal cortex (PFC)) (Hayes *et al* 2012; Rauch*, et al* 2006). Recently, research with structural and functional magnetic resonance imaging (MRI) has started to disentangle whether neurobiological alterations found in PTSD change after successful treatment. Some studies have shown that treatment potentially normalizes activity in limbic system and regulatory brain areas (e.g. amygdala, ACC (Aupperle*, et al* 2013; Fani*, et al* 2011; Roy*, et al* 2010)). In addition, functional neuroimaging studies have reported treatment outcome to be related to pre-treatment structure and activity of limbic and regulatory regions, such as the ACC (Aupperle*, et al* 2013; Bryant*, et al* 2008; Bryant*, et al* 2008; Dickie*, et al* 2013; van Rooij*, et al* 2014; van Rooij*, et al* 2015). These results indicate the possibility of using brain based biological markers as pretreatment outcome predictors, and suggest the possibility that there are potential differences in the neurobiology of remitted PTSD patients compared to those that fail to respond to treatment.

In cross-sectional studies using diffusion tensor imaging (DTI), white matter microstructure alterations have been reported in PTSD (Daniels*, et al* 2013). From these studies, Fractional Anisotropy (FA) is most frequently obtained as a parameter of interest. FA is a measure sensitive to alterations in axonal directionality and white matter organization (Beaulieu 2009). Reduced FA in the cingulum bundle has frequently been reported in PTSD patients (Fani*, et al* 2012; Kim*, et al* 2005; Sanjuan*, et al* 2013; Schuff*, et al* 2011), although heightened FA in the cingulum bundle has also been reported (Abe*, et al* 2006; Zhang*, et al* 2012). One longitudinal study has investigated white matter microstructure in a small sample of only eight PTSD patients, 10 and 24 months after experiencing a traumatic event (Zhang*, et al* 2011). An increase in FA in the posterior cingulum bundle over time was reported (Zhang*, et al* 2011). However, no control group was included in this study and the relation to symptom improvement was not directly assessed. Thus, it remains unclear as to whether or not white matter microstructure of the cingulum bundle changes in relation to PTSD treatment outcome.

In addition, we were interested in investigating other structures. The stria terminalis and fornix are important association pathways of the limbic system, which are involved in the formation of emotional memory, fear, and anxiety (Avery*, et al* 2014; Gray 1982). The stria terminalis comprises connections between the amygdala and the bed nucleus of the stria terminalis (BNST), while the fornix connects the hippocampi with the septal area and hypothalamus (Mori*, et al* 2008). Although literature is abundant on altered functioning of the amygdala and hippocampus in PTSD, to our knowledge the stria terminalis and the fornix, tracts that form crucial connections among these brain areas, have not been systematically investigated in PTSD patients.

In the current study, we investigate trauma-focused therapy effects on white matter microstructure of the cingulum bundle, stria terminalis, and fornix in PTSD patients versus combat controls with diffusion tensor imaging, which provides information about axonal orientation and density (Beaulieu 2009). In addition, magnetization transfer images are investigated, which can provide additional information on density of macromolecules, and can be sensitive to white matter degradation (Henkelman*, et al* 2001). Scans were acquired before treatment (baseline) and after approximately six to eight months of trauma-focused therapy (post-treatment). In addition, whole-brain analyses were performed to provide a comprehensive unrestricted survey of potential treatment-related white matter differences. We included a deployed, trauma-exposed comparison group to control for the effects of time and deployment (Van Wingen*, et al* 2012). Using treatment outcome as an indicator, patients with remitted PTSD were compared with patients that still had a PTSD diagnosis after treatment (persistent PTSD), and with combat controls. We expected to observe: (a) an interaction effect caused by differences between PTSD patients and combat controls at baseline with remitted PTSD patients becoming comparable with combat controls after treatment (recovery-related changes; normalization), and (b) treatment outcome related differences (remitted and persistent PTSD differences). More specifically, based on previous research we expected lower baseline FA values in the cingulum bundle that may restore to control levels after treatment, and lower cingulum FA in persistent versus remitted PTSD patients.

