Boosting oxytocin after trauma: Effects of oxytocin on fear neurocircuitry in patients with post-traumatic stress disorder

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About 10 percent of trauma-exposed individuals develop a post-traumatic stress disorder (PTSD). Since a substantial part of PTSD patients does not fully benefit from existing psychotherapies, new interventions boosting treatment response in PTSD are highly needed. The neuropeptide oxytocin has been suggested as promising pharmacological agent to enhance treatment response in PTSD, because of its anxiolytic and pro-social properties. As a first step to explore the clinical potential of oxytocin in PTSD, the effects oxytocin administration on fear neurocircuitry were investigated in male and female police officers with and without PTSD.

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BOOSTING OXYTOCIN AFTER TRAUMA

Effects of oxytocin on fear neurocircuitry in patients with post-traumatic stress disorder

Saskia Barbara Josephina Koch
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Faculteit der Geneeskunde
‘If you can meet with Triumph and Disaster
And treat those two impostors just the same’
Rudyard Kipling (1895)

Voor mijn ouders
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CHAPTER 1

General Introduction
Today it is widely recognized that individuals exposed to a traumatic event may develop psychiatric disorders (American Psychiatric Association, 2013). One hundred years ago, however, this idea was not yet accepted. During the First World War a new medical condition called ‘shell shock’ emerged (Myers, 1915). Frontline soldiers were stuck in trenches and exposed to (threat of) mortar attacks, heavy artillery fire, poisonous gas and mines. A significant proportion of military combat casualties (e.g. 10% in the British army) suffered from shell shock, including symptoms of dizziness, headaches, hypersensitivity to noise, tremors and amnesia (Jones et al, 2007; Mitchell and Smith, 1931). Resembling the symptoms of head injury, shell shock was first thought of as neurological lesion, resulting from blast exposure to exploding artillery shells. However, as many patients were not blast-exposed or did not suffer from head injury, shell shock was increasingly recognized as a psychological, rather than physical combat injury (Jones et al, 2007).

A traumatic event consists of direct or indirect exposure to actual or threatened death, serious injury or sexual violence (American Psychiatric Association, 2013). It has been estimated that approximately 80% of the general Dutch population will experience at least one traumatic event during their lives (de Vries and Olff, 2009). After experiencing a traumatic event, initial feelings of fear and distress usually constitute adaptive behavioral and physiological responses to cope with environmental threat. Across species, the ability to learn, adapt and flexibly respond to danger signals in the environment is a prerequisite for survival (Rodrigues et al, 2009). In the majority of trauma-exposed individuals, initial feelings of fear and distress gradually decline in the aftermath of a traumatic event, when the individual returns to safe, unthreatening contexts. However, in a minority of individuals, trauma exposure results in psychiatric disorders, such as post-traumatic stress disorder (PTSD), depression and anxiety disorders (Goldstein et al, 2016). By definition, PTSD is the only psychiatric disorder for which trauma exposure represents an identifiable precipitating event.

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder, which develops in approximately 10% of individuals after experiencing a traumatic event, resulting in an estimated life-time prevalence of about 7.4% in the Dutch general population (de Vries and Olff, 2009). Symptoms of PTSD include intrusive re-experiencing, such as intrusive thoughts, nightmares and flashbacks about the traumatic event, as well as avoidance of intrinsic and external reminders of the traumatic event (American Psychiatric Association, 2013). In addition, PTSD symptoms include negative alterations in cognition and mood, such as negative (distorted) cognitions, negative trauma-related emotions and diminished interest in significant activities. Finally, PTSD symptoms consist of alterations in arousal and reactivity, including aggressive behavior, hypervigilance and sleep disturbances (American Psychiatric Association, 2013). According to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013), PTSD symptoms should be present for at least one
month and cause significant functional impairment and distress. PTSD has been associated with high individual and societal burden: PTSD is highly comorbid with physical and psychiatric health problems, such as major depressive disorder (MDD), and is associated with impaired well-being and increased mortality rates (Goldstein et al., 2016; Kessler, 2000).

