The Neural Web of War

Het Neurale Oorlogsweb

Mitzy Kennis

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Proefschrift

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Prologue

'While I was looking into his gun barrel, he asked: "What do you carry?"

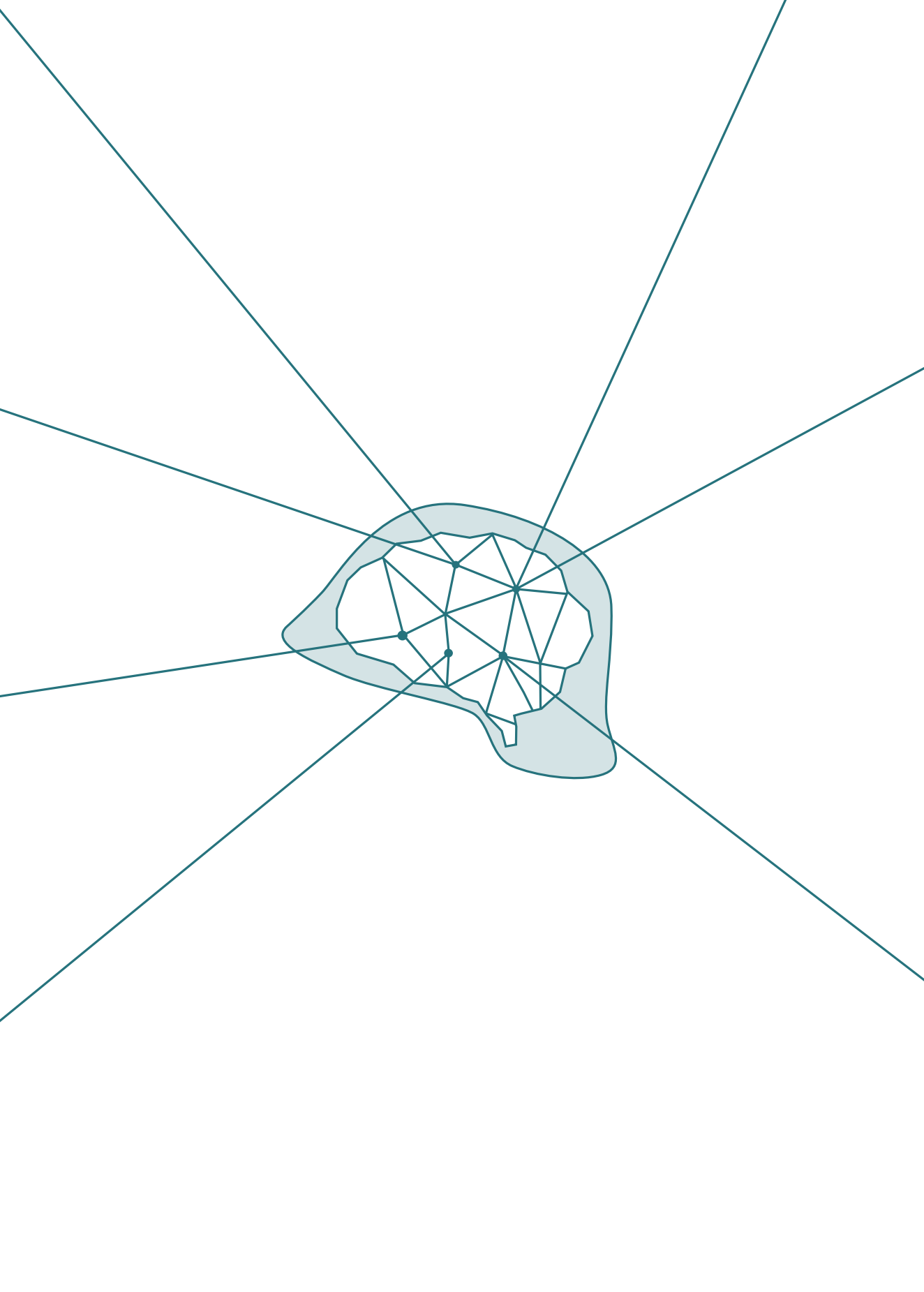
"Supplies", I replied. I felt my heart beat, the adrenalin rushing through my veins and drops of sweat forming on my forehead. I knew that many other soldiers called the road I had taken *the road of death*, but it was the only road from the airport back to camp. I had driven 90 km per hour, the maximum speed of a loaded truck in 40 degrees Celsius, without blowing up the engine.

"Stay", were my instructions. While waiting is one of the things that we train a lot, these minutes felt like hours. I could hear them opening my truck, and getting in to inspect the load. I tried to think of ways to make contact or send out a signal, but the others were still watching me. I heard the truck doors closing and could hear footsteps coming closer to the front of the truck.

"Go!" I stared straightforward through the windscreen and was not able to respond.

"Go, go, go!" he shouted, while he kicked the door of the truck. *Bang.*
The loud noise brought me back.

That moment I realized I was sitting in my car in front of the supermarket.'



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General introduction

Introduction

Posttraumatic stress disorder (PTSD) is the only psychiatric disorder that requires an external factor for diagnosis: the experience of a traumatic event. During my PhD project I interviewed over a hundred participants about their stressful and potentially traumatic life experiences. Whether participants were controls or patients, stories were touching. Interestingly, some of our participants were involved in the *same* traumatic event, but reported a completely different experience of the situation. How is it possible that some people develop PTSD while others do not after a similar event? Are the behavioral changes associated with changes in the brain? And what kinds of neural changes occur that underlie the behavioral changes? Having written a review on personality and neuroimaging studies, I thought I knew the answer: individual differences in neural networks. Thus, the first aim of my dissertation was to show that neural networks differ between veterans with and without PTSD, and healthy civilian controls.

During my internship at the MGGZ, I heard success stories from psychologists about a relatively new treatment method: eye movement desensitization and reprocessing (EMDR), which is a form of trauma-focused therapy. This method was shown to be effective in a short time period, and has even been applied during deployment to get soldiers ready to get back into the field after experiencing a traumatic event. However, it remains unknown *how* trauma-focused therapy works. To disentangle how trauma-focused therapeutic strategies work in practice it is important to investigate what happens in the brain over the course of treatment. Which features are related to symptom improvement, and which features are markers of PTSD persistence? The second aim of my dissertation was therefore to investigate neural networks in PTSD patients before and after treatment. Below, I will introduce the basic concepts necessary for understanding this dissertation.

Posttraumatic stress disorder

Anyone who has experienced a stressful or traumatic event can probably recognize some of the following symptoms: having nightmares, unexpected intrusions of the event, avoiding places related to the event, loss of positive feelings, attentional problems, sleep difficulties, and being easily irritated and startled. These are all PTSD symptoms. It is normal to experience (some of) these after a traumatic event, but they should fade out in the first months following the event. When the number of symptoms becomes problematic for daily functioning, and a combination of re-experience, avoidance, emotional numbing, and hyperarousal symptoms is present, PTSD is diagnosed according to the DSM-IV criteria (American Psychiatric Association, 1994; see box 1).

During deployment, veterans have a high risk of being exposed to traumatic events, such as enemy fire, general threat, bombings (IED), or witnessing serious injuries or death

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Box 1. Posttraumatic stress disorder (PTSD) facts

- Diagnostic criteria for PTSD (DSM-IV (American Psychiatric Association, 1994)):
  A. Experiencing a traumatic event with intense fear or helplessness
  B. One re-experience symptoms (e.g. flashbacks, nightmares)
  C. Three avoidance/emotional numbing symptoms (e.g. avoid places associated with trauma, feeling emotionally numb, having reduced interest in pleasant activities)
  D. Two hyperarousal symptoms (e.g. irritable, exaggerated startle response)
  E. The symptoms are present for at least one month
  F. The symptoms cause significant distress or impairment in important areas of functioning (e.g. social, occupational)
- 6-9 % of the Dutch veterans develop a high level of PTSD symptoms after deployment to Afghanistan (Reijnen et al., 2014)
- 50% of the PTSD patients have comorbid depression (Brady et al., 2000)
- Treatment: trauma-focused therapy; effective for 50% of the patients (Bisson et al., 2007; Bradley et al., 2005)
  - Eye movement desensitization and reprocessing (EMDR)
  - Trauma-focused cognitive behavioral therapy (TFCBT)

of a colleague. About 6-9% of the Dutch veterans deployed to Afghanistan report high levels of PTSD symptoms (Reijnen et al., 2014). In order to provide better care for these veterans, it is of importance to study the psychopathology of PTSD so we can contribute to the improvement of interventions, early recognition, and perhaps the prevention of the development of PTSD.

Neurobiology of PTSD

Many cross-sectional studies have been performed investigating the structure and function of the brain of PTSD patients versus controls (Patel et al., 2012; Rauch, Shin, Phelps, 2006; Shin and Liberzon, 2010). Most consistently, hyperactivation of the amygdala, and hypoactivation of the ventral medial prefrontal cortex (vmPFC) have been reported during symptom provocation or the presentation of emotional stimuli. Differences are also reported in the hippocampus, insula, anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (see Figure 1). Based on these studies, it has been suggested that reduced inhibitory connectivity of the prefrontal cortex with the amygdala may underlie PTSD (Etkin and Wager, 2007; Rauch, Shin, Phelps, 2006). However, inconsistent results have been reported in studies directly investigating structural and functional *connectivity* between these brain regions in PTSD patients versus controls. For example, increased and decreased structural connectivity was found in the cingulum white matter

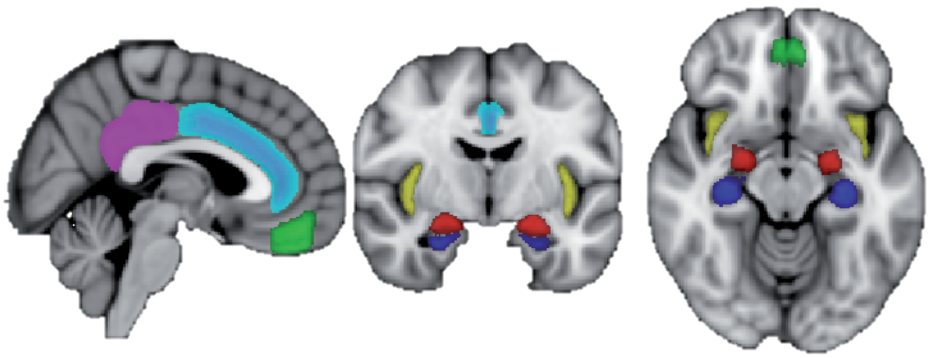


Figure 1. Neurobiological alterations are found in PTSD with fMRI and resting state fMRI in these brain regions: Amygdala (red), hippocampus (blue), insula (yellow), medial frontal cortex (green), anterior cingulate cortex (cyan), and posterior cingulate (violet).

(Abe et al., 2006; Bierer et al., 2015; Fani et al., 2012; Kim et al., 2007; Schuff et al., 2011; Zhang et al., 2011; Zhang et al., 2012). In addition, increased and decreased functional connectivity of the ACC was reported (Daniels et al., 2010; Sripada et al., 2012a; Sripada et al., Schneider, 2013; St. Jacques, Kragel, Rubin, 2013; Yin et al., 2011). Therefore, more research is needed to investigate connectivity alterations in PTSD patients, in particular in the cingulum.

Trauma-focused therapy

The current golden standard for treating PTSD is trauma-focused therapy, including trauma-focused cognitive behavioral therapy (TFCBT) and in the Netherlands it also includes eye movement desensitization and reprocessing (EMDR; Balkom et al., 2013). Both therapies seem to have similar efficiency (Bisson et al., 2007). However, only about half of the PTSD patients recover after treatment (Bradley et al., 2005). In order to improve treatment efficiency and response rates, it is important to understand both the psychopathology of PTSD as well as to determine the neurobiological alterations related to treatment outcome.

Trauma-focused therapy is thought to stimulate fear habituation and to induce fear extinction of trauma-related memories (Foa and Kozak, 1986; Rothbaum and Davis, 2003). Therefore, trauma-focused therapy is expected to alter brain regions that are involved in fear, memory and extinction: the amygdala, hippocampus, and vmPFC. Over the last years research has started to disentangle treatment effects on the brain with imaging research. Potential recovery of brain function and structure has been reported over the course of treatment (Aupperle et al., 2013; Lindauer et al., 2005; Roy et al., 2010;

Roy et al., 2014; Thomaes et al., 2012; Vermetten et al., 2003). Pre-treatment differences were also related to treatment outcome (Bryant et al., 2008a; Falconer et al., 2013; van Rooij et al., 2015a). However, no previous studies investigated connectivity alterations over the course of treatment while controlling for the effects of time by including a control group. Thus, to determine markers of treatment outcome and recovery related changes longitudinal research is needed on the neural web of PTSD.

Box 2. Gap

Limited studies investigated neural network connectivity in PTSD. In addition, no previous studies investigated treatment effects on PTSD neural network connectivity. This dissertation therefore aims to provide more insight in *The Neural Web of War*, by comparing PTSD patients with controls before and after treatment.

Methods

Design

This dissertation comprises results from the resting state and diffusion MRI scans included in the project BETTER: *Biological Effects of Traumatic Experiences, Treatment and Recovery.* Veterans with and without PTSD, and healthy civilian controls were included in this project. By including both veteran and civilian controls, the effects of deployment and/or military training and PTSD can be separated. Furthermore, PTSD patients with and without comorbid major depressive disorder (MDD) were included, providing the possibility to investigate the effects of comorbid MDD on neural network connectivity.

After an interval of 6-8 months, during which patients received trauma-focused therapy, all veterans (PTSD patients and combat controls) were reassessed to investigate treatment effects. Based on their clinical interview after treatment, PTSD patients were subdivided in a remitted PTSD group (no PTSD diagnosis) and a persistent PTSD group (PTSD diagnosis, see Box 1) to investigate treatment outcome related changes.

Diffusion tensor imaging (DTI)

A non-invasive way to investigate *structural connectivity* is diffusion tensor imaging. This technique is based on the flow of water molecules in the brain: diffusion. Large white matter fiber bundles form a physical barrier for water to cross, thus more water molecules will flow along the white matter bundles as opposed to crossing the bundle (Alexander et al.,

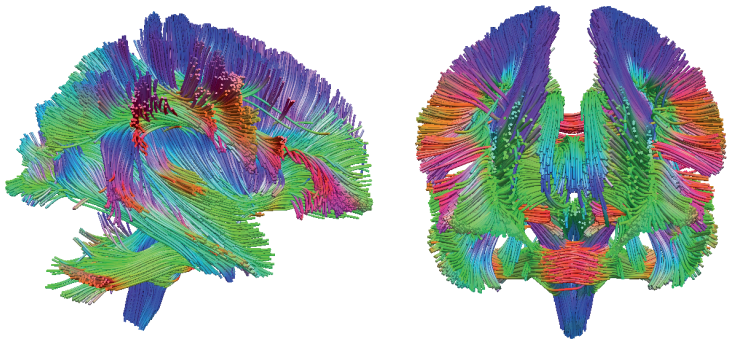


Figure 2. Diffusion tensor image based whole brain tractography, showing the fiber tracts that run throughout the brain. The colors represent the direction of the fibers. A side view (sagittal) is presented on the left, and a frontal view (coronal) is presented in the right.

2011; see Figure 2). The advantage of DTI is that it provides a comprehensive, noninvasive, anatomy mapping that is sensitive to the direction of white matter bundles, and this is currently the most optimal imaging method to visualize white matter in the brain (Assaf and Pasternak, 2008). The most frequently obtained parameter of interest in DTI research is Fractional Anisotropy (FA), which provides an estimation of microstructural organization of white matter tissue, as it is sensitive to axonal direction, and myelination (Alexander et al., 2011). *Section 1* of this dissertation comprises DTI studies.

Resting state functional connectivity

Resting state functional magnetic resonance imaging (fMRI) can be utilized to investigate co-activation of brain regions, and is a useful tool to study neural networks. During the resting state fMRI scan participants are asked to look at a fixation cross, and let their mind wander. During this "resting" period, we measure the Blood Oxygenation Level Dependent (BOLD) signal: a measure reflecting neural activity. By calculating the correlations between the activation of different brain regions, we quantify the co-activation of brain regions. This indicates that these brain regions may be involved in the same process, and we therefore call this *functional connectivity (*see Figure 3). Using resting state functional connectivity distinct neural networks can be identified, that are similar to anatomical networks (Biswal et al., 1995; Greicius et al., 2009; Raichle et al., 2001). Furthermore, resting state networks reflect networks that were also identified with task-based fMRI studies (Damoiseaux et al., 2006). The advantage of resting state as opposed to task-based fMRI is that factors that could influence task performance (attentional problems) are not a problem during rest. *Section 2* of this dissertation describes resting state functional connectivity studies. Two different methods were applied: seed-based analyses and graph analyses.

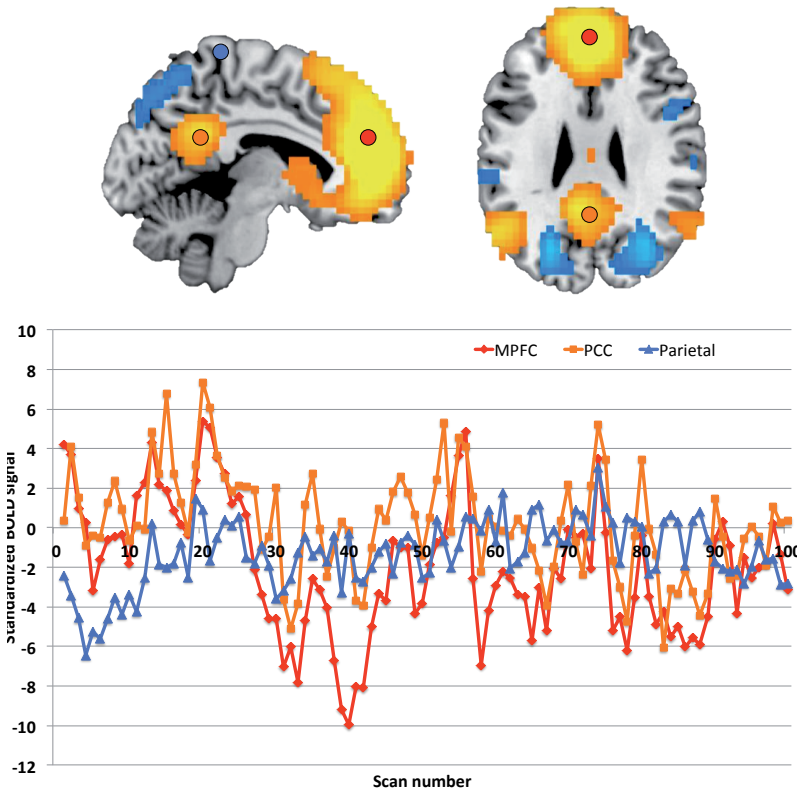


Figure 3. Resting state functional connectivity maps of the medial prefrontal cortex (MPFC) are presented on the top. The resting state BOLD signals of the MPFC (red), posterior cingulate cortex (PCC, orange), and a parietal region (Parietal, blue) are presented on the bottom, over 100 scans. It is visible that the MPFC and PCC are correlated, but the parietal region has an uncorrelated activation pattern. Thus, the MPFC and PCC show *functional connectivity.*

Seed-based analysis

With seed-based analyses a *region of interest* (ROI or seed) is predefined, and a whole brain functional network correlation map of that seed is calculated and compared between groups. This method has the advantage of being specific for the seed region, and provides a hypothesis driven investigation.

Graph analysis

A more exploratory approach investigating the whole brain network is graph analysis. By calculating correlations between the patterns of activity of brain regions, functional whole brain network properties can be investigated. For example, calculating the number of connections of a brain region can be informative for the importance of that brain region in the whole brain network. The advantage of this analysis is that it provides a whole brain analysis, while the pitfall is that it requires a strict multiple comparison correction.

Outline

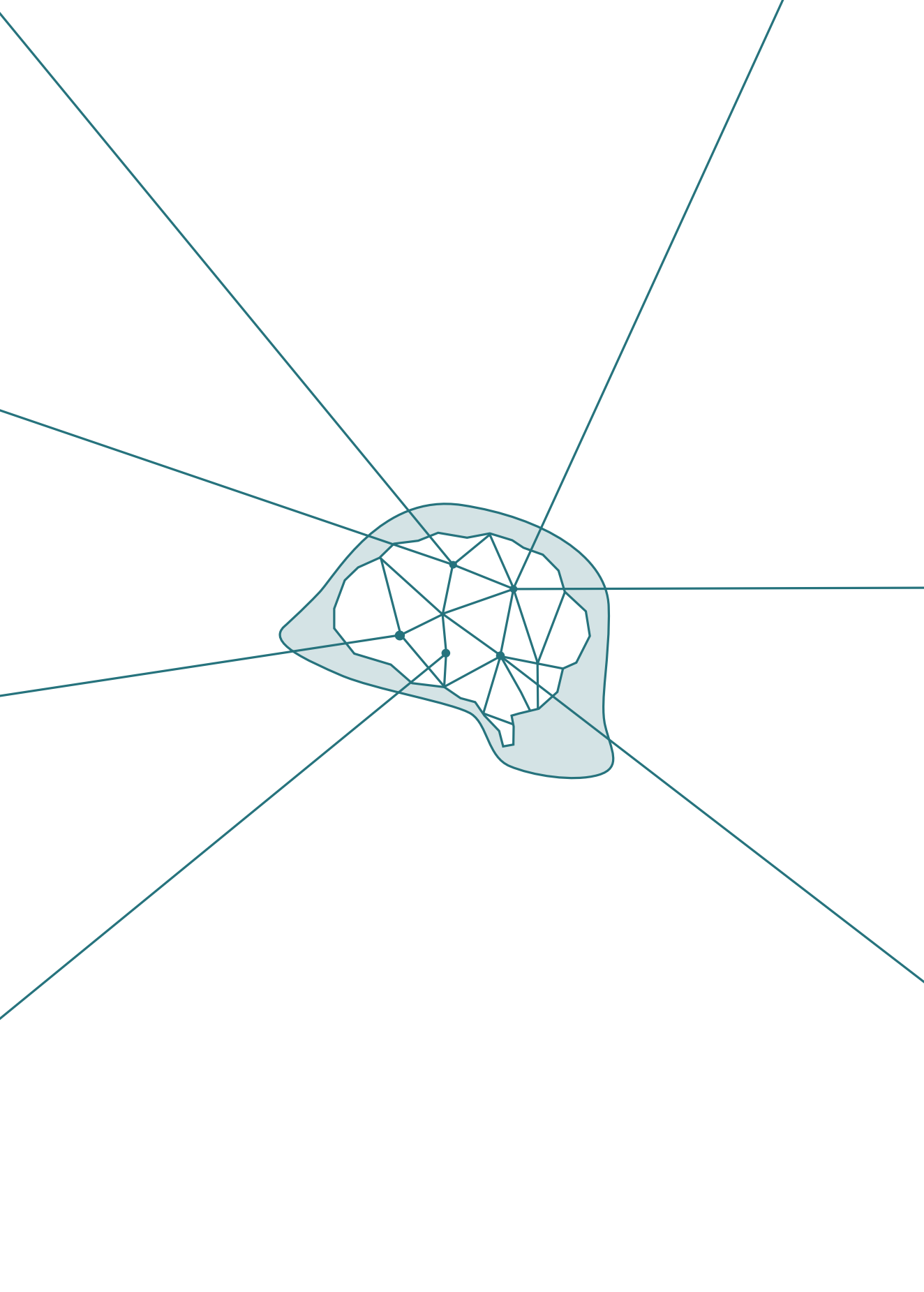
Box 3. Aim

The aim of this dissertation is to gain more insights in the neural networks alterations that may underlie PTSD and trauma-focused therapy.
Section 1 comprises **structural connectivity** studies, investigating white matter microstructure integrity with diffusion tensor imaging.
Section 2 describes **functional connectivity** studies, investigating co-activation patterns during resting state fMRI.

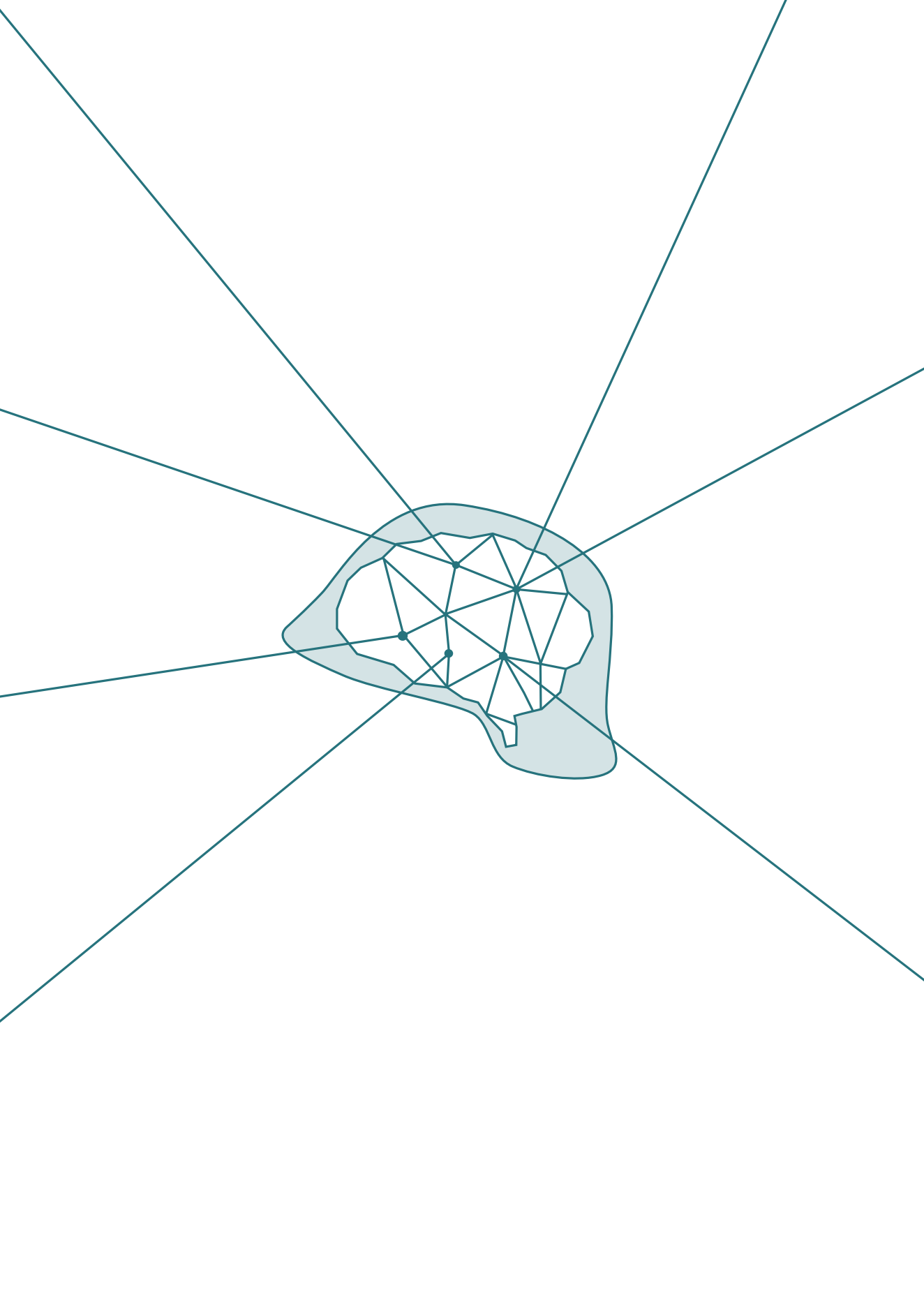
Section 1 describes two structural connectivity studies using DTI investigating differences between PTSD patients that recover after treatment (remitted PTSD), patients that are still diagnosed with PTSD after treatment (persistent PTSD) and combat controls. **Chapter 2** describes treatment effects on white matter fiber bundles that connect brain areas involved in emotion and memory: the cingulum bundle, stria terminalis, and fornix.

A main confounding factor that differs between DTI studies and may potentially explain the inconsistency in current findings is the polarity of phase encoding direction during image acquisition. However, it is yet unknown what the effect of this acquisition parameter is on the outcome of a clinical research question. This was investigated in **Chapter 3**.

In *Section 2* resting state functional connectivity is investigated with seed analysis (**Chapter 4 and 5**) and graph analysis (**Chapter 6**). In **Chapter 4** pretreatment differences in anterior cingulate cortex (ACC) resting state connectivity were investigated between PTSD patients, healthy controls, and combat controls. In **Chapter 5** resting state functional connectivity of the subgenual ACC and insula was investigated pre-treatment between PTSD patients with and without comorbid depression. Finally, whole brain functional network properties are compared before and after treatment with graph analysis between combat controls, remitted, and persistent PTSD patients in **Chapter 6**.



Section 1
Structural connectivity



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Treatment outcome related white matter differences in veterans with posttraumatic stress disorder

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Abstract

Posttraumatic 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 posttreatment 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 vs persistent PTSD patients. This study contributes to a better understanding of the neural underpinnings of PTSD treatment outcome.

Introduction

Posttraumatic 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 psychopathology 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, Hayes, Mikedis, 2012; Rauch, Shin, Phelps, 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., 2008a; Bryant et al., 2008b; Dickie et al., 2013; van Rooij et al., 2015a; van Rooij et al., 2015b). 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., 2012). An increase in FA in the posterior cingulum bundle over time was reported (Zhang et al., 2012). 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, Stanisz, Graham, 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., 2011a). 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, Keane, Friedman, 2000). Treatment selection was part of treatment as usual, a pplied 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).

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.

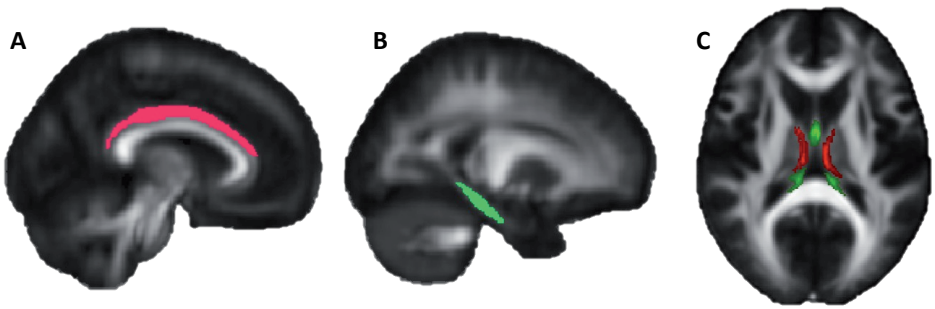

Figure 1. Regions of interest are presented that are investigated in the tract-based analysis: (A, left) Dorsal cingulum (pink), (B, left) hippocampal cingulum (light green), and (C) fornix (green) and stria terminalis (red).

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

Table 1. Demographical and Clinical Characteristics of the Groups.

	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)		$t_{(33)} = -0.00$	$p = 0.99$
(<5 / 5-10 / >10)	(4 / 6 / 5)	(3 / 8 / 9)			
Clinical scores at baseline					
CAPS total score	63.25 (± 10.55)	73.00 (±14.37)		$t_{(36)} = -2.31$	$p = 0.03$
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		$\chi^2_{(1)} = 0.07$	$p = 1.00$

Table 1. Demographical and Clinical Characteristics of the Groups. (*Continued*)

	Remitted PTSD (mean ± SD)	Persistent PTSD (mean ± SD)	Combat Control (mean ± SD)	Test-value (df)	Sig. (two-tailed)
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		$\chi^2_{(1)} = 1.68$	$p = 0.49$

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.

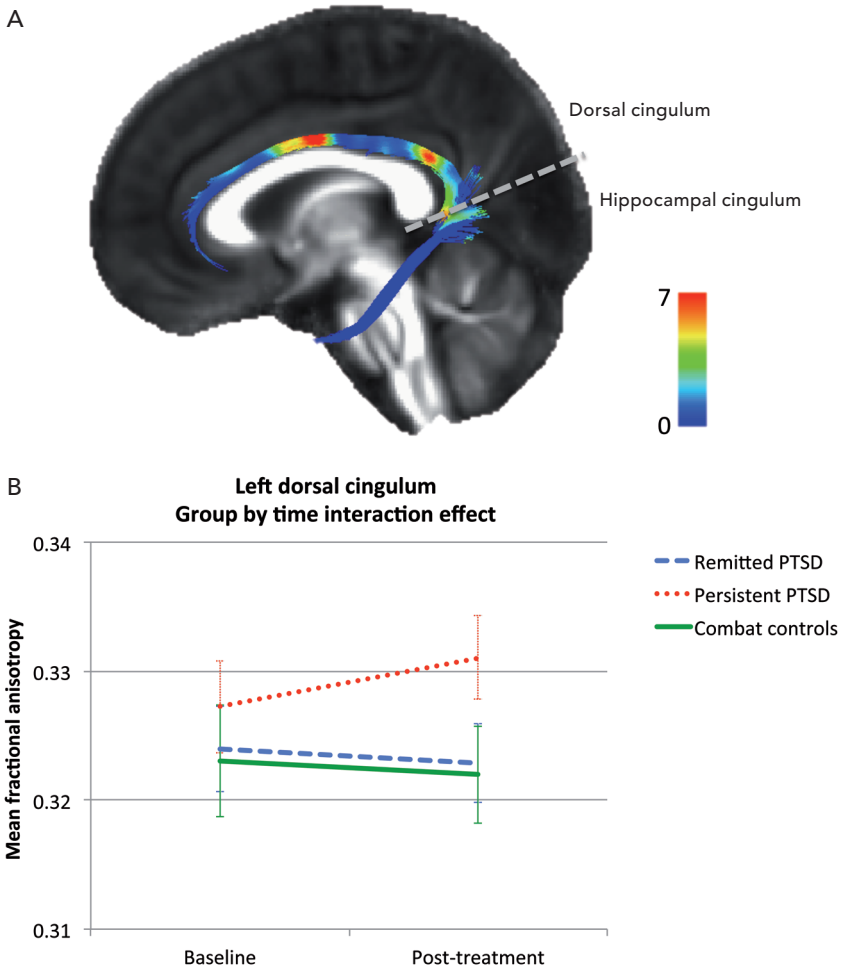

Figure 2. A group-by-time interaction effect was found in the left dorsal cingulum. (A) F-values overlaid on left cingulum bundle and (B) left dorsal cingulum fractional anisotropy (FA) at baseline and posttreatment for the combat controls (green solid line), remitted (blue dashed line), and persistent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

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.

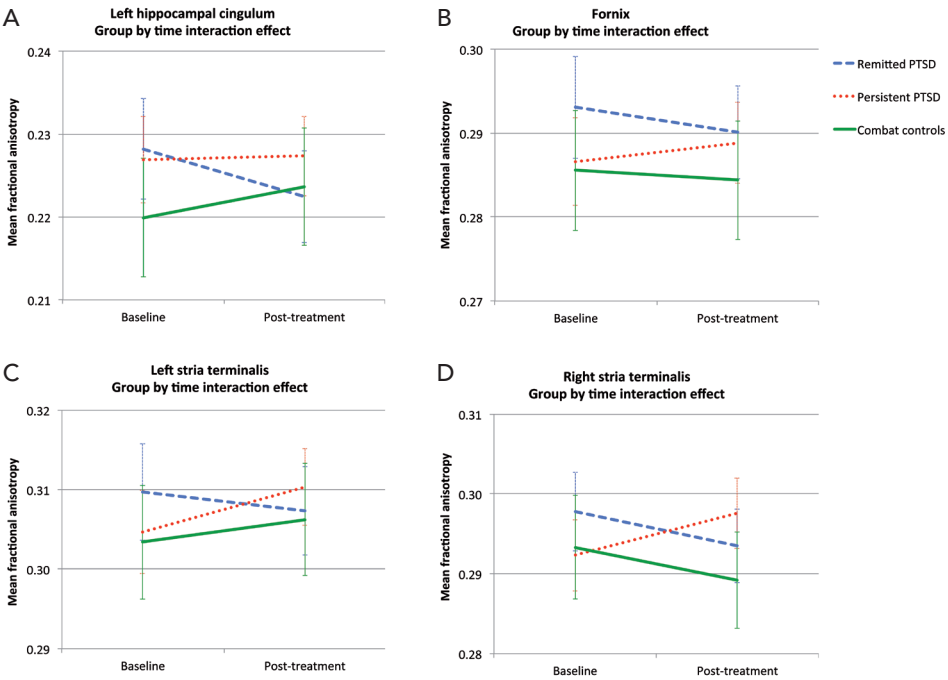

Figure 3. Group-by-time interaction effects in mean fractional anisotropy (FA) values in the left hippocampal cingulum bundle (A), fornix (B), and stria terminalis (C and D). The lines presents the mean FA values for the combat controls (green solid line), remitted (blue dashed line), and persistent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

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, $p = 0.004$).