Materials and Methods

Participants and clinical assessment

In total, 41 male veterans with PTSD and 24 male veterans without PTSD (combat controls) were included in this study. PTSD patients were recruited from one of four outpatient clinics of the Military Mental Healthcare Organization. PTSD was diagnosed by a clinician according to DSM-IV criteria (American Psychiatric Association 1994), and PTSD severity was assessed with the clinician administered PTSD scale (CAPS, (Blake*, et al* 1995)). A clinician or trained researcher administered the interviews. Control participants were recruited via advertisements. For all participants, the presence of (comorbid) disorders or lifetime disorders was assessed with the Structured Clinical Interview for DSM IV (SCID-I (First*, et al* 1997)). At the time of inclusion, all PTSD patients had current PTSD (CAPS≥45), no current alcohol or substance dependence, and no neurological disorder. Combat controls included in the study had no clinical PTSD symptoms (CAPS≤15), no current psychiatric disorder, no alcohol or substance dependency, and no neurological disorder. After inclusion and a baseline MRI scan (baseline), patients underwent trauma-focused therapy, which consisted of trauma-focused cognitive behavioral therapy (TFCBT) with exposure and/or eye movement desensitization and reprocessing (EMDR), in accordance with Dutch and international treatment guidelines (Balkom*, et al* 2013; Foa and Meadows 1997). Treatment selection was part of "treatment as usual", applied by a clinician. The clinician decided whether TFCBT or EMDR was applied as initial therapy. TFCBT and EMDR have been shown to have similar efficacy (Bisson*, et al* 2007). After an interval of six to eight months, all participants were reassessed with clinical interviews (CAPS and SCID-I) and MRI protocol (post-treatment). PTSD patients were divided into a remitted group (when no PTSD

diagnosis was present at the second clinical assessment according to DSM-IV criteria (First, *et al* 1997)), and a symptom persistent group (PTSD patients who still had a diagnosis of PTSD at the second assessment; persistent PTSD).

After written and verbal explanation of the study was given, all participants gave informed consent. This study was approved by the medical ethical committee of the University Medical Center Utrecht and was performed in accordance with the Declaration of Helsinki (World Medical Association 2013).

Image acquisition and processing

Diffusion and magnetic transfer images were obtained using a 3.0 Tesla magnetic resonance imaging scanner (Philips Medical System, Best, The Netherlands) at both time-points (for scan parameters see supplementary information). Quality of these images was assessed and scans with bad quality were excluded from further analysis (PTSD patients n = 1, control n = 2). One PTSD patient was excluded from all analyses because normalization was not possible. Preprocessing steps for the diffusion images were performed with FSL, CAMINO and DTI-TK (see supplementary information). Briefly, processing included distortion correction, tensor model fitting, normalization to MNI space. Scalars of the tensor image were calculated (fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD) and mean diffusivity (MD)), and smoothed (FWHM 8 mm) to increase the signal to noise ratio. FA is a fraction of diffusion in all directions, which is sensitive to axonal directionality relative to radial diffusivity, and can be regarded as a summary measure for microstructural integrity (Alexander, *et al* 2011). FA was the initial scalar of interest. To specify which process is potentially altered, RD, AD, and MD were additionally investigated. RD represents the diffusivity in the direction perpendicular to the white matter tract and is sensitive to

demyelination and axonal diameter (Alexander, *et al* 2011). AD represents diffusion parallel to white matter and is sensitive to general axonal damage (Alexander, *et al* 2011). MD is the average diffusivity in all directions and represents isotropic diffusivity, which is high in cerebrospinal fluid, and is sensitive to cellular damage (e.g. edema and necrosis (Alexander, *et al* 2011)).

The magnetization transfer images were registered to the unweighted diffusion image (b0). The magnetization transfer ratio (MTR) was calculated by subtracting the image with magnetization prepulse from the baseline image and then dividing the residual by the baseline image. The resulting MTR images were normalized to the diffusion group template using DTI-TK.

Statistical analysis

Tract-based analyses

The cingulum bundle is a C-shaped bundle that runs between the ACC and the entorhinal cortex, and can be subdivided into a dorsal and hippocampal region (see Figure 1A,B). Regions of interest (ROIs) were created for the bilateral dorsal and hippocampal cingulum bundle subdivisions, derived from the JHU-ICBM-81 atlas template (Mori, *et al* 2005).

To extract tracts not available in the JHU white matter atlas we ran whole-brain deterministic tractography, using the tensor template in MNI space. The stria terminalis was iteratively traced with respect to its known anatomical boundaries by placing ROI's in the amygdala and BNST (Avery, *et al* 2014; Mori, *et al* 2005). Tracing was verified by two researchers, MK and DPMT. The fornix was dissected following manual dissecting protocols by placing an ROI in the body of the fornix ((Mori, *et al* 2005) see Figure 1C,D).