According to international guidelines, exposure therapy, cognitive behavioral therapy (CBT) and eye-movement desensitization and reprocessing (EMDR) are treatments of choice for PTSD (Foa et al., 2009; Schnyder et al., 2015). These psychotherapies are trauma-focused, consisting of (in vivo) exposure to reminders of the traumatic event, thereby extinguishing or inhibiting fear responses (Rothbaum and Davis, 2003). Although generally effective, a substantial part of PTSD patients drops out of treatment or relapses after initial successful response: according to a meta-analysis approximately one-third of PTSD patients still meet the criteria for PTSD after treatment completion (Bradley et al., 2005). Because of the high individual and societal burden of PTSD, it is of paramount importance to investigate new interventions, either pharmacological or psychological, to boost treatment response in PTSD. The neuropeptide oxytocin has been suggested as promising pharmacological agent to enhance treatment response in PTSD, because of its anxiolytic and pro-social properties (Olff et al., 2010).

BACKGROUND ON PTSD AND OXYTOCIN

Neurobiology of PTSD

According to meta-analyses on functional neuroimaging studies comparing PTSD patients to healthy controls, PTSD is associated with amygdala hyperactivity towards negative stimuli (Hayes et al., 2012), as well as with hyperactivity of the anterior insula and dorsal anterior cingulate cortex (dACC) (Patel et al., 2012). The amygdala, anterior insula and dACC are core nodes of the salience network (SN), an intrinsic brain connectivity network aimed at detecting biologically relevant information in the environment and orchestrating subsequent behavioral adaptation to environmental demands (Menon, 2011; Seeley et al., 2007). Especially the amygdala plays a pivotal role in the detection of biologically relevant information (i.e. such as threat and emotional faces) and orchestrates subsequent expression of fear, via excitatory connections to the brainstem and hypothalamus (Hariri et al., 2002; LeDoux, 2007; Phelps and LeDoux, 2005). Additionally, PTSD has been characterized by increased communication between these core nodes of the SN (Sripada et al., 2012b). Taken together, increased activity in and connectivity between the amygdala, anterior insula and dACC in PTSD may represent the underlying neural correlate of hypervigilance symptoms frequently observed in PTSD (Sripada et al., 2012b).
Although generally adaptive in threatening situations, fear expression of the amygdala should be controlled in safe, unthreatening contexts. The ventromedial prefrontal cortex (vmPFC) is involved in top-down control over the fear response, via inhibitory projections to the amygdala (Jovanovic and Ressler, 2010). Not surprisingly, hypoactivity of the vmPFC has been implicated in the pathophysiology of PTSD, possibly underlying deficient top-down prefrontal control over the fear response (Rauch et al, 2006). In line with the suggestion of diminished top-down control in PTSD, decreased functional connectivity between the vmPFC, amygdala and hippocampus has been observed in PTSD patients (Sripada et al, 2012a). Additionally, PTSD was associated with grey matter reductions in the vmPFC and left hippocampus according to a meta-analysis on structural grey matter alterations in PTSD (Kühn and Gallinat, 2013a). The hippocampus is especially important in discriminating between safe and threatening contexts (Kalisch et al, 2006; Pohlack et al, 2012), and has been suggested to underlie impaired contextual extinction learning in PTSD (Rauch et al, 2006). In conclusion, PTSD has been associated with increased activity in and connectivity between salience processing areas, as well as decreased prefrontal top-down control over the fear response, presumably resulting in generalization of the fear responses to safe, unthreatening contexts (Rauch et al, 2006).

Neurobiology of oxytocin

The neuropeptide oxytocin is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and is projected to various brain areas involved in fear regulation and social behavior, such as the amygdala, brainstem, hippocampus and prefrontal cortex (Meyer-Lindenberg et al, 2011). Additionally, oxytocin is released into the bloodstream via the posterior pituitary gland, where it acts as a hormone on peripheral targets involved in lactation and parturition. Animal studies have highlighted the involvement of oxytocin in maternal behavior, pair bonding and social behavior (Neumann, 2008; Wang and Aragona, 2004). Rodent studies observed increased peripheral and central endogenous oxytocin release in response to stressors (Engelmann et al, 2004), as well as anxiolytic effects of both synthetic and endogenous oxytocin (Neumann and Slattery, 2015). In line with these observations, intranasal oxytocin administration studies in healthy humans also indicated pro-social and anxiolytic properties of oxytocin (Heinrichs et al, 2003; Van IJzendoorn and Bakermans-Kranenburg, 2012). For example, oxytocin administration in healthy individuals resulted in enhanced (in-group) trust (Van IJzendoorn and Bakermans-Kranenburg, 2012), as well as diminished subjective anxiety during public speaking stress (Heinrichs et al, 2003; de Oliveira et al, 2012). Given the pro-social and anxiolytic effects of oxytocin in animal and human literature, a great interest has recently emerged in the therapeutic potential of oxytocin in various psychiatric disorders characterized by deficits in fear regulation or social functioning, such as autism spectrum disorder, schizophrenia, depression and generalized anxiety disorder (Cochran et al, 2013). According to a recent meta-analysis of randomized, placebo-controlled clinical
trials, oxytocin administration reduced symptoms of various psychiatric disorders, such as symptoms of anxiety, depression, autism and psychosis (Hofmann et al., 2015).