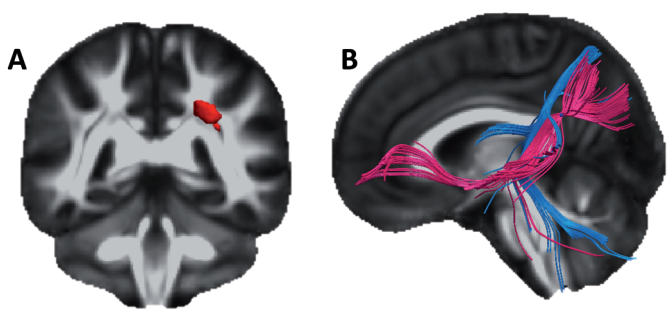

Figure 4. Whole brain time-by-group interaction effect in the left posterior corona radiata and superior longitudinal fasciculus (A: TFCE-corrected $p < 0.05$). The tracts that run through this cluster are visualized in (B).

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, Hayes, Mikedis, 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., 2009b). 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., 2015c). 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., 2012). 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, Hayes, Mikedis, 2012) in particular in persistent PTSD patients (van Rooij et al., 2015c). 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.

In 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 (Phillips and LeDoux, 1992). 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 information). 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.

Acknowledgements and disclosure

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Supplementary information

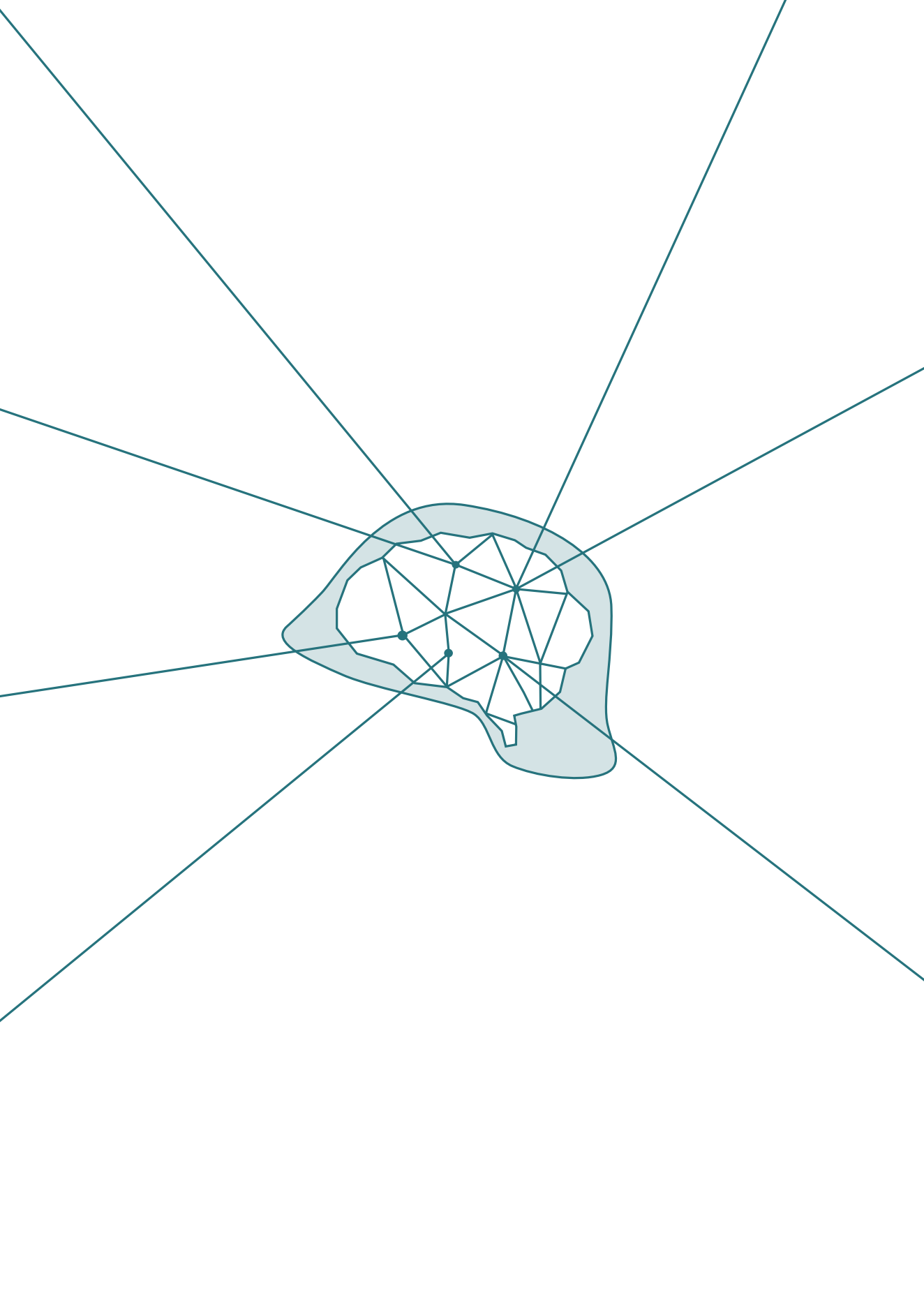
Scan parameters

A set of two transverse DTI scans including one diffusion un-weighted image (b = 0 s mm^{-2}) and 30 diffusion weighted images (b = 1000 s mm^{-2}) were acquired with reversed phase-encode blips, in order to correct for distortions (TR = 7057 ms, TE 68 ms, matrix 128 x 99, resolution 1,875 x 1,875 x 2, no gap, EPI factor 35, SENSE factor 3, FOV = 240 mm, 75 slices, slice thickness 2 mm).

Two transverse magnetization transfer images were acquired for calculation of the magnetization transfer ratio (TR = 66 ms, TE= 2.2ms, matrix 128 x 96, resolution 1.88 x 1.88 x 2 mm, SENSE factor 2, FOV 240, 95 slices, flip angle 18, off-resonance MT prepulse 10.5 microT).

Preprocessing diffusion images

For each subject, the susceptibility-induced off-resonance field was estimated from the set of scans, and the two images were combined into a single corrected image (Andersson, Skare, Ashburner, 2003; Smith et al., 2004). Affine co-registration and geometrical unwarping was performed in order to correct for distortions (e.g. eddy currents, magnetic field in-homogeneities, and head motion (Smith et al., 2004)). Tensor maps were acquired by fitting a model of the spin displacement density using nonlinear optimization, constrained to be positive semi-definite as implemented in CAMINO (Cook et al., 2006). Tensor images were registered to a group template and normalized to MNI space, using an advanced DTI special normalization and atlas construction tool (DTI_TK: http://www.nitrc.org/projects/dtitk (Zhang et al., 2006; Zhang et al., 2007)).



3

Choosing the polarity of the phase-encoding direction in diffusion MRI: Does it matter for group analysis?

Submitted

Co-authors: **S.J.H. van Rooij, R.S. Kahn, E. Geuze, A. Leemans.**

Abstract

Notorious for degrading diffusion MRI data quality are so-called susceptibility-induced off-resonance fields, which cause non-linear geometric image deformations. While acquiring additional data to correct for these distortions alleviates the adverse effects of this artifact drastically – e.g., by reversing the polarity of the phase-encoding (PE) direction – this strategy is often not an option due to scan time constraints. Especially in a clinical context, where patient comfort and safety are of paramount importance, acquisition specifications are preferred that minimize scan time, typically resulting in data obtained with only one PE direction. In this work, we investigated whether choosing a different polarity of the PE direction would affect the outcome of a specific clinical research study. To address this methodological question, fractional anisotropy (FA) estimates of white matter brain regions were obtained in civilian and combat controls, remitted posttraumatic stress disorder (PTSD) patients, and persistent PTSD patients before and after trauma-focused therapy and were compared between diffusion MRI data sets acquired with different polarities of the PE direction (posterior-to-anterior, PA and anterior-to-posterior, AP). Our results demonstrate that regional white matter FA estimates differ 5% on average between AP and PA PE data. In addition, when comparing FA estimates between different subject groups for specific cingulum subdivisions, the conclusions for AP and PA PE data were not in agreement. These findings increase our understanding of how one of the most pronounced data artifacts in diffusion MRI can impact group analyses and should encourage users to be more cautious when interpreting and reporting study outcomes derived from data acquired along a single PE direction.

Introduction

Diffusion tensor imaging (DTI) is a popular approach for studying white matter microstructural characteristics (Basser, Mattiello, LeBihan, 1994; Jones and Leemans, 2011) and has been applied in a wide range of clinical applications (Menon 2011; O'Hanlon et al., 2015; Reijmer et al., 2015; Verhoeven et al., 2012; Wang, Hsu, Leemans, 2012). To minimize scan times, diffusion MRI data are generally acquired with echo-planar imaging (EPI) (Turner and Le Bihan, 1990). A major disadvantage of acquiring DTI data with EPI, however, is the presence of susceptibility-induced geometric distortions (Andersson, Skare, Ashburner, 2003; Gallichan et al., 2010; Jones and Cercignani, 2010; Ruthotto et al., 2012). These distortions are generally visible as geometric image deformations in combination with signal expansion (signal loss) or compression (signal pile up) in the phase-encoding (PE) direction and have been shown to affect global fractional anisotropy (FA) values (Wu et al., 2008) and tractography results (Irfanoglu et al., 2012).

As susceptibility-induced distortions can be more harmful in data acquired along the left-to-right PE orientation (blurring signals across the midline and hampering the natural symmetry of the left and right brain hemispheres) than in data with anterior-to-posterior (AP) or posterior-to-anterior (PA) PE directions, the latter is most frequently applied in diffusion MRI of the brain (Glover et al., 2012). To correct for EPI distortions, diffusion images can be normalized to an anatomical scan without EPI distortions (e.g., to a T_1 or T_2 weighted image as described in (Irfanoglu et al., 2012). Although more advanced methods to correct for distortions are currently available, these come at the cost of requiring additional information (e.g., two sets of diffusion images, acquired with opposite PE, or a B0-field map characterizing the magnetic field inhomogeneity (Irfanoglu et al., 2015). Especially in a clinical context, where scan times are kept minimal, it is therefore common practice to obtain only one set of diffusion images with one specific PE direction in anterior-to-posterior (AP) or posterior-to-anterior (PA) direction and, subsequently, to apply a registration-based procedure for correcting EPI distortions. However, whether FA estimates derived with a typical analysis pipeline differ significantly between scans with a different PE direction remains unclear. Investigating this potential confound is particularly relevant for clinical research applications, where such type of image artifact could affect conclusions.

To determine the magnitude and significance of the effect of PE direction on FA estimates in specific brain regions, we included 342 DTI data sets (i.e., 171 with AP and 171 with PA PE directions) from healthy civilian controls, veterans with posttraumatic stress disorder (PTSD), and combat controls (veterans without PTSD). For 61 veterans DTI data were acquired at two time points with the PTSD patients receiving trauma-focused therapy in between scans. In addition to exploring regional WM FA differences

between the PA and AP PE DTI data, we investigated whether the outcome of a specific clinical research question would be in agreement between PA and AP PE scans. In particular for this study, we questioned whether the observed FA changes in specific cingulum subdivisions – brains areas known to be affected in PTSD (e.g., Abe et al., 2006; Daniels et al., 2013; Fani et al., 2012; Kim et al., 2007; Zhang et al., 2011) – were different between (a) PTSD patients who recovered after treatment (remitted PTSD); (b) veterans who still had a PTSD diagnosis after treatment (persistent PTSD); and (c) combat controls. Our study shows that the polarity of the PE direction (AP or PA) can significantly affect WM regional FA estimates and that the choice of PE polarity can modulate the outcome of a clinical research question.

Material and Methods

Participants and clinical assessment

PTSD patients were recruited from one of the four outpatient clinics of the Military Mental Healthcare Organization, after a clinician diagnosed PTSD. Healthy civilian and combat controls were recruited with advertisements. After written and verbal explanation of the study was given, all participants gave informed consent. In total, 342 sets of DTI scans (i.e., 171 scans with PA PE direction and 171 scans with AP PE direction) were obtained to investigate the effect of the polarity of the PE on the FA estimates. This included scans from 25 healthy civilian controls, 28 healthy veterans and 51 PTSD patients at the first time point, and scans at reassessment of 22 healthy veterans and 45 PTSD patients.

All veterans (with and without PTSD) were reassessed after 6-8 months, during which PTSD patients received treatment as usual (see supplementary material A for an overview of the clinical assessment, the inclusion and exclusion criteria, and details of the demographics of the participants). Based on PTSD diagnosis at reassessment, PTSD patients were subdivided into a remitted group (no PTSD diagnosis at reassessment, N=16), and a persistent PTSD group (PTSD diagnosis at reassessment, N=23). 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).

Data acquisition

Two transverse DTI data sets with opposite polarity of the PE direction (i.e., PA and AP) were acquired, each consisting of one non-diffusion weighted image (b = 0 s/mm^2) and 30 diffusion-weighted images (b = 1000 s/mm^2) (Jones, 2004). Other acquisition settings were: TR = 7057 ms, TE = 68 ms, matrix size = 128 x 128, voxel size = 1.875 x 1.875 x 2 mm^3, no gap, EPI factor = 35, SENSE factor = 3, FOV = 240 x 240mm^2, 75 slices, slice

thickness = 2 mm, scan time = 4:21 min. The acquisition details for the T1-weighted high-resolution scan, obtained during the same scan session, are TR = 10 ms, TE = 4.6 ms, flip angle = 8°, 200 sagittal slices, FOV = 240 x 240 mm^2, matrix size = 304 x 299, voxel size = 0.8 x 0.8 x 0.8 mm^3.

Data processing

ExploreDTI (v4.8.4) (Leemans et al., 2009) was used to process each DTI data set, which consisted of correcting for subject motion, eddy current-induced distortions, and susceptibility artifacts (Irfanoglu et al., 2012; Leemans and Jones, 2009). The diffusion tensor was estimated with a robust fitting routine (Tax et al., 2015; Veraart et al., 2013). For both PA and AP PE DTI scans, mean FA values were extracted for 70 cortical white matter regions and 27 subcortical regions as derived with *Freesurfer* (Fischl et al., 2002, 2012) (see Fig. 1).

Statistical analyses

Regional FA differences

The absolute difference and the percentage difference in FA values between PA and AP PE directions was calculated for each *Freesurfer* region over all available scans. For each region, a paired samples t-test was performed using the FA values of the PA and AP PE scans for all groups and time points combined (N=171). Bonferroni correction was applied ($p < 0.05/97 = 0.0005$ is deemed significant) to correct for testing multiple brain areas. The absolute and percentage FA differences were also displayed on the "FS_cvs_avg35_inMNI152" *Freesurfer* template (Fischl 2012).

Clinical research question

To answer the clinical research question, i.e., whether the observed FA changes in specific cingulum subdivisions were different between (a) remitted PTSD (b) persistent PTSD and (c) combat controls over the course of treatment, repeated measures ANOVAs (group (3) by time (2) by hemisphere (2)) were performed to compare the rostral, caudal, posterior, isthmus and hippocampal cingulum subdivisions, for the two sets of reversed PE diffusion images, using age as covariate. Since we were not interested in asymmetry of the cingulum, hemisphere was modeled as a parameter of non-interest to provide overall statistics for the left and right cingulum subdivisions combined. To correct for testing five subdivisions of the cingulum, Bonferroni correction was applied ($p < 0.05/5 = 0.01$ is deemed significant).

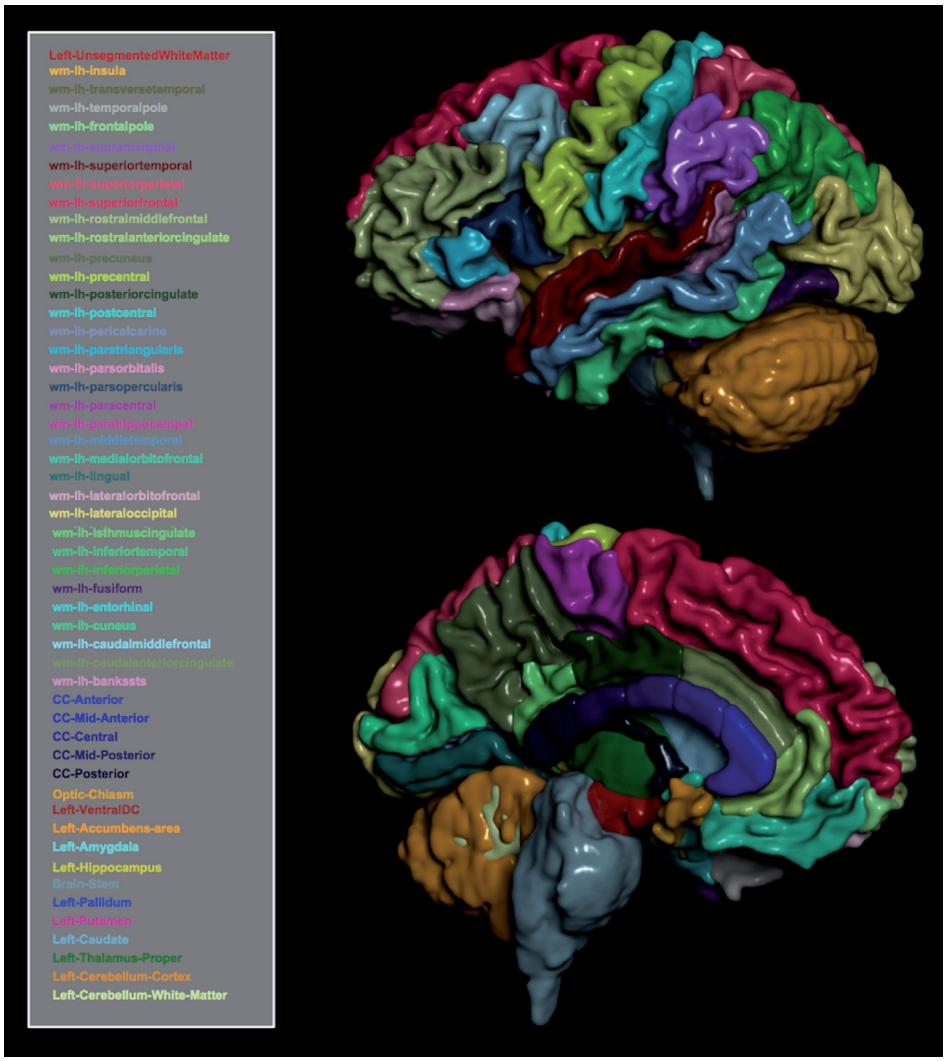


Figure 1. Surface rendering of *Freesurfer* parcellations for the left hemisphere of a representative subject. A list of *Freesurfer* white matter and subcortical brain regions included in the analyses is shown on the left with color-coding corresponding to the surface rendering.

Results

Correction of susceptibility artifacts

Fig. 2 presents an overview of the procedure to correct for susceptibility-induced artifacts. The top row shows the color-encoded FA maps after correcting for subject motion and eddy current-induced distortions, but *before* the susceptibility correction step (left: PA PE direction; right: AP PE direction). On these maps – and their enlarged regions in the middle – one can easily appreciate the differences in geometry of the brain stem area between the AP and PA scans. Also frontal brain areas are heavily affected as can be seen on the non-diffusion-weighted images (middle row). By registering the dMRI data to the T1 weighted data with *ExploreDTI*, whereby the deformation field is

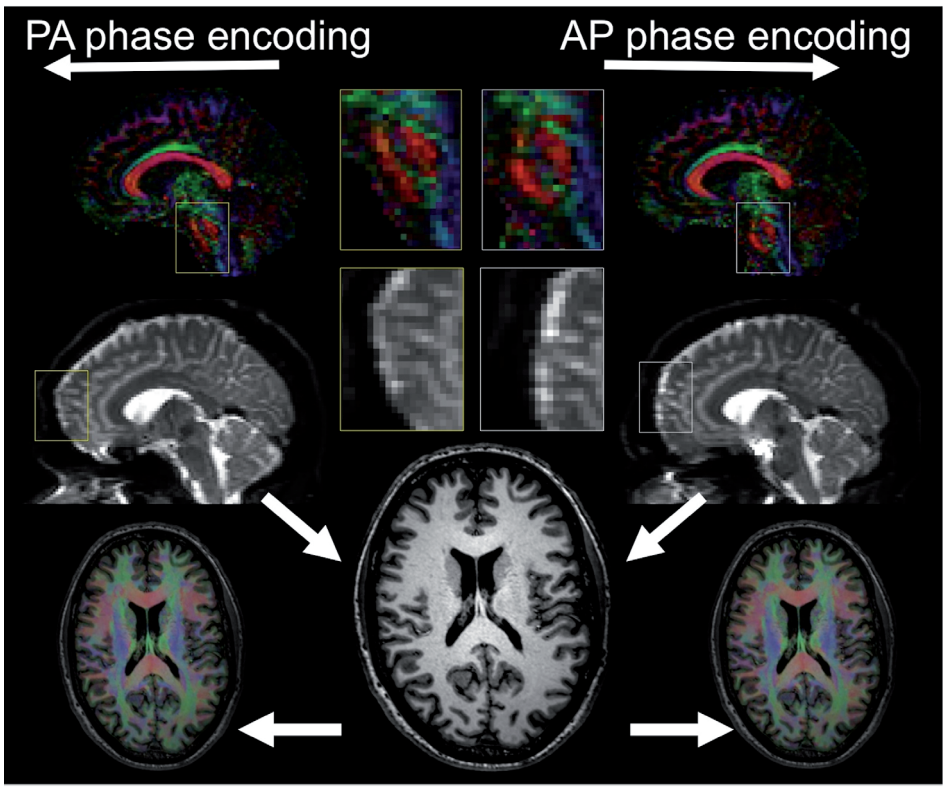


Figure 2. Susceptibility-induced artifacts and their differences due to polarity of PE direction (PA: left vs. AP: right). Top and middle rows show the color-encoded fractional anisotropy and non-diffusion-weighted images, respectively, which were already corrected for subject motion and eddy current distortions. Notice the difference in geometry between the AP and PA PE scans as shown in the enlargements. The bottom row shows the color-encoded diffusion orientation fused with the T1 weighted image, which was used for correcting the susceptibility-induced artifacts.

constrained to the PE direction (for details, see Irfanoglu et al., 2012), one can correct the susceptibility-induced artifacts (bottom row). The bottom left and bottom right images show the color-encoded FA maps *after* the susceptibility correction step and fused with the T1 weighted image.

While correcting for EPI deformations improves the quality of the data geometry, residual misalignment between the T1 weighted and diffusion-weighted data can still often be observed. Fig. 3 shows an example where such spatial correspondence is not optimal. Especially in the frontal area, where these artifacts are quite pronounced, the difference in geometry between AP and PA PE data is clearly visible (see enlarged regions in Fig. 3).

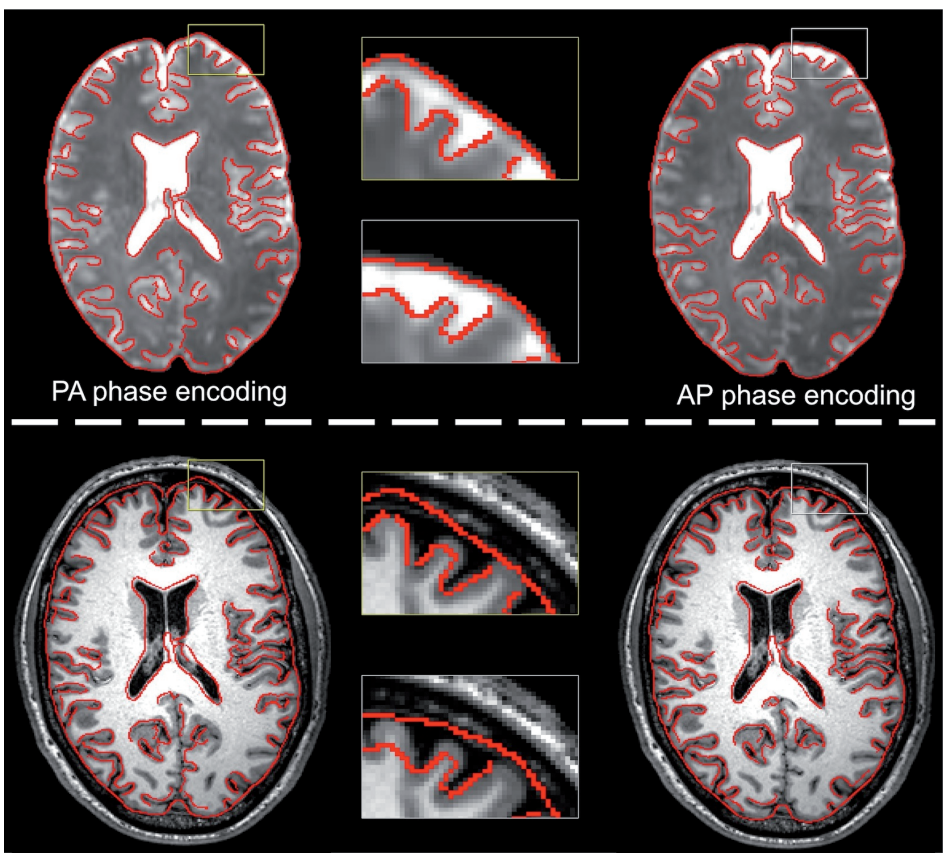


Figure 3. Illustration of the difference in residual spatial misalignment between AP and PA PE data after distortion correction. Edges (grey/white matter boundary) of the non-diffusion-weighted image (top) are displayed in red and overlaid on the T1 weighted image (bottom) for both PA (left) AP (right) PE for a representative subject.

Regional FA differences

The spatial distribution of FA estimates of *Freesurfer* brain regions of PA and AP PE scans for all subjects at both time points (N=171) is presented in Figure 4, from which similar patterns can be observed. The FA magnitude difference between PA and AP PE direction scans (N=171 for each PE direction) ranged from 0.001 to 0.06 or, equivalently, from 0.4% to 30% across all *Freesurfer* regions (Fig. 5). The FA values were significantly different between PA and AP scans for many of the *Freesurfer* regions (for a complete list see supplementary material B). Regions that showed the largest positive "PA minus AP" differences in FA (i.e., with differences > 0.03) were the optic chiasm (0.06 or 30%), left inferior parietal (0.04 or 12%), left lateral occipital (0.03 or 12%), and left bankSSTS (0.03 or 9%). Regions with the largest negative "PA minus AP" differences in FA (i.e., with differences < -0.03) were the middle posterior corpus callosum (-0.04 or -7%), posterior

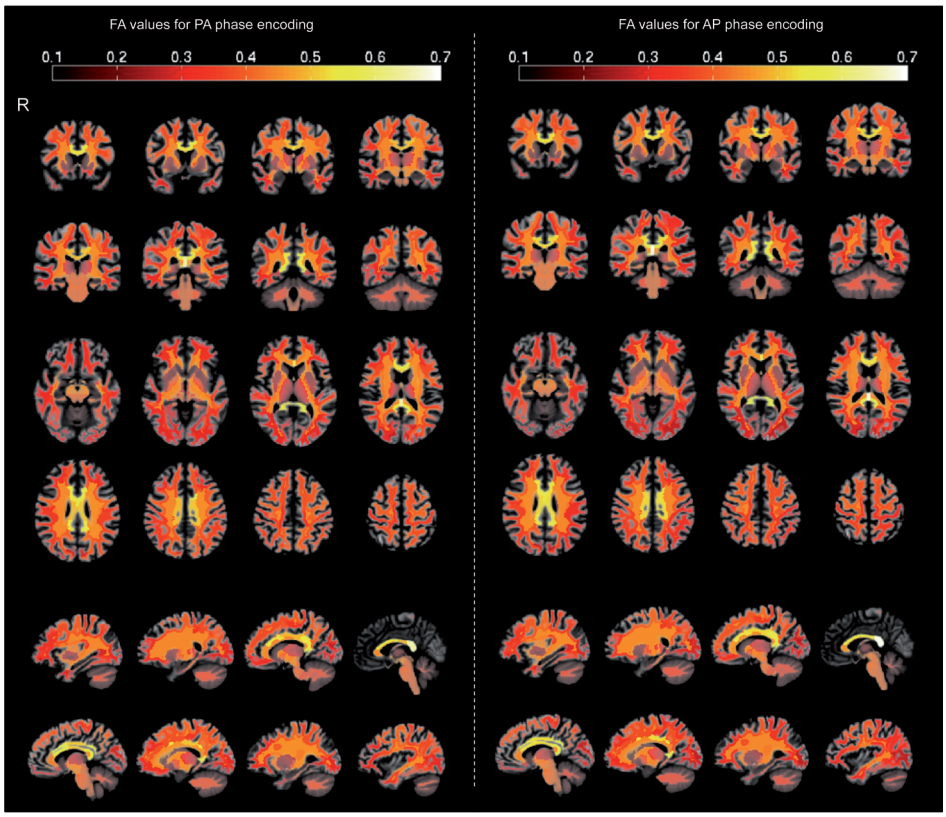


Figure 4. Spatial distribution of FA estimates of PA and AP PE scans for all subjects at both time points (N=171).

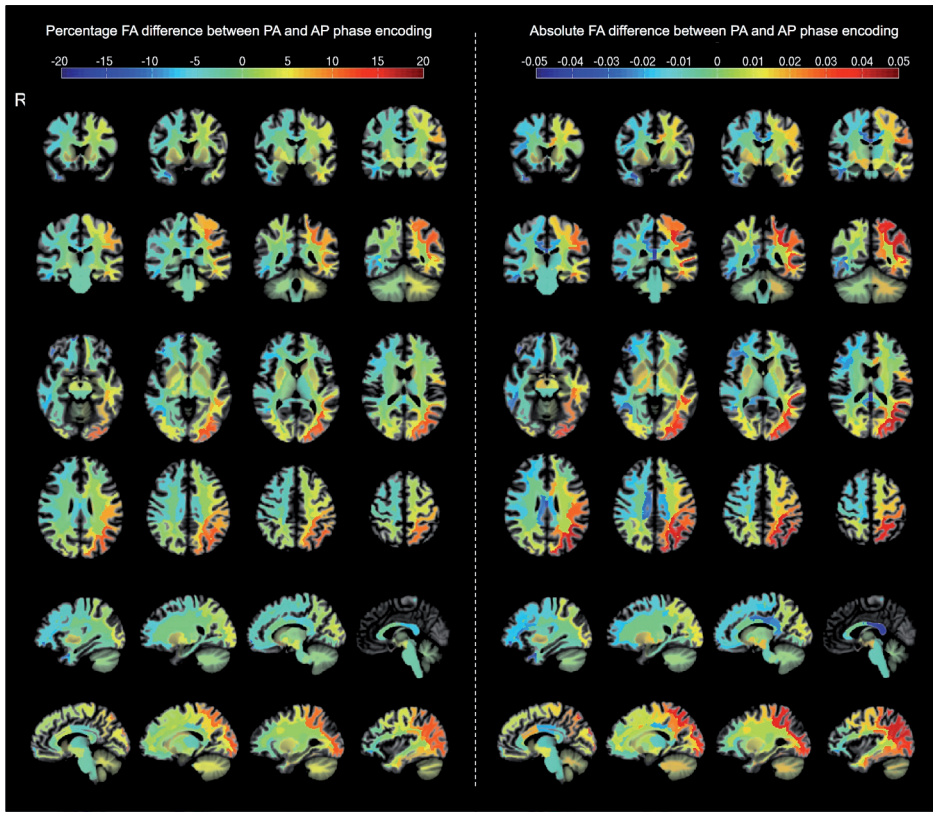


Figure 5. Percentage and absolute FA difference between PA and AP PE over all participants (N=171 for each PE direction).

corpus callosum (-0.04 or -6%), right temporal pole (-0.03, -11%), right pars orbitalis (-0.03 or -11%), and right frontal pole (-0.03 or -15%).

From Fig. 4 one can observe that the largest FA differences between the PA and PA PE direction scans are located in regions closest to the interface between brain and non-brain tissue. In addition, positive FA differences (PA > AP) tend to be located more frequently in the left hemisphere than in the right hemisphere (Fig. 5).

Clinical research question

The DTI scans with AP PE direction showed a main effect of group for the FA of the isthmus cingulum ($F_{(2,56)} = 5.318$, $p = 0.008$), where remitted PTSD patients had significantly lower FA values than persistent PTSD patients and controls (Fig. 6). The scans with PA PE direction showed a similar pattern, i.e., a main effect of group for the FA of the isthmus cingulum ($F_{(2,56)} = 4.490$, $p = 0.016$), but this effect did not survive

Bonferroni correction (Fig. 6). No interaction effects were observed for the FA of the isthmus cingulum. No group or group-by-time interaction effects were found for either PA or AP PE data for the FA of the other cingulum white matter subdivisions.

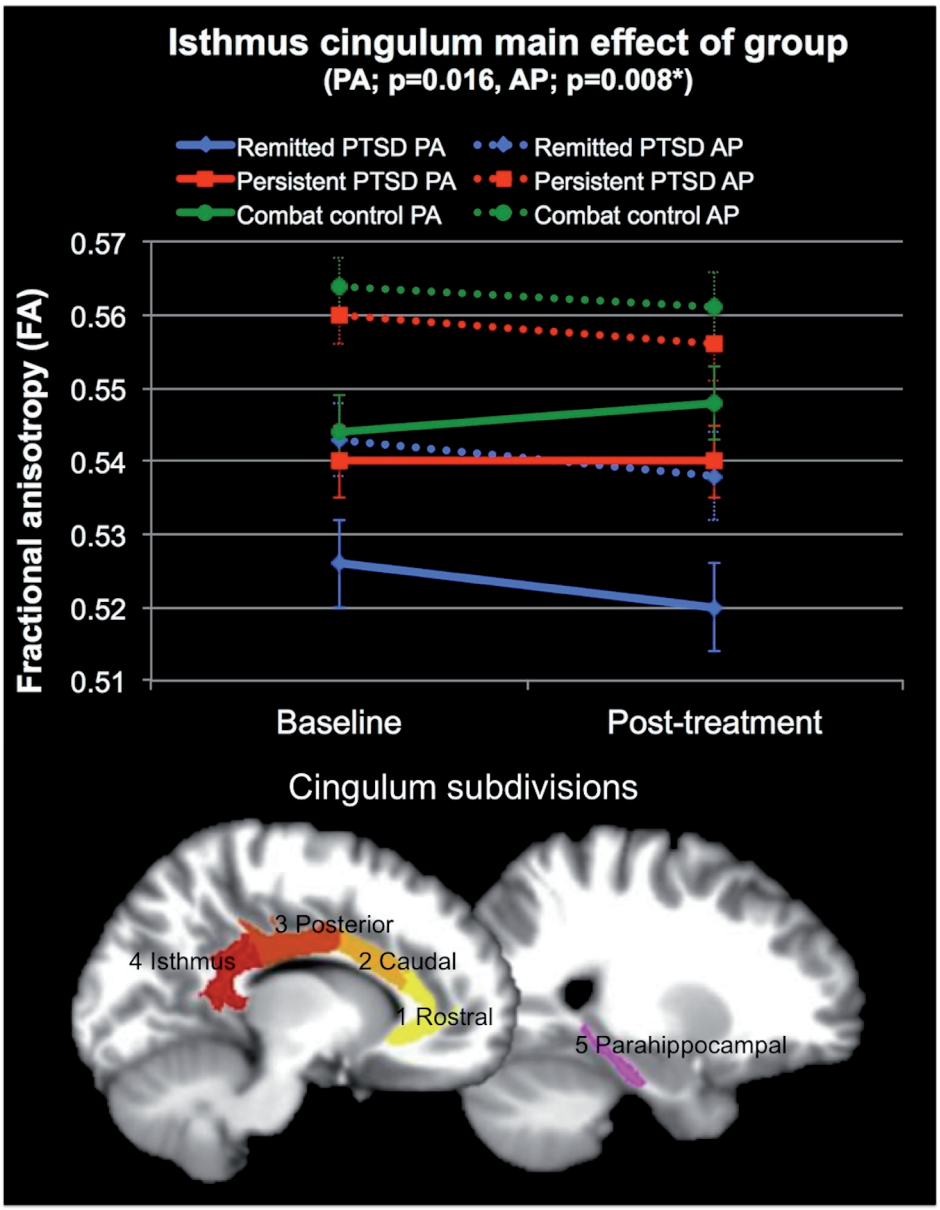


Figure 6. Main effect of group for the isthmus cingulum subdivision for PA and AP PE (top). The investigated subdivisions of the cingulum are also presented (bottom).