Mean FA and MTR values were extracted for these ROIs and exported into IBM SPSS Statistics for Windows Version 21.0 (Armonk, New York, USA; IBM Corporation) for statistical testing. A general linear model for repeated measures was applied for all ROIs (fornix and left and right dorsal cingulum, hippocampal cingulum, stria terminalis) for FA and MTR to compare the patients with remitted PTSD, the patients with persistent PTSD and the combat controls at both time points. Additional analyses of RD, AD, and MD were applied when an effect for FA was found, to specify which processes were altered. Post-hoc tests were performed when multivariate interaction effects were found. Analyses were covaried for the whole brain baseline mean of the eigenvalue tested, and age.

Voxel-wise analyses

From the individual pairs of FA maps (baseline and post-treatment), difference in FA maps (ΔFA maps) and mean FA maps were created to explore the interaction between time and group, and the group effect respectively using FSL randomize. Threshold free cluster enhancement (TFCE-corrected $p < 0.05$; (Smith and Nichols 2009)) was used to correct for multiple comparison, using a white matter mask.

Results

Participants

An overview of demographical and clinical information is presented in Table 1. After treatment, 16 PTSD patients recovered from PTSD (remitted PTSD); 23 PTSD patients had not recovered and still fulfilled DSM-IV criteria for PTSD (persistent PTSD). The combat controls, and the remitted and persistent PTSD groups did not differ in age ($F_{(2, 56)} = 0.520$, $p = 0.597$), educational level ($F_{(2,.56} = 1.47$, $p = 0.863$), the number of times they were deployed ($\chi^2_{(14)} = 13.343$, $p = 0.500$), time since last deployment ($F_{(2,56)} = 0.291$, $p = 0.749$), and interval between scans ($F_{(2,56)} = 1.112$, $p = 0.337$). The number of subjects that (self-) reported being exposed to blast during deployment was more prevalent in the persistent PTSD group ($\chi^2_{(1)} = 6.306$, $p = 0.043$).

No difference between the remitted PTSD patients and persistent PTSD patients was found in the total number of treatment sessions between scans ($t_{(33)} = -0.008$, $p = 0.993$). More specifically, no difference was found between the remitted PTSD patients and persistent PTSD patients in the number of TFCBT sessions ($t_{(33)} = 0.11$, $p = 0.91$), or the number of EMDR sessions between scans ($t_{(33)} = -0.15$, $p = 0.88$). The persistent PTSD group had a higher CAPS score at baseline ($t_{(36)} = -2.31$, $p = 0.027$), as well as after treatment ($t_{(37)} = -7.295$, $p = 0.000$). Control participants had a mean CAPS score of 4.5 (±4.3) at both time points. One control participant used psychotropic medication (Ritalin), all the others did not use psychotropic medication. Comorbidity of anxiety disorders was more prevalent in the persistent PTSD group versus the remitted PTSD group at baseline ($\chi^2_{(1)} = 5.30$, $p = 0.037$), and a trend was observed for mood disorders ($\chi^2_{(1)} = 3.95$, $p = 0.059$). Post-treatment comorbidity was only present in the patients with persistent PTSD. The PTSD groups did not

differ on psychotropic medication use. None of the participants was physically injured during deployment.

Tract-based analyses

A significant multivariate group by time interaction effect was found for FA values (Wilks' Lambda = 0.589, $F_{(14,100)}$ = 2.167, p = 0.014). The interaction effect was driven by interactions in the left dorsal cingulum, left hippocampal cingulum, bilateral stria terminalis, and fornix FA, which will be described below (see Figure 2 and 3). There were no significant correlations between the differences in tract FA values over time and symptom improvement within the groups. No significant effects were observed for MTR, AD, RD, and MD.

Dorsal cingulum

A group by time interaction effect was found for the left dorsal cingulum ($F_{(2,56)}$ = 3.932, p = 0.026). After treatment persistent PTSD patients had higher FA in the left dorsal cingulum compared to combat controls (p = 0.026), and remitted PTSD patients (p = 0.062). The groups did not differ significantly at baseline. A significant increase in left dorsal cingulum FA over time was found in persistent PTSD patients (p = 0.008). This indicates that higher FA develops over the course of treatment in persistent PTSD patients.