At the neural level, intranasal oxytocin fMRI studies in healthy males have shown dampened amygdala reactivity towards emotional stimuli (Domes et al., 2007; Kirsch et al., 2005) and previously fear conditioned faces (Petrovic et al., 2008), although contradictory findings were observed in healthy females (Domes et al., 2010; Lischke et al., 2012). Furthermore, intranasal oxytocin administration resulted in enhanced resting-state functional connectivity between the amygdala and vmPFC in healthy males (Sripada et al., 2013). Additionally, oxytocin administration resulted in decreased amygdala reactivity towards emotional faces, both in male patients with generalized social anxiety disorder (GSAD) (Labuschagne et al., 2010) and in female patients with borderline personality disorder (Bertsch et al., 2013), as well as in increased functional connectivity between the amygdala and vmPFC in male GSAD patients (Dodhia et al., 2014). Taken together, these previous findings indicate that oxytocin may be a promising pharmacological adjuvant to enhance treatment response in PTSD, by targeting (neural correlates of) excessive fear and diminished prefrontal top-down control over the fear response.

**THE BOOSTER STUDY**

It has been suggested that oxytocin may exert beneficial effects on treatment response in PTSD via a dual mechanism: (1) oxytocin may dampen the excessive fear response commonly observed in PTSD patients, (2) oxytocin may enhance reward sensitivity and thereby the therapeutic alliance (Olff et al., 2010). Although promising, the therapeutic potential of oxytocin administration in PTSD patients has scarcely been studied. As a first step to investigate the clinical potential of oxytocin in PTSD, we investigated the effects of a single oxytocin administration on fear and reward-related neural processes in PTSD patients: the BOOSTER (BOosting the Oxytocin System after Trauma: neurobiological effects on Emotion and Reward-processing in PTSD) study.

The BOOSTER study is a randomized placebo-controlled cross-over functional magnetic resonance imaging (fMRI) study in 77 male and female police officers with (n=37, 21 males) and without PTSD (n=40, 20 males). Police officers are first responders during emergency work, during which they are frequently confronted with various traumatic events, such as shooting incidents, dead bodies, traffic accidents and severely injured victims. Not surprisingly, police work has been regarded as one of the most stressful jobs (Carlier et al., 1997). The incidence of PTSD in Dutch police officers ranges from 7% after critical incidents (Carlier et al., 1997) up to 19% after shooting incidents (Gersons, 1989). We included trauma-exposed police officers...
in the BOOSTER study, resulting in a relatively homogeneous participant sample and thereby controlling for the potential effect of trauma exposure on neurobiology (van Wingen et al., 2011). Additionally, we included both male and female participants to investigate possible sex-differential effects on the neurobiology of PTSD and the effects of oxytocin on fear circuitry.

The BOOSTER study consisted of three assessments. During the first assessment (T0), written informed consent was obtained and in- and exclusion criteria were checked. Hereafter, two fMRI sessions were conducted (T1 and T2), which were each separated by approximately one week (See Figure 1.1 for the study design). Each fMRI session started with questionnaires assessing current PTSD, anxiety and depression symptom severity, followed by intranasal spray administration under experimenter supervision. Placebo was administered during one and oxytocin during the other session. Medication-order was counterbalanced and double-blinded. Approximately 40 minutes after intranasal spray administration, the MRI scanning session started with the acquisition of a structural image: a T1-weighted image was obtained during the first scanning session and a diffusion-weighted image (DWI) was obtained during the second session. Hereafter, functional scanning began, consisting of several tasks assessing the neurobiology underlying fear and reward processing (See Figure 1.1 for a description and tasks).