Discussion

Susceptibility-induced artifacts are known to hamper diffusion scan quality, though it is unclear to what extent the polarity of PE direction matters for FA estimates, especially for group analyses. Here, a large set of diffusion images with opposing polarity of PE direction (AP or PA) was utilized to examine effects of the choice of PE direction on FA estimates, and on the outcome of a specific clinical research question. Although there are other ways proposed to correct for susceptibility-induced artifacts, including mapping the B_0 magnetic field inhomogeneity (Jezzard and Balaban, 1995), and collecting diffusion scans with reversed PE direction (Andersson, Skare, Ashburner, 2003; Andersson and Sotiropoulos, 2015; Gallichan et al., 2010; Irfanoglu et al., 2015; Morgan et al., 2004; Ruthotto et al., 2012), we chose to compare scans with the reversed polarity of PE direction using a registration-based distortion correction to a T1-weighted scan, since this reflects a typical clinical setting, where scan time is kept minimal.

Correction of susceptibility artifacts

An identical processing pipeline was applied for the PA and AP datasets, which included corrections for subject motion, eddy current distortions, and EPI deformations. Although EPI distortions were clearly visible in the scans (Fig. 2), the AP and PA scans showed similar regions with high and low FA values (e.g., the corpus callosum has higher FA values compared to the subcortical structures; see Fig. 4).

Despite the similarities in FA estimates, residual misalignment was still visible between the diffusion scans and the anatomical T1-weighted scan (Fig. 3). Since a *Freesurfer* template-based method was applied here, the misalignment between the *Freesurfer* brain areas and the FA maps may be the driving force behind the observed FA differences between the PA and AP PE scans. A *Freesurfer* template-based method was chosen to circumvent effects of intersubject registration, which is necessary for voxelwise analyses, and effects of fiber orientation deconvolution, which is necessary for tractography. Therefore, this atlas-based method has minimal sensitivity to confouncing factors, other than the effect of PE direction. The effects of PE direction on FA estimates in a the *Freesurfer* template-based analysis will be aligned in the next sections.

Regional FA differences

Differences in FA values between PA and AP PE scans were observed for 85 of the 97 investigated *Freesurfer* brain regions and were on average in the order of 5%. The magnitude of this effect is similar to and even larger than the magnitude of differences in FA estimates between clinical groups (e.g., Phan et al., 2009; Tromp et al., 2012). Therefore, the effect of interest (e.g., a group difference) is generally similar or even smaller than the effect of the choice in PE direction shown here.

Another observation was that the difference in FA estimates between opposing PE direction scans was not the same across regions. In some regions, scans with PA PE direction provided higher FA estimates than scans with AP PE direction (see positive difference, i.e., the "red-ish" brain areas in Fig. 5), whereas the opposite was found in other brain regions (see negative difference, i.e., the "blue-ish" brain areas in Fig. 5). Furthermore, higher FA in PA versus AP PE scans was more frequently present in the left hemisphere, and lower FA in PA versus AP PE was observed in the right hemisphere. Therefore, PE direction might influence lateralization measures, although this was beyond the scope of this study, and was not directly investigated here. Possibly, the natural asymmetry of the brain may explain why asymmetry was observed in the correction of the EPI distortions (Büchel et al., 2004; de Groot et al., 2009). Future studies can investigate the relation between the effect of PE direction, brain asymmetry, and lateralization measures to further elucidate this observation.

Clinical research question

The effect of the choice of the polarity of PE direction (AP or PA) on the outcome of the clinical research question, i.e., whether the observed FA changes in specific cingulum subdivisions were different between (a) remitted PTSD (b) persistent PTSD and (c) combat controls over the course of treatment, was also investigated. Both PA and AP PE diffusion scans showed an uncorrected group difference in the isthmus cingulate. However, for the PA PE data the isthmus group effect did not survive Bonferroni correction for multiple comparisons. Therefore, the conclusions drawn from both group analyses for data acquired with different polarity of PE direction are not in agreement. Because of the publication bias for significant results (Easterbrook et al., 1991), and the importance of correcting for multiple comparisons in neuroimaging studies, it can be assumed that publishing the significant group difference (AP PE direction) would be much easier than publishing the trend-significant group difference (PA PE direction).

The trend observed for AP PE showed a non-significant group effect in the isthmus cingulum similar to the significant group difference found for PA PE (i.e. lower FA values in remitted PTSD patients versus controls and persistent PTSD patients), but AP PE results did not survive Bonferroni correction (see Fig. 5). One may therefore infer that the FA values from both PE directions show similar patterns, as was also noted for FA estimates above (see Fig. 4). Yet, results are marred by the choice in PE direction. Although previous studies already highlighted the effect of the direction of PE polarity, and our results complement this by showing a direct effect of PE direction choice on a clinical research question, PE direction is not always clearly reported in (clinical) diffusion MRI research (Irfanoglu et al., 2012; Wu et al., 2008). We encourage researchers to report the PE direction, in order to be able to compare the effects of PE polarity direction between studies in the future.

Methodological considerations

The two sets of DTI scans were acquired in the same order: the PA before the AP PE direction. Therefore, multiple slow scanner drifts may have affected the quality of the scans, and possibly have interacted with the PE effect. The difference in FA estimates was compared between scans with opposing polarity of PE using a similar processing pipeline, and not between different susceptibility distortion correction methods (e.g., compare registration-based methods with reversed PE polarity methods such as Top-up of FSL (Andersson, Skare, Ashburner, 2003)). However, this approach was particularly chosen to minimize differences between the processing pipelines of AP and PA PE direction scans, allowing a direct comparison of FA estimates obtained with a typical analysis pipeline, which was the main goal of this study.

The number of subjects in the remitted (N=16) group was relatively small for investigating the clinical research question. However, previous studies also reported differences between these patients groups, and therefore this research question represents a typical clinical investigation. Furthermore, methodological effects are commonly tested on a limited number of (representative) subjects, and not in a large sample or in clinical groups (Irfanoglu et al., 2015; Wu et al., 2008). Therefore, our investigation contributes to the currently available methodological studies by providing investigation of the effect of PE direction in a large sample of scans.

Conclusion

In this study, choosing a different polarity of the PE direction (AP versus PA) was shown to affect the estimation of FA values in 85 of the 97 investigated *Freesurfer* brain regions. In addition, we have shown that the conclusions for the clinical research question outcome of the AP and PA PE data did not concur. Our study highlights the importance of choice of the polarity of the PE direction in a DTI group analysis. These findings increase our understanding of how one of the most pronounced data artifacts in diffusion MRI can impact group studies and should encourage users to be more cautious when interpreting and reporting study outcomes derived from data acquired along a single PE direction.

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Supplementary Information A

Clinical assessment and inclusion/exclusion criteria

Participants were assessed with the clinician administered PTSD scale (CAPS; Blake et al., 199)) interview, and the Structured Clinical Interview for DSM IV (SCID-I; First et al., 1997) to asses comorbidity of axis I disorders. Psychologists or a trained PhD student administered the interviews. Inclusion criteria for patients were CAPS≥45, no alcohol of substance dependency, and no known neurological disorder. Inclusion criteria for controls were no clinical PTSD symptoms (CAPS≤15), no current psychiatric disorder, no alcohol or substance dependency, and no neurological disorder. DTI data were obtained from all participants after inclusion, and after 6-8 months. In between assessments, PTSD patients received trauma-focused therapy as part of "treatment as usual", consisting of trauma focused cognitive behavioral therapy and/or eye movement desensitization and reprocessing, in accordance with treatment guidelines (Balkom et al., 2013; Foa, Keane, Friedman, 2000). At the reassessment, clinical interviews were also repeated.

Participants included for addressing the clinical research question

In total, 39 PTSD patients and 21 combat controls were included. At reassessment, 16 PTSD patients were remitted and 23 had persistent PTSD. These groups did not differ in age, education, the number of left, right and ambidextrous participants, the number of times deployed, and time since deployment (see Table 1 below for a detailed overview). There was a trend found for the time between scans, where the interval was slightly smaller for controls versus PTSD patients ($p = 0.066$). Comparing the remitted and persistent PTSD patients showed no difference in total treatment between scans, comorbidity of mood and somatoform disorders, and medication use. Baseline CAPS score ($p = 0.005$) and comorbidity of anxiety disorders ($p = 0.005$) were higher in the persistent PTSD group compared to the remittent PTSD group. At reassessment, CAPS scores were higher in the persistent PTSD patients compared to remitted PTSD. At reassessment, trends for more comorbidity of mood an anxiety disorders were also observed for the persistent PTSD group compared to the remitted PTSD group.

Table S1. Demographical and clinical characteristics of the remitted and persistent PTSD patients and combat controls.

	Remitted PTSD (mean ± SD)	Persistent PTSD (mean ± SD)	Combat Control (mean ± SD)	Test-value (df)	Sig. (two-tailed)
Number of participants	16	23	21		
Age (years) (range 21-57)	33.81 (±9.77)	37.70 (±9.20)	36.43 (±10.71)	$F_{(2)}$=0.733	*p*=0.485
Education (ISCED)					
Own	3.81 (±1.17)	3.55 (±1.14)	4.19 (±1.69)	$F_{(2)}$=1.198	*p*=0.309
Mother	2.36 (±0.63)	2.52 (±1.63)	3.29 (±1.49)	$F_{(2)}$=2.384	*p*=0.102
Father	3.27 (±1.62)	3.20 (±2.04)	4.05 (±1.79)	$F_{(2)}$=1.266	*p*=0.290
Handedness (Left / Ambidextrous/ Right)	(1 / 0 / 15)	(2 / 3 / 17)	(1 / 2 / 18)	$\chi^2_{(4)}$=2.681	*p*=0.612
Number of times deployed (1 / 2 / 3 / >3)	(4 / 5 / 4 / 3)	(10 / 2 / 6 / 3)	(6 / 6 / 4 / 5)	$\chi^2_{(14)}$=14.657	*p*=0.550
Time since last deployment (years)	6.94 (±8.05)	7.62 (±7.88)	5.43 (±5.60)	$F_{(2)}$=0.507	*p*=0.605
Time between scans in (months)	6.06 (±1.12)	6.36 (±0.85)	5.19 (±2.44)	$F_{(2)}$=2.859	*p*=0.066
Total trauma-focused treatment sessions between assessments	9.33 (±7.20)	9.94 (±5.03)		$t_{(32)}$=-0.293	*p*=0.772
(<5 / 5-10 / >10)	(4 / 6 / 5)	(3 / 7 / 9)			
Clinical scores at baseline					
PTSD severity (CAPS total score)	63.25 (± 10.55)	75.35 (±13.50)		$t_{(37)}$=-3.00	*p*=0.005
Current comorbid disorder baseline (SCID)					
Mood disorder	6	15		$\chi^2_{(1)}$=2.917	*p*=0.112
Anxiety disorder	1	12		$\chi^2_{(1)}$=8.955	*p*=0.005
Somatoform disorder	1	2		$\chi^2_{(1)}$=0.079	*p*=1.000
Medication					
SSRI/SARI	4	8		$\chi^2_{(2)}$=0.424	*p*=0.726
Benzodiazepines	5	5		$\chi^2_{(1)}$=0.448	*p*=0.711
Antipsychotics	1	1		$\chi^2_{(1)}$=0.070	*p*=1.000
Other	1	3		$\chi^2_{(1)}$=0.473	*p*=0.631
Clinical scores post-treatment					
CAPS total score	22.56 (±14.63)	60.23 (±18.02)		$t_{(35)}$=-6.869	*p*=0.000
Current comorbid disorder after treatment (SCID)					
Mood disorder	-	5		$\chi^2_{(2)}$=5.182	*p*=0.075
Anxiety disorder	1	7		$\chi^2_{(2)}$=4.704	*p*=0.095
Somatoform disorder	-	2		$\chi^2_{(2)}$=2.369	*p*=0.306
Alcohol dependency	-	2		$\chi^2_{(2)}$=1.611	*p*=0.495

Table S1. Demographical and clinical characteristics of the remitted and persistent PTSD patients and combat controls. (*Continued*)

	Remitted PTSD (mean ± SD)	Persistent PTSD (mean ± SD)	Combat Control (mean ± SD)	Test-value (df)	Sig. (two-tailed)
Medication					
SSRI/SARI	4	9		$\chi^2_{(1)}$=1.541	p=0.301
Benzodiazepines	3	1		$\chi^2_{(1)}$=0.689	p=0.613
Antipsychotics	4	2		$\chi^2_{(1)}$=1.440	p=0.226
Other	1	3		$\chi^2_{(1)}$=1.694	p=0.492

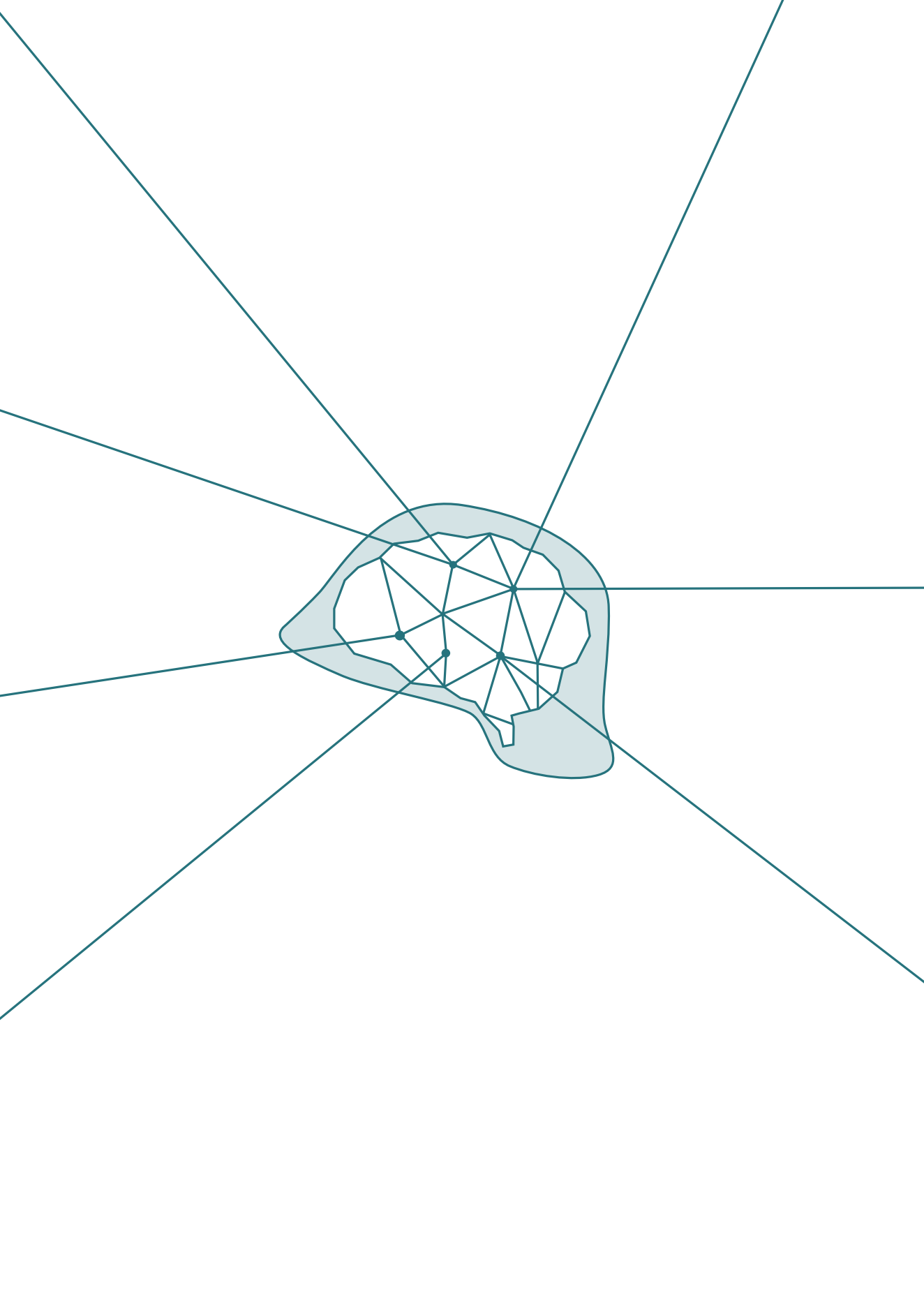
Supplementary Information B

Overview of the regional brain FA differences (mean across all subjects and time points) between the AP and PA PE DTI data. Bonferroni correction was applied ($p < 0.0005$ is deemed significant).

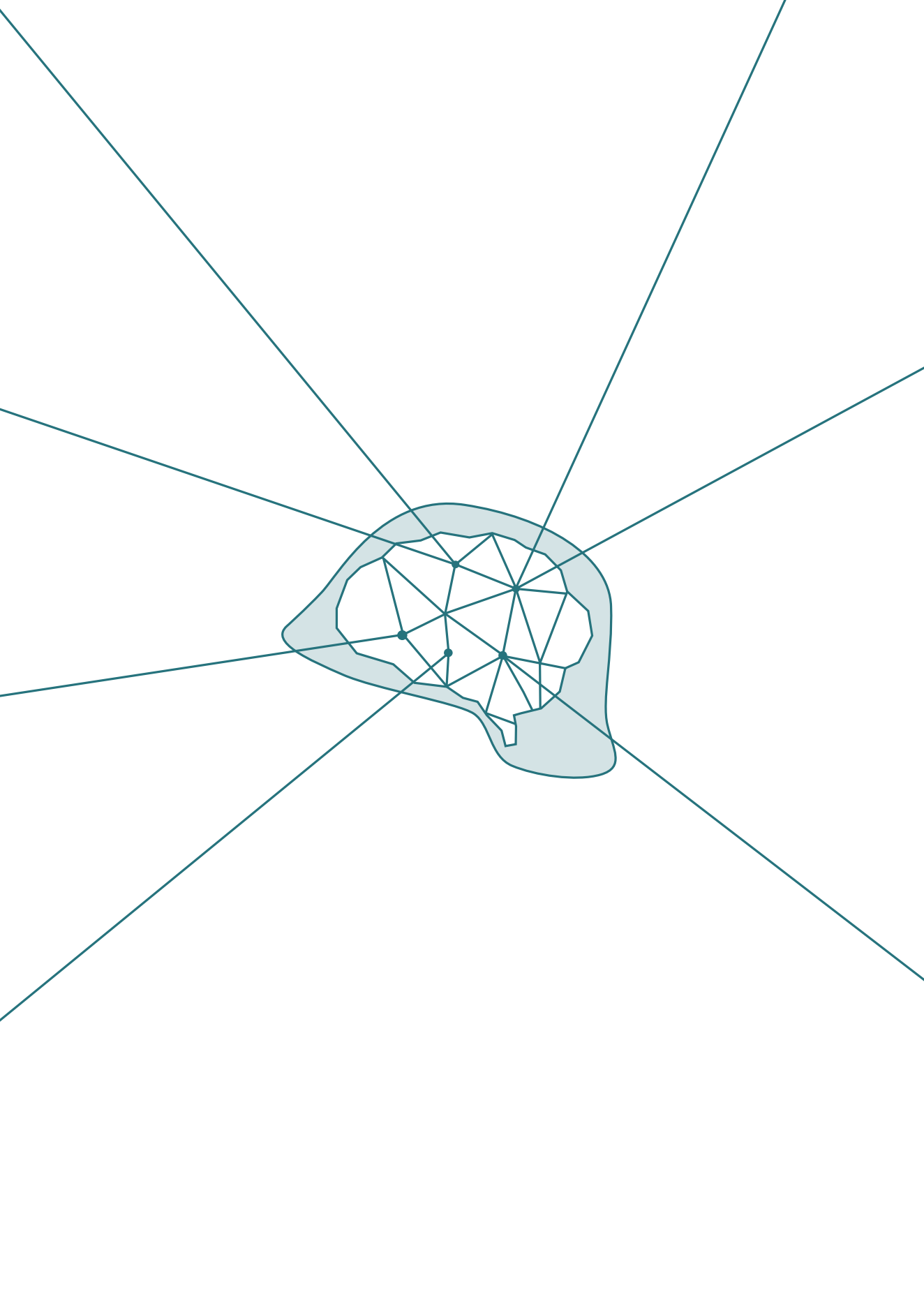
	Freesurfer label	Mean FA difference ("PA minus AP")	Mean percentage FA difference ("PA minus AP")	$t_{(170)}$	Sig. (2-tailed)
1	left cerebellum white matter	0.02	5.2	24	$p<0.0005$
2	left cerebellum cortex	0.01	3.7	21	$p<0.0005$
3	left thalamus proper	-0.01	-3.2	-6	$p<0.0005$
4	left caudate	0.01	6.7	15	$p<0.0005$
5	left putamen	0.01	5.3	14	$p<0.0005$
6	left pallidum	<0.01 & >0	0.4	1	$p = 0.434$
7	left hippocampus	<0.01 & >0	2.8	-9	$p<0.0005$
8	left amygdala	0.01	4.4	5	$p<0.0005$
9	left accumbens area	0.01	4.6	9	$p<0.0005$
10	left ventraldc	0.01	2.9	6	$p<0.0005$
11	right cerebellum white matter	<0.01 & >0	1.1	8	$p<0.0005$
12	right cerebellum cortex	<0.01 & >0	0.6	5	$p<0.0005$
13	right thalamus proper	<0.01 & >0	0.7	2	$p = 0.033$
14	right caudate	0.01	2.6	1	$p = 0.168$
15	right putamen	0.02	5.9	5	$p<0.0005$
16	right pallidum	0.02	4.5	11	$p<0.0005$
17	right hippocampus	>-0.01 & <0	-0.9	7	$p<0.0005$
18	right amygdala	0.01	3.4	-2	$p = 0.054$
19	right accumbens area	0.02	9.2	6	$p<0.0005$
20	right ventraldc	0.02	4.3	13	$p<0.0005$
21	optic chiasm	0.06	29.6	13	$p<0.0005$
22	brain stem	-0.01	-2.1	23	$p<0.0005$
23	cc posterior	-0.04	-6.4	-18	$p<0.0005$
24	cc mid posterior	-0.04	-7.4	-16	$p<0.0005$
25	cc central	-0.01	-1.0	-3	$p = 0.005$
26	cc mid anterior	<0.01 & >0	0.8	2	$p = 0.016$
27	cc anterior	-0.01	-1.0	-4	$p<0.0005$
28	wm lh bankssts	0.03	8.8	41	$p<0.0005$
29	wm lh caudalanteriorcingulate	0.02	3.7	19	$p<0.0005$
30	wm lh caudalmiddlefrontal	0.02	3.9	20	$p<0.0005$
31	wm lh cuneus	0.01	4.8	15	$p<0.0005$

	Freesurfer label	Mean FA difference ("PA minus AP")	Mean percentage FA difference ("PA minus AP")	$t_{(170)}$	Sig. (2-tailed)
32	wm lh entorhinal	0.02	7.0	14	$p<0.0005$
33	wm lh fusiform	0.03	7.6	-3	$p = 0.001$
34	wm lh inferiorparietal	0.04	11.7	28	$p<0.0005$
35	wm lh inferiortemporal	0.02	5.0	67	$p<0.0005$
36	wm lh isthmuscingulate	-0.01	-1.8	20	$p<0.0005$
37	wm lh lateraloccipital	0.03	11.9	2	$p = 0.018$
38	wm lh lateralorbitofrontal	-0.01	-1.8	-7	$p<0.0005$
39	wm lh lingual	0.01	3.2	53	$p<0.0005$
40	wm lh medialorbitofrontal	0.01	3.6	-7	$p<0.0005$
41	wm lh middletemporal	0.01	4.1	10	$p<0.0005$
42	wm lh parahippocampal	0.01	3.6	12	$p<0.0005$
43	wm lh paracentral	0.02	3.8	19	$p<0.0005$
44	wm lh parsopercularis	<0.01 & >0	0.8	12	$p<0.0005$
45	wm lh parsorbitalis	-0.02	-8.5	5	$p<0.0005$
46	wm lh parstriangularis	-0.01	-3.3	4	$p<0.0005$
47	wm lh pericalcarine	0.02	4.8	-20	$p<0.0005$
48	wm lh postcentral	0.03	7.7	-14	$p<0.0005$
49	wm lh posteriorcingulate	-0.02	-4.0	20	$p<0.0005$
50	wm lh precentral	0.02	4.3	36	$p<0.0005$
51	wm lh precuneus	0.02	6.2	-13	$p<0.0005$
52	wm lh rostralanteriorcingulate	-0.01	-1.6	29	$p<0.0005$
53	wm lh rostralmiddlefrontal	>-0.01 & <0	-1.2	31	$p<0.0005$
54	wm lh superiorfrontal	0.01	2.3	-8	$p<0.0005$
55	wm lh superiorparietal	0.04	10.6	-5	$p<0.0005$
56	wm lh superiortemporal	0.01	3.7	14	$p<0.0005$
57	wm lh supramarginal	0.03	8.2	51	$p<0.0005$
58	wm lh frontalpole	-0.02	-7.5	20	$p<0.0005$
59	wm lh temporalpole	>-0.01 & <0	-0.9	36	$p<0.0005$
60	wm lh transversetemporal	0.01	1.8	-2	$p = 0.119$
61	wm lh insula	<0.01 & >0	0.5	4	$p<0.0005$
62	wm rh bankssts	-0.01	-3.7	-11	$p<0.0005$
63	wm rh caudalanteriorcingulate	>-0.01 & <0	-0.4	-2	$p = 0.042$
64	wm rh caudalmiddlefrontal	-0.02	-4.1	-16	$p<0.0005$
65	wm rh cuneus	0.01	3.2	9	$p<0.0005$
66	wm rh entorhinal	>-0.01 & <0	-0.8	-1	$p = 0.208$
67	wm rh fusiform	-0.01	-2.0	-8	$p<0.0005$
68	wm rh inferiorparietal	<0.01 & >0	1.2	-10	$p<0.0005$
69	wm rh inferiortemporal	-0.03	-7.8	8	$p<0.0005$

	Freesurfer label	Mean FA difference ("PA minus AP")	Mean percentage FA difference ("PA minus AP")	$t_{(170)}$	Sig. (2-tailed)
70	wm rh isthmuscingulate	-0.02	-4.3	-28	p<0.0005
71	wm rh lateraloccipital	0.01	4.7	-7	p<0.0005
72	wm rh lateralorbitofrontal	-0.02	-5.0	-17	p<0.0005
73	wm rh lingual	>-0.01 & <0	-1.3	20	p<0.0005
74	wm rh medialorbitofrontal	-0.01	-3.4	-18	p<0.0005
75	wm rh middletemporal	-0.02	-4.9	-4	p<0.0005
76	wm rh parahippocampal	-0.01	-2.4	-9	p<0.0005
77	wm rh paracentral	-0.02	-4.2	-15	p<0.0005
78	wm rh parsopercularis	-0.02	-5.6	-20	p<0.0005
79	wm rh parsorbitalis	-0.03	-11.2	-5	p<0.0005
80	wm rh parstriangularis	-0.02	-7.2	-17	p<0.0005
81	wm rh pericalcarine	0.01	3.9	-24	p<0.0005
82	wm rh postcentral	-0.01	-2.2	-19	p<0.0005
83	wm rh posteriorcingulate	-0.03	-5.2	16	p<0.0005
84	wm rh precentral	-0.01	-3.2	-8	p<0.0005
85	wm rh precuneus	0.01	1.7	-22	p<0.0005
86	wm rh rostralanteriorcingulate	-0.01	-2.9	-15	p<0.0005
87	wm rh rostralmiddlefrontal	-0.02	-5.2	8	p<0.0005
88	wm rh superiorfrontal	-0.01	-3.5	-12	p<0.0005
89	wm rh superiorparietal	0.01	2.7	-26	p<0.0005
90	wm rh superiortemporal	-0.01	-3.4	-18	p<0.0005
91	wm rh supramarginal	-0.02	-4.4	15	p<0.0005
92	wm rh frontalpole	-0.03	-14.8	-9	p<0.0005
93	wm rh temporalpole	-0.03	-11.3	-20	p<0.0005
94	wm rh transversetemporal	-0.02	-5.0	-18	p<0.0005
95	wm rh insula	-0.01	-1.5	-7	p<0.0005
96	left unsegmentedwhitematter	0.01	1.6	12	p<0.0005
97	right unsegmentedwhitematter	<-0.01	-0.5	-3	p = 0.001



Section 2
Functional connectivity



4

Resting state functional connectivity of the anterior cingulate cortex in veterans with and without posttraumatic stress disorder

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Abstract

Posttraumatic stress disorder (PTSD) is an anxiety disorder that is associated with structural and functional alterations in several brain areas, including the anterior cingulate cortex (ACC). Here, we examine resting state functional connectivity of ACC subdivisions in PTSD, using a seed-based approach. Resting state magnetic resonance images were obtained from male veterans with (n = 31) and without (n = 25) PTSD, and healthy male civilian controls (n = 25).

Veterans with and without PTSD (combat controls) had reduced functional connectivity compared to healthy controls between the caudal ACC and the precentral gyrus, and between the perigenual ACC and the superior medial gyrus and middle temporal gyrus. Combat controls had increased connectivity between the rostral ACC and precentral/middle frontal gyrus compared to PTSD patients and healthy civilian controls. The resting state functional connectivity differences in the perigenual ACC network reported here indicate that veterans differ from healthy controls, potentially due to military training, deployment and/or trauma exposure. In addition, specific alterations in the combat controls may potentially be related to resilience. These results underline the importance of distinguishing trauma-exposed (combat) controls from healthy civilian controls when studying PTSD.

Introduction

Posttraumatic stress disorder (PTSD) is an anxiety disorder that may develop after experiencing a traumatic event (American Psychiatric Association, 1994). Since many veterans experience traumatic events during deployment, veterans are at increased risk for developing PTSD. The prevalence of PTSD among veterans assessed with questionnaires is 6.2-12.9% in American veterans deployed to Iraq and Afghanistan (Hoge et al., 2004) and 6.7-8.9% in Dutch veterans deployed to Afghanistan (Reijnen et al., 2014), but prevalence rates vary in different studies depending on sampling strategies (Richardson, Frueh, Acierno, 2010). PTSD is characterized by symptoms of re-experiencing of the event, avoidance of trauma-related stimuli and emotional numbing, and hyperarousal (American Psychiatric Association, 1994). Neuroimaging techniques have been utilized to investigate the biology of PTSD. These studies have revealed anatomical and functional alterations in brain areas such as the amygdala, hippocampus, ventromedial prefrontal cortex (vmPFC), and anterior cingulate cortex (ACC) (Pitman et al., 2012; Shin and Liberzon, 2010).

Alterations in the ACC seem to be more pronounced in PTSD compared to social anxiety or specific phobia (Etkin and Wager, 2007), although other anxiety disorders are also associated with alterations in the ACC, such as obsessive-compulsive disorder (Fitzgerald et al., 2005; Melcher, Falkai, Gruber, 2008; Ursu et al., 2003). Studies report hypoactivation of the rostral ACC in PTSD versus controls during symptom provocation using script-driven imagery (Britton et al., 2005; Lanius et al., 2001; Lanius et al., 2007), during the presentation of trauma-related stimuli (Hou et al., 2007; Yang et al., 2004), and the presentation of negative stimuli (Kim et al., 2008; Lanius et al., 2003; Shin et al., 2001; Shin et al., 2005; Williams et al., 2006). Several studies have reported that nonthreatening salient stimuli induce dorsal ACC hyperactivity in PTSD versus controls (Bryant et al., 2005; Felmingham et al., 2009; Milad et al., 2009; Rougemont-Bücking et al., 2011; Shin et al., 2011). Thus, depending on the tasks investigated in these studies, there seems to be a tendency that the rostral ACC is hypoactive in PTSD, while the dorsal ACC is hyperactive in PTSD. Investigating subdivisions of the ACC is therefore of substantial importance when investigating the neurobiology of PTSD.

In functional magnetic resonance imaging (fMRI) studies investigating resting state functional connectivity in PTSD specific subdivisions of the ACC were shown to have reduced connectivity with different brain areas. First, the rostral/perigenual ACC showed reduced connectivity with the PCC/precuneus (Sripada et al., 2012), and resting state functional connectivity of these regions correlated with symptom severity (Lanius et al., 2010). Second, the dorsal ACC had reduced connectivity with the thalamus (Yin et al., 2011). Third, dorsal and rostral ACC showed reduced negative functional

connectivity with the amygdala (Sripada et al., 2012b). However, these studies applied seed-based analysis using the PCC/precuneus (and vmPFC), thalamus, or amygdala as regions of interest respectively. None of these studies have taken the ACC or subdivisions of the ACC as a region of interest during resting state functional connectivity analysis in PTSD. Therefore, it remains unclear whether more ACC abnormalities in functional connectivity are present in PTSD or whether these abnormalities are restricted to connectivity between the ACC and PCC/precuneus, thalamus and amygdala. A further comprehensive analysis of resting state functional connectivity of the subdivisions of the ACC is thus required.

Studies investigating cytoarchitecture and function of the ACC have generally distinguished four subdivisions (Etkin, Egner, Kalisch, 2011; Palomero-Gallagher et al., 2009; Shackman et al., 2011; Vogt, Berger, Derbyshire, 2003). A systematic examination of subdivisions in the ACC with resting state functional connectivity showed that five distinct networks were separable within the ACC (posterior to anterior: caudal, dorsal, rostral, perigenual, and subgenual; see Table 1 and Fig. 1 for the seed locations of these subdivisions (Kelly et al., 2009; Margulies et al., 2007)). The existence of five subdivisions of the ACC has also been confirmed with diffusion tensor imaging, investigating structural connectivity (Beckmann, Johansen-Berg, Rushworth, 2009). In addition, the ACC subserves separable functions along its axis, although the relationship is complex and functions overlap amongst regions. The caudal ACC is involved in motor control (Dum and Strick, 1991), the dorsal ACC in cognitive control (Chouinard and Paus, 2006; Paus, 2001), the rostral ACC in conflict monitoring (Botvinick, Cohen, Carter, 2004), the perigenual ACC in self-referential and social processing (Amodio and Frith, 2006; Kelley et al., 2002), and the subgenual ACC in emotional regulation (Drevets et al., 1997; Phan et al., 2002).

Table 1. Coordinates for the five left and right seeds are given in coordinates defined in Montreal Neurological Institute space.

Seed		MNI coordinates		
	ACC region	x	y	z
Seed 1	Caudal ACC	±5	–10	47
Seed 2	Dorsal ACC	±5	14	42
Seed 3	Rostral ACC	±5	34	28
Seed 4	Perigenual ACC	±5	47	11
Seed 5	Subgenual ACC	±5	25	–10

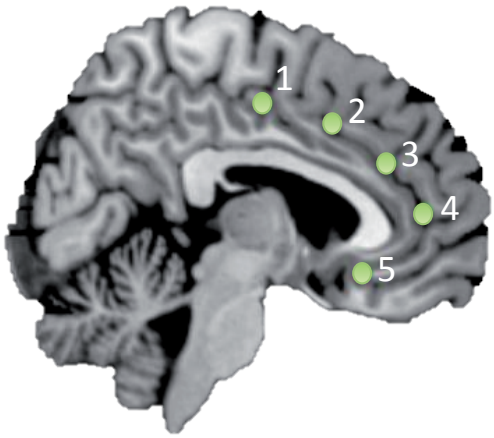

Figure 1. Location of the ACC seeds.