Of note, a univariate main effect of group (uncorrected) was observed in the right dorsal cingulum ($F_{(2,.56)}$ = 4.614, p = 0.014), where patients with persistent PTSD had higher FA in the dorsal cingulum compared to the remitted PTSD group, and combat controls across both time points.

Hippocampal cingulum

An interaction between time and group was found for left hippocampal cingulum FA ($F_{(2, 56)}$ = 4.491, p = 0.016). There were no main effects for group or time. Remitted PTSD patients showed a non-significant reduction in FA over time towards the FA values of combat controls, the combat controls had a non-significant increase in FA over time, and persistent PTSD patients show stable (heightened) FA levels. This pattern suggests that changes in hippocampal cingulum FA may be recovery-related.

Stria terminalis

A significant interaction between time and group was found for bilateral stria terminalis FA ($F_{(2,56)}$ = 3.379, p = 0.041; $F_{(2,56)}$ = 6.690, p = 0.002), in the absence of main effects for group or time. Persistent PTSD patients showed a non-significant increase in FA over time and the remitted PTSD patients showed a non-significant decrease in FA over time, while controls showed stable lower FA values.

Fornix

A group by time interaction was found for fornix FA ($F_{(2,56)}$ = 3.908, p = 0.026), in the absence of main effects for group or time. Persistent PTSD patients had a non-significant increase in FA versus remitted PTSD and controls who displayed a non-significant decrease in FA.

Voxel-wise analyses

Exploration of whole brain effects revealed a significant group by time interaction in two clusters of voxels. The largest cluster was located in the left posterior corona radiata (k =

218, p = 0.004. Peak voxel: F = 19.37, MNI coordinates x = -22, y = -40, z = 35; see Figure 4). A second cluster was located in the superior longitudinal fasciculus (k = 16, p = 0.049. Peak voxel: F=10.47, MNI coordinates x = -31, y = -43, z = 26; see Figure 4). The interaction effect for both clusters was driven by a significant decrease in FA in the patients with remitted PTSD versus a significant increase in FA in combat controls, while the persistent PTSD group did not differ over time. The change in FA in the posterior corona radiata correlated with the percentage change in CAPS score (Pearsons r = 0.451, p = 0.004).

Discussion

This is the first longitudinal study to report treatment-related differences in white matter microstructure between remitted and persistent PTSD patients, and combat controls. After treatment, higher FA values in the dorsal cingulum were found in patients with persistent PTSD versus patients with remitted PTSD and combat controls, indicating that white matter microstructure in the dorsal cingulum may be an acquired feature of persistent PTSD that develops over time**.** In addition, group by time interaction effects were found for the left hippocampal cingulum, fornix, stria terminalis, posterior corona radiata, and superior longitudinal fasciculus.

Cross-sectional studies have previously found higher dorsal cingulum FA in PTSD patients compared to controls (Abe*, et al* 2006; Zhang*, et al* 2012). We showed that this heightened FA was specific to patients with persistent PTSD, who differed from both combat controls and remitted PTSD patients after treatment. The dorsal cingulum runs from subcallosal frontal cortex to the posterior cingulate cortex (PCC), forming connections between the cingulate cortex and frontal and parietal brain areas (Mori*, et al* 2005). Heightened functional activation of the dorsal ACC and PCC has been reported in a meta-analysis of PTSD studies (Hayes*, et al* 2012). Moreover, altered PCC-medial PFC connectivity has been shown in PTSD patients both during a working memory task (increased) (Daniels*, et al* 2010), and at rest (decreased) (Bluhm*, et al* 2009). Interestingly, in a recent study by our group, persistent PTSD patients showed increased dorsal ACC activity towards negative images, while remitted PTSD patients did not (van Rooij*, et al* 2015). In line with these studies, our results show increased white matter microstructural integrity in the cingulum bundle near the PCC and dorsal ACC (see Figure 2). Together with previous findings, our

results suggest that altered dorsal cingulum structure may complement altered cingulate function and be specific for treatment-resistant PTSD that develops or progresses over time.