**FIGURE 1.1 Design of the BOOSTER study and tasks**

Schematic representation of the design of the BOOSTER study. Male and female police officers were scanned twice, receiving intranasal placebo during one and oxytocin during the other session. The order of intranasal spray administration was double-blind and randomized. Scanning began with the acquisition of a structural scan (T1-weighted image during first session, and diffusion-weighted image (DTI-scan) during second session), approximately 40 minutes after intranasal spray administration. Functional scanning consisted of an emotional face-matching task (Chapter 3), resting-state scan (Chapter 4) and distraction task (Chapter 5).
of the relevant tasks). This PhD thesis focuses on the acute effects of intranasal oxytocin administration on fear-related neurocircuitry in PTSD patients.

AIMS AND OUTLINE

The aim of this PhD thesis was to investigate the neurobiological effects of a single oxytocin administration on fear neurocircuitry in male and female PTSD police officers with and without PTSD. More specifically, we focused on oxytocin administration effects on subjective anxiety, amygdala activity and functional connectivity between the amygdala and vmPFC in PTSD patients. Additionally, we investigated neurobiological alterations in PTSD, focusing on resting-state brain activity, functional connectivity, as well as structural connectivity of major white matter tracts.

This thesis can be divided in two parts. The first part consists of Chapters 2-5, in which the effects of oxytocin administration on amygdala activity and connectivity in male and female police officers with and without PTSD are investigated. In the second part of this thesis (Chapters 6-7), we investigated baseline (i.e. oxytocin unrelated) alterations in resting-state brain activity, as well as functional and structural connectivity in male and female PTSD patients, compared to trauma-exposed controls.

Part 1: Oxytocin effects on fear neurocircuitry in PTSD

Chapter 2 is a literature review, outlining the fear neurocircuitry in healthy individuals and current knowledge on its alterations in PTSD. Additionally, the hypothesized effects of oxytocin administration on the fear neurocircuitry in PTSD are discussed.

In Chapter 3 we investigated the effects of a single oxytocin administration on amygdala activity towards emotional faces in male and female PTSD patients and trauma-exposed controls.

In Chapter 4 the effects of oxytocin administration on resting-state functional connectivity of the amygdala subregions with the prefrontal cortex and salience processing areas were investigated in male and female PTSD patients and trauma-exposed controls.

In Chapter 5 we investigated behavioral and neural oxytocin effects on distraction as a cognitive emotion regulation strategy in male and female PTSD patients and trauma-exposed controls.
**Part 2: Neurobiological alterations in PTSD**

In **Chapter 6** we aimed at providing a comprehensive overview of all resting-state studies conducted to date in PTSD. This chapter consists of both a quantitative meta-analysis on whole-brain resting-state studies and a systematic review of all seed-based (i.e. functional connectivity) resting-state studies in PTSD patients, compared to healthy controls.

In **Chapter 7** we conducted a diffusion tensor imaging study to investigate alterations in white matter integrity of several major anatomical white matter paths, including the uncinate fasciculus, cingulum, and superior longitudinal fasciculus (both parietal and temporal projections) in PTSD patients, compared to trauma-exposed controls.

Finally, **Chapter 8** consists of a summary and general discussion in which findings from previous chapters are integrated.
Chapter 2

Intranasal oxytocin as strategy for medication-enhanced psychotherapy in PTSD: salience processing and fear inhibition processes

Invited review

Saskia B.J. Koch, Mirjam van Zuiden, Laura Nawijn, Jessie L. Frijling, Dick J. Veltman & Miranda Olff