Here, we investigate resting state functional connectivity of these five functionally diverse ACC subdivisions in PTSD in order to provide a thorough investigation of cingulate dysfunction in PTSD. These five seeds have been selected since they exhibit distinct resting state functional connectivity patterns in healthy subjects (Margulies et al., 2007), and have been related to other psychiatric disorders and human development (Camchong et al., 2011; Davey et al., 2012a; Kelly et al., 2009). We compare PTSD patients with two control groups: a combat control group, consisting of deployed veterans who have experienced similar traumatic events as the PTSD patients, and a healthy civilian control group. By including two control groups, general effects of military training and deployment, which includes trauma exposure, can be investigated. To rule out any effects of medication, only PTSD patients that were medication naive or patients that occasionally used benzodiazepines, but had not taken benzodiazepines at least 48 hours prior to scanning were included. Differences found in resting state connectivity with subdivisions of the ACC have shown alterations in the rostral, dorsal and perigenual subdivisions in PTSD patients (Lanius et al., 2010; Sripada et al., 2012a; Sripada et al., 2012b; Yin et al., 2011). Thus, we hypothesized that resting state functional connectivity of the dorsal, rostral, perigenual, and subgenual networks were reduced in PTSD versus controls. No differences in the caudal ACC network were expected. Furthermore, we expected that PTSD patients deviate the most from healthy controls, while combat controls were expected to have an intermediate phenotype.

Methods

Participants

In total, 37 male veterans with PTSD, 27 male veterans without PTSD (combat controls), and 26 healthy never deployed male civilian controls (healthy controls) were included in this study. All patients were recruited from one of four outpatient clinics of the Military Mental Healthcare Organization in Utrecht, The Netherlands. Patients were included if they met diagnostic criteria for PTSD according to the DSM-IV (American Psychiatric Association, 1994). PTSD severity was assessed with the clinician administered PTSD scale (CAPS) (Blake et al., 1995). Control participants were recruited via advertisements. The veterans (with or without PTSD) were mostly deployed to Afghanistan (PTSD patients: n = 18; combat controls: n = 17). Most patients (n = 33) were medication naive, and four patients occasionally used benzodiazepines, but had not taken benzodiazepines in the 48 h prior to the scan. After receiving a complete written and verbal description of the study all participants gave informed consent. Participants received financial compensation for their participation. The Medical Ethical Committee of the UMC Utrecht approved the study, and the study was performed in accordance with the Declaration of Helsinki (World Medical Association, 2013).

Data acquisition

Functional and structural images were obtained using a 3.0 Tesla magnetic resonance imaging scanner (Philips Medical System, Best, the Netherlands). In order to allow the participants to adapt to the scanner environment a T1-weighted high resolution image was acquired before the resting state scan (TR = 10 ms TE = 4.6 ms flip angle 8, 200 slices sagittal orientation, FOV 240 x 240 x 160, matrix of 304 x 299). This image was utilized for coregistration and segmentation purposes. For the resting state scan participants were asked to relax, to let their mind wander and to focus on the fixation cross. Three hundred and twenty images were collected (T2* weighted echo planar interleaved images, repetition time TR = 1600 ms, TE = 23 ms, flip angle = 72.5°, field of view FOV 256 x 208 x 120, 30 transverse slices, 64 x 51 matrix, total scan time 8 min and 44.8 sec, 0.4 mm gap, acquired voxel size 4 x 4 x 3.60 mm).

Image analyses

Preprocessing was conducted with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) and comprised slice-timing correction, realignment, coregistration with the anatomical scan, normalization, and spatial smoothing (8 mm FWHM). Scans were resliced to 4 mm^3 isotropic voxel size. Participants were excluded when motion parameters (derived from the realignment step) during the acquisition of the resting state images exceeded 2 mm in any direction (x, y, or z) and 2 degrees rotation (pitch,

roll or yaw). In addition, participants were excluded when small movements (0.5 mm frame displacement) were detected in more than 173 images. Six PTSD patients, two combat controls, and one healthy control were excluded from further analysis due to excessive motion. In addition, mean motion and the number of movements were compared between these groups.

The Data Processing Assistant for Resting-State fMRI (DPARSF) was utilized for further analyses (restfmri.net) (Song et al., 2011), which is based on MRIcroN (http://www.mricro.com), SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/), and the Resting-State fMRI Data Analysis Toolkit (Song et al., 2011). Resting state images were band-pass filtered (0.01 - 0.08 Hz), and detrended. To correct for physiological processes and motion, nine nuisance parameters were included as covariates in the analysis (six motion parameters from the realignment step, mean global signal, white matter signal, and cerebral spinal fluid signal).

Functional connectivity analysis

Five bilateral seed points in the ACC were selected, the same as in Kelly et al. (2009). Thus, ten spherical seeds (3.5 mm radius) were created around each seed point coordinate (see Table 1 and Fig. 1) (Kelly et al., 2009; Margulies et al., 2007). The mean time series for each of those seeds was extracted for each individual and correlated with the time series of every voxel in the brain, in order to create functional connectivity maps. These correlation maps were normalized using Fishers z-transform. The individual z-maps were used for second-level group analysis (full factorial design, SPM). Since global mean signal was used as nuisance regressor, negative correlations could be induced (Murphy et al., 2009). Therefore, analyses were restricted to regions with positive functional connectivity. A positive functional connectivity map was created per seed over all participants, which was used as an inclusive mask (caudal left k = 9701, right k= 9343; dorsal left k = 10437, right k = 10876; rostral left k = 9951, right k = 9714; perigenual left k = 9558, right k = 9788; subgenual left k = 7445, right k = 7194). For every 10 seeds an F-test was applied to determine whether there were differences between the three groups. Cluster-level multiple comparison correction was applied according to Gaussian Random Field theory (Cox 1996). A initial voxel detection height threshold of $p<0.001$ was applied and a subsequent cluster threshold extent was calculated for each F-test, corresponding to a Bonferroni corrected $p<0.0001$, based on Monte Carlo simulations as implemented in the REST toolbox (restfmri.net) (Song et al., 2011). A whole brain mask was used for calculating these thresholds (25,622 voxels). The minimum corrected cluster sizes for the right hemisphere networks were k = 25 (caudal), k = 28 (dorsal), k = 25 (rostral), k = 33 (perigenual), and k = 25 (subgenual). The critical cluster sizes for the left hemisphere networks were k = 24

(caudal), k = 29 (dorsal), k = 23 (rostral), k = 32 (perigenual) and k = 26 (subgenual). False discovery rate (FDR) and family wise error (FWE) were also determined for the peak voxels within clusters of significant difference. Post-hoc t-tests were performed (p<0.001, restricted to the clusters of group differences from the F-test) to investigate the direction of group differences.

Post-hoc analyses

In addition, the effects of the diagnosis of comorbid depression and the effects of educational level, measured with the international standard classification of education (ISCED (Schneider, 2013)) on the clusters of significant differences were investigated by including these variables as covariates.

Results

Participants

Patients, healthy controls, and combat controls did not differ in age and handedness. There were also no significant differences between patients and combat controls in the number of times they were deployed and the time since their last deployment. There was no significant difference between patients and combat controls in educational level as measured with the ISCED (Schneider, 2013). The healthy control group had higher educational level than both veterans with and without PTSD (F = 5.916, p = 0.004). PTSD patients had significant higher CAPS scores than both combat controls and healthy controls (F = 630.925, p = 0.000). Twenty PTSD patients met the current diagnostic criteria for the following comorbid disorders, as assessed with the SCID I (First et al., 1997): major depressive disorder (MDD, n = 9), MDD and an anxiety disorder combined (n = 6), anxiety disorder (n = 2), MDD and a somatoform disorder combined (n = 2), and a somatoform disorder (n = 1). An overview of demographical and clinical data is presented in Table 2.

Functional connectivity

Spatial connectivity maps

Significant resting state functional connectivity for the seeds located in the right hemisphere is presented in Figure 2 for each group. Similar networks were found with the left hemisphere seeds. The spatial connectivity maps of the ACC subdivision network revealed that PTSD and control groups had overlapping regions that were functionally connected with the ACC seeds. Supporting Information Table S1-S3 lists the locations of the peak functional connectivity of the right ACC seeds for the three groups separately (height threshold P < 0.001). There were no significant differences in motion parameters between the groups.

Table 2. Demographical and clinical data.

	PTSD patient (mean ± SD)	Combat Control (mean ± SD)	Civilian Control (mean ± SD)	F or t or X^2 value	Sig. (two-tailed)
N	31	25	25		
Age (range 21-57)	35.58 (± 9.66)	36.04 (± 10.15)	34.16 (± 9.32)	$F_{(2)}$=0.256	0.775
Education (ISCED)	3.90 (± 1.47)	4.20 (± 1.50)	5.16 (± 1.18)	$F_{(2)}$=5.916	0.004*
Frequencies ISCED (2 / 3 / 4 / 6 / 7)	(7 / 4 / 13 / 6 / 1)	(3 / 5 / 10 / 5 / 2)	(0 / 0 / 12 / 10 / 3)		
Handedness (left / right)	(27 / 4)	(22 / 3)	(25 / 0)	$X^2_{(4)}$=3.875	0.423
Number of times deployed (range 1-15)	2.61 (± 3.68)	2.44 (± 1.47)	-	t=-0.221	0.826
Number of times deployed (1 / 2 / 3 / >3)	(16 / 5 / 6 / 4)	(9 / 6 / 4 / 6)	-		
Time since last deployment (years)	8.03 (± 9.22)	5.52 (± 6.38)	-	t=-1.202	0.235
Country of last deployment					
Afghanistan	18	17	-		
Former Yugoslavia	6	4	-		
Other	7	4	-		
CAPS total score	67.09 (± 11.01)	5.00 (± 4.42)	4.92 (±4.37)	$F_{(2)}$=630.925	0.000*
Current comorbid disorder (SCID)	20	-	-		
Major depressive disorder	9				
Major depressive & anxiety disorder	6				
Anxiety disorder	2				
Major depressive & somatoform disorder	2				
Somatoform disorder	1				

ISCED: international scale for education; EHI: Edinburgh Handedness Inventory; CAPS: clinician administered PTSD scale; SCID: structured clinical interview for DSM IV Axis II disorders.

Group differences

Significant differences between the groups were found in the caudal, rostal, and perigenual ACC network (see Fig. 3, Table 3, and Fig. 4). Resting state functional connectivity of the bilateral dorsal, and subgenual ACC did not differ significantly between the groups.

Caudal ACC

Group differences were found in resting state functional connectivity between the right caudal ACC seed and the right precentral gyrus (73 voxels; peak value z = 4.67; peak MNI coordinates 28, -24, 56), and left caudal ACC with the left precentral gyrus (25 voxels; peak value z = 4.32; peak MNI coordinates -28, -24, 64). Post-hoc t-test showed that the

caudal ACC seeds had reduced functional connectivity with the right precentral gyrus in both veterans with and without PTSD as compared to the healthy control group. The patients and combat controls did not differ in resting state functional connectivity of the right caudal ACC.

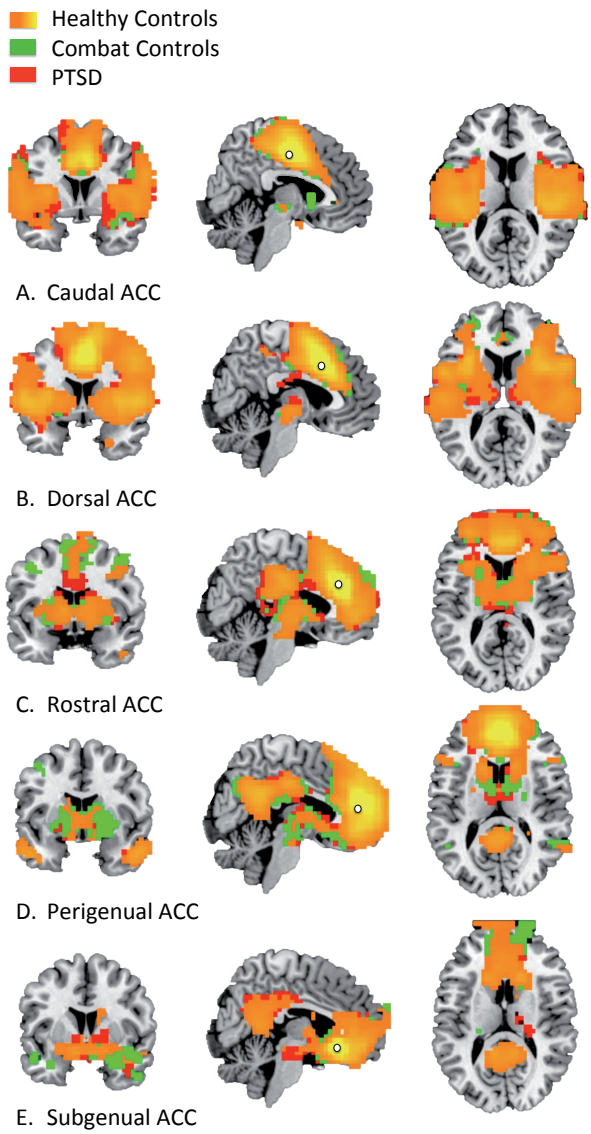

Figure 2. Positive resting state functional connectivity of the ACC seeds located in the right hemisphere. Orange–yellow: healthy controls, green: combat controls, red: PTSD. The white circle represents the seed location.

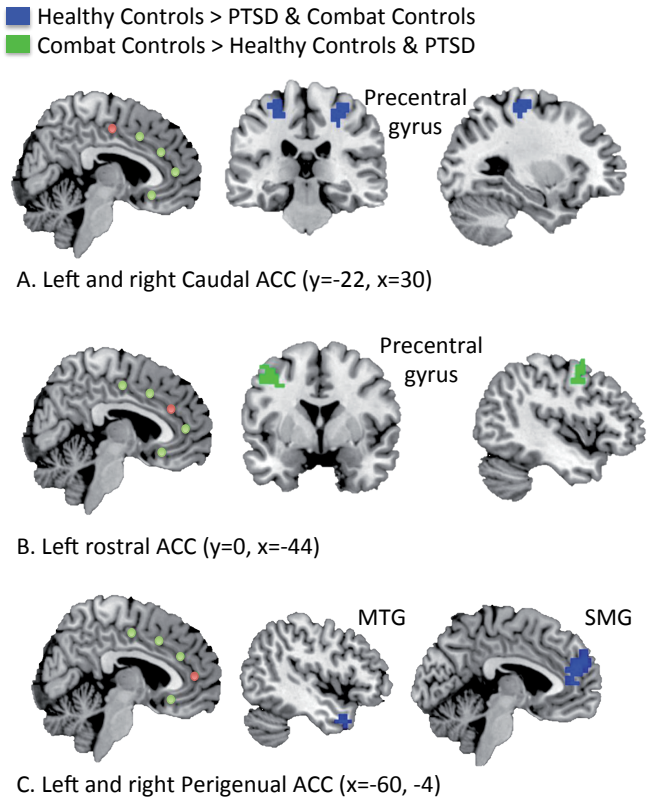

Figure 3. Clusters of significant differences between the PTSD patients (PTSD), combat controls, and healthy controls (corrected P<0.0001). The left sagittal slice shows the seed locations (in green circles), and the red circle represents the seed of the particular network. A: Differences in the right caudal ACC network are found in the precentral gyrus. B: Differences in the left rostral ACC network are found in the precentral gyrus, extending into the MFG. C: Differences in the perigenual ACC network are found in the left MTG, and SMG.

Rostral ACC

The left rostral ACC network differed in connectivity with the left precentral gyrus (30 voxels; peak value z = 4.22; peak MNI coordinates -40, 0, 44). The cluster was located on the precentral gyrus and extended into the middle frontal gyrus (MFG). Combat controls showed increased connectivity between these regions versus both PTSD patients and controls. No differences in the right rostral ACC network were found.

Perigenual ACC

Alterations in the right perigenual ACC network were found in the right superior medial gyrus (SMG; 123 voxels; peak value z = Inf; peak MNI coordinates 4, 64, 10). Reduced functional connectivity with the left SMG was found in both veterans with and without PTSD as compared to the healthy controls.

Table 3. Location and z-value of the peaks within the clusters of significant different resting state functional connectivity between the PTSD, combat control and civilian control groups per seed (F-test).

Seed	Minimum corrected-cluster size (voxels)*	Number of voxels	Peak Value (z)	MNI coordinates x	y	z	BA	Brain area	FDR-corrected *p*-value	FWE-corrected *p*-value
Right										
Caudal ACC	k=25	73	4.67	28	-24	56	6	Right Precentral Gyrus	0.027	0.039
Perigenual ACC	k=33	123	3.71	4	64	0	10	Right Superior Medial Gyrus	0.000	0.000
Left										
Caudal ACC	k=24	25	4.32	-28	-24	64	4	Left Precentral Gyrus	0.087	0.163
Rostral ACC	k=23	30	4.22	-40	0	44	6	Left Precentral Gyrus	0.315	0.230
Perigenual ACC	k=32	109	4.43	-8	64	4	10	Left Superior Medial Gyrus	0.000	0.000
		55	4.14	-56	0	-28	21	Left Middle Temporal Gyrus	0.002	0.047

*Initial height thresholded at p<0.001, minimum cluster size (k) corresponding to Bonferroni-corrected p<0.0001, using a whole brain mask (25622 voxels).

The left perigenual ACC also showed differences between the groups in resting state functional connectivity with the left SMG (109 voxels; peak value z = 4.43; peak MNI coordinates -8, 64, 4), and the left middle temporal gyrus (MTG; 55 voxels, peak value z = 4.14; peak MNI coordinates -56, 0, -28). Veterans with and without PTSD patients had reduced connectivity between the left perigenual ACC and the left SMG and MTG compared to healthy controls.

Post-hoc analyses

Educational level and comorbid depression were included as covariates in post-hoc analyses. Covarying for educational level did not affect significance of the clusters that were significantly different. Covarying for comorbid depression reduced the clustersize of the left precentral cluster in the left caudal and left rostral ACC network below the minimum cluster size threshold.

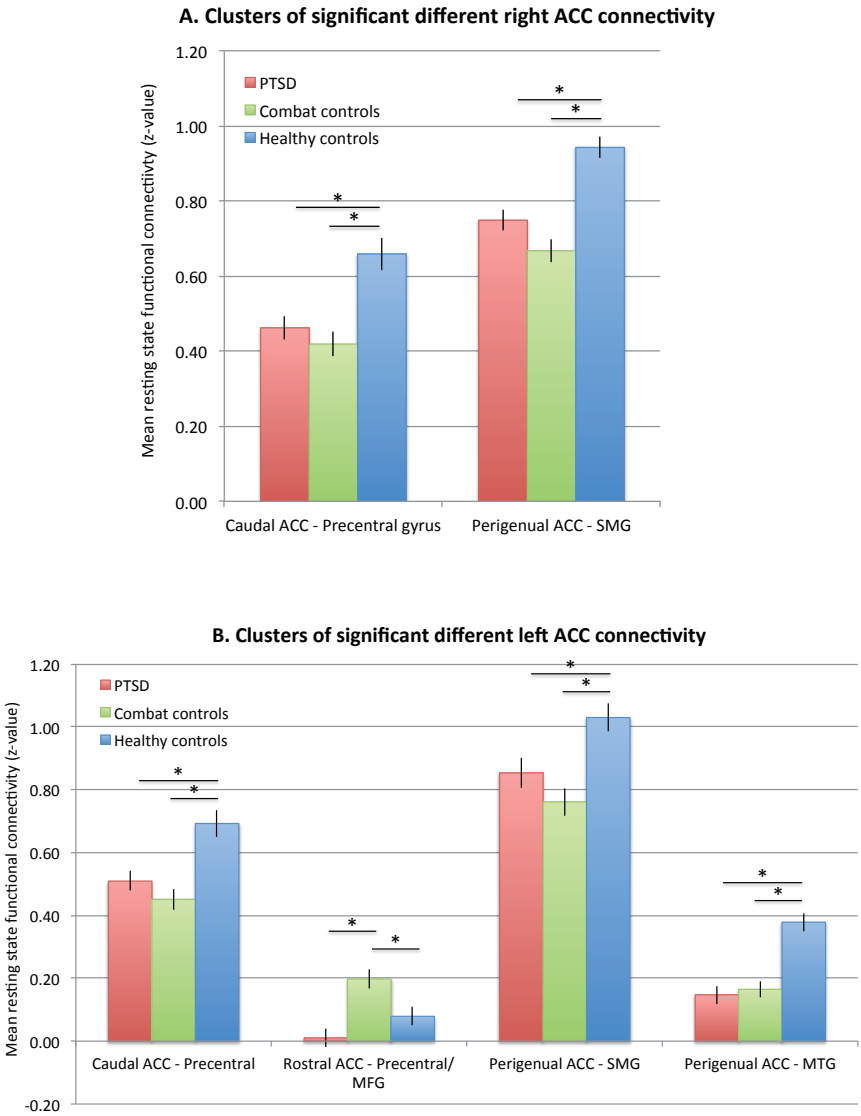

Figure 4. A: Mean resting state functional connectivity (mean z-value) for the PTSD patients (red), combat controls (green), and healthy controls (blue) for the clusters that differed between the groups for the ACC seeds located in the right hemisphere. B: Mean resting state functional connectivity (mean z-value) for the PTSD patients (blue), combat controls (red), and healthy controls (green) for the clusters that differed between the groups for the ACC seeds located in the left hemisphere.

Discussion

In this study resting state functional connectivity of five regions of the ACC were examined to determine differences between PTSD patients, combat controls and healthy controls. The three groups showed similar functional connectivity patterns, comparable with previous studies (Camchong et al., 2011; Davey et al., 2012a; Kelly et al., 2009; Margulies et al., 2007), but there were some regional differences found between groups. Differences were found in resting state functional connectivity of the caudal, rostral, and perigenual ACC. Veterans with and without PTSD showed reduced functional connectivity of the caudal ACC with the precentral gyrus and perigenual ACC with the superior medial gyrus (SMG) and middle temporal gyrus (MTG) as compared to the healthy controls. In addition, combat controls showed increased functional connectivity between the rostral ACC and precentral gyrus (extending into the middle frontal gyrus; MFG) versus PTSD patients and healthy controls.

Decreased functional connectivity between the caudal ACC and the precentral gyrus in veterans with and without PTSD compared to healthy controls suggests that military training or deployment, including trauma exposure, influence the caudal ACC network. Both the caudal ACC and precentral gyrus are involved in motor control (Chouinard and Paus, 2006; Dum and Strick, 1991; Paus, 2001). In addition, activity of the (anterior) precentral gyrus has been related to attention and memory in humans (Simon et al., 2002), and to defensive behavior in monkeys (Graziano and Cooke, 2006). Physical exercise and vigilance and alertness training that are part of military training may thus be related to the reported reduced caudal ACC connectivity with the precentral gyrus in the veteran groups (Jolles et al., 2013; Kelly and Garavan, 2005; Ma et al., 2011). Conversely, there is some evidence that supports the hypothesis that alterations in brain connectivity can occur after deployment in healthy veterans; sustained altered functional connectivity after deployment has been reported, including dorsal ACC coupling with the amygdala (Van Wingen et al., 2011b; Van Wingen et al., 2012). This indicates that differences in functional connectivity can be induced by deployment. Thus, our results in the caudal ACC network may indeed represent military training or deployment effects. However, this interpretation is still speculative, since other factors such as personality, or substance and alcohol use may also influence the results. For example, personality dimensions have also been related to altered resting state functional connectivity (Adelstein et al., 2011; Kennis, Rademaker, Geuze, 2013).

The differences between healthy controls and veterans with PTSD in connectivity of the perigenual ACC with the left middle temporal gyrus (MTG) and superior medial gyrus (SMG) are in line with studies investigating the default mode network. This is a network that is active during rest and deactivated during task performance (Greicius et

al., 2003). Reduced default mode network connectivity in PTSD patients versus healthy controls of these brain areas has been reported (Bluhm et al., 2009a; Daniels et al., 2010), as well as reduced default mode network activation during self-referential processing (Bluhm et al., 2012). Furthermore, the medial prefrontal cortex (mPFC), including the perigenual ACC and SMG, has been reported to show reduced activation in PTSD versus controls during emotional tasks (Pitman et al., 2012; Shin et al., 2005; Shin and Liberzon, 2010). Reductions of gray matter of the MTG and medial PFC have also been reported in PTSD patients versus healthy controls (Kühn and Gallinat, 2013). In line with these studies, our results also suggest alterations in the default mode network in PTSD.

However, whether these results are due to trauma exposure remains unclear, since these described studies investigated either trauma exposed or healthy controls and not both control groups. Alterations in medial PFC have been reported during negative emotional experience in veterans with and without PTSD versus healthy controls (Phan et al., 2002), although alterations in the medial PFC during exposure to traumatic memories have also been reported specifically for PTSD patients versus combat and healthy controls (Britton et al., 2005). Differences between trauma exposed healthy controls and nontrauma exposed controls in structure and connectivity of the default mode network have been reported (Ganzel et al., 2008; Geuze et al., 2008; Phan et al., 2002; Philip et al., 2013). Similarly, our results showed differences between the combat controls and healthy controls in perigenual ACC connectivity with the SMG. In addition, two resting state studies found a relation between a reduction in functional connectivity measures in the PFC after exposure to traumatic events (measured with reduced synchronisation with magnetoencephalography (James et al., 2013), and with reduced global connectivity and hub-like properties of the ventrolateral PFC and decreased local network connectivity of the dorsal ACC (Cisler et al., 2014). In line with these studies, we report decreased resting state functional connectivity between prefrontal regions (SMG-perigenual ACC) in combat exposed veterans versus healthy controls. It is therefore plausible that this reduction in connectivity during rest is related to trauma exposure. The perigenual ACC network is generally related to social processing and self-referential processing (Amodio and Frith, 2006; Kelley et al., 2002). These results suggest that experiencing a period of deployment including many stressful situations may alter the network that subserves these processes. Alternatively, military training may alter social processing and self-referential processing as well. For example being trained to follow orders, which is a social process, may alter the default mode network. In addition, a selection bias, induced by self-selection to join military service, may also be related to these differences in default mode network connectivity.

Specific differences for the combat controls versus the PTSD and healthy control group were found. Combat controls showed reduced left rostral ACC connectivity with

the precentral gyrus, extending into the MFG, as compared to the patients and healthy controls. It has been argued that differences found in trauma exposed controls in particular can provide information on resilience to developing PTSD after experiencing trauma (van der Werff et al., 2013). Therefore, it is tempting to hypothesize that increased connectivity of the precentral gyrus/MFG with the rostral ACC that is specific for combat control may be a protective factor for developing PTSD, and may be a measure of resilience. There are some studies providing support for this interpretation. Two fMRI studies have reported increased prefrontal cortex and ACC activation during attentional tasks in trauma-exposed controls specifically versus both PTSD patients and healthy nontraumatized controls (Blair et al., 2013; New et al., 2009). They argue that recruitment of the medial PFC and regions of the ACC during attentional tasks may be an effective strategy to cope with negative emotions, and thus also with traumatic experiences. Furthermore, increased MFG activation during symptom provocation paradigms has been correlated to resilience as measured with a resilience questionnaire (Daniels et al., 2012). Therefore, we complement these findings by showing reduced connectivity in the precentral gyrus/MFG with the rostral ACC in combat controls specifically. Thus, increased precentral gyrus/MFG –rostral ACC connectivity may be related to successful coping with trauma exposure (resilience). However, this interpretation remains speculative since other confounding factors, such as personality or drug and alcohol use may also influence the findings.

Several limitations need to be addressed. First, the healthy control group differed from the PTSD patients on educational level. However, it is unlikely that this influenced the results, since including ISCED level as covariate did not affect the results. Second, this study only included male veterans with PTSD. Thus, the results may not be generalized to females and to the healthy population. Third, the majority of our PTSD group had comorbid major depressive disorder (as is consistent with most PTSD studies). Differences between PTSD patients with and without major depressive disorder have been reported (Kemp et al., 2007; Kennis et al., 2013; Lanius et al., 2007). Whether these results are specific to PTSD as compared to other major depressive disorder remains unclear. However, post-hoc analysis, in which current diagnosis of depression was included as a covariate, did not change the majority of results. Fourth, we restricted analyses to positive functional connectivity, since global signal regression was applied which can induce spurious negative functional connectivity. Therefore, this study is limited to the regions that show similar activation patterns, and does not investigate regions that are anticorrelated to our seeds. Finally, we resliced our scans into 4 mm^3 isovoxels, as this is the closest round number to our original acquired voxel size. Although, this is a relatively large voxel size for functional connectivity analyses (e.g. 1 mm^3 (Kelly et al., 2009)). Therefore, our results carry a higher risk for partial volume effects. Though, functional

connectivity studies on the default mode network in PTSD apply similar methods (Bluhm et al., 2009; 4 mm^3 isovoxels, Daniels et al., 2010; 5 mm^3 isovoxels).

In order to gain a complete image of the alterations in the brain network in PTSD and after trauma exposure, whole brain complex network analysis, such as graph theory-based network analysis (Rubinov and Sporns, 2010), should be applied. In contrast with seed-based analysis, where only a limited number of interactions are investigated, whole brain interaction analyses may identify key brain areas that are altered in functional connectivity in patients versus controls. By mapping out alterations of brain networks in patients with PTSD versus trauma-exposed and non trauma exposed controls, better treatments for the disorder can be developed in the future. For example, mindfulness and meditation have been shown to alter resting state functional connectivity of the default mode network (Kilpatrick et al., 2011; Taylor et al., 2011; Taylor et al., 2013). In addition, resting state networks can be modulated by brief transcranial magnetic stimulation (TMS (Chen et al., 2013)). Thus, when deviations in resting state networks are mapped (that differ from both trauma-exposed controls as healthy controls), methods to modify these networks can be applied in order to treat psychiatric disorders such as PTSD. In addition, resting state parameters in fMRI have been shown to be predictive of the (development of) symptom severity in PTSD (Lanius et al., 2010; Zhou et al., 2012), and resting state parameters measured with fMRI or positron emission tomography have been related to treatment outcome in depression (Guo et al., 2013; Mayberg et al., 1997). Thus, further exploration of resting state characteristics of PTSD versus trauma-exposed controls and healthy non trauma exposed controls may potentially be helpful for diagnostic and predictive purposes.

Conclusion

This was the first study that focussed on resting state networks of the ACC in PTSD. The results show that the caudal and perigenual ACC network differed between veterans with and without PTSD and healthy controls. Furthermore, combat controls had increased connectivity in the rostral ACC network compared to PTSD patients and healthy controls. These results indicate that military training, deployment or trauma exposure may alter resting state functional connectivity. The regional ACC connectivity differences we demonstrated underline the importance of distinguishing trauma-exposed combat controls and healthy civilian controls when studying PTSD.

Acknowledgements and disclosure

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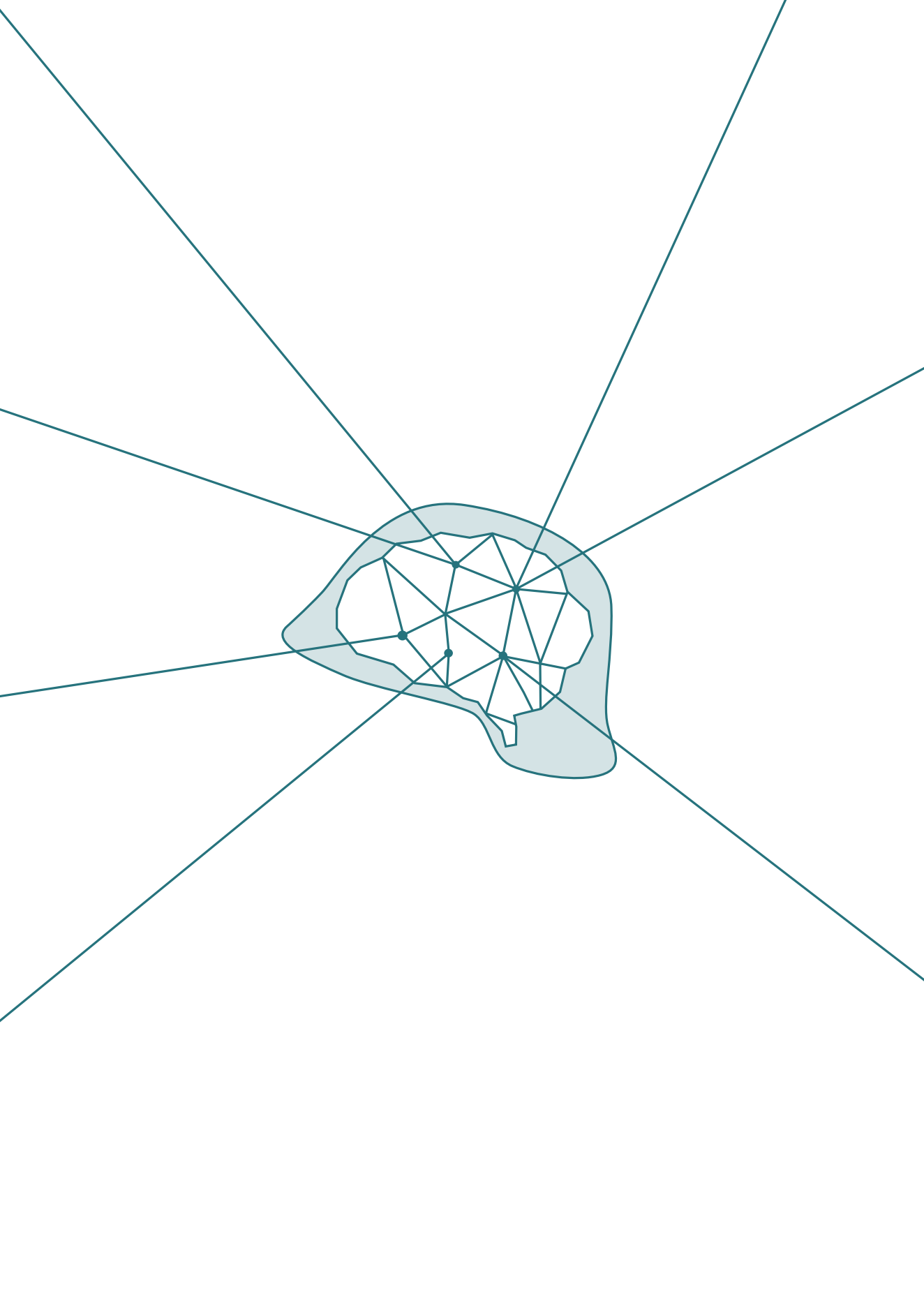

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Altered functional connectivity in posttraumatic stress disorder with versus without comorbid major depressive disorder: a resting state fMRI study

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Abstract

Posttraumatic stress disorder (PTSD) is an anxiety disorder that is often diagnosed with comorbid depressive disorder. Therefore, neuroimaging studies investigating PTSD typically include both patients with and without comorbid depression. Differences in activity of the anterior cingulate cortex (ACC) and insula have been shown to differentiate PTSD patients with and without major depressive disorder (MDD). Whether or not comorbid MDD affects resting state functional connectivity of PTSD patients has not been investigated to our knowledge. Here, resting state functional connectivity of PTSD patients with (PTSD+MDD; n=27) and without (PTSD-MDD; n=23) comorbid MDD was investigated. The subgenual ACC and insula were investigated as seed regions. Connectivity between the subgenual ACC and perigenual parts of the ACC was increased in PTSD+MDD versus PTSD-MDD, which may reflect the presence of depressive specific symptoms such as rumination. Functional connectivity of the subgenual ACC with the thalamus was reduced, potentially related to more severe deficits in executive functioning in the PTSD+MDD group versus the PTSD-MDD group. In addition, the PTSD+MDD group showed reduced functional connectivity of the insula with the hippocampus compared to the PTSD-MDD group. However, this cluster was no longer significantly different when PTSD patients that were using medication were excluded from analyses. Thus, resting state functional connectivity of the subgenual ACC can distinguish PTSD+MDD from PTSD-MDD, and this may therefore be used as a neurobiological marker for comorbid MDD in the presence of PTSD. As PTSD+MDD are more treatment resistant, these findings can also guide treatment development, for example by targeting the subgenual ACC network with treatment.

Introduction

Posttraumatic stress disorder (PTSD) is an anxiety disorder that can develop after a traumatic event. It is characterized by re-experiencing the traumatic event, avoidance of trauma reminders and emotional numbing symptoms, and increased arousal (American Psychiatric Association, 1994). PTSD frequently co-occurs with other Axis I psychiatric disorders, such as major depressive disorder (MDD (Kessler et al., 1995)). Patients with both PTSD and depression were found to have more psychological distress and are also more treatment resistant than patients with PTSD or depression alone (Campbell et al., 2007; Chan et al., 2009; Morina et al., 2013). Studies have demonstrated that comorbidity between mood and anxiety disorders increases risk for cardiovascular disease, autoimmune diseases and mortality (Boscarino, 2004; Phillips et al., 2009). In addition, depressive symptom severity and comorbidity of MDD are related to poorer executive functioning in PTSD (Olff et al., 2014; Polak et al., 2012). In order to better prevent, diagnose or treat these disorders it is of importance to determine biological overlap and differences between mood and anxiety disorders, and also the effect of comorbidity. About 48% of PTSD patients were found to have comorbid MDD in a large national survey in the United States (Kessler et al., 1995). Therefore, studies investigating the neurobiology of PTSD often comprise patients with and without comorbid MDD. Neuroimaging studies have demonstrated dysfunction of similar brain regions in both PTSD and MDD. That is, PTSD and MDD are both associated with alterations in structure and function of the medial prefrontal cortex (mPFC), amygdala, insula, and anterior cingulate cortex (ACC (Mayberg 1997; Pitman et al., 2012; Shin and Liberzon, 2010)). To what extent comorbid MDD contributes to the reported neurobiological alterations of PTSD is yet to be determined.