The only previous longitudinal DTI study that was performed in a small sample of PTSD subjects found an increase in (posterior) cingulum FA values over time in PTSD patients with persistent symptoms, though no control group was included (Zhang*, et al* 2011). We complement these findings by showing that persistent PTSD patients had increasing FA in the dorsal cingulum over time, and higher FA values after treatment compared to remitted PTSD patients and controls. Interestingly, a correlation between state anxiety and an increase in left cingulum FA over time has been reported in recently traumatized subjects (Sekiguchi*, et al* 2014), suggesting that some individuals develop heightened FA early after trauma. In the current study there were indications (that is an uncorrected group difference in right cingulum) that FA was already heightened at baseline. Therefore, future studies should follow up recently traumatized subjects during the development of PTSD (and compare these with controls over time) to investigate if FA increases before or after the onset of PTSD. These studies will help determine if altered cingulum FA is a biomarker or, perhaps more interestingly, a mechanism that underlies persistent PTSD, and can be the target of early interventions to prevent persistent PTSD.

The interaction effect in the dorsal cingulum may be related to neural plasticity. As noted, previous studies reported increased cingulum cortex activity (Hayes*, et al* 2012) in particular in persistent PTSD patients (van Rooij*, et al* 2015). Cortical activity has been reported to modulate myelination (Wang and Young 2014), and increased FA values have been reported after learning (Concha 2014). Therefore, we can speculate that hyperactivity of the cingulate cortex (for example during intrusions) may augment a kind of 'fear learning' by initiating dorsal cingulum bundle myelination, resulting in higher FA. Some studies

support this suggestion; higher cingulum bundle FA in particular has been reported after fear conditioning in rats (Ding*, et al* 2013), and higher cingulum bundle FA has been related to state anxiety after an earthquake (Sekiguchi*, et al* 2014). Further studies could confirm this suggestion by investigating the relation between heightened FA and heightened activity in PTSD.

This study using a longitudinal design and a non-PTSD combat control group to account for trauma exposure and deployment effects, we found increased dorsal cingulum FA in PTSD patients. In contrast, previous studies have reported decreased cingulum FA of PTSD patients (Fani*, et al* 2012; Kim*, et al* 2005; Sanjuan*, et al* 2013; Schuff*, et al* 2011). These inconsistencies in cingulum FA are likely due to differences in study design (e.g. cross sectional, no control group), or inclusion of non-deployed controls. These differences, along with the observation that deployment has been shown to reduce white matter microstructure integrity in the brainstem (Van Wingen*, et al* 2012), suggest that future studies aimed at understanding the neurobiology of PTSD in combat-deployed PTSD patients must include a combat-exposed control group.

The hippocampal cingulum FA values of remitted PTSD patients showed a pattern for recovery, as remitted PTSD patients show non-significant increased baseline FA values that are more comparable to controls after treatment. This could reflect normalization of hippocampal cingulum FA values in remitted PTSD patients, although no group effects were observed at either time point and none of the groups showed a significant change over time. The hippocampal cingulum comprises connections between the cingulate cortex and the temporal lobe, including the hippocampus and amygdala (Mori*, et al* 2005). Restoration of hippocampal and ACC structure and function has previously been reported in PTSD after treatment (Lindauer*, et al* 2005; Roy*, et al* 2010). Furthermore, altered connectivity

between temporal regions and the PCC and ACC has been reported in PTSD during a working memory task (Daniels*, et al* 2010), and resting state (Kennis*, et al* 2014). Potentially, our results, suggesting normalization of increased hippocampal cingulum FA, may be related to restoration of hippocampal and ACC structure and function, and connectivity from medial temporal brain areas to the cingulate cortex.

The interaction effects in stria terminalis and fornix were characterized by differential FA time related patterns between remitted (non-significant decrease) and persistent (non-significant increase) PTSD patients. This might indicate that different processes take place during a period of treatment that differentially alter these limbic tracts. For example, we could speculate that processes of fear extinction take place in remitted PTSD during exposure therapy, while fear reinstatement processes take place in persistent PTSD patients, which are processes that involve the fornix and stria terminalis (Philip*, et al* 2013). However, there were no significant changes in any group over time, and no group differences at any time point. Therefore, caution should be taken with interpreting these effects, as partial voluming effects and delineation of the stria terminalis could confound our results. Further studies should investigate the time course of the hippocampal cingulum, stria terminalis and fornix in order to confirm the observed patterns.