ABSTRACT

About ten percent of people experiencing a traumatic event will subsequently develop post-traumatic stress disorder (PTSD). PTSD is characterized by an exaggerated fear response which fails to extinguish over time and cannot be inhibited in safe contexts. The neurobiological correlates of PTSD involve enhanced salience processing (i.e. amygdala, dorsal anterior cingulate cortex (dACC) and anterior insula (AI) hyperactivity), and reduced top-down inhibitory control over this fear response (i.e. dorsal and ventromedial prefrontal cortex (vmPFC) hypoactivity and diminished structural and functional connectivity between the vmPFC, hippocampus and amygdala). Therefore, dampening the exaggerated fear response (i.e. by reducing amygdala hyperactivity) and enhancing top-down inhibitory control (i.e. by promoting prefrontal control over the amygdala) during psychotherapy is an important target for medication-enhanced psychotherapy (MEP) in PTSD patients. Since the neuropeptide oxytocin (OT) has been found to act on these two processes, we propose that oxytocin is a promising pharmacological agent to boost treatment response in PTSD. Human fMRI studies indicate that intranasal oxytocin attenuates amygdala (hyper)activity and enhances connectivity of the amygdala with the vmPFC and hippocampus, resulting in increased top-down control over the fear response. In addition, intranasal oxytocin was found to attenuate amygdala – brainstem connectivity and to change activity and connectivity in nodes of the salience network (i.e. AI and dACC). Furthermore, oxytocin administration may modulate hypothalamus-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) function and may enhance social behavior, which could be beneficial in the therapeutic alliance. We also discuss contextual and inter-individual factors (e.g. gender and social context) which may influence the effectiveness of oxytocin in MEP. In all, we propose that intranasal oxytocin given prior to each psychotherapy session may be an effective additive treatment to boost treatment response in PTSD.
1. INTRODUCTION

About 80 percent of the general population will experience a potentially traumatic event (i.e. a situation involving actual or threatened death, actual or threatened serious injury or actual or threatened sexual violence) during their lifetime (American Psychiatric Association, 2013; de Vries and Olff, 2009). Typically, initial feelings of distress will gradually reduce in the aftermath of the event. Approximately ten percent of trauma-exposed individuals, however, will develop a post-traumatic stress disorder (PTSD) (de Vries and Olff, 2009), which is characterized by an excessive fear response to reminders of the traumatic event. PTSD consists of four clusters of symptoms: intrusions (i.e. nightmares, flashbacks and intrusive thoughts about the traumatic event), avoidance (i.e. avoidance of thoughts and reminders of the traumatic event), negative alterations in cognitions and mood (i.e. diminished interest in significant activities, emotional numbing, persistent negative beliefs and trauma-related emotions and distorted blame) and alterations in arousal and reactivity (i.e. increased self-destructive and aggressive behavior and increased alertness and physiological arousal) (American Psychiatric Association, 2013).

Regarding treatment of PTSD, exposure therapy and trauma-focused cognitive behavioral therapy (TF-CBT) are recommended by international guidelines as treatments of choice (Foa et al., 2009). Exposure therapy consists of exposure to imaginary or in vivo reminders of the traumatic event and ideally results in attenuated fear responses towards these reminders. Imaginary exposure is also an important active component of TF-CBT, together with cognitive restructuring (Foa et al., 2009). Unfortunately, not all PTSD patients fully benefit from these psychotherapies, as a considerable percentage of patients fails to continue treatment and treatment completers may suffer from substantial residual symptoms or relapse after initial response to treatment (Cloitre, 2009): a meta-analysis showed that approximately one third of PTSD patients completing psychotherapy did still meet the diagnostic criteria of PTSD (Bradley et al., 2005). Therefore, focusing on new strategies to augment treatment response in PTSD seems highly relevant. A recent review on new medication strategies for PTSD indicated that medication-enhanced psychotherapy (MEP) is a promising new venue for treatment in PTSD (Dunlop et al., 2012). Notably, new pharmacological agents that inhibit excessive fear and facilitate therapeutic engagement seem especially appropriate to enhance treatment response in PTSD (Dunlop et al., 2012). Although several pharmacological agents (including MDMA and D-cycloserine) have been investigated (de Kleine et al., 2012; Mithoefer et al., 2011), none of these can be used in routine clinical practice yet. We propose that the neuropeptide oxytocin (OT), which can be administered intranasally, is a promising candidate for MEP in PTSD. First of all, oxytocin has strong anxiolytic properties, which could dampen the excessive fear observed in PTSD. Furthermore, oxytocin may increase sensitivity to cues from the social environment, possibly leading to improved social functioning (e.g. increased...
feelings of trust) within safe environments (Olff et al, 2013), which could be beneficial in the therapeutic alliance (Olff et al, 2010).

This review will focus on the neurobiological mechanisms by which oxytocin is hypothesized to dampen the excessive fear response observed in PTSD. We will