Thus far, two neuroimaging studies have directly investigated differences in PTSD patients with and without comorbid MDD. First, reduced activity of the mPFC and amygdala was found in PTSD patients with comorbid MDD versus PTSD patients without MDD, when fearful faces were presented (Kemp et al., 2007). Second, during a symptom provocation paradigm PTSD patients with comorbid MDD had decreased activity in the insula, and increased ACC and posterior cingulate cortex (PCC) activation versus PTSD patients without MDD (Lanius et al., 2007). In addition, decreased insula activation remained significant after controlling for PTSD severity. One other study has investigated the effects of depressive symptoms in PTSD patients. A positive correlation between depressive symptoms and (para)hippocampal and ventral ACC activity during an emotional memory task was observed in PTSD patients. A fourth fMRI study involving PTSD patients versus both controls and MDD patients found increased activity in several brain areas of PTSD patients including the insula when emotional pictures were presented (Whalley et al., 2009).

The four studies discussed above were limited by small sample sizes (8 PTSD-MDD, 8 PTSD+MDD (Kemp et al., 2007), 11 PTSD-MDD and 15 PTSD+MDD (Lanius et al., 2007), 21 PTSD+MDD and 12 PTSD-MDD (Thomaes et al., 2013), 16 PTSD and 16 MDD (Whalley et al., 2009)). In addition, these studies investigated neurobiological alterations during emotional tasks, potentially inducing PTSD (and/or depressive) symptoms. It is expected that PTSD and/or MDD symptom provocation induces an altered state in PTSD with or without MDD, which is reflected by alterations in brain activity. Whether regular functioning of the brain in the absence of symptom-inducing stimuli deviates in PTSD with versus without comorbid MDD remains unclear. To our knowledge, functioning of the brain during resting state, without presenting stimuli or requiring task performance, has not been investigated in PTSD patients with and without comorbid MDD. Thus, the effect of comorbid MDD on brain functioning at baseline of PTSD patients deserves further investigation.

Here, we investigate the effects of comorbid MDD on resting state functional connectivity in PTSD patients. Since the studies described above indicated that functioning of the ACC distinguishes PTSD with and without MDD during emotional tasks (Kemp et al., 2007; Lanius et al., 2007; Thomaes et al., 2013), this brain area was chosen as a region of interest. MDD has been associated with alterations in structure (Drevets, Savitz, Trimble, 2008), function (Gotlib et al., 2005), structural connectivity (Cullen et al., 2010), and reduced resting state functional connectivity (Davey et al., Schneider, 2013; Greicius et al., 2007; Sheline et al., 2010) of the subgenual ACC in particular, which is a subdivision of the ventral ACC. In addition, subgenual ACC activation and cortical thickness have been associated with symptom improvement in PTSD (Dickie et al., 2011; Dickie et al., 2013). Therefore, the subgenual ACC was selected as a more specific region of interest. Second, alterations in activation of the insula also differed between PTSD patients with and without PTSD, even when controlling for PTSD severity (Lanius et al., 2007). Furthermore, insula activation distinguished PTSD patients from MDD patients (Whalley et al., 2009). Alterations in structure (Shin and Liberzon, 2010; Sprengelmeyer et al., 2011), function (Shin and Liberzon, 2010; Sliz and Hayley, 2012), and resting state functional connectivity (Manoliu et al., 2014; Sripada et al., 2012a) have been reported in PTSD patients and MDD patients respectively. Thus, the insula was chosen as a second region of interest. As increased ACC activity was found in PTSD with comorbid MDD, as well as a positive correlation of ACC activity with depressive symptoms, we hypothesize that functional connectivity of the subgenual ACC is increased in PTSD with versus without comorbid MDD. Since insula activity is increased in PTSD versus MDD and insula activity was reduced in PTSD with comorbid MDD versus PTSD without MDD, we expected to find lower insula functional connectivity in PTSD with MDD as compared to PTSD without MDD. In summary, in order to provide more insights into the potential

effects of MDD on the neurobiology of PTSD, the present study examined the effects of comorbid MDD on subgenual ACC and insula resting state functional connectivity in PTSD patients.

Methods

Participants

In total, 30 male veterans with PTSD with comorbid MDD (PTSD+MDD, mean age 34.2 ± 8.5), and 25 male veterans with PTSD without comorbid MDD (PTSD-MDD, mean age 37.4 ± 10.1) were included in this study. All patients were recruited from the Military Mental Healthcare Center, the Netherlands. Patients were included after a clinician (psychologist or psychiatrist) diagnosed PTSD with or without MDD. PTSD and MDD diagnoses were confirmed using the Clinician Administered PTSD scale (CAPS (Blake et al., 1995)) and the Structural Clinical interview for DSM-IV (SCID (First et al., 1997)). A clinician, a trained PhD student or a trained research assistant administered the interviews. Training included a CAPS training, and additionally observing at least five interviews, and performing at least five interviews under supervision of an experienced clinician. Several patients were medication naive (PTSD+MDD; n=15, PTSD-MDD; n=13), some patients were currently taking antidepressants (e.g. selective serotonin reuptake inhibitors; PTSD+MDD; n=4, PTSD-MDD; n=5), and some patients used benzodiazepines (PTSD+MDD; n=4, PTSD-MDD; n=1), or both antidepressants and benzodiazepines (PTSD+MDD; n=2, PTSD-MDD; n=2). One patient from the PTSD+MDD group used both antipsychotics and antidepressants. Most of the veterans had been deployed to Afghanistan (n=28) and to the former Yugoslavia (n=10). After receiving a complete written and verbal description of the study, all participants gave informed consent. Participants received financial compensation of €250 for their participation. The Medical Ethical Committee of the UMC Utrecht approved the study (protocol number NL29550.041.09), and the study was performed in accordance with the Declaration of Helsinki (World Medical Association, 2004).

Data acquisition

Functional and structural images were obtained using a 3.0 Tesla magnetic resonance imaging scanner (Philips Medical System, Best, the Netherlands). Before the resting state scan, a ten minute T1-weighted high-resolution image (TR = 10 ms TE = 4.6 ms flip angle 8, 200 slices sagittal orientation, FOV 240 × 240 × 160, 304 × 299 matrix) was acquired. This image was utilized for co-registration and segmentation purposes and also allowed the participants to adapt to the scanner environment. During the nine minute resting state scan participants were asked to relax, to let their mind wander and to focus on a fixation cross. Three hundred and twenty T2* echoplanar interleaved images were

collected (TR = 1600 ms, TE = 23 ms, flip angle = 72.5°, 30 transverse slices, FOV 256 × 208 × 120, 64 × 51 matrix).

Image analyses

Preprocessing was conducted with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/), which included slice-timing correction, realignment, co-registration with the anatomical scan, normalization, and spatial smoothing (8 mm FWHM). Five participants (2 PTSD+MDD, 3 PTSD-MDD) were excluded due to excessive motion (more than 2 mm displacement in any direction (x, y or z) or 2 degrees rotation (pitch, roll or yaw)).

The Data Processing Assistant for Resting-State fMRI (DPARSF) was utilized for further analyses (restfmri.net (Song et al., 2011)), which is based on MRIcroN (http://www.mricro.com), SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/), and the Resting-State fMRI Data Analysis Toolkit (Song et al., 2011). Resting state images were band-pass filtered (0.08-0.01 Hz) to reduce low-frequency drift and high-frequency noise, and detrended to correct for general signal drift. In order to correct for physiological processes and motion, the motion parameters from the realignment step, mean global signal, white matter signal, and cerebral spinal fluid signal were included as covariates in the analysis. In addition, motion scrubbing was applied to scans that surrounded a minimum of 0.5 mm frame displacement (one scan before displacement, two scans after displacement), using nearest neighbour interpolation (Power et al., 2012). A minimum of approximately 5 minutes of resting state (183 unscrubbed resting state images) was set as a required threshold for correct scrubbing. One participant was excluded due to excessive scrubbing, resulting in the following groups: 27 PTSD+MDD, and 22 PTSD-MDD.

Functional connectivity analysis

For the subgenual ACC two spherical seeds (left and right, 3.5 mm radius) were created around two seed point coordinates, as previously described by Kelly *et al.* (2009). The anterior insula seed was created from two distinct anterior insula subdivisions that were described as the insula regions involved in emotion and cognition, as reported by Kelly *et al.* (2012). The mean time series for each of those seeds was extracted for all individuals and correlated with the time series of every voxel in the brain in order to create functional connectivity maps. These correlation maps were normalized using Fishers z-transform, resulting in a z-map for each ACC network per participant. The individual z-maps were used for second-level group analysis (full factorial design, SPM). A general effect of group (F-test) was investigated to determine group differences within the positive and negative network of the seed pairs.

Cluster-level multiple comparison correction was applied according to Gaussian Random Field theory (Cox 1996). A height threshold of $p<0.001$ was applied and combined with an cluster threshold,extent that corresponds to a corrected $p<0.05$ (as determined with 1000 Monte Carlo simulations using Alphasim, implemented in the REST toolbox).

In addition, functional connectivity values (z-values) were extracted from the peak voxels of clusters of significant differences in order to perform post-hoc correlations with PTSD and MDD symptom severity. Post-hoc correlation analyses were performed including the total CAPS score and the signal extracted from the peaks of clusters of significant connectivity differences, in order to assess whether the results are related to PTSD severity. In addition, the relation of positive affect (PA) score from the mood and anxiety questionnaire (MASQ (Watson and Clark, 1991)), which has been reported to reflect a core feature of MDD (de Beurs et al., 2007), to the functional connectivity of the peak of the clusters of significant difference was assessed. Subsequently, correlations between whole brain functional connectivity and CAPS and inverse PA scores were calculated respectively. Finally, we performed a post-hoc analysis on a subsample of medication naive patients and patients that occasionally used benzodiazepines, but had not taken benzodiazepines at least 48 hours prior to scanning.

Results

Participants

Groups did not differ significantly in age, handedness, the number of times they were deployed, the time since their last deployment, and educational level as measured with the international standard classification of education (ISCED (Schneider, 2013)). The PTSD+MDD group differed from the PTSD-MDD group in total PTSD severity (CAPS score; p=0.008), which appeared to be largely driven by differences in avoidance and emotional numbing symptom scores (cluster C; p=0.001). In addition, the PTSD+MDD group had lower PA scores versus the PTSD-MDD group (p=0.012), while negative affect and somatic anxiety did not differ between groups. In the PTSD+MDD group 10 patients were diagnosed with a comorbid anxiety disorder (n=10), and one patient had a comorbid somatoform disorder. In the PTSD-MDD group seven patients met the current diagnostic criteria for a comorbid anxiety disorder, one patient had a somatoform disorder only, and one patient was diagnosed with both a comorbid anxiety and somatoform disorder. An overview of demographical and clinical data is presented in Table 1.

Functional connectivity

Spatial connectivity maps

Figure 1 shows the positive and negative networks for the bilateral insula and the bilateral subgenual ACC. Positive functional connectivity of the subgenual ACC was found with the ventromedial PFC, temporal regions (including the hippocampus) and a posterior cluster comprising the PCC/precuneus. Positive functional connectivity of the insula was found around the insular lobe, extending into the temporal and parietal lobe. A medial cluster around the dorsal ACC showed positive functional connectivity with the insula.

Table 1. Demographic and clinical characteristics of the PTSD+MDD and the PTSD-MDD group.

	PTSD + MDD (mean ± SD)	PTSD - MDD (mean ± SD)	df	Sig. (two-tailed)
N	27	22		
Age (range 21-57)	37.41 (±10.12)	33.87 (±8.43)	47	0.239
Education (ISCED level)	4.00 (±1.20)	3.65 (±1.23)	46	0.311
Handedness (Right/Left/Ambidexter)	(21 / 4 / 2)	(20 / 0 / 2)	2	0.169
Number of times deployed (range 1-15)	2.16 (±1.43)	3.18 (±4.22)	45	0.898
Time since last deployment (years)	8.00 (±8.537)	7.05 (±8.72)	45	0.706
Country of last deployment				
Afghanistan	13	17		
Former Yugoslavia	6	3		
Other	8	5		
CAPS total score	75.15 (±12.45)	65.09 (±12.87)	47	0.008*
Cluster B	22.67 (±5.61)	22.64 (±5.43)	47	0.985
Cluster C	27.48 (±8.76)	18.59 (±8.30)	47	0.001*
Cluster D	25.00 (±4.47)	23.86 (±4.97)	47	0.404
Negative Affect (MASQ)	52.12 (±14.91)	46.00 (±10.50)	42	0.130
Positive Affect (MASQ)	40.87 (±15.80)	51.70 (±10.50)	42	0.012*
Somatic Anxiety (MASQ)	44.75 (±13.52)	41.50 (±10.91)	42	0.392
Current comorbid disorder (SCID)				
Major depressive disorder	27	-		
Anxiety disorder	10	7		
Anxiety disorder & somatoform disorder	-	1		
Somatoform disorder	1	1		

*Significant differences between groups; $p<0.05$

Group differences

Subgenual ACC

Reduced functional connectivity of the PTSD+MDD group versus the PTSD-MDD group was found in functional connectivity of the subgenual ACC with the bilateral thalamus (Left thalamus; 29 voxels; peak value F=25.71; peak MNI-coordinates x=-12, y=-16, z=4. Right thalamus; 16 voxels; peak value F=34.37; peak MNI-coordinates x=20, y=-12, z=4). Increased functional connectivity was found between the subgenual ACC and perigenual regions of the ACC (peak in left perigenual ACC; 100 voxels; peak value F=25.71; peak MNI-coordinates x=-12, y=40, z=-4) in the PTSD+MDD group versus the PTSD-MDD group (see Figure 2, Figure 3 and Table 2).

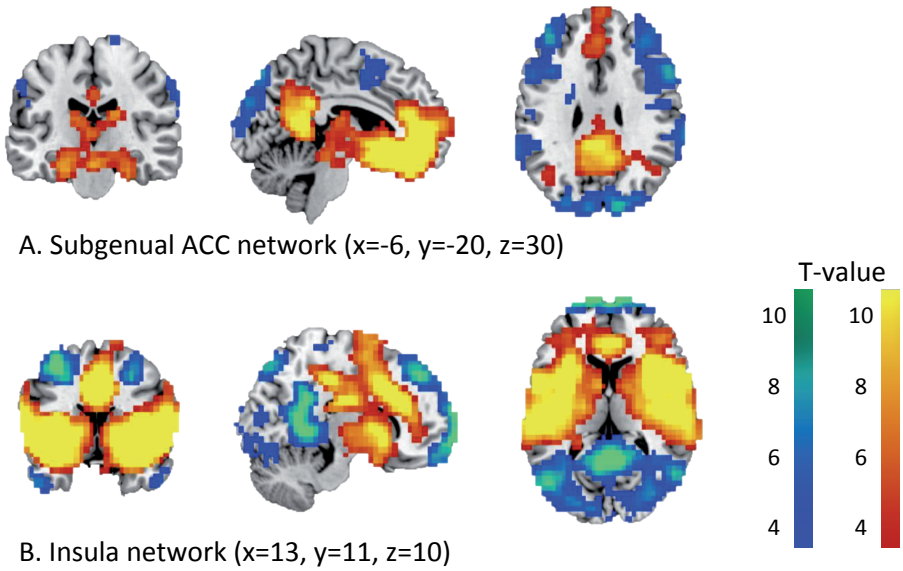


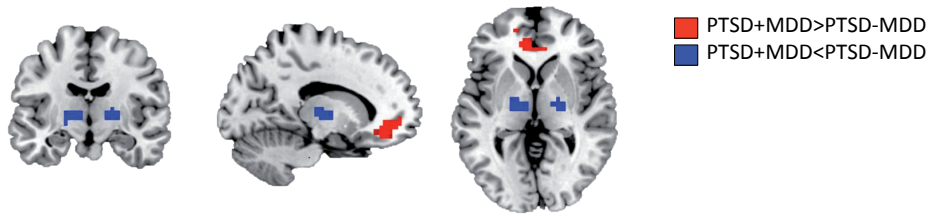
Figure 1. Functional connectivity of the subgenual ACC (A), and insula (B) seeds. Positive connectivity is represented in red-yellow and negative connectivity in blue-green. The effects were FDR corrected $p<0.001$ for illustrative purposes.

Insula

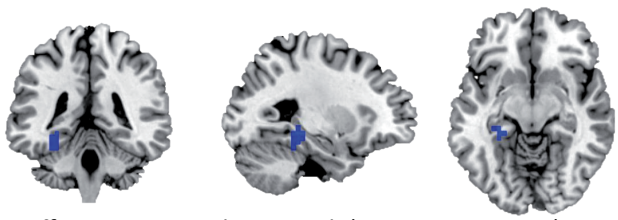
Functional connectivity of the bilateral insula with the left hippocampus (17 voxels; peak value F=19.05; peak MNI-coordinates x=-28, y=-32, z=-8) was reduced in the PTSD+MDD group as compared to the PTSD-MDD group, which showed no functional connectivity between these regions (see Figure 2, Figure 3, and Table 2).

Post-hoc analyses

Post-hoc correlation analyses of the peak voxels of significant functional connectivity difference with CAPS total, CAPS symptom cluster, and inverse PA scores were performed within both groups separately. No significant correlations were found between the peak voxels and total CAPS score and inverse PA scores. Correlations with symptom clusters revealed two significant correlations and these correlations are also represented over all participants for illustrative purposes (Figure 4). Within the PTSD+MDD group CAPS cluster B scores correlated negatively with connectivity of the subgenual ACC with the peak voxel of significant difference in the perigenual ACC (r = -0.396, p=0.041; Figure 4a). CAPS cluster C scores correlated negatively with connectivity of the subgenual ACC with the peak voxel of significant difference in the left thalamus (r = -0.523, p=0.012) within the PTSD-MDD group (Figure 4b). No correlations were found between CAPS cluster D scores or inverse PA scores and the peak voxels of difference in connectivity.



A. Differences in subgenual ACC network (x=-11, y=-15, z=-1)



B. Differences in insula network (x=-27, y=-40, z=-9)

Figure 2. Clusters of significant different functional connectivity of the insula (A) and subgenual ACC (B) seeds. Increased functional connectivity in PTSD+MDD versus PTSD-MDD is shown in red and reduced connectivity in blue (FDR corrected $p<0.05$).

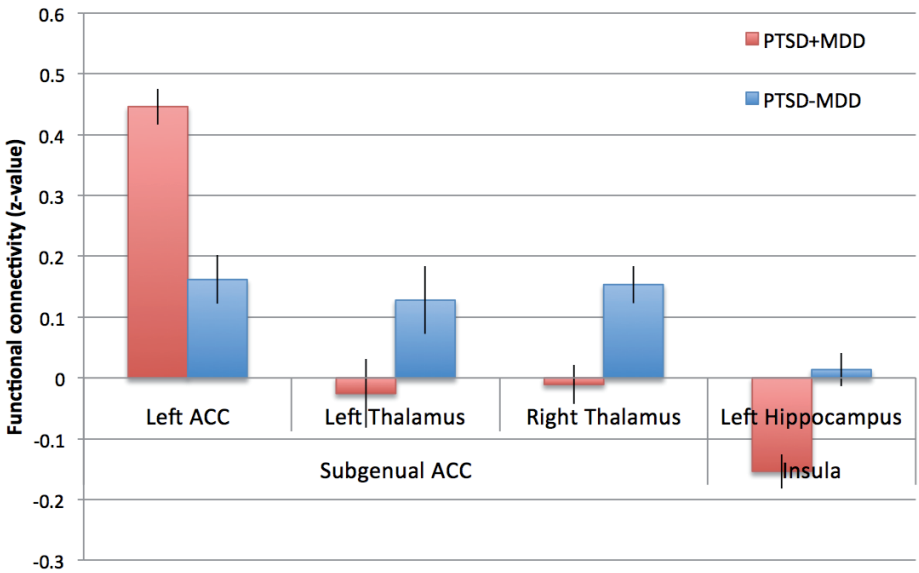


Figure 3. Functional connectivity of peak voxels of significant differences for the subgenual ACC and insula network. Z-values of the peak voxels for the PTSD-MDD group (red) and the PTSD+MDD (blue) group are presented. Error bars represent the standard error of the mean. Z-values of the peak voxels for the PTSD-MDD group (red) and the PTSD+MDD (blue) group are presented.

Table 2. Peak voxels of significant differences between PTSD+MDD and PTSD-MDD for the subgenual ACC and insula.

Network	Number of voxels (k)	Peak Value (F)	MNI coordinates x	y	z	Brain area
Subgenual ACC	100	25.71	-12	40	-4	Left Anterior Cingulate Cortex
	29	23.79	-12	-16	4	Left Thalamus
	16	34.37	20	-12	4	Right Thalamus
Insula	17	19.05	-28	-32	-8	Left Hippocampus

Exploring the relation of whole brain subgenual ACC connectivity with CAPS and inverse PA scores revealed a negative correlation of CAPS and inverse PA scores with subgenual ACC-PCC/precuneus connectivity, amongst other regions (see Supplementary Figure S1). In addition, a negative correlation was found between CAPS and inverse PA scores and negative functional connectivity of the insula with the PCC/precuneus (see Supplementary Figure S1).

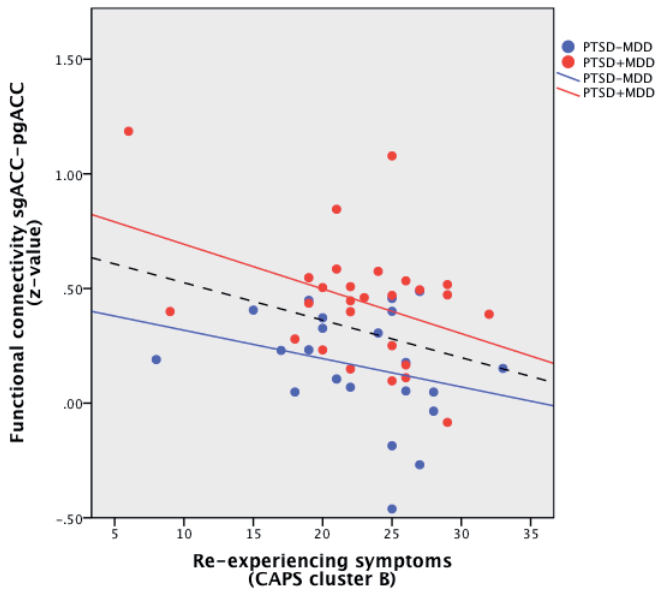
Finally, when PTSD patients that were taking medication were excluded from analyses (PTSD+MDD n = 20, PTSD-MDD n = 15) similar clusters of significant differences for the subgenual ACC network were found. The cluster of significant differences in functional connectivity between the hippocampus and insula was no longer significant in this subsample.

Discussion

This study compared PTSD patients with and without comorbid depressive disorder and revealed differences in resting state functional connectivity of the subgenual ACC with the perigenual ACC and thalamus, and of the insula with left hippocampus. This study complements previous task-based studies (Kemp et al., 2007; Lanius et al., 2007) by showing that differences in the subgenual ACC and insula between PTSD patients with and without comorbid MDD are already apparent during resting state functional connectivity, in the absence of symptom-inducing stimuli or task performance. Based on these findings, it can be hypothesized that MDD comorbidity in the context of PTSD is related to general alterations in subgenual ACC and insula functioning.

Increased subgenual ACC connectivity with the perigenual ACC was found in PTSD+MDD versus PTSD-MDD, which is in line with neuroimaging studies that have found increased resting state functional connectivity between the subgenual ACC and perigenual ACC in MDD versus controls (Davey et al., Schneider, 2013; Greicius et al., 2007; Sheline et al., 2010). Reduced functional connectivity of ACC regions has been shown in PTSD patients versus controls (Bluhm et al., 2009a). Thus, the current finding

A



B

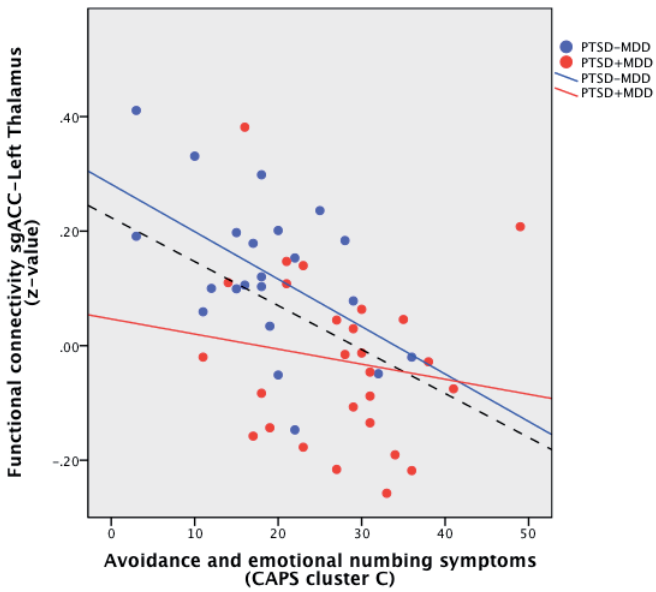


Figure 4. Correlations between CAPS symptom cluster scores and resting state functional connectivity of peak voxels of the significant different clusters within the PTSD+MDD group (red) and the PTSD-MDD group (blue). A correlation line for the whole group is also represented for illustrative purposes (dashed black line). Connectivity of the subgenual ACC with the perigenual ACC correlated with cluster B symptoms (re-experiencing; 4A). Connectivity of the subgenual ACC with the left thalamus correlated with cluster C symptoms (avoidance and emotional numbing; 4B). Abbreviations: sgACC: subgenual ACC, pgACC: perigenual ACC.

of increased connectivity of the subgenual ACC with the perigenual ACC may indeed be a marker of the presence of MDD in the context of PTSD. The perigenual ACC, which is part of the medial PFC, has been related to self-referential processing (Amodio and Frith, 2006), which underlies depressive symptoms such as helplessness, self-reproach and (guilt) rumination (Davey et al., Schneider, 2013; Lemogne et al., 2012). Increased resting state functional connectivity in the medial PFC (including the perigenual ACC) has been directly related to rumination in MDD (Nejad, Fossati, Lemogne, 2013) while decreased functional connectivity with the medial PFC has been related to autobiographical memory recall in PTSD versus controls (St. Jacques, Kragel, Rubin, 2013). Altered functioning of the medial PFC during self-referential processing tasks has also been found in MDD patients versus controls (Grimm et al., 2011; Johnson et al., 2009; Lemogne et al., 2012) (reduced medial PFC deactivation), and in PTSD versus controls (Bluhm et al., 2012) (reduced medial PFC activation). Increased subgenual-perigenual ACC connectivity in the PTSD+MDD group versus the PTSD-MDD group could thus reflect a difference in self-referential processing, and potentially reflects symptoms such as rumination. However, this was not directly investigated here, and is subject to further investigation.

A negative correlation between re-experiencing symptoms and functional connectivity of the subgenual ACC and perigenual ACC was found within the PTSD+MDD group (and across all patients). The same pattern was visible in the PTSD-MDD group, although this correlation was not significant. These correlations indicate that stronger functional connectivity between the subgenual ACC and perigenual ACC is related to lower (PTSD-specific) re-experiencing symptoms. This is in line with a previous study describing reduced connectivity in midline structures during autobiographical memory recall in PTSD versus controls (St. Jacques, Kragel, Rubin, 2013), indicating that the medial PFC can indeed be involved in re-experiencing autobiographical traumatic events. Thus, stronger functional connectivity between the subgenual ACC and perigenual ACC may reflect the presence of MDD, and is also negatively related to (PTSD specific) re-experiencing symptoms.

Connectivity between the thalamus and subgenual ACC was reduced in PTSD+MDD versus PTSD-MDD, which was also reported in previous studies in both depression (Anand et al., 2005) and PTSD (Yin et al., 2011) versus healthy controls. The thalamus is the relay station of the brain (Sherman and Guillery, 2002), and can modulate attention and arousal (Portas et al., 1998). Therefore, reduced thalamus-subgenual ACC connectivity may explain the more severe problems with executive function that are prevalent in PTSD with comorbid MDD (Olff et al., 2014; Polak et al., 2012). Functional connectivity between the subgenual ACC and thalamus was negatively correlated with avoidance and emotional numbing symptoms in the PTSD-MDD group (and across all participants). Emotional numbing is a shared PTSD and MDD symptom. A weaker

connection between the subgenual ACC and thalamus, that was found in PTSD+MDD versus PTSD-MDD, may therefore reflect the presence of depression related symptoms. Thus, reduced thalamus-subgenual ACC connectivity is a marker for comorbid MDD in the context of PTSD, and also relates to avoidance and emotional numbing symptoms.

Insula connectivity with the hippocampus was reduced in the PTSD+MDD group versus PTSD-MDD. The hippocampus is a brain region that is often associated with PTSD (Geuze, Vermetten, Bremner, 2005; Pitman et al., 2012; Shin, Rauch, Pitman, 2006) and is involved in memory (Squire, 1992). Therefore, differences found in connectivity between the insula and hippocampus can be related to more severe difficulties in executive functioning that are prevalent in PTSD+MDD versus PTSD-MDD (Olff et al., 2014; Polak et al., 2012). However, the cluster was no longer significant when patients that were taking medication were excluded from analyses. Thus, hippocampus-insula connectivity differences may have been induced by medication use.

In our whole brain post-hoc correlation analysis negative correlations were found between symptom severity scores and subgenual ACC connectivity with the PCC/precuneus (see Supplementary Figure S1a). Specific correlations between CAPS scores and subgenual ACC-PCC/precuneus connectivity were also present, while controlling for inverse PA scores. The medial PFC (including ACC regions) and PCC/precuneus are regions of the default mode network (DMN), which is the network that is active during rest and deactivated during task performance (Greicius et al., 2003). DMN functional connectivity has been negatively correlated with general symptom severity in PTSD in previous studies, even when correcting for depression diagnosis (Lanius et al., 2010) and depression severity (Yoshimura et al., 2010), which is in line with our results. In addition, a negative correlation was found between symptom severity scores and negative functional connectivity (anticorrelation) between the insula and PCC/precuneus (see Supplementary Figure S1d). Alterations in anticorrelation between the insula network and the DMN has been described in PTSD and depression (Daniels et al., 2010; Manoliu et al., 2014; Sripada et al., 2012a). In healthy subjects the insula-PCC/precuneus anticorrelation represents a dynamic equilibrium between engagement of networks during different circumstances, and dysfunctional anticorrelation is thought to underlie attentional problems (Greicius and Menon, 2004). Thus, the negative correlation between symptom severity and anticorrelation between DMN and insula may reflect a disequilibrium between networks and can potentially be related to attentional problems in PTSD patients (with or without comorbid depression).

Unravelling the neurobiological features of MDD and PTSD during rest can provide insights into which specific brain areas could be targeted for effective treatments. For example, tasks, psychotherapy, or brain stimulation methods that alter functional connectivity between the regions with dysfunctional connectivity may be effective (Chen

et al., 2013; Kilpatrick et al., 2011). Future studies should investigate long-term effects of training, transcranial magnetic stimulation, transcranial direct current stimulation, or deep brain stimulation on functional connectivity. In addition, in severe treatment resistant PTSD+MDD surgical treatment may be considered, targeting the regions with altered functional connectivity. The thalamus for example has already been implicated as a target for deep brain stimulation of severe MDD (Velasco et al., 2005) and can therefore be a candidate for treatment in PTSD+MDD as well. This is particularly relevant for treatment of PTSD patients with comorbid MDD, since patients with this combination of psychiatric disorders tend to be more treatment resistant.

Limitations

This study has some limitations. First, no MDD only group or control group was included for analyses in the current study. Thus, this study does not show whether subgenual ACC and insula connectivity differs from patients with MDD only nor does it show if the patients deviate from controls. The current results only give insight in the effects of comorbid MDD in the context of PTSD, and not on general effects of PTSD or MDD. Inclusion of more control groups in future research can provide more insight in the specific effects of PTSD, MDD, and their neurobiological overlap or differences. Second, no validated measure of the severity of all MDD symptoms was included in the study. If MDD severity was measured, it would have been possible to determine common and distinct factors of PTSD symptom severity and MDD symptom severity by including both measures in a single model (as attempted in the Supplementary Figure S1). Here, MDD diagnosis was determined with the SCID, and depressive symptom severity was approximated with the positive affect scale of the MASQ, which is only representative of a subset of symptoms (reduced positive affect). Future studies should investigate the specific effect of MDD symptom severity in the presence of comorbid PTSD, measured with more sensitive and comprehensive instruments.

Conclusion

This study revealed differences between PTSD+MDD and PTSD-MDD in resting state functional connectivity of the subgenual ACC with the perigenual ACC and bilateral thalamus. Reduced connectivity of the perigenual ACC with the subgenual ACC may be related to specific depressive symptoms, such as rumination. A negative relation was found with PTSD-specific re-experiencing symptoms, indicating that reduced subgenual ACC connectivity with the perigenual ACC is a marker of MDD and negatively related to PTSD-specific symptoms. Increased thalamus connectivity with the subgenual ACC can potentially be related to deficits in executive functioning in PTSD+MDD versus

PTSD-MDD. Differences in connectivity of the insula and hippocampus were also found, but may have been induced by confounding effects of medication. The current study shows the potential of resting state analyses to differentiate between PTSD patients with versus without MDD, provides more insight in the neurobiological differences between these subgroups. These findings provide neurobiological markers for the presence of comorbid MDD in the context of PTSD and may potentially be targeted with treatment.

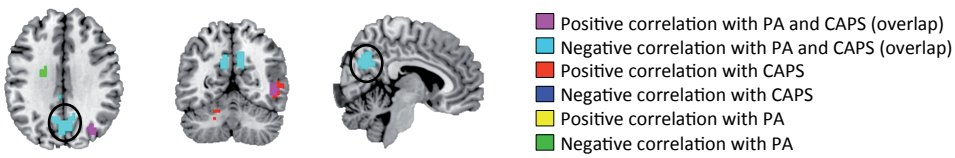
Acknowledgements and disclosure

This study was funded by the Dutch Ministry of Defence. In addition, we thank Jonathan van Leeuwen for his help with data acquisition and preprocessing.

Supplementary information

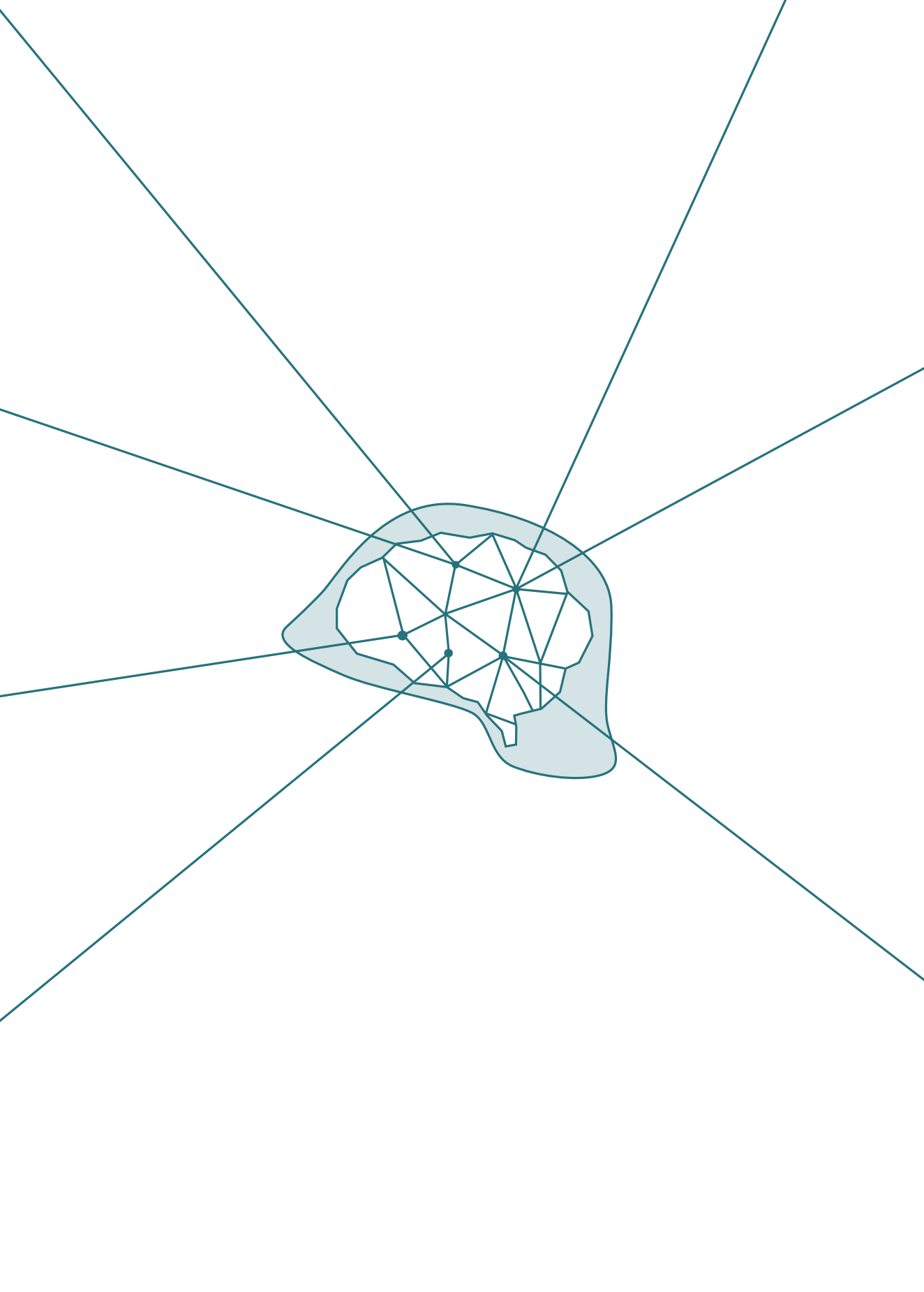
B. Subgenual ACC negative network (z=47, y=-7, x=17)

C. Insula positive network (z=13, y=-45, x=52)



D. Insula negative network (z=33, y=-58, x=6)

Figure S1. Correlations of PTSD symptom severity and reduced positive affect with subgenual ACC (a, b) and insula (c, d) functional connectivity. Violet = positive correlation with both CAPS scores and reduced PA, cyan = negative correlations with both CAPS scores and reduced PA, red = positive correlations with CAPS scores, blue = negative correlations with CAPS, yellow = positive correlations with reduced PA, and green = negative correlation with reduced PA (corrected $p<0.05$).



6

Functional network topology associated with posttraumatic stress disorder in veterans

Submitted

Co-authors: **S.J.H. van Rooij, M.P. van den Heuvel, R.S. Kahn, E. Geuze.**

Abstract

Posttraumatic stress disorder (PTSD) is a disabling disorder associated with resting state functional connectivity alterations. However, whether specific brain regions are altered in PTSD or that the whole brain network organisation differs remains unclear. PTSD can be treated with trauma-focused therapy, although only half of the patients recover after treatment. In order to better understand PTSD psychopathology, our aim was to study resting state networks in PTSD before and after treatment. Resting state functional magnetic resonance images were obtained from veterans with PTSD (n = 50) and controls (combat and civilian controls; n = 54) to explore which network topology properties (degree and clustering coefficient) of which brain regions are associated with PTSD. Then, PTSD-associated brain regions were investigated before and after treatment. PTSD patients were subdivided in persistent (n = 22) and remitted PTSD patients (n = 17) and compared with combat controls (n = 22), who were also reassessed. Prior to treatment, associations with PTSD were found for the degree of orbitofrontal, and temporoparietal brain regions, and for the clustering coefficient of the anterior cingulate cortex. No significant effects were found over the course of treatment. Our results are in line with previous resting state studies, showing resting state connectivity alterations in the salience network and default mode network in PTSD, and also highlight the importance of other brain regions. However, network metrics do not seem to change over the course of treatment. This study contributes to a better understanding of the psychopathology of PTSD.

Introduction

Posttraumatic stress disorder (PTSD) is a trauma- and stressor-related disorder that can develop after experiencing a traumatic event (American Psychiatric Association 2013). Since many veterans are exposed to traumatic events during deployment, they are at risk for developing PTSD. About 6-12% of the veterans who have been deployed to Afghanistan and Iraq develop a high level of PTSD symptoms (Hoge et al., 2004; Reijnen et al., 2014). Trauma-focused therapy is shown to be an effective therapeutic strategy for PTSD, which stimulates fear habituation and induces fear extinction of trauma-related memories (Rothbaum and Davis, 2003). However, only half of the PTSD patients recover after trauma-focused therapy (Bradley et al., 2005). In order to improve response rates it is important to understand the psychopathology of PTSD, and to determine biological markers for treatment outcome. Therefore, we investigated neurobiological alterations in PTSD and combat controls in a longitudinal design, before and after trauma-focused therapy.

PTSD has been associated with hyperactivity of limbic brain regions, such as the amygdala, and hypo-activity of brain areas involved in emotional regulation, such as the ventromedial prefrontal cortex (vmPFC; (Liberzon and Sripada, 2007; Rauch, Shin, Phelps, 2006). Over the last decade, alterations in resting state functional connectivity have also been reported in PTSD in cross-sectional studies. Resting state functional connectivity refers to a correlation between brain activation of different regions, indicating synchronization of neural activation of those regions during rest (Greicius et al., 2009). Alterations in functional connectivity between the amygdala and vmPFC have been reported in PTSD compared to controls during resting state (Bluhm et al., 2009; Brown et al., 2014; Daniels et al., 2010; Sripada et al., 2012), as well as alterations in functional connectivity between other regions (Chen and Etkin, 2013; Dunkley et al., 2014; Kennis et al., 2014; Yin et al., 2011). However, it remains unclear whether resting state functional connectivity is altered in these brain areas only in PTSD versus controls, or whether the whole brain network is altered. Moreover, it has been suggested that normalization of resting state network connectivity may be related to a reduction in (specific) PTSD symptoms (Lanius et al., 2015). For example, changes in arousal level may be related to alterations in a network including the insula and dorsal anterior cingulate cortex (ACC), and an altered sense of self can be related to alterations in a network including the medial PFC and posterior cingulate cortex (PCC; Tursich et al., 2015). However, the effect of treatment on resting state functional connectivity has not been investigated. Therefore, it is relevant to study which brain regions are in particular altered in PTSD, and if treatment effects functional connectivity of these regions.

Recently, functional magnetic resonance imaging studies have emerged investigating neurobiological effects of treatment in PTSD. Task-based activation studies reported pre-treatment differences in the prefrontal cortex, anterior cingulate cortex and

amygdala activation that normalized to control levels after treatment (Fani et al., 2011; Felmingham et al., 2007; Roy et al., 2010; Simmons et al., 2013). Pre-treatment differences in hippocampal and anterior cingulate structure (Bryant et al., 2008b; van Rooij et al., 2015c) and amygdala, ACC and inferior parietal lobule function (Aupperle et al., 2013; Bryant et al., 2008a; van Rooij et al., 2015b) have been shown to be markers of treatment outcome. This suggests that some neurobiological characteristics of PTSD may restore after treatment, while other features are stable markers for treatment outcome.

Here, we investigated resting state functional brain network topology in PTSD before and after treatment using graph theoretical analysis (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). Graph theoretical analysis applied on whole brain resting state functional connectivity provides a data driven methodology for whole brain analyses, without specific a priori seed selection (Bullmore and Sporns, 2009; van den Heuvel and Hulshoff Pol, 2010). We investigate two network metrics: degree and clustering coefficient. The degree of a brain region (a node) is the number of connections of a node, and represents the importance of a node in the network by functionally interacting with many other nodes (Rubinov and Sporns, 2010). The clustering coefficient reflects the interconnectedness of a group of nodes surrounding a node, and when this is high the nodes forms a cluster. A high clustering coefficient is indicative of functional segregation (Rubinov and Sporns, 2010). First, we investigated which whole brain functional network properties are associated with PTSD prior to treatment (baseline) using backward regression on PTSD patients and controls (including combat and civilian controls). Based on previous resting state studies, we expected that network metrics of the amygdala, hippocampus, thalamus, insula, mPFC, PCC, and precuneus are associated with PTSD.

Second, a follow up scan was acquired for the patients and combat controls six to eight months after the first scan. During that interval PTSD patients received trauma-focused therapy. To investigate treatment effects we compared the network metrics associated with PTSD between patients who still had a PTSD diagnosis after treatment (persistent PTSD), patients who recover from PTSD (remitted PTSD), and combat controls. We expected to observe normalization of the network alterations to combat control levels in remitted PTSD patients, and treatment outcome-related differences.

Materials and Methods

Participants

Fifty-three PTSD patients, 29 veteran controls (combat controls) and 26 civilian controls (healthy controls) were included, who were all male. Patients were recruited from one of four outpatient clinics of the Military Mental Healthcare Organization, The Netherlands. Patients were included after a psychologist or psychiatrist diagnosed PTSD. PTSD diagnosis was confirmed using the Clinician Administered PTSD scale (CAPS ≥ 45;

Blake et al., 1995). The Structural Clinical interview for DSM-IV (SCID-I; First et al., 1997) was applied to diagnose comorbid disorders. A trained psychologist or PhD student administered the interviews. Control participants were recruited via advertisements, and the interviews (SCID and CAPS) were also applied to investigate PTSD symptoms and psychiatric disorders. Inclusion criteria for controls were no current psychiatric or neurological disorder, and the no presence of current PTSD symptoms (CAPS ≤ 15).

After an interval of six to eight months, 39 PTSD patients and 22 combat controls were reassessed with interviews and MRI. In order to match the civilian sample to the veterans, the civilian controls followed the inclusion of the veterans. However, due to scanner updates during our protocol, and altered scan parameters, re-assessment of the civilian controls was not performed. During the 6 to 8 months interval patients received trauma-focused therapy, in line with Dutch and international treatment guidelines (Balkom et al., 2013; Bisson et al., 2007; Foa, Keane, Friedman, 2000). Trauma-focused therapy included trauma-focused cognitive behavioural therapy (TFCBT) and/or eye-movement desensitization and reprocessing (EMDR), which are both effective therapeutic strategies that have similar efficacy (Bisson et al., 2007). A clinician applied the treatment (treatment as usual), and decided which strategy was applied initially. Based on PTSD diagnosis at the reassessment according to DSM-IV criteria (American Psychiatric Association 1994), PTSD patients were divided into a remitted group (no PTSD diagnosis at reassessment; n = 17), and a symptom persistent group (PTSD diagnosis at reassessment; n = 22). After receiving a complete written and verbal description of the study all participants gave written informed consent. The Medical Ethical Committee of the UMC Utrecht approved the study, and the study was performed in accordance with the Declaration of Helsinki (World Medical Association 2013).

Image acquisition and pre-processing

Resting state functional magnetic resonance images were obtained (T2*-weighted echo planar interleaved images, repetition time TR = 1600 ms, TE = 23 ms, flip angle = 72.5°, field of view (FOV) 256 x 208 x 120, 30 transverse slices, 64 x 51 matrix, total scan time 8 min and 44.8 sec, 0.4 mm gap, acquired voxel size 4 x 4 x 3.60 mm), where participants were asked to focus on a fixation cross, while letting their mind wander and relax. Images were pre-processed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/), and the Resting-State fMRI Data Analysis Toolkit (restfmri.net; Song et al., 2011). Pre-processing included slice-timing correction, realignment, co-registration with a T1-weighted high resolution scan acquired during the same scan session (TR = 10 ms, TE = 4.6 ms, flip angle 8°, 200 sagittal slices, FOV 240 x 240 x 160, matrix of 304 x 299), normalization, spatial smoothing (8 FWHM), de-trending, and band-pass filtering (0.08-0.1 Hz). Individuals that showed excessive motion (>2mm

in x, y, z direction or >2° in pitch, roll, yaw rotation) were excluded from analyses (3 PTSD patients, 1 healthy control), resulting in baseline data of 50 PTSD patients and 54 controls, and data at reassessment of 39 PTSD patients and 22 combat controls. To correct for physiological noise and motion, nuisance parameters were included as regressors in the analyses (cerebrospinal fluid signal, white matter signal, and individual realignment parameters). Using the automated anatomical labelling (AAL) template (Tzourio-Mazoyer et al., 2002), the mean time-series of 90 anatomical structures were extracted and correlated with each other (Pearson's correlation) to create individual subject correlation matrices. The cerebellar regions were excluded, since the cerebellum was not included in the FOV for all subjects. The correlation matrices were used for calculation of network measures.

Network metrics

Network metrics were calculated with the brain connectivity toolbox (https://sites.google.com/site/bctnet/Home; Rubinov and Sporns, 2010). The individual correlation matrices were thresholded over a range of initial height thresholds (ranging from 0-0.9 in steps of 0.1), where a 0.1 threshold indicates that only correlations higher than 0.1 are preserved in the weighted correlation matrix. For each of the matrices node-specific degree and clustering coefficient were calculated (undirected). The degree of a node is the number of connections of a node that link the node to the rest of the network, indicating the importance of a node in the network (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). The clustering coefficient is the number of connections to the nearest neighbours of a node as a fraction of the maximum number of possible connections between the nearest neighbours, which is a measure of functional segregation (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010)

Statistics

To explore which pre-treatment (baseline) network properties were related to PTSD, backward Wald regression was applied (IBM SPSS statistics version 21). Backward Wald regression determines the most optimal fitted model, with a minimum number variables, that explains the factor of interest (group: PTSD versus non-PTSD). Backward regression also provides a data-driven method without a priori specified variables of interest. To circumvent co-linearity the left and right hemisphere were analysed separately, as well as the degree and clustering coefficient. In a case where the model did not run due to convergence of the variables in the algorithm, one of two variables with the highest correlation was removed from the regression model, and regarded as representing both variables in the final model. Bonferroni correction was applied for the number of backward regression models investigated ($p < 0.05/40 = 0.00125$ is deemed significant).

The brain regions that were consistently associated with PTSD in the optimal fitted model over at least four height thresholds were further investigated over the course of treatment. A minimum of four thresholds was chosen to reduce false positives found (e.g. results for a single threshold), and to retain sensitivity for detecting connectivity variables related to PTSD. To give an indication for the direction of the relation the mean b-value was calculated. For these regions repeated measure ANOVA's were utilized to assess treatment-related changes over time between remitted and persistent PTSD patients and combat controls (3 groups x 2 time points). Bonferroni correction was applied to correct for the number of brain regions that were investigated. Furthermore, correlational analyses between symptom improvement (ΔCAPS = baseline CAPS – reassessment CAPS) and change in network characteristics for the PTSD associated brain regions were explored within the PTSD group.

Results

Demographics

An overview of the demographical and clinical data can be found in Table 1 and 2. Age and handedness did not differ between PTSD patients (n = 50) and controls (n = 54; see Table 1). Educational level as measured with the international standard classification of education (ISCED; Schneider, 2013) was higher in controls than in PTSD patients, but parental education did not differ. PTSD severity as measured with the CAPS was higher in PTSD patients than in controls.

Table 1. Demographical characteristics for PTSD patients and controls at baseline. ISCED = international standard classification of education; CAPS = clinician-administered PTSD scale; SSRI = selective serotonin re-uptake inhibitor; SARI = serotonin antagonist and reuptake inhibitor.

	PTSD (mean ± SD)	Controls (mean ± SD)	Test-value (df)	*p*-value
Number of participants	50	54		
Veterans/civilian	50/0	29/25		
Age (range 21-57)	36.30 (±9.64)	35.74 (±9.68)	$t_{(102)} = -0.29$	0.769
Education (ISCED)				
Own	3.80 (±1.24)	4.53 (±1.58)	$t_{(98)} = 2.59$	0.010
Mother	2.54 (±1.35)	3.02 (±1.63)	$t_{(98)} = 1.60$	0.114
Father	3.50 (±1.92)	3.28 (±1.82)	$t_{(97)} = -0.58$	0.566
Edinburgh Handedness Inventory (Left / Ambidextrous / Right)	(4 / 4 / 41)	(2 / 4 / 48)	$\chi^2_{(2)} = 0.98$	0.614
CAPS total score	70.44 (± 13.42)	5.06 (± 4.56)	$t_{(102)} = -32.75$	$p < 0.001$

Table 2. Demographical and clinical characteristics of combat controls, remitted PTSD and persistent PTSD at baseline and at the reassessment.

	Remitted PTSD (mean ± SD)	Persistent PTSD (mean ± SD)	Combat Control (mean ± SD)	Test-value (df)	*p*-value
Number of participants	17	22	22		
Age (range 21-57)	35.12 (±9.53)	38.82 (±9.74)	36.73 (±10.67)	$F_{(2,58)} = 0.67$	0.516
Education (ISCED)					
Own	3.88 (±1.27)	3.55 (±1.14)	4.14 (±1.67)	$F_{(2,58)} = 1.00$	0.374
Mother	2.44 (±0.73)	2.48 (±1.66)	3.18 (±1.47)	$F_{(2,56)} = 1.86$	0.165
Father	3.41 (±1.66)	3.60 (±2.56)	3.90 (±1.84)	$F_{(2,55)} = 0.31$	0.732
Handedness (Left / Ambidexter / Right)	(1 / 0 / 16)	(3 / 2 / 17)	(2 / 2 / 18)	$\chi^2_{(4)} = 2.17$	0.700
Number of times deployed (1 / 2 / 3 / >3)	(4 / 4 / 4 / 5)	(9 / 3 / 6 / 3)	(7 / 6 / 4 / 5)	$F_{(2,57)} = 0.88$	0.420
Time since last deployment (years)	6.53 (±7.95)	8.86 (±9.31)	5.95 (±6.83)	$F_{(2,57)} = 0.78$	0.464
Country of last deployment					
Afghanistan	12	12	15		
Former Yugoslavia	2	6	4		
Other	3	3	3		
Time between scans in (months)	6.12 (±1.11)	6.23 (±1.07)	6.32 (±0.48)	$F_{(2,58)} = 1.88$	0.161
Total trauma-focused treatment sessions between assessments	9.18 (±6.78)	9.50 (±4.88)		$t_{(37)} = -1.73$	0.863
(<5 / 5-10 / >10)	(4 / 7 / 4)	(3 / 10 / 10)			
Clinical scores at baseline					
PTSD severity (CAPS total score)	65.00 (± 12.45)	72.95 (±14.39)		$t_{(37)} = -1.81$	0.078
Current comorbid disorder baseline (SCID)					
Mood disorder	6	16		$\chi^2_{(1)} = 5.47$	0.019
Anxiety disorder	2	11		$\chi^2_{(1)} = 6.31$	0.012
Somatoform disorder	1	1		$\chi^2_{(1)} = 0.04$	0.851
Medication					
SSRI/SARI	4	6		$\chi^2_{(2)} = 0.07$	0.791
Benzodiazepines	4	3		$\chi^2_{(1)} = 0.64$	0.425
Antipsychotics	1	1		$\chi^2_{(1)} = 0.04$	0.851
Other	1	2		$\chi^2_{(1)} = 0.14$	0.709
Clinical scores post-treatment					
CAPS total score	21.29 (±14.11)	61.36 (±17.14)		$t_{(37)} = -7.80$	$p < 0.001$

Table 2. Demographical and clinical characteristics of combat controls, remitted PTSD and persistent PTSD at baseline and at the reassessment. (*Continued*)

	Remitted PTSD (mean ± SD)	Persistent PTSD (mean ± SD)	Combat Control (mean ± SD)	Test-value (df)	*p*-value
Current comorbid disorder after treatment (SCID)					
Mood disorder	-	4		$\chi^2_{(2)} = 4.43$	0.109
Anxiety disorder	-	7		$\chi^2_{(2)} = 7.78$	0.020
Somatoform disorder	-	1		$\chi^2_{(2)} = 1.63$	0.443
Alcohol dependency	-	2		$\chi^2_{(2)} = 1.71$	0.191
Medication					
SSRI/SARI	3	9		$\chi^2_{(1)} = 3.14$	0.077
Benzodiazepines	3	1		$\chi^2_{(1)} = 1.52$	0.217
Antipsychotics	-	3		$\chi^2_{(1)} = 2.78$	0.096
Other	-	2		$\chi^2_{(1)} = 1.80$	0.180

At the reassessment, 17 PTSD patients were remitted and 22 still had a PTSD diagnosis, in line with previously response rates of 50% (Bisson et al., 2013). The remitted and persistent PTSD patients and combat controls (n = 22) did not differ in age, education, handedness, number of times deployed, time since last deployment, or time between scans (see Table 2). The remitted and persistent PTSD groups showed no difference in number of treatment sessions, and psychotropic medication (see Table 2). Persistent PTSD patients had more comorbid mood disorders at baseline, and more comorbid anxiety disorders at both time points. At baseline persistent PTSD showed a trend significant higher symptom severity at baseline compared to remitted PTSD patients.

PTSD versus controls – baseline associations

Results from the backward regression models ($p < 0.00125$) can be found in the supplementary information (Supplementary Table S1-S4). Baseline PTSD was significantly associated with degree and clustering coefficient of a variety of brain regions. Brain areas that were associated with PTSD in the optimal fitted models for at least four thresholds will be discussed below (see Table 3).

A positive mean b-value for predicting PTSD group membership was consistently (≥4 thresholds) found for the bilateral olfactory gyrus, right precuneus and left fusiform gyrus. This might indicate that PTSD had on average higher degree in these brain regions compared to controls. A negative mean b-value for predicting PTSD group membership was consistently (≥4 thresholds) found for the degree of the bilateral rolandic operculum, left orbital inferior frontal gyrus, left orbital superior frontal gyrus, right superior temporal gyrus, right inferior temporal gyrus, left angular gyrus, left superior parietal

Table 3. Frequency of PTSD-associated network metrics of brain regions that were consistently (≥4 thresholds) included in significant optimal fitted backward regression models. The mean, minimum and maximum b-values are also presented for each brain region. On average positive associations (positive mean b-value) with PTSD are presented on the top and on average negative associations (negative mean b-value) on the bottom.

	Lobe	Brain region	Mean b	Min. b	Max. b	Frequency
Positive	Frontal	Right olfactory gyrus	0.240	0.030	0.810	5
		Left olfactory gyrus	0.080	0.060	0.120	4
	Parietal	Right precuneus	0.276	-0.170	1.610	4
	Occipital	Left fusiform gyrus	0.037	-0.194	0.130	4
Negative	Central	Right rolandic operculum	-0.262	-1.020	-0.080	6
		Left rolandic operculum	-0.063	-0.440	0.460	4
	Frontal	Left orbital inferior frontal gyrus	-0.235	-0.460	-0.060	5
		Left orbital superior frontal gyrus	-0.137	-0.178	-0.090	4
	Temporal	Right superior temporal gyrus	-0.098	-0.900	0.200	6
		Right inferior temporal gyrus	-0.106	-0.520	0.260	5
	Parietal	Left angular gyrus	-0.090	-0.160	-0.040	4
		Left superior parietal gyrus	-0.160	-0.290	-0.070	4
	Limbic	Left anterior cingulate gyrus (Clustering coefficient)	-0.763	-3.040	2.450	4
		Left posterior cingulate gyrus	-0.294	-0.880	-0.040	4
		Left middle temporal pole	-0.078	-0.110	-0.050	4
	Subcortical	Left pallidum	-0.004	-0.118	0.150	5

gyrus, left posterior cingulate gyrus, left middle temporal pole, and left pallidum. This might indicate that PTSD had on average lower degree of these brain areas versus controls. The clustering coefficient from the left anterior cingulate cortex was also negatively associated with PTSD for four thresholds.

Treatment effects

There were no significant (Bonferroni corrected) group or group by time interaction effects found with the repeated measures ANOVAs ($p < 0.05/16 = 0.003$ is deemed significant). Post-hoc analysis of the remitted versus persistent PTSD patients showed a significant group by time interaction effect of the pallidum degree (threshold 0.4 and 0.5, $p < 0.003$), where remitted PTSD showed an increase in degree or clustering coefficient while persistent PTSD patients did not change over time or showed an increase. No significant correlations were observed between the difference in network metrics and the symptom improvement on the CAPS.

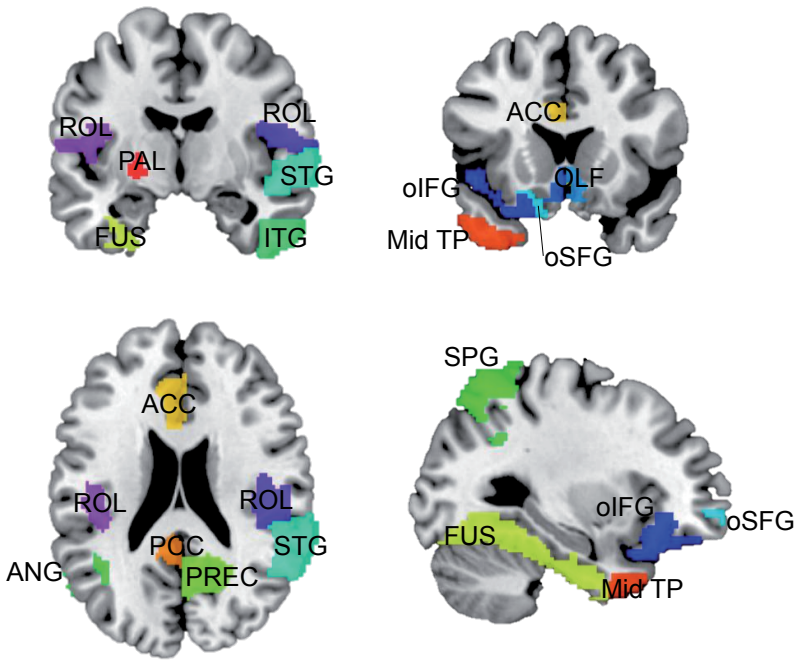


Figure 1. Brain regions with PTSD-associated clustering coefficient (ACC) and degree (all other regions). Slices y = 7 & 18; z = 22; z = -3. Abbreviations: ANG = angular gyrus, ACC = anterior cingulate cortex, FUS = fusiform gyrus, ITG = inferior temporal gyrus, Mid TP = middle temporal pole, oIFG = orbital inferior frontal gyrus, OLF = Olfactory gyrus, oSFG = orbital superior frontal gyrus, PAL = Pallidum, PCC = posterior cingulate cortex, PREC = Precuneus, ROL = rolandic operculum, SPG = superior parietal gyrus, STG = superior temporal gyrus.

Discussion

In this resting state functional MRI study, baseline PTSD-related functional whole brain network properties were investigated, and followed up after treatment. Prior to treatment, we observed that network topology of orbitofrontal regions, the left cingulate cortex, parietal regions, and temporal regions was associated with PTSD over several thresholds. This indicates that PTSD is associated with aberrant information integration in these brain regions. Longitudinal analyses showed no main effects of group or group by time interaction effects over the course of treatment in these brain regions.

Our results are in line with previous cross-sectional resting state whole brain fMRI network studies, reporting decreased orbitofrontal connectivity (Jin et al., 2013), decreased frontal and temporal degree (Suo et al., 2015), and a trend for increased precuneus degree (Lei et al., 2015). A magneto encephalography (MEG) study also reported increased

connectivity of the precuneus (amongst other regions) in PTSD (Dunkley et al., 2014). In addition, a state specific network comprising the cingulate cortex network can differentiate patients from controls (Li et al., 2014). Seed analyses have also shown reduced resting state functional connectivity of the precuneus/posterior cingulate cortex and temporoparietal regions during rest in PTSD patients versus controls, which are regions involved in the default mode network (DMN; Bluhm et al., 2009; Chen and Etkin, 2013; Sripada et al., 2012). Our results also indicate that DMN regions have reduced degree in PTSD, but on average an increased degree for the precuneus. This indicates that the DMN regions are less integrated in and of less importance for the whole brain network, except for the precuneus, which is more integrated in the whole brain network. These findings altogether suggest that reduced connectivity is present in DMN regions in PTSD, but that the number of connections of the precuneus is increased, which warrants further investigation.

Furthermore, we found associations with PTSD in the degree of the pallidum, rolandic operculum, and middle temporal pole, and in the clustering coefficient of the ACC. These are regions that may be regarded as nodes of the salience network (SN; Lei et al., 2015; Menon, 2011). Previous resting state fMRI studies indicated increased functional connectivity between SN brain regions (Daniels et al., 2010; Lei et al., 2015; Sripada et al., 2012b). A structural graph analysis also indicated higher pallidum centrality in PTSD (Long et al., 2013). This is in line with our results, showing increased importance of the pallidum in the whole brain network. However, other salience network regions had on average lower degree in PTSD (by showing a negative average b-value). This indicates that these regions are less important in the whole brain network in PTSD. Increased connectivity may therefore only be present between specific regions (such as the pallidum) or with limbic brain regions such as the amygdala and the insula, which were regions of interest in the previous resting state studies. Our results do, however, subscribe the importance of SN regions for PTSD. In addition, the average lower clustering coefficient in PTSD observed here, suggests that the ACC neighbours have reduced connectivity with each other. This may indicate that information integration in the ACC network is reduced in PTSD. Reduced ACC resting state function connectivity with the thalamus, amygdala, PCC/precuneus, and prefrontal regions has been reported in PTSD versus controls (Kennis et al., 2014; Sripada et al., 2012a; Sripada et al., 2012b; Yin et al., 2011). Thus, our results together with previous findings indicate altered connectivity and potentially information processing of the SN is associated with PTSD.

In addition to the DMN and SN, it has been suggested that the central executive network (CEN) is a third important network that can be related to dysfunction in psychiatric disorders, and this model is described as a triple network model (Menon, 2011). Our results support this model by showing an association between PTSD and

the degree of important nodes of the CEN, i.e. the superior parietal gyrus, and the orbital part of the IFG and SFG, are associated with PTSD (Menon, 2011). Future studies should investigate if resting state alterations in PTSD are specific to these three networks compared to other networks.

Furthermore, network metrics in the fusiform gyrus and olfactory cortex were on average higher in PTSD, suggesting that these brain areas are more important in the whole brain network in PTSD patients versus controls. Interestingly, altered olfactory perception has been reported in PTSD, which is strongly related to activity of the olfactory cortex (Vasterling, Brailey, Sutker, 2000; Vermetten et al., 2007; Zald and Pardo, 2000). Furthermore, increased activation of the fusiform gyrus in PTSD versus both trauma-exposed and non-trauma exposed controls was reported in a meta-analysis (Patel et al., 2012). In addition, previous studies reported higher activity of occipital brain areas in response to trauma-related pictures in PTSD (Hendler, Rotshtein, Hadar, 2001; Hendler et al., 2003), and during dissociative responses (Lanius et al., 2005; Whalley et al., 2013). Thus, we could hypothesize that altered network topology of the fusiform gyrus and the olfactory cortex may be related to altered visual and olfactory perception in PTSD, and potentially to dissociative symptoms. However, future research should establish the importance of these brain regions in PTSD.

Although we expected to find differences over the course of treatment between controls, remitted and persistent PTSD, our results did not show any group or group by time interaction effects in the longitudinal analysis. Only when comparing remitted and persistent PTSD patients only a significant interaction effect was observed in the pallidum. This indicates that treatment may alter network topology in relation to remission from PTSD, although caution should be taken when interpreting these results. Interestingly, the regions previously related to remission from PTSD or treatment outcome were not associated with PTSD at baseline in our sample (e.g. amygdala, hippocampus, medial PFC; Roy et al., 2010; Simmons et al., 2013; van Rooij et al., 2015a). Alternative approaches (e.g. using treatment-theory driven *a priori* specified seeds), potentially focussing on pallidum functional connectivity, may provide more sensitivity to treatment related alterations in neural networks.

A number of limitations has to be taken into account when interpreting the findings of this study. First, dividing the PTSD group into a persistent and remitted group resulted in two small samples. However, analysing the PTSD patient group as a whole did not reveal any general treatment effects, indicating group subdivision did not underlie the null findings. In addition, by applying whole brain analyses (and not investigating a selection of a priori regions of interest) strong multiple comparison correction was required. Therefore, to confirm that treatment may not alter PTSD-related network metrics, additional research with larger samples of PTSD patients is needed before and after treatment to investigate (heterogeneity in) remission from PTSD. Patients and controls

differed on educational level. However, since we did not find correlations with ISCED level and PTSD-related network metrics and their parental education did not differ, educational level is not likely to influence our results. Furthermore, the healthy controls were not followed up after treatment, due to scanner updates. Therefore, including them at baseline may influence the results. However, exploration of backward regression without the healthy controls showed similar brain regions (threshold 0.3), indicating that the effects were not (fully) driven by inclusion of healthy controls at baseline. Also, only one female participant applied for this study, and therefore we did not include women here. This hampers generalization of our results to women. The remitted and persistent PTSD group differed in comorbidity. However, there were no significant correlations between PTSD-related network metrics and comorbidity. Therefore, it is not expected that including patients with comorbidity majorly affects our results.

Despite the great care taken to minimize the effects of motion by including regressors (realignment parameters, cerebrospinal fluid signal and white matter signal), the BOLD signal measured to calculate resting state functional connectivity can still be confounded by other temporal patterns, such as cardiac and respiratory patterns, and motion (Van Dijk et al., 2010). Furthermore, we selected only positive connections by starting the thresholding at 0, which may influence our results by introducing a selection bias. However, this was chosen to circumvent interpreting negative correlations, which can be induced by preprocessing steps (Van Dijk et al., 2010). In addition, the methodology to create a neural network representation or connectome is relatively new, is still developing, and has many analytical degrees of freedom (e.g. thresholding, initial parcellation; Rubinov and Sporns, 2010). Therefore, we presented results of several applied thresholds. Future maturation of the methodology should provide more standard approaches in order to better compare results between studies.

Conclusion

This study indicates that resting state network measures of orbitofrontal, temporal and parietal brain regions, and the cingulate cortex are associated with PTSD. This is in line with previous studies, reporting alteration in resting state functional connectivity in the salience network and default mode network. In addition, some regions (orbitofrontal, superior parietal) of the central executive network were also found to be associated with PTSD. Therefore, our results may be interpreted from the triple network model perspective, indicating that indeed the salience, default mode and central executive network are of importance for PTSD psychopathology. However, these PTSD associated network metrics do not seem to change over the course of treatment. This study contributes to a better understanding of the psychopathology of PTSD, and PTSD treatment.

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Supplementary Information

Supplementary Table S1. Outcome of the backward regression models for 10 thresholds for the degree of left hemisphere brain regions, using group (PTSD vs control) as dependent variable.

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
0	**Model**	**Step 26**			45.04	20	$p < 0.00125$*
	Frontal_Sup_Orb_L		-0.18	0.06	9.16	1	0.002
	Frontal_Mid_Orb_L		0.11	0.06	3.30	1	0.069
	Frontal_Inf_Orb_L		-0.32	0.12	6.81	1	0.009
	Supp_Motor_Area_L		-0.24	0.14	3.24	1	0.072
	Olfactory_L		0.07	0.04	3.42	1	0.065
	Frontal_Sup_Medial_L		0.17	0.08	4.94	1	0.026
	Frontal_Med_Orb_L		0.08	0.04	3.48	1	0.062
	Cingulum_Ant_L		-0.10	0.06	2.28	1	0.131
	Cingulum_Post_L		-0.13	0.04	10.02	1	0.002
	Calcarine_L		0.06	0.04	1.80	1	0.179
	Occipital_Mid_L		0.34	0.12	7.87	1	0.005
	Occipital_Inf_L		0.29	0.11	6.29	1	0.012
	Fusiform_L		-0.19	0.10	3.89	1	0.049
	Parietal_Sup_L		-0.17	0.07	5.47	1	0.019
	Paracentral_Lobule_L		-0.12	0.07	2.61	1	0.106
	Putamen_L		0.33	0.11	9.44	1	0.002
	Pallidum_L		-0.12	0.07	2.71	1	0.100
	Thalamus_L		0.03	0.02	3.95	1	0.047
	Temporal_Mid_L		-0.25	0.15	2.81	1	0.094
	Temporal_Inf_L		-0.32	0.17	3.40	1	0.065
	Constant		58.83	18.61	10.00	1	0.002
0.1	**Model**	**Step 27**			**65.24**	**1**	$p < 0.00125$*
	Precentral_L		0.16	0.08	4.19	1	0.041
	Frontal_Sup_L		0.15	0.07	4.73	1	0.03
	Frontal_Sup_Orb_L		-0.13	0.04	8.95	1	0.003
	Frontal_Inf_Orb_L		-0.46	0.14	11.15	1	0.001
	Olfactory_L		0.12	0.04	11.31	1	0.001
	Rectus_L		0.10	0.04	6.05	1	0.014
	Insula_L		-0.23	0.09	6.48	1	0.011
	Cingulum_Mid_L		0.25	0.12	4.03	1	0.045
	Calcarine_L		0.28	0.09	10.74	1	0.001
	Lingual_L		-0.21	0.09	5.92	1	0.015

Supplementary Table S1. Outcome of the backward regression models for 10 thresholds for the degree of left hemisphere brain regions, using group (PTSD vs control) as dependent variable. (*Continued*)

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
	Occipital_Inf_L		-0.14	0.07	4.66	1	0.031
	Parietal_Sup_L		-0.29	0.09	9.56	1	0.002
	Parietal_Inf_L		0.16	0.08	4.38	1	0.036
	Angular_L		-0.12	0.04	8.01	1	0.005
	Precuneus_L		0.26	0.11	6.17	1	0.013
	Caudate_L		-0.02	0.02	2.75	1	0.097
	Temporal_Sup_L		0.41	0.13	10.23	1	0.001
	Temporal_Pole_Sup_L		-0.11	0.06	2.96	1	0.085
	Temporal_Pole_Mid_L		-0.09	0.05	3.25	1	0.071
	Constant		-10.09	6.77	2.22	1	0.136
0.2	**Model**	**Step 28**			**59.75**	**1**	***p* < 0.00125***
	Frontal_Sup_L		0.16	0.06	7.32	1	0.007
	Frontal_Sup_Orb_L		-0.09	0.03	7.83	1	0.005
	Frontal_Inf_Orb_L		-0.21	0.06	10.84	1	0.001
	Rolandic_Oper_L		-0.44	0.14	10.49	1	0.001
	Olfactory_L		0.07	0.02	10.35	1	0.001
	Cingulum_Post_L		-0.04	0.02	2.77	1	0.096
	Calcarine_L		0.09	0.04	5.34	1	0.021
	Occipital_Sup_L		0.20	0.06	9.97	1	0.002
	Occipital_Inf_L		-0.11	0.05	4.54	1	0.033
	Fusiform_L		0.11	0.06	3.11	1	0.078
	Postcentral_L		-0.14	0.05	6.34	1	0.012
	Parietal_Sup_L		-0.11	0.05	5.79	1	0.016
	SupraMarginal_L		0.19	0.07	7.64	1	0.006
	Angular_L		-0.04	0.03	2.91	1	0.088
	Pallidum_L		-0.05	0.03	2.78	1	0.096
	Heschl_L		0.18	0.08	5.51	1	0.019
	Temporal_Sup_L		0.41	0.12	11.91	1	0.001
	Temporal_Pole_Mid_L		-0.11	0.05	5.91	1	0.015
	Constant		-7.51	2.97	6.41	1	0.011
0.3	**Model**	**Step 33**			**42.89**	**1**	***p* < 0.00125***
	Frontal_Mid_L		0.08	0.04	5.09	1	0.024
	Frontal_Inf_Oper_L		0.12	0.04	7.65	1	0.006
	Frontal_Inf_Orb_L		-0.12	0.04	10.79	1	0.001
	Rolandic_Oper_L		-0.12	0.04	8.32	1	0.004
	Supp_Motor_Area_L		-0.09	0.05	3.83	1	0.050

Supplementary Table S1. Outcome of the backward regression models for 10 thresholds for the degree of left hemisphere brain regions, using group (PTSD vs control) as dependent variable. (*Continued*)

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
	Olfactory_L		0.06	0.02	12.38	1	$p < 0.001$
	Frontal_Sup_Medial_L		0.07	0.03	4.20	1	0.041
	Cingulum_Ant_L		-0.06	0.03	3.77	1	0.052
	Cingulum_Mid_L		0.20	0.06	10.67	1	0.001
	Angular_L		-0.04	0.02	3.37	1	0.066
	Paracentral_Lobule_L		0.05	0.03	3.21	1	0.073
	Pallidum_L		-0.07	0.03	9.01	1	0.003
	Temporal_Pole_Mid_L		-0.06	0.03	4.24	1	0.040
	Constant		-2.78	1.51	3.37	1	0.067
0.4	NS						
0.5	NS						
0.6	Model	Step 31			37.10	1	$p < 0.00125$*
	Precentral_L		-0.08	0.04	4.34	1	0.037
	Frontal_Sup_Orb_L		-0.15	0.05	8.01	1	0.005
	Frontal_Mid_Orb_L		0.16	0.05	10.29	1	0.001
	Frontal_Inf_Orb_L		-0.06	0.03	3.34	1	0.067
	Rolandic_Oper_L		-0.15	0.05	8.46	1	0.004
	Frontal_Med_Orb_L		0.10	0.04	5.12	1	0.024
	Insula_L		0.11	0.04	8.77	1	0.003
	Cingulum_Post_L		-0.13	0.05	5.73	1	0.017
	Cuneus_L		0.12	0.05	7.31	1	0.007
	Lingual_L		-0.09	0.04	4.03	1	0.045
	Fusiform_L		0.13	0.04	10.22	1	0.001
	Parietal_Sup_L		-0.07	0.04	3.11	1	0.078
	Parietal_Inf_L		0.07	0.04	4.49	1	0.034
	Paracentral_Lobule_L		0.05	0.04	2.30	1	0.129
	Pallidum_L		0.07	0.03	3.85	1	0.050
	Temporal_Pole_Mid_L		-0.05	0.04	2.27	1	0.132
	Constant		-0.07	0.55	0.02	1	0.899
0.7	Model	Step 36			36.05	1	$p < 0.00125$*
	Frontal_Inf_Oper_L		0.11	0.06	2.94	1	0.086
	Frontal_Inf_Tri_L		-0.29	0.09	11.16	1	0.001
	Frontal_Sup_Medial_L		0.13	0.05	6.61	1	0.01
	Insula_L		0.09	0.05	2.91	1	0.088
	Cingulum_Mid_L		-0.08	0.03	5.19	1	0.023
	Hippocampus_L		0.19	0.10	4.01	1	0.045

Supplementary Table S1. Outcome of the backward regression models for 10 thresholds for the degree of left hemisphere brain regions, using group (PTSD vs control) as dependent variable. (*Continued*)

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
	ParaHippocampal_L		-0.17	0.06	8.14	1	0.004
	Fusiform_L		0.10	0.04	7.71	1	0.005
	Angular_L		-0.16	0.07	4.89	1	0.027
	Pallidum_L		0.15	0.07	4.74	1	0.030
	Constant		-0.30	0.42	0.52	1	0.472
0.8	Model	Step 44			22.37	4	$p < 0.00125$*
	Frontal_Mid_Orb_L		0.29	0.15	3.68	1	0.055
	Frontal_Inf_Tri_L		-0.60	0.17	12.24	1	$p < 0.001$
	Cingulum_Ant_L		0.28	0.12	5.71	1	0.017
	SupraMarginal_L		0.13	0.06	4.22	1	0.040
	Constant		-0.13	0.36	0.14	1	0.713
0.9	Model	Step 36			30.90	1	$p < 0.00125$*
	Frontal_Inf_Oper_L		-0.89	0.39	5.21	1	0.022
	Rolandic_Oper_L		0.46	0.21	5.00	1	0.025
	Frontal_Med_Orb_L		-1.17	0.45	6.88	1	0.009
	Rectus_L		1.00	0.41	6.11	1	0.013
	Cingulum_Post_L		-0.88	0.46	3.67	1	0.055
	Temporal_Sup_L		-0.42	0.20	4.38	1	0.036
	Temporal_Inf_L		0.64	0.27	5.57	1	0.018
	Constant		0.97	0.50	3.73	1	0.053

Supplementary Table S2. Outcome of the backward regression models for 10 thresholds for the degree of right hemisphere brain regions, using group (PTSD vs control) as dependent variable.

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
0	**Model**	**Step 38**			**33.11**	**8**	***p* < 0.00125***
	Precentral_R		0.17	0.08	4.29	1	0.038
	Frontal_Inf_Tri_R		0.15	0.06	6.88	1	0.009
	Rolandic_Oper_R		-0.14	0.06	6.67	1	0.010
	Rectus_R		-0.05	0.03	4.18	1	0.041
	Amygdala_R		-0.14	0.06	5.20	1	0.023
	Occipital_Inf_R		0.12	0.05	6.01	1	0.014
	Temporal_Sup_R		0.20	0.11	3.19	1	0.074
	Temporal_Inf_R		-0.52	0.15	12.34	1	*p* < 0.001
	Constant		18.33	7.13	6.62	1	0.010
0.1	**Model**	**Step 40**			**26.18**	**6**	***p* < 0.00125***
	Cingulum_Mid_R		0.14	0.07	3.54	1	0.060
	ParaHippocampal_R		-0.09	0.04	5.19	1	0.023
	Occipital_Inf_R		0.06	0.03	5.25	1	0.022
	Paracentral_Lobule_R		0.08	0.03	5.58	1	0.018
	Pallidum_R		-0.04	0.02	4.41	1	0.036
	Temporal_Pole_Mid_R		-0.09	0.04	5.14	1	0.023
	Constant		-4.36	3.60	1.47	1	0.225
0.2	**Model**	**Step 42**			**18.39**	**4**	***p* < 0.00125***
	Rolandic_Oper_R		-0.08	0.03	5.48	1	0.019
	Occipital_Inf_R		0.04	0.02	5.75	1	0.017
	Temporal_Sup_R		0.13	0.05	6.63	1	0.010
	Temporal_Inf_R		-0.11	0.05	6.05	1	0.014
	Constant		1.35	2.24	0.36	1	0.548
0.3	**Model**	**Step 40**			**22.58**	**6**	***p* < 0.00125***
	Rolandic_Oper_R		-0.09	0.03	7.33	1	0.007
	Olfactory_R		0.03	0.01	6.05	1	0.014
	Occipital_Sup_R		0.06	0.03	6.44	1	0.011
	Angular_R		-0.04	0.02	6.38	1	0.012
	Temporal_Sup_R		0.13	0.05	8.20	1	0.004
	Temporal_Inf_R		-0.10	0.04	8.70	1	0.003
	Constant		0.43	1.40	0.09	1	0.760
0.4	**NS**						
0.5	**Model**	**Step 34**			**32.40**	**1**	***p* < 0.00125***
	Frontal_Mid_R		-0.06	0.03	5.66	1	0.017
	Frontal_Inf_Oper_R		0.06	0.03	4.63	1	0.031

Supplementary Table S2. Outcome of the backward regression models for 10 thresholds for the degree of right hemisphere brain regions, using group (PTSD vs control) as dependent variable. (*Continued*)

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
	Frontal_Inf_Orb_R		-0.04	0.02	4.76	1	0.029
	Rolandic_Oper_R		-0.08	0.03	5.18	1	0.023
	Olfactory_R		0.05	0.02	7.41	1	0.006
	Fusiform_R		0.07	0.03	3.56	1	0.059
	Temporal_Sup_R		0.08	0.04	4.94	1	0.026
	Temporal_Inf_R		-0.06	0.03	4.36	1	0.037
	Constant		-0.34	0.63	0.29	1	0.591
0.6	**Model**	**Step 34**			**35.75**	**1**	***p* < 0.00125***
	Frontal_Mid_R		-0.13	0.04	8.44	1	0.004
	Frontal_Inf_Oper_R		0.11	0.04	6.82	1	0.009
	Frontal_Inf_Orb_R		-0.10	0.03	8.29	1	0.004
	Rolandic_Oper_R		-0.16	0.06	6.54	1	0.011
	Olfactory_R		0.15	0.05	10.32	1	0.001
	Frontal_Sup_Medial_R		0.06	0.03	3.40	1	0.065
	Hippocampus_R		-0.11	0.04	7.48	1	0.006
	Calcarine_R		-0.12	0.06	3.69	1	0.055
	Cuneus_R		0.11	0.04	6.27	1	0.012
	Lingual_R		0.11	0.05	5.29	1	0.021
	Precuneus_R		-0.06	0.03	3.10	1	0.078
	Heschl_R		0.11	0.05	5.47	1	0.019
	Constant		0.20	0.54	0.14	1	0.713
0.7	**Model**	**Step 39**			**27.50**	**7**	***p* < 0.00125***
	Frontal_Inf_Orb_R		-0.14	0.05	8.29	1	0.004
	Olfactory_R		0.16	0.06	7.75	1	0.005
	Frontal_Sup_Medial_R		0.13	0.05	7.40	1	0.007
	Cuneus_R		0.10	0.05	5.13	1	0.024
	Precuneus_R		-0.17	0.05	11.69	1	0.001
	Paracentral_Lobule_R		0.08	0.04	4.88	1	0.027
	Thalamus_R		0.19	0.08	5.04	1	0.025
	Constant		-1.11	0.47	5.45	1	0.020
0.8	**Model**	**Step 35**			**38.70**	**1**	***p* < 0.00125***
	Frontal_Inf_Tri_R		-0.54	0.17	9.61	1	0.002
	Supp_Motor_Area_R		0.16	0.07	5.44	1	0.02
	Olfactory_R		0.81	0.35	5.48	1	0.019
	Frontal_Sup_Medial_R		-0.35	0.14	6.36	1	0.012
	Cingulum_Ant_R		0.50	0.16	9.41	1	0.002

Supplementary Table S2. Outcome of the backward regression models for 10 thresholds for the degree of right hemisphere brain regions, using group (PTSD vs control) as dependent variable. (*Continued*)

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
	Occipital_Sup_R		-0.29	0.11	6.67	1	0.010
	Precuneus_R		-0.17	0.08	4.16	1	0.041
	Pallidum_R		-0.54	0.27	4.13	1	0.042
	Temporal_Sup_R		-0.23	0.09	6.95	1	0.008
	Temporal_Mid_R		0.22	0.09	6.14	1	0.013
	Temporal_Inf_R		0.26	0.10	6.44	1	0.011
	Constant		2.61	0.85	9.47	1	0.002
0.9	Model	Step 26			67.23	2	$p < 0.00125$*
	Frontal_Sup_R		1.14	0.63	3.30	1	0.069
	Frontal_Sup_Orb_R		2.07	0.94	4.85	1	0.028
	Frontal_Inf_Oper_R		2.98	1.68	3.15	1	0.076
	Frontal_Inf_Tri_R		-4.98	1.78	7.87	1	0.005
	Rolandic_Oper_R		-1.02	0.47	4.74	1	0.029
	Frontal_Med_Orb_R		-2.27	0.65	12.11	1	0.001
	Insula_R		2.13	0.87	6.08	1	0.014
	Cingulum_Ant_R		2.78	1.04	7.11	1	0.008
	Cingulum_Mid_R		-1.27	0.55	5.41	1	0.020
	Occipital_Sup_R		-0.97	0.32	9.17	1	0.002
	Parietal_Sup_R		-2.01	0.85	5.52	1	0.019
	Parietal_Inf_R		1.69	0.71	5.60	1	0.018
	Precuneus_R		1.61	0.73	4.84	1	0.028
	Paracentral_Lobule_R		1.73	0.57	9.22	1	0.002
	Putamen_R		3.05	1.09	7.87	1	0.005
	Pallidum_R		-6.50	1.91	11.60	1	0.001
	Thalamus_R		-1.74	0.88	3.89	1	0.049
	Heschl_R		1.33	0.45	8.74	1	0.003
	Temporal_Sup_R		-0.90	0.45	4.06	1	0.044
	Temporal_Mid_R		1.63	0.62	6.96	1	0.008
	Constant		0.69	0.99	0.49	1	0.486

Supplementary Table S3. Outcome of the backward regression models for 10 thresholds for the clustering coefficient of left hemisphere brain regions, using group (PTSD vs control) as dependent variable.

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
0	Step 16	Step 16			77.12	3	$p < 0.00125$*
	Frontal_Sup_Orb_L		31.66	13.03	5.90	1	0.015
	Frontal_Mid_L		-60.80	22.60	7.24	1	0.007
	Frontal_Inf_Oper_L		62.05	23.80	6.80	1	0.009
	Frontal_Inf_Orb_L		-29.03	12.62	5.30	1	0.021
	Rolandic_Oper_L		-117.48	37.15	10.00	1	0.002
	Supp_Motor_Area_L		-29.11	17.39	2.80	1	0.094
	Olfactory_L		59.23	16.87	12.32	1	$p < 0.001$
	Frontal_Sup_Medial_L		87.93	24.59	12.79	1	$p < 0.001$
	Rectus_L		-48.91	17.13	8.16	1	0.004
	Insula_L		42.81	17.93	5.70	1	0.017
	Cingulum_Ant_L		-29.73	13.67	4.73	1	0.03
	Cingulum_Post_L		-30.98	11.98	6.69	1	0.01
	Amygdala_L		-28.22	9.73	8.41	1	0.004
	Lingual_L		-31.34	13.25	5.60	1	0.018
	Occipital_Sup_L		31.09	13.72	5.13	1	0.023
	Occipital_Mid_L		-40.49	15.62	6.72	1	0.010
	Occipital_Inf_L		-32.57	14.52	5.03	1	0.025
	Fusiform_L		45.78	15.81	8.38	1	0.004
	Postcentral_L		89.41	30.93	8.36	1	0.004
	Parietal_Sup_L		-38.04	16.59	5.26	1	0.022
	Parietal_Inf_L		80.42	23.78	11.43	1	0.001
	SupraMarginal_L		51.18	20.53	6.22	1	0.013
	Angular_L		-31.42	12.03	6.83	1	0.009
	Precuneus_L		34.82	14.46	5.80	1	0.016
	Paracentral_Lobule_L		-56.76	21.09	7.25	1	0.007
	Putamen_L		-64.05	23.08	7.70	1	0.006
	Pallidum_L		32.13	15.90	4.08	1	0.043
	Thalamus_L		14.75	6.36	5.39	1	0.020
	Heschl_L		52.58	20.94	6.30	1	0.012
	Temporal_Inf_L		-27.66	15.33	3.26	1	0.071
	Constant		-7.08	2.61	7.33	1	0.007
0.1	NS						
0.2	NS						
0.3	NS						
0.4	NS						

Supplementary Table S3. Outcome of the backward regression models for 10 thresholds for the clustering coefficient of left hemisphere brain regions, using group (PTSD vs control) as dependent variable. (*Continued*)

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
0.5	Model	Step 32			35.59	1	$p < 0.00125$*
	Frontal_Mid_L_		-8.41	3.41	6.10	1	0.014
	Rolandic_Oper_L		9.66	4.45	4.72	1	0.030
	Supp_Motor_Area_L		8.28	3.83	4.68	1	0.031
	Insula_L		-11.59	4.55	6.48	1	0.011
	Cingulum_Post_L		4.58	2.62	3.05	1	0.081
	Pallidum_L		5.00	2.74	3.32	1	0.068
	Heschl_L		-8.47	3.93	4.64	1	0.031
	Constant		0.66	1.35	0.24	1	0.624
0.6	Model	Step 39			27.99	7	$p < 0.00125$*
	Frontal_Mid_L		-7.84	2.80	7.82	1	0.005
	Supp_Motor_Area_L		9.15	3.22	8.07	1	0.004
	Olfactory_L		1.61	0.98	2.71	1	0.100
	Cingulum_Ant_L		-3.04	1.60	3.62	1	0.057
	Occipital_Inf_L		-3.03	1.59	3.63	1	0.057
	Parietal_Sup_L		4.71	2.17	4.72	1	0.030
	Temporal_Sup_L		-6.91	2.91	5.62	1	0.018
	Constant		2.93	1.27	5.35	1	0.021
0.7	Model	Step 38			33.03	8	$p < 0.00125$*
	Frontal_Inf_Tri_L		2.92	1.27	5.32	1	0.021
	Olfactory_L		2.06	0.94	4.77	1	0.029
	Cingulum_Ant_L		-1.70	0.98	3.03	1	0.082
	Lingual_L		-6.35	1.85	11.83	1	0.001
	Fusiform_L		-2.70	1.25	4.69	1	0.030
	Postcentral_L		3.76	1.38	7.44	1	0.006
	Precuneus_L		-3.10	1.25	6.13	1	0.013
	Pallidum_L		-2.00	0.83	5.83	1	0.016
	Constant		4.58	1.54	8.89	1	0.003
0.8	Model	Step 35			53.28	1	$p < 0.00125$*
	Frontal_Mid_Orb_L		2.42	1.12	4.69	1	0.030
	Frontal_Inf_Oper_L		-3.71	1.61	5.33	1	0.021
	Frontal_Inf_Tri_L		3.39	1.46	5.42	1	0.020
	Rolandic_Oper_L		-2.70	1.22	4.89	1	0.027
	Frontal_Sup_Medial_L		-5.37	1.69	10.08	1	0.001
	Cingulum_Ant_L		2.45	1.04	5.60	1	0.018
	Occipital_Sup_L		-2.74	1.29	4.49	1	0.034

Supplementary Table S3. Outcome of the backward regression models for 10 thresholds for the clustering coefficient of left hemisphere brain regions, using group (PTSD vs control) as dependent variable. (*Continued*)

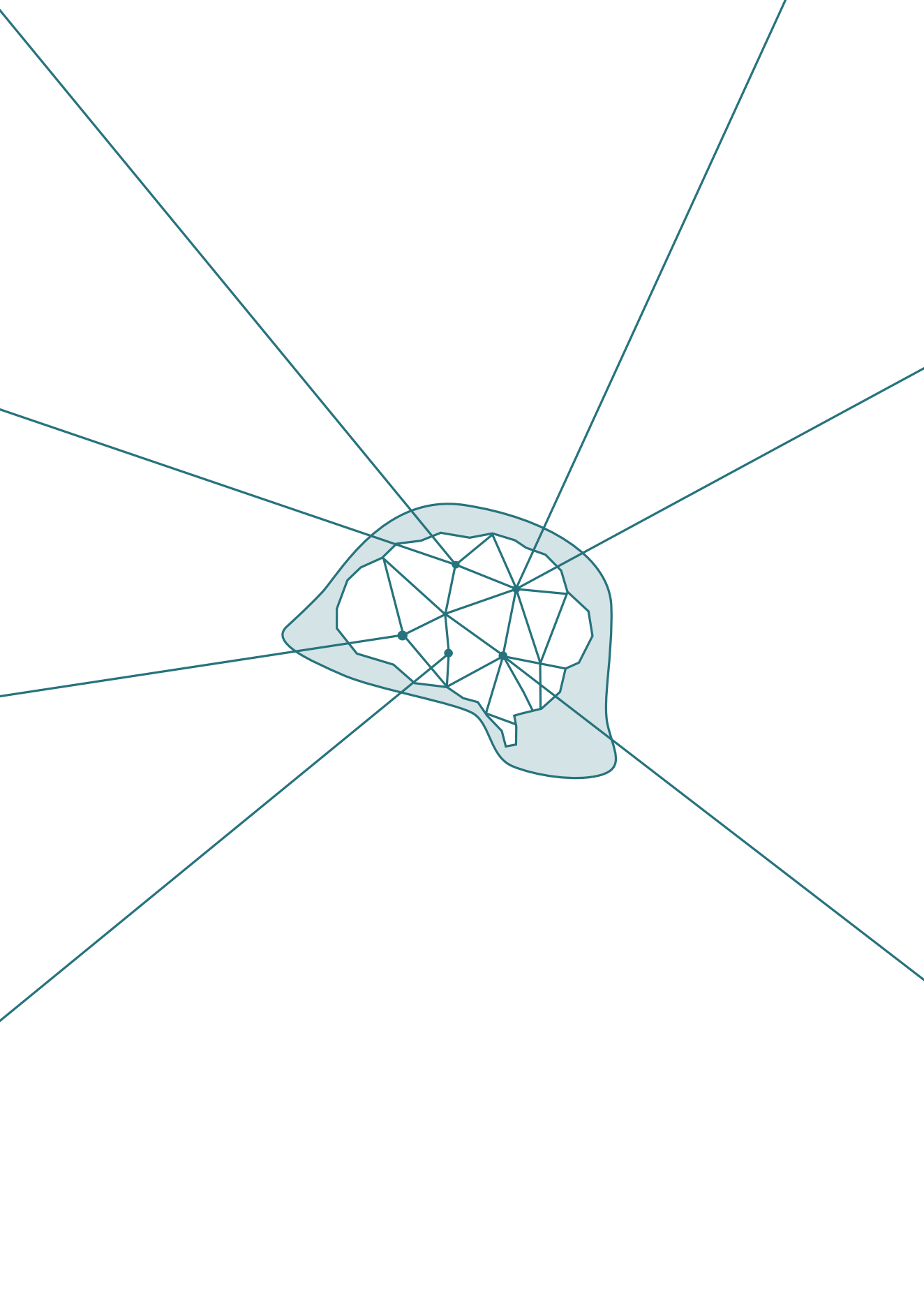
Threshold	Model or brain area (AAL) Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
	Parietal_Inf_L	4.42	1.62	7.45	1	0.006
	Temporal_Pole_Sup_L	-6.59	2.37	7.70	1	0.006
	Temporal_Mid_L	9.06	2.66	11.58	1	0.001
	Temporal_Inf_L	-2.74	1.46	3.52	1	0.061
	Constant	2.15	0.92	5.48	1	0.019
0.9	NS					

Supplementary Table S4. Outcome of the backward regression models for 10 thresholds for the clustering coefficient of right hemisphere brain regions, using group membership (PTSD vs control) as dependent variable.

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
0.1	NS						
0.2	NS						
0.3	Model	Step 32			3.00	14	$p < 0.00125$*
	Frontal_Sup_Orb_R		10.71	5.88	3.32	1	0.068
	Frontal_Mid_R		-23.22	8.37	7.69	1	0.006
	Frontal_Inf_Tri_R		23.35	10.74	4.73	1	0.030
	Frontal_Inf_Orb_R		-30.26	9.36	10.45	1	0.001
	Olfactory_R		14.84	5.12	8.40	1	0.004
	ParaHippocampal_R		-16.06	7.28	4.87	1	0.027
	Amygdala_R		-11.97	6.93	2.98	1	0.084
	Lingual_R		-12.13	6.10	3.96	1	0.047
	Fusiform_R		17.65	7.60	5.40	1	0.020
	Parietal_Sup_R		-15.21	7.77	3.83	1	0.050
	Parietal_Inf_R		14.66	7.56	3.76	1	0.053
	Paracentral_Lobule_R		17.11	8.18	4.38	1	0.036
	Heschl_R		-12.92	6.23	4.30	1	0.038
	Temporal_Pole_Mid_R		23.28	7.83	8.85	1	0.003
	Constant		0.81	1.40	0.34	1	0.562
0.4	Model	Step 15			84.67	3	$p < 0.00125$*
	Precentral_R		42.80	19.18	4.98	1	0.026
	Frontal_Sup_R		80.38	30.16	7.10	1	0.008
	Frontal_Mid_R		-98.72	34.87	8.01	1	0.005
	Frontal_Inf_Oper_R		-93.95	35.99	6.81	1	0.009
	Frontal_Inf_Tri_R		64.09	27.16	5.57	1	0.018
	Frontal_Inf_Orb_R		-72.64	24.27	8.96	1	0.003
	Supp_Motor_Area_R		-76.85	27.96	7.55	1	0.006
	Olfactory_R		42.84	15.70	7.45	1	0.006
	Frontal_Sup_Medial_R		67.71	22.62	8.96	1	0.003
	Insula_R		-67.92	30.24	5.05	1	0.025
	Cingulum_Ant_R		-60.33	22.72	7.05	1	0.008
	Cingulum_Post_R		-40.95	15.26	7.21	1	0.007
	ParaHippocampal_R		-20.59	8.29	6.17	1	0.013
	Amygdala_R		-42.46	15.69	7.33	1	0.007
	Calcarine_R		58.61	19.90	8.68	1	0.003
	Cuneus_R		-79.30	26.66	8.85	1	0.003
	Lingual_R		-79.20	26.63	8.85	1	0.003

Supplementary Table S4. Outcome of the backward regression models for 10 thresholds for the clustering coefficient of right hemisphere brain regions, using group membership (PTSD vs control) as dependent variable. (*Continued*)

Threshold	Model or brain area (AAL)	Step	B	SE	Chi-square (for model) Wald (for brain area)	df	*p*-value
	Occipital_Sup_R		56.91	24.01	5.62	1	0.018
	Occipital_Mid_R		-38.13	18.17	4.41	1	0.036
	Occipital_Inf_R		29.34	11.53	6.48	1	0.011
	Postcentral_R_		-123.10	40.14	9.40	1	0.002
	Parietal_Sup_R		-44.15	22.94	3.70	1	0.054
	Parietal_Inf_R		142.00	46.24	9.43	1	0.002
	Angular_R		-75.72	22.15	11.68	1	0.001
	Precuneus_R		26.12	13.57	3.71	1	0.054
	Paracentral_Lobule_R		90.31	29.27	9.52	1	0.002
	Caudate_R		5.07	3.06	2.74	1	0.098
	Pallidum_R		76.81	25.78	8.88	1	0.003
	Thalamus_R		6.62	3.94	2.83	1	0.093
	Heschl_R		38.53	15.33	6.32	1	0.012
	Temporal_Sup_R		-60.54	27.82	4.74	1	0.030
	Temporal_Pole_Sup_R		164.02	53.28	9.48	1	0.002
	Temporal_Inf_R		52.84	22.68	5.43	1	0.020
	Constant		22.91	11.17	4.21	1	0.040
0.5	NS						
0.6	Model	Step 41			21.37	5	$p < 0.00125$*
	Frontal_Inf_Tri_R		-5.00	2.03	6.04	1	0.014
	Rolandic_Oper_R		5.65	2.56	4.85	1	0.028
	Cingulum_Ant_R		-2.63	1.48	3.17	1	0.075
	Amygdala_R		2.75	1.34	4.24	1	0.040
	Heschl_R		-6.16	2.46	6.29	1	0.012
	Constant		2.73	1.45	3.54	1	0.060
0.7	NS						
0.8	NS						
0.9	NS						



7

Summary and general discussion

The aim of this thesis was to gain more insights in the neural network alterations that may underlie PTSD and trauma-focused therapy outcome. To investigate *The Neural Web of War* brain scans of healthy civilians, and veterans with and without PTSD were assessed. Structural and functional connectivity studies were performed and several neural network indicators for PTSD and treatment outcome were found. The results will be summarized and discussed below.

Summary

In **Chapter 2** structural connectivity of the cingulum bundle was compared between remitted and persistent PTSD patients and combat controls. The results indicate that the cingulum bundle white matter may be related to treatment outcome: higher dorsal cingulum fractional anisotropy (FA) was found in persistent PTSD patients, which further increased over time. Interaction effects were found for hippocampal cingulum, stria terminalis, and fornix FA, where remitted PTSD patients potentially restore to control levels after treatment. **Chapter 3** also shows treatment outcome related differences in the isthmus cingulum, but we show that results are marred by phase encoding direction during scan acquisition. Thus, *Section 1* indicated that treatment outcome is related to alterations in the microstructural integrity of the cingulum bundle, and there are some indications that this changes over time.

Chapter 4 shows that functional connectivity of the anterior cingulate cortex (ACC) subdivisions differs between veterans (with and without PTSD) and combat controls. This indicates that ACC connectivity differences may be related to deployment, military training or selection bias. In addition, specific differences in the perigenual ACC network were found for the veteran controls, which may potentially be related to resilience. In addition, in **Chapter 5** differences in the subgenual ACC and insula network were found between PTSD patients with and without comorbid depression. Therefore, resting state functional connectivity provides indicators for deployment and/or military training, but can also indicate the presence of comorbid depression.

In **Chapter 6** whole brain functional connectivity was explored and the results indicate that network measures of orbitofrontal, temporoparietal, and the anterior cingulate cortex are associated with PTSD. These network measures did not change over the course of treatment and did not correlate with change in symptoms. This provides more insight in the psychopathology of PTSD, and provides a first investigation of treatment effects on the functional whole brain network in PTSD. Thus, *Section 2* indicated that functional connectivity measures were associated with PTSD and also with other factors, such as deployment or military training, and comorbidity of depression.

The neural web of PTSD

Although neurocircuitry models of PTSD have been proposed, at the start of this project limited studies on neural networks of PTSD were performed. Therefore, the first aim of this dissertation was to further investigate neural networks in PTSD. We observed differences between PTSD patients and controls, and found PTSD associated functional neural network metrics. These results may be integrated with two proposed neurocircuitry models: the triple network model for psychopathology and the conventional neurocircuitry model for PTSD.

The triple network model for psychopathology

Alterations in three major networks of the brain have been proposed to underlie psychopathology in the triple network model (Menon, 2011): the default mode network, the salience network, and the central executive network. The default mode network (DMN) is the network that is active during rest and deactivated during task performance, and has been related to self-referential mental states (Greicius et al., 2003; Seeley et al., 2007). Core nodes of this network are the ventromedial prefrontal cortex (PFC), the posterior cingulate cortex (PCC), and precuneus (Raichle et al., 2001). PTSD has been associated with decreased DMN connectivity (Bluhm et al., 2009b; Daniels et al., 2010; Sripada et al., 2012a), and reduced activity of these regions has also been reported during self-referential processing in PTSD (Bluhm et al., 2012). Our results from **Chapter 6** complement these findings by showing that the number of functional connections (degree) of regions of the DMN are associated with PTSD. This may imply that information flow or communication between brain regions in the DMN is disturbed, or that functional segregation of this network is altered. In addition, PTSD patients also had reduced DMN connectivity compared to healthy controls (**Chapter 4**).

The salience network (SN) is the network that is related to detection and mapping of salient external and internal events. Core nodes of this network are the insula, and dorsal ACC (Seeley et al., 2007). PTSD has been associated with increased SN connectivity (Daniels et al., 2010; Sripada et al., 2012a). Furthermore, a meta-analysis showed that alterations in activity are also reported in these regions in PTSD (Patel et al., 2012). In line with previous studies, the importance of SN brain regions and functional segregation of SN brain regions was associated with PTSD in **Chapter 6**. Furthermore, the rolandic operculum was associated with PTSD, which is a central brain region. Exploratory analyses of the functional connectivity pattern of the rolandic operculum with 1000 resting brains in neurosynth (neurosynth.org) showed that the rolandic operculum is a node in the salience network. Thus, our results indeed indicate that the SN is altered in PTSD.

The central executive network (CEN) is the network that is activated during working memory and attention tasks, and deactivated during rest. Core nodes of this network

are the bilateral dorsolateral PFC and the posterior parietal cortex. Alterations in these brain regions are shown in PTSD patients during executive tasks, and difficulties with performing these tasks are also reported (Daniels et al., 2010; Polak et al., 2012). Here, PTSD was also associated with reduced CEN connectivity (**Chapter 6**), and PTSD patients had reduced CEN connectivity compared to healthy veterans (**Chapter 4**). Therefore, CEN alterations are also related to PTSD.

The triple network model has its origin in observations from functional (connectivity) studies. Therefore, our structural connectivity results (**Chapter 2**) are harder to interpret from a triple network model. It may be speculated that the hippocampal cingulum is a crucial connection between the hippocampus and the rest of the DMN. In this view, finding a trend for higher baseline hippocampal cingulum FA in remitted PTSD patients may reflect that increased baseline DMN connectivity may be beneficial for recovery. On the other hand, persistent PTSD patients developed increased dorsal cingulum FA. The dorsal cingulum has also been related to the DMN, comprising connections between the medial PFC and PCC (Van Den Heuvel et al., 2008). However, the dorsal cingulate cortex is also a core node in the salience network (Seeley et al., 2007). Since increased cross network connectivity has been reported in PTSD (Daniels et al., 2010; Sripada et al., 2012a), it can perhaps be suggested that increased dorsal cingulum connectivity represents this increased cross-network connectivity, which may hamper recovery.

Finally, it must be noted that we applied a priori seed-analyses and a bottom up exploratory whole brain graph analysis. Therefore, we did not directly investigate the involvement of these networks as opposed to other networks. Further research is needed with techniques such as independent component analysis to investigate the involvement of other brain networks, in comparison with the DMN, SN and CEN. However, our results provide additional confirmation of the triple network model, and indicate that PTSD is related to disturbances in three core networks: the DMN, SN, and CEN.

The conventional neurocircuitry model for PTSD

The conventional neurocircuitry model for PTSD first described by Rauch et al. (2006) states that hypoactivation of medial prefrontal control over subcortical brain regions together with increased amygdala and altered hippocampal activation underlie most deficits in PTSD. In accordance with this model, we found an association between PTSD and disturbances in functional connections of the orbitofrontal cortex and anterior cingulate cortex regions (**Chapter 6**). Furthermore, we found increased functional connectivity in medial prefrontal regions in particular in healthy controls, suggesting that experiencing a traumatic event may already alter the medial PFC. This is in line with a meta-analysis of PTSD showing alterations in the medial PFC in PTSD patients versus healthy controls, but not versus trauma exposed controls (Patel et al., 2012).

Second, over the course of treatment interaction effects were found for the white matter tracts that are important for amygdala (stria terminalis) and hippocampal (fornix) functioning (**Chapter 2**). These patterns may be related to the previous reported hyperactivity of the amygdala and hippocampus in PTSD (Patel et al., 2012), which may induce an increase in FA by a potential increase in myelination (Pape and Pare, 2010). In PTSD patients that remit after treatment, these alterations in FA may restore to normative after treatment. This is in line with the conventional neurocircuitry model for PTSD (Rauch, Shin, Phelps, 2006), and with previous treatment studies with structural and functional MRI studies, indicating recovery of alterations after treatment (Aupperle et al., 2013; Lindauer et al., 2005; Roy et al., 2010; Roy et al., 2014; Thomaes et al., 2012; Vermetten et al., 2003). Our results show neurobiological markers in amygdala and hippocampal white matter fiber tracts for recovery from PTSD, in line with the conventional neurocircuitry model for PTSD.

Out of the box

Besides findings that could be interpreted from a neural network model perspective, other brain areas, not noted in current models for PTSD, were also related to PTSD (**Chapter 6**). For example, the fusiform gyrus degree was positively associated with PTSD. The fusiform gyrus has not received particular interest yet, although two meta-analyses of PTSD both report significant alterations in this brain region (Hayes, Hayes, Mikedis, 2012; Patel et al., 2012). The fusiform gyrus is involved in processing visual information, and alterations in the fusiform gyrus during visual information processing are reported in PTSD patients (Hendler et al., 2003). Furthermore, we observed associations with the olfactory gyrus, which is important for smell. Aberrant activation of the olfactory gyrus has been reported in PTSD when smelling aversive odors, and activity was related to dissociative symptoms (Vasterling, Brailey, Sutker, 2000; Vermetten et al., 2007). It has been argued that salient and emotional visual information may enhance processing in sensory regions by expectation-based feedback (Morey et al., 2009; Vuilleumier et al., 2001). Also, for olfactory perception it has been shown that top down expectations can modulate perception and response of primary sensory areas (De Araujo et al., 2005). Altered connectivity of the olfactory gyrus and fusiform gyrus may thus be related to higher expectation-based modulation of perceptual processes. Future studies should explore this idea and establish the importance of these regions in PTSD psychopathology.

Blurred lines – PTSD diagnosis and comorbidity

In this dissertation PTSD patients were subdivided into remitted and persistent PTSD patients after treatment. This subdivision is made based on PTSD diagnostic criteria

(DSM-IV). However, other subdivisions have also been proposed, such as 30% symptom reduction or CAPS score below 45 (van Rooij et al., 2015b; van Rooij et al., 2015c). Furthermore, the diagnostic criteria have slightly changed in the DSM-5 (American Psychiatric Association, 2013). In order to investigate effects of the revised diagnostic criteria, we added the new DSM-5 criteria to the CAPS. Exploring the differences in diagnosis revealed that after treatment two patients switch from persistent to remitted PTSD, indicating that the DSM-5 criteria comprise a more strict PTSD diagnosis (Miller et al., 2013). Of note, the new DSM-5 group division did not affect the DTI results. The switch for these two patients can be related to splitting the avoidance and emotional numbing symptoms into separate clusters. Thus, although we interpret PTSD as a singular diagnosis based on DSM-IV criteria, these rules are not as clear and diagnostic criteria change over the years.

In **Chapter 5** differences between PTSD patients with and without comorbid major depressive disorder were found in insula and subgenual ACC network. In addition, in **Chapter 2** and **6** a greater number of comorbid anxiety disorders was observed in persistent PTSD patients, and a trend was found for more mood disorders. This indicates that PTSD patients are not a homogeneous group, but that specific differences in neural networks are related to particular symptoms (e.g. depressive symptoms), and treatment outcome. Since about half of the PTSD patients have comorbid MDD, and these patients are known to benefit less from treatment (Brady et al., 2000; Morina et al., 2013), it is important to further investigate the neurobiology of comorbid disorders. Here, persistent PTSD patients had higher comorbidity and were indeed harder to treat. Furthermore, we attempted to investigate PTSD (with some comorbidity) as one construct with strict borders and compare remitted from persistent PTSD patients, while a chronic *pure* PTSD patient may not exist in reality, according to clinical experts. For example, personality disorders may be more present in chronic PTSD, which is not accounted for in our paradigm. This may further complicate the recovery process. Preliminary analysis investigating the personality profile of persistent PTSD patients showed reduced self-directedness, which indicates that personality differences may be present in persistent versus remitted PTSD. So there are factors that *blur the lines* of PTSD diagnosis, and are shown to further complicate investigation of the neural network of PTSD.

The neural web of treatment

As noted in the introduction, only 50% of the PTSD patients recover after treatment. Our study showed similar rates of recovery. During data collection we noted that 6-8 months for trauma-focused therapy was not sufficient for patients to complete therapy. Therefore,

a long term follow-up measurement has been performed with 25 PTSD patients (Kennis et al., *in preparation*), indicating that the PTSD patients that fully recovered after treatment remained recovered (except one), and many chronic patients showed chronic symptoms. Five patients recovered from PTSD after 4 years, although symptoms were still present. Therefore, studying differences between remitted and persistent PTSD after 6-8 months of treatment seems to be appropriate for distinguishing remitted versus persistent or chronic PTSD. Determining differences between these groups may provide future targets for treatment of persistent PTSD.

Indeed, when comparing remitted and persistent PTSD patients, and combat controls, we observed patterns that may reflect recovery in remitted PTSD patients in important white matter tracts that are related to emotional processes (stria terminalis, fornix, hippocampal cingulum, **Chapter 2**). Nevertheless, no group differences were found pre- or post treatment, so caution has to be taken with interpretations. However, this is the first study to investigate white matter integrity over the course of treatment in PTSD, and shows that changes in white matter microstructure may be part of the therapeutic response.

Furthermore, treatment outcome related differences were observed for the cingulum bundle, where the persistent PTSD patients showed increased dorsal cingulum bundle connectivity and remitted PTSD patients reduced isthmus cingulum bundle connectivity (**Chapter 2**). This may be related to the increased dorsal ACC activity found in the same sample of persistent PTSD patients (van Rooij et al., 2015a). In addition, the measures for structural connectivity further increased over time. This makes it tempting to hypothesize that increased FA in the dorsal cingulum is an acquired factor, which progresses over time. However, we cannot distinguish vulnerability factors from acquired factors. It would be interesting to follow recently traumatized subjects to see if this change occurs after trauma. If this is indeed an acquired factor, early interventions may prevent this aberration to progress.

Bridging a gap – connecting disciplines

The future goal of the studies performed here, is to contribute to developing better treatment strategies and select the appropriate treatment for each individual (personalized treatment). Before these treatment strategies can be developed and optimized, more research is necessary to unravel which specific processes underlie effective treatment, and which biological processes hamper recovery. Therefore, studies investigating neural networks *during* therapy need to be performed. Few studies have started to disentangle which neurobiological processes take place during therapy sessions (Cisler et al., 2014; Lansing et al., 2005; Ohta ni et al., 2009). Future studies should investigate further enhancement of the processes that are positively related to

symptom improvement in PTSD. Furthermore, pharmacological MRI can contribute to determine the effects of medication on brain functioning directly. In addition, if specific medication effects are known, personalized medicine can also become a realistic treatment option to specifically target symptom related brain aberrations in PTSD.

In addition, large-scale PTSD research cohorts need to be set up, in order to increase power and investigate the whole spectrum of PTSD symptoms, and not only categorical groups "with and without" PTSD. With large samples, dimensional analyses can be performed based on biological measures and symptom scales rather than arbitrary subdivisions. Furthermore, correlations with specific symptoms can be investigated. When specific symptoms can be related to specific network alterations as has been proposed (Lanius et al., 2015), personalized treatment can be applied to restore the balance in large-scale networks.

When we know how successful treatment (generally) works, *new* strategies to enhance these processes can also be developed. For example, it is thought that extinction of the traumatic memories occurs during treatment. If neural correlates show which process takes place during treatment, pharmacological compounds influencing the processes for successful treatment can be administered to enhance improvement. For example, D-cycloserine can facilitate extinction and may enhance treatment (De Kleine et al., 2012). Alternatively, blocking reconsolidation after reactivating fear memories with propranolol may also be a promising intervention to dampen the emotional response to traumatic memories (Kindt, Soeter, Vervliet, 2009). In addition, to investigate the causal relation between symptomatology and network connectivity trans cranial stimulation can temporarily stimulate or inhibit core nodes of specific networks. If symptoms are then worsened or relieved, a causal relation between these brain regions and symptomatology can be established. If you speculate even further, stimulating (or inhibiting) brain regions that needed to be (de)activated during treatment sessions to influence the balance between neural networks may help to enhance treatment efficiency.

Furthermore, translating science into psychotherapeutic advice and practice can also improve in the future. A narrow collaboration between the clinician and scientist is necessary to bridge the gap between these fields of expertise (Craske, 2012; Peres et al., 2008). For example, integrating factors that can enhance extinction learning, such as surprise, may facilitate treatment. Important input from clinicians in a study design for future research may therefore be very valuable. Hence, optimal collaboration between disciplines - clinicians and scientist - is necessary to develop optimal treatment strategies, and to exert research that can lead to applications in clinical practice.

The neural web of combat and resilience

Besides PTSD specific differences and treatment outcome related alterations, we encountered other factors that are related to neural network aberrations in **Chapter 4**. First, we observed differences in ACC connectivity for both veterans with and without PTSD versus civilian controls. This may reflect effects of war experience or military training, but may also be related to a selection bias. Second, increased connectivity between the ACC and precentral gyrus was observed in the combat controls specifically. Previous studies reported increased medial PFC activity in healthy traumatized subjects during attentional tasks (Blair et al., 2013; New et al., 2009) and resilience was correlated to PFC activity during symptom provocation (Daniels et al., 2012). Therefore, this characteristic specific for combat controls may be related to resilience, as also argued elsewhere (van der Werff et al., 2013). However, for both war and resilience related features we cannot distinguish vulnerability factors from acquired features. Therefore, it is of importance to acquire data before trauma, as is possible with soldiers before and after deployment (Reijnen et al., 2014; Van Wingen et al., 2012). When pre-deployment factors can be determined to identify participants at risk for developing PTSD, these individuals can be monitored and perhaps early intervention is then possible before PTSD onset. Though, a gap in research currently exist investigating (neuro)biology *during the experience of a stressful life event*, for example during deployment. When more information is available about the specific time-course of biological changes, specific targeted interventions may be developed to prevent these alterations, perhaps by enhancing processes such as extinction (De Kleine et al., 2012). Of note, this information is also of relevance for patients' understanding of what happened: many PTSD patients asked *what has changed in my head during my deployment?* It would be of particular value for them to further investigate this. In addition, when more is known about resilience related factors prior a traumatic event, it can be proposed to train these resilience networks prior to deployment. It has been shown that for example mindfulness training can alter resting state connectivity (Kilpatrick et al., 2011; Taylor et al., 2011). In summary, network alterations may occur after military training and/or combat experience, some of which can be protective resilience factors.

Box

Highlights:

- Aim: investigate neural networks before and after treatment in PTSD
- Markers for PTSD and treatment outcome are found
- Indicators for comorbidity, military deployment/training, and resilience are also observed

Future research:

- Study neural alterations *during* treatment
- Study biological changes *during* deployment
- Large-scale multidisciplinary research
- Pharmacological MRI
- Developing personalized treatment

Take home message:

- Experiencing a traumatic life event can alter the brain
- These alterations can be both protective factors (resilience) or markers for PTSD
- Treatment outcome is related to brain alterations

Strengths and limitations

In this dissertation a longitudinal design was applied before and after treatment to investigate neural network alterations over the course of PTSD treatment. This longitudinal design included a combat control group to control for effects of time and aging. This is a unique design and provides a reliable basis for investigating the effect of treatment. In addition, a group of healthy controls was measured pretreatment, which provides possibilities for distinguishing deployment and/or military training related differences from PTSD specific differences. Furthermore, markers for resilience after experiencing a traumatic event could also be investigated with this paradigm.

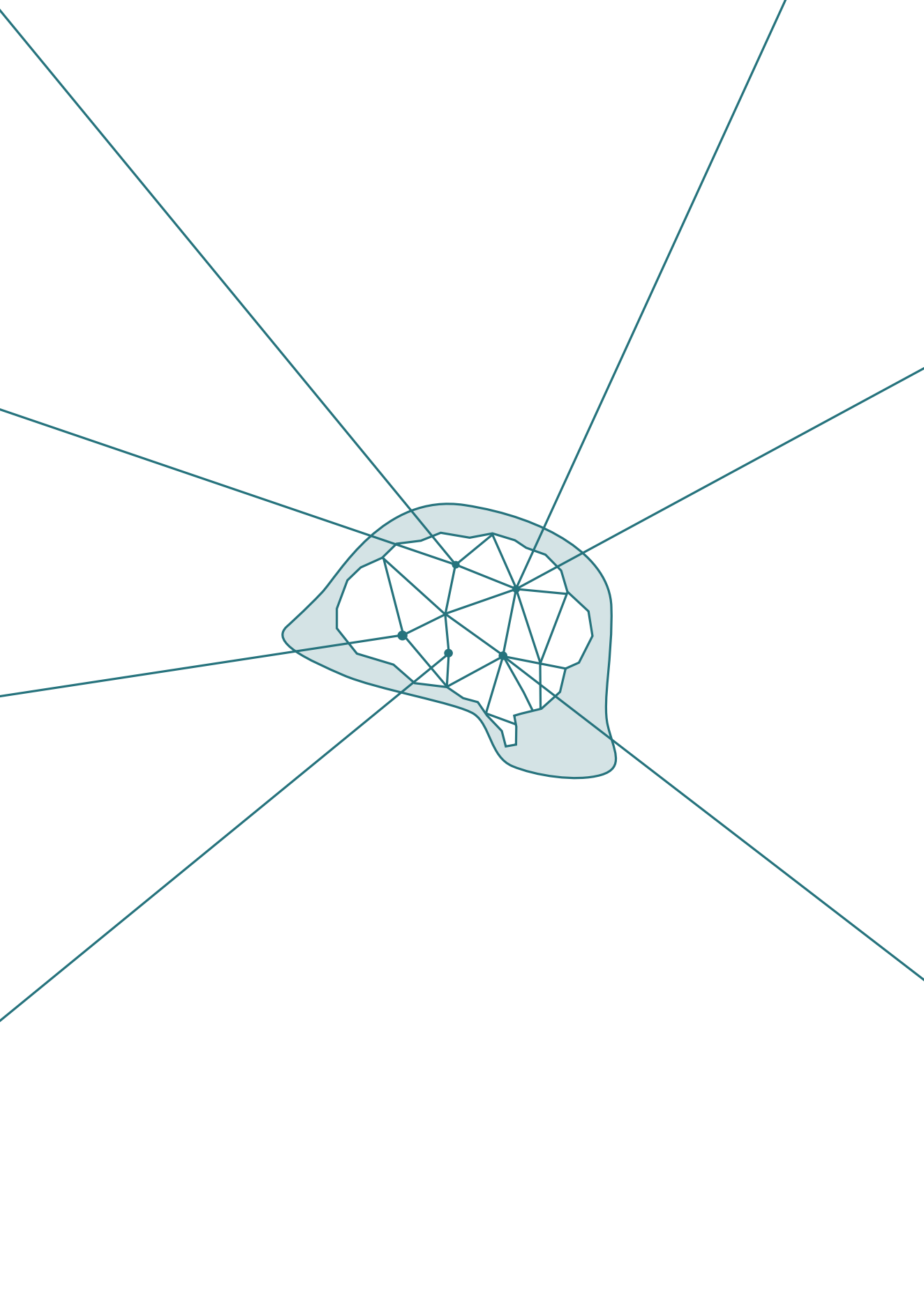
As with all research, our studies had limitations. As discussed in every chapter, each technique has its own methodological issues. In short, the measures investigated here are derived after many steps (and choices) of preprocessing, correlating, thresholding, and extracting signals. In addition, different methodological degrees of freedom can be applied with respect to correction for multiple comparisons. This should be kept in mind when interpreting the results.

In our protocol we investigated treatment as usual and do not distinguish between specific effects of EMDR, TFCBT and pharmacotherapy. A recent meta-analysis suggests that EMDR may be subordinate to other exposure treatments in veterans with PTSD

(Haagen et al., 2015). However, due to our observational design we cannot distinguish these effects or compare efficiency of particular therapies. We also did not include a "waitlist" patient control group, so we cannot distinguish "placebo" effects from additional therapeutic effects, which have been shown to be similar to psychopharmacological effects (Brambilla 2010). Future studies should further investigate and distinguish the effects of specific therapies. Here, we reported novel observational results showing neurobiological alterations before and after treatment in PTSD, whilst including a control group, which is a first step in unraveling the neural web of PTSD treatment.

Conclusion

To conclude, this dissertation contributes to our understanding of *The Neural Web of War*. We showed alterations in connectivity related to PTSD, persistence of PTSD, acquisition method, comorbid depression, deployment and/or military training, and resilience. Furthermore, we found indicators for treatment outcome related changes that occur over the course of treatment. However, it was evident that neural networks do not simply recover after treatment, when a patient recovers from PTSD. Our results do shed light on the psychopathology of PTSD and showed that there are many individual differences that may be related to differences in neural networks, not only the presence of PTSD. Furthermore, we confirmed that PTSD patients were a heterogeneous group consisting of patients that recover and patients that have persistent symptoms, potentially related to comorbid disorders. Therefore, our studies provided insight in individual differences that may be used in the future for personalized treatment. To conclude, in this dissertation we applied advanced neuroimaging techniques to study connectivity in veterans with and without PTSD and civilians, which revealed essential information for disentangling *The Neural Web of War*.



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Nederlandse samenvatting – Het Neurale Oorlogsweb

Inleiding

Van de Nederlandse militairen die zijn uitgezonden naar Afghanistan ontwikkelt 6-9% symptomen van een posttraumatische stress-stoornis (PTSS) (Reijnen et al., 2014). Deze stoornis wordt onder andere gekenmerkt door nachtmerries, flashbacks, vermijding van herinneringen, emotionele vervlakking en prikkelbaarheid. De gouden standaard voor behandeling van PTSS is trauma-gerichte therapie zoals 'cognitieve gedragstherapie met exposure' en 'eye-movement desensitisation and reprocessing' (EMDR), waarbij de traumatische herinnering centraal staat en het doel is dat de emotionele reactiviteit tijdens het behandelingsproces langzaam afneemt (Foa and Kozak, 1986). Eerdere studies hebben laten zien dat er verschillen in de hersenen van PTSS patiënten en gezonde controles te zien zijn op magnetic resonance imaging (MRI) scans, zowel wat betreft de mate van activiteit als de structuur van hersengebieden die betrokken zijn bij emotie en geheugen zoals de amygdala, de hippocampus en de anterieure cingulate cortex (Liberzon en Sripada, 2007; Rauch, Shin, Phelps, 2006). Daarnaast zijn er ook verschillen in connectiviteit tussen deze hersengebieden gevonden. Zowel de structurele als de functionele verbindingen tussen specifieke hersengebieden van mensen met PTSS verschilden van die van mensen zonder PTSS (Daniels et al., 2013; Daniels, Bluhm, Lanius, 2013). Het is echter nog niet duidelijk of deze verschillen in connectiviteit specifiek zijn voor de onderzochte hersengebieden of dat het gehele brein van deze twee groepen verschilt in connectiviteit. Daarnaast is nog niet duidelijk of de connectiviteit bij mensen met PTSS herstelt na een succesvolle behandeling en of de gevonden verschillen het behandelresultaat kunnen voorspellen. Om dit te onderzoeken werd het project BETER opgezet (Biologische Effecten van Traumatische ERvaringen, behandeling en herstel). Tijdens het BETER project zijn 58 veteranen met PTSS, 29 veteranen zonder PTSS en 26 gezonde burgers onderzocht door middel van interviews, vragenlijsten en hersenscans op twee momenten: voor behandeling en 6-8 maanden later. Het doel van mijn onderzoek, dat resulteerde in dit proefschrift, was meer inzicht te verkrijgen in *Het Neurale Oorlogsweb* door de structurele en functionele connectiviteit van mensen met PTSS en mensen zonder PTSS met elkaar te vergelijken.

Structurele verbindingen – Sectie 1

Met diffusie tensor imaging (DTI) scans kunnen de zenuwbanen in het brein, de 'witte stof', in kaart worden gebracht. Met DTI scans wordt beweging van water gemeten en omdat water eerder langs de zenuwbaan loopt dan dat het de zenuwbaan kruist,

kunnen structurele connecties in het brein in beeld worden gebracht. *Sectie 1* beschrijft de verschillen die we hebben gevonden tussen de structurele connectiviteit van veteranen zonder PTSS, veteranen met PTSS die herstellen na behandeling en veteranen met PTSS die chronisch klachten houden.

In het onderzoek dat beschreven wordt in **Hoofdstuk 2** hebben we specifieke zenuwbanen in de hersenen bekeken die belangrijk zijn voor de emotieregulatie en het autobiografisch geheugen: de cingulum bundel, de fornix en de stria terminalis. We ontdekten dat veteranen met chronische PTSS een sterkere cingulum bundel hebben. De cingulum bundel werd zelfs sterker in de loop der tijd. Dit kan erop wijzen dat de verbinding sterker wordt naarmate er langer PTSS klachten zijn. Er werden ook veranderingen in de andere zenuwbanen gevonden die mogelijk markers zijn voor het herstel van PTSS.

In **Hoofdstuk 3** beschrijven we de verschillen in de isthmus cingulum die we hebben gevonden bij PTSS patiënten die herstellen in vergelijking met PTSS patiënten die niet herstellen en controles. In hetzelfde hoofdstuk laten we zien dat de resultaten beïnvloed werden door de keuze van de fasecoderingsrichting tijdens de dataverzameling. De keuze voor een bepaalde acquisitieparameter kan grote invloed hebben. Wij zouden onderzoekers willen aanraden om hier alert op te zijn en de fasecoderingsrichting te rapporteren in nieuwe artikelen.

Functionele verbindingen – Sectie 2

Met een functionele magnetic resonance imaging (fMRI) scan tijdens rust kunnen hersengebieden die met elkaar communiceren in kaart worden gebracht. Door te kijken naar patronen van activatie van hersengebieden en te berekenen in hoeverre hersengebieden tegelijkertijd actief zijn (co-activatie), wordt functionele connectiviteit gemeten (zie Figuur 2 in **Hoodstuk 1**).

In **Hoofdstuk 4** wordt beschreven welke verschillen gevonden zijn in de functionele connectiviteit van subgebieden van de anterieure cingulate cortex (ACC) van militairen met en zonder PTSS en gezonde burgers. Veteranen (met en zonder PTSS) vertonen een lagere connectiviteit dan de gezonde burgers tussen motorische controlegebieden (de caudale ACC en de precentrale gyrus) en tussen gebieden die betrokken zijn bij zelfreflectie (de perigenuale ACC en de superiore mediale gyrus en de middelste temporale gyrus). Deze verschillen hebben mogelijk te maken met militaire training, uitzending en/of blootstelling aan trauma. Naast verschillen tussen veteranen en burgers werden ook specifieke kenmerken van gezonde veteranen gevonden. Veteranen zonder PTSS hebben meer connectiviteit tussen aandachtsgebieden (de rostrale ACC en de precentrale/middelste frontale gyrus) dan veteranen met PTSS en gezonde

burgers. Dit zou samen kunnen hangen met veerkracht. Deze resultaten laten zien dat het belangrijk is om in PTSS onderzoek zowel een controlegroep mee te nemen die is blootgesteld aan eenzelfde trauma (bijv. uitzending) als een groep zonder trauma blootstelling.

Een posttraumatische stressstoornis wordt vaak gediagnostiseerd in combinatie met een huidige depressieve stoornis. Daarom is het onduidelijk in hoeverre neurobiologisch onderzoek naar PTSS wordt gekleurd door de aanwezigheid van een depressie. In **Hoofdstuk 5** is de connectiviteit van twee hersengebieden die zowel met PTSS als met depressie zijn geassocieerd (de insula en de subgenuale ACC) vergeleken tussen militairen met PTSS met een depressie en militairen met PTSS zonder depressie. De connectiviteit van de ACC met omliggende prefrontale regio's was hoger bij PTSS patiënten met depressie dan bij PTSS patiënten zonder depressie. Dit verschil hangt mogelijk samen met de depressieve klachten. De connectiviteit tussen de ACC en de thalamus en tussen de insula en hippocampus was lager in het brein van PTSS patiënten met depressie dan in het brein van PTSS patiënten zonder depressie. Dit impliceert dat er bij patiënten met PTSS met depressie minder communicatie tussen deze regio's is.

In **Hoofdstuk 6** is het functionele netwerk van het gehele brein in kaart gebracht met een 'graph analyse'. Via deze methode worden de hoeveelheid connecties van een bepaald hersengebied (degree) en de hoeveelheid connecties van aangrenzende hersengebieden die met elkaar een verbinding hebben, berekend in verhouding tot het totaal aantal mogelijke verbindingen (de clusteringscoëfficiënt). De degree van een hersengebied geeft aan hoe belangrijk het gebied is in het netwerk en de clusteringscoëfficiënt geeft aan hoe sterk aangrenzende hersengebieden met elkaar zijn verbonden en is daarmee een maat voor functionele specialisatie.

Met de complete brein netwerk analyse werden voor behandeling verschillende hersengebieden geïdentificeerd waarvan het aantal connecties samenhing met de aanwezigheid van PTSS. Het aantal connecties van onder andere de precuneus en posteriore cingulate cortex, pallidum, rolandic operculum en orbitofrontale cortex hing samen met de aanwezigheid van PTSS (**Hoofdstuk 6**). Deze gebieden spelen een rol bij processen als zelfreflectie, aandacht en executieve functies. Mogelijk zijn deze processen verstoord bij PTSS patiënten en spelen de connecties van deze hersengebieden daar een rol bij. Daarnaast was de clusteringscoëfficiënt van de ACC van patiënten vaak lager dan die van controles. Dit kan samenhangen met een verminderde functionele specialisatie van het lokale ACC netwerk. Deze gebieden werden vervolgens vergeleken voor en na behandeling tussen PTSS patiënten die herstelden, patiënten die niet herstelden en controles. Er werden geen verschillen gevonden die samenhingen met herstel. Deze studie biedt meer inzicht in de psychopathologie van PTSS.

Discussie

Het neurale web van PTSS

Er zijn verschillende neurale modellen beschreven die PTSS klachten zouden kunnen verklaren, waarvan er hier twee worden besproken: het drievoudige netwerkmodel voor psychopathologie (Menon, 2011) en het conventionele model voor PTSS (Rauch, Shin, Phelps, 2006). Het drievoudige netwerkmodel stelt dat er veranderingen in drie belangrijke netwerken samenhangen met allerlei soorten psychopathologie, waaronder PTSS. In **Hoofdstuk 6** werd de connectiviteit van hersengebieden van deze drie netwerken geassocieerd met PTSS, hetgeen deze theorie ondersteunt. Het conventionele model voor PTSS veronderstelt dat een verhoogde activatie van het emotiecentrum (amygdala) samen met verminderde controle daarvan door de prefrontale cortex ten grondslag ligt aan PTSS klachten. Onze resultaten lieten verschillen zien tussen PTSS patiënten en gezonde mensen (burgers en veteranen) in prefrontale gebieden (**Hoofdstuk 6**) en gaven ook aanwijzingen voor veranderingen in de structurele verbinding van deze regio's (in de stria terminalis en fornix, **Hoofdstuk 2**), wanneer patiënten herstelden van de klachten.

Vage lijnen – co-morbide depressie

Er zijn factoren die een duidelijke indeling van PTSS bemoeilijken. Zo kleurt de aanwezigheid van een co-morbide depressie het beeld van PTSS en verschillen tussen PTSS patiënten met en zonder co-morbide depressie zijn ook zichtbaar in functionele connectiviteit (**Hoofdstuk 5)**. Daarnaast wordt vaak beschreven dat co-morbide depressieve klachten samenhangen met een slechtere prognose. In **Hoofdstuk 2 en 6** vonden wij inderdaad dat de PTSS patiënten die niet herstelden na behandeling vaker een co-morbide depressie of angststoornis hadden.

Daarnaast zijn er vage grenzen rondom de diagnose PTSS en het definiëren van herstel. Zo zijn nieuwe diagnostische criteria voor PTSS ontwikkeld in de DSM-5, waarbij zowel vermijdings- als stemmingssymptomen aanwezig moeten zijn om PTSS vast te stellen. In dit proefschrift is gebruik gemaakt van de DSM-IV criteria, maar wanneer de resultaten uit **Hoofdstuk 2** werden bekeken met DSM-5 criteria werden dezelfde resultaten gevonden. Dit lijkt dus niet uit te maken voor deze resultaten. Echter, naast de diagnostische criteria kan ook gekozen worden voor een afkappunt op de interviewscores (CAPS: 45) of voor een percentage afname in klachten (>30%) om herstel van PTSS te definiëren. Kortom, verschillende keuzes en indelingen zijn mogelijk die het vergelijken van resultaten bemoeilijken, de lijnen van PTSS vervagen en die in het achterhoofd gehouden moeten worden wanneer over PTSS en behandeling gesproken wordt.

Behandeling van PTSS

Er zijn verschillende maten gevonden die samenhingen met behandelresultaat. Er werd bijvoorbeeld een sterkere dorsale cingulum gevonden in **Hoofdstuk 2** bij patiënten met aanhoudende klachten, die verder ontwikkelde in de loop der tijd. Het is daarom van belang te bepalen wanneer deze verbinding voor het eerst sterker wordt na het meemaken van een traumatische ervaring. Wanneer we dit weten, zou mogelijk voorkomen kunnen worden dat PTSS chronisch wordt, door bijvoorbeeld vroege interventies te doen.

Er is echter meer onderzoek nodig om de effecten en werkingsmechanismen van behandeling beter in kaart te brengen. Zo zou onderzoek tijdens behandeling of sessies kunnen bijdragen aan een beter inzicht in het neurale mechanisme dat ten grondslag ligt aan de subjectieve verandering in klachten. Daarnaast zijn er factoren van invloed die in de klinische setting vaak worden gezien, maar niet worden meegenomen in onderzoek (zoals het meten van persoonlijkheidsstoornissen). Om een compleet beeld te krijgen van de stoornis en om dit soort toekomstig onderzoek te kunnen uitvoeren is een nauwe samenwerking tussen behandelaren en onderzoekers van belang.

Oorlog en veerkracht

In **Hoofdstuk 4** werden verschillen gevonden in de connectiviteit tussen veteranen en burgers. Vervolgstudies die metingen voor uitzending vergelijken met metingen na uitzending zouden meer inzicht kunnen bieden in wanneer deze verschillen ontstaan. Daarmee zou onderscheiden kunnen worden welke verschillen ontstaan na uitzending en welke eerder al aanwezig zijn. Daarnaast werden er ook specifieke verschillen gevonden in de groep gezonde veteranen, die mogelijk kunnen samenhangen met veerkracht. Toekomstig onderzoek met metingen voor uitzending zou ook meer inzicht kunnen bieden in veerkrachtsfactoren. Dit kan bijdragen aan het ontwikkelen van trainingen voor veerkracht (specifiek gericht op de neurale netwerken die te maken hebben met veerkracht) of het ontwikkelen van vroege interventies om het ontwikkelen van een 'misconnectie' in het netwerk te voorkomen zodat er geen PTSS ontstaat.

Conclusie

Dit proefschrift draagt bij aan ons begrip van *Het Neurale Oorlogsweb*. We vonden verschillen in connectiviteit die samenhingen met PTSS, persistentie van de klachten, acquisitie methode, co-morbide depressie, uitzendervaring/militaire training en veerkracht. Daarnaast vonden we indicatoren voor behandelresultaat, die mogelijk nog duidelijker worden wanneer klachten langere tijd aanhouden. Onze resultaten bieden inzicht in de psychopathologie van PTSS en behandeling en laten ook zien dat andere factoren samenhangen met connectiviteit. Verder hebben we bevestigd dat niet iedere

PTSS patiënt hetzelfde is, maar dat individuele verschillen bijvoorbeeld in co-morbiditeit de behandeluitkomst kunnen beïnvloeden. De inzichten van deze studies kunnen in de toekomst gebruikt worden voor 'behandeling op maat'. Concluderend, in dit proefschrift zijn geavanceerde technieken toegepast om de connectiviteit van het brein te bestuderen in veteranen met en zonder PTSS en burgers en dit heeft essentiële informatie opgeleverd voor het ontwarren van *Het Neurale Oorlogsweb*.

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Beste Elbert, *we go way back*. In 2008 kwam ik als 21-jarig studentje slaaponderzoek doen. De printer stond toen nog in de stagiaire kamer (de vissenkom), dus daar kwam jij regelmatig buurten. Ik leerde je kennen als enthousiaste onderzoeker die alle manieren om PTSS te onderzoeken inzet: neuroimaging/ neuropsychologische taken/ vragenlijsten/ hormonen/ (epi)genetica. Toen ik na mijn stage op zoek was naar een scriptieproject (ik wilde een review schrijven over de neurale correlaten van EMDR) hebben jij en Arthur mij gestrikt en ging ik uiteindelijk (bij gebrek aan literatuur) jullie vakgebieden combineren: neuroimaging en persoonlijkheidsleer. Wat een feestje: de afspraken op maandagochtend begonnen met een extra dark roast senseo en jij en Arthur wisselden van good cop naar bad cop. Toen jullie me tijdens

mijn tweede stage in een skype-gesprek vroegen terug te komen uit Australië voor een onderzoeksassistent-baan, kon ik dan ook alleen maar volmondig ja zeggen. En vervolgens hebben jullie een PhD plek voor me gecreëerd. Bedankt voor deze kansen! Ik heb veel van je geleerd als copromotor, zoals wetenschappelijk schrijven, maar ook het bestaan van een grijs gebied tussen goed en fout. Ik wens je veel plezier met het verbouwen van je nieuwe huis en hoop dat we elkaar in de toekomst nog vaak zullen tegenkomen in het vakgebied!

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Dear Professor Kalin, dear Ned, Thank you for giving me the opportunity to visit your lab and including me in the Kalin lab. You gave me a warm welcome and I learned a lot about translational research, the fear circuit and diffusion tensor imaging.

Dear Dr. Fox, Drew! You really impress me with your knowledge about methodology, neuroanatomy, programming, writing and more to mention. I am happy that you and Do found each other and I want to thank you for making my stay in Madison a success.

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Claire, ik snap ook niet waarom ze die bestekbak niet vóór de borden hebben gezet bij de kantine ☺. Ik hoop dat je een baan vindt in jouw straatje.

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M Kennis, SJH van Rooij, MP van den Heuvel, RS Kahn, E Geuze. Functional network topology associated with posttraumatic stress disorder in veterans. *Submitted*.

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Epilogue

When gaining knowledge, you become aware of the unknown. Trying to resolve questions often results in having more unanswered questions. This is perhaps the pitfall but also the beauty of research, that has frustrated and inspired me during my PhD. More research is definitely needed in this field, since there is still so much to discover. Feeling personally attached to my research field, I am determined to gain more insights in the neurobiology of posttraumatic stress disorder, in order to help people that experience trauma in the (near or far) future. The positive response of many participants drives me to find better ways to help people that experienced trauma. For them and others I will continue to do my best to gain knowledge to disentangle the *Neural Web of War*. Hence, I will try to do right to my last name: *Kennis* - knowledge.