Whole brain voxel-wise correlation analyses revealed a significant decrease over time in the posterior corona radiata and superior longitudinal fasciculus FA of remitted PTSD patients. The posterior corona radiata comprises thalamo-cortical and corticospinal projections, which are postulated to be important in the psychopathology of PTSD (Lanius*, et al* 2003). Alterations in superior longitudinal fasciculus FA values have previously been reported in PTSD patients compared to trauma-exposed controls (Daniels*, et al* 2013). However, the pattern of the interactions found in the current study was not consistent with

a normalization of function as was expected, but rather showed more deviation of the remitted PTSD patients from combat controls at reassessment. In addition, it was not expected that the combat controls would demonstrate time related increases in FA, as was found for the posterior corona radiata. Therefore, it is unclear how to interpret these results and replication of this finding is necessary.

Blast exposure during deployment was more prevalent in persistent PTSD patients in our study. Blast-exposure may induce mild traumatic brain injury, which has been suggested to increase vulnerability to develop PTSD and potentially reinforces PTSD symptoms (Bazarian*, et al* 2013; Costanzo*, et al* 2014). However, mild traumatic brain injury has been related to white matter lesions and reductions in white matter microstructure integrity (Bazarian*, et al* 2013). Since we found higher FA values in our persistent PTSD patients after treatment, it is unlikely that blast exposure affects our results. Post-hoc analyses excluding participants with blast exposure yielded similar results (see supplementary material). Future studies should further investigate the contributing effects of blast exposure to PTSD symptoms.

This study has some limitations. First, we included a small number of PTSD patients currently taking medication, and a number of patients (in particular persistent PTSD patients) had comorbid disorders. However, this is representative for PTSD (Brady*, et al* 2000), and makes our results more generalizable. Post-hoc correlations between change in FA values and comorbidity only revealed a correlation between change in fornix FA and baseline comorbidity within the persistent PTSD group, indicating that (only) this tract may be influenced by comorbidity. Treatment type was not randomised, but represented treatment as usual. No differences in the number of EMDR versus TFCBT sessions were present between groups. In addition, there were no correlations within the groups between

number of EMDR or TFCBT sessions with CAPS improvement, or with differences in tract FA values. Therefore, it is not expected that the type of treatment influenced our results. Furthermore, our remitted and persistent PTSD group differed in initial symptom severity, which may confound our results. However, there were no correlations between baseline CAPS scores and tract FA values within the PTSD group, and it is therefore not expected that the difference in baseline CAPS scores directly influenced the results. Though, it could be argued that the persistent PTSD group represents a more 'complex' PTSD group (more comorbidity and severity), and is therefore more treatment resistant (Morina*, et al* 2013). Hence, when studying PTSD treatment, comorbidity, medication and higher symptom severity will generally be confounding factors in these studies, when not used as exclusion criteria. In order to address the effects of these factors in treatment response, large-scale studies need to be performed to understand the heterogeneity within PTSD and in treatment response.

In summary, we observed differences in white matter microstructure of the dorsal cingulum between patients with persistent PTSD, and patients with remitted PTSD and combat controls at reassessment. In the persistent PTSD patients dorsal cingulum FA increased over time. Treatment may be accompanied with white matter microstructure changes of the left hippocampal cingulum bundle, stria terminalis, fornix, posterior corona radiata, and superior longitudinal fasciculus, but the interaction patterns observed need to be replicated. In addition, future studies should investigate recently traumatized subjects longitudinally to determine whether dorsal cingulum differences develop before the onset of PTSD (vulnerability factor) or are acquired after onset. This study provides first steps in order to help in a better understanding of the neural underpinnings of PTSD and identifying

potential markers of treatment resistance can help to develop targeted treatments for these persistent PTSD patients.

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Table captions

Table 1. Demographical and clinical characteristics of the groups.

Figure captions

Figure 1. Regions of interest are presented that are investigated in the tract-based analysis: A. (left) dorsal cingulum (pink), B. (left) hippocampal cingulum (light green), C. fornix (green) and stria terminalis (red).

Figure 2. A group by time interaction effect was found in the left dorsal cingulum. A: F-values overlaid on left cingulum bundle and B: left dorsal cingulum fractional anisotropy at baseline and post-treatment for the combat controls (green solid line), remitted (blue dashed line), and persistent PTSD patients (red dotted line).

Figure 3. Group by time interaction effects in mean FA values in the left hippocampal cingulum bundle (A), fornix (B), and stria terminalis (C and D). The lines presents the mean FA values for the combat controls (green solid line), remitted (blue dashed line), and persistent PTSD patients (red dotted line).

Figure 4. Whole brain time by group interaction effect in the left posterior corona radiata and superior longitudinal fasciculus (A: TFCE corrected $p<0.05$). The tracts that run through this cluster are visualized in B.

	Remitted PTSD (mean ± SD)	Persistent PTSD (mean ± SD)	Combat Control (mean ± SD)	Test-value (df)	Sig. (two-tailed)
N	16	23	22		
Age (range 22-57)	34.38 (±9.58)	36.61 (±8.74)	37.64 (±10.97)	$F_{(2, 56)} = 0.52$	$p = 0.60$
Education (ISCED)	3.81 (±1.17)	3.61 (±1.27)	4.04 (±1.86)	$F_{(2, 56)} = 0.15$	$p = 0.86$
Edinburgh handedness Inventory (Left / Ambidextrous / Right)	(1 / 0 / 15)	(3 / 4 / 16)	(1 / 2 / 19)	$\chi^2_{(4)} = 4.77$	$p = 0.31$
Number of times deployed (1 / 2 / 3 / >3)	(4 / 5 / 4 / 3)	(11 / 3 / 4 / 5)	(6 / 8 / 4 / 4)	$\chi^2_{(14)} = 13.34$	$p = 0.50$
Time since last deployment (years)	6.50 (±8.17)	7.23 (±7.73)	5.50 (±6.83)	$F_{(2, 56)} = 0.29$	$p = 0.75$
Country of last deployment					
Afghanistan	12	11	16		
Former Yugoslavia	1	7	2		
Other	4	3	4		
Number of subjects exposed to a blast during deployment	1	5	0	$\chi^2_{(1)} = 6.31$	$p = 0.04$
Time between scans in (months)	6.25 (±0.73)	6.61 (±0.77)	6.0 (±0.82)	$F_{(2, 56)} = 1.11$	$p = 0.34$
Total treatment sessions between scans	9.33 (±7.20)	9.35 (±4.63)		$t_{(33)} = -0.00$	$p = 0.99$
Total number of sessions (<5 / 5-10 / >10)	(4 / 6 / 5)	(3 / 8 / 9)			
Trauma-focused cognitive behavioral therapy	4.63 (±6.34)	4.40 (±4.27)		$t_{(33)} = 0.11$	$p = 0.91$
Number of sessions (<5 / 5-10 / >10)	(7 / 6 / 2)	(11 / 6 / 3)			
Eye movement desensitization and reprocessing	4.73 (±3.63)	4.95 (±4.54)		$t_{(33)} = -0.15$	$p = 0.88$
Number of sessions (<5 / 5-10 / >10)	(10 / 2 / 3)	(10 / 7 / 3)			
Clinical scores at baseline					
CAPS total score	63.25 (± 10.55)	73.00 (±14.37)		$t_{(37)} = -2.31$	$p = 0.03$
Current comorbid disorder baseline (SCID)					
Mood disorder	6	16		$\chi^2_{(1)} = 3.95$	$p = 0.06$
Anxiety disorder	2	11		$\chi^2_{(1)} = 5.30$	$p = 0.04$
Somatoform disorder	1	2		$\chi^2_{(1)} = 0.08$	$p = 0.64$
Medication					
SSRI/SARI	4	5		$\chi^2_{(2)} = 0.06$	$p = 1.00$
Benzodiazepines	5	4		$\chi^2_{(1)} = 1.02$	$p = 0.44$
Antipsychotics	1	1		$\chi^2_{(1)} = 0.07$	$p = 1.00$
Other	1	1		$\chi^2_{(1)} = 0.07$	$p = 1.00$
Clinical scores post-treatment					
CAPS total score	22.56 (±14.63)	58.91 (±15.75)		$t_{(37)} = -7.30$	$p = 0.00$
Current comorbid disorder after treatment (SCID)					
Mood disorder	-	3		$\chi^2_{(2)} = 4.04$	$p = 0.13$
Anxiety disorder	-	4		$\chi^2_{(2)} = 4.91$	$p = 0.09$
Somatoform disorder	-	1		$\chi^2_{(2)} = 1.76$	$p = 0.42$
Medication					
SSRI/SARI	3	7		$\chi^2_{(2)} = 1.15$	$p = 0.45$
Benzodiazepines	3	1		$\chi^2_{(1)} = 1.75$	$p = 0.30$
Antipsychotics	-	2		$\chi^2_{(1)} = 1.68$	$p = 0.49$
Other	-	2		$\chi^2_{(1)} = 1.68$	$p = 0.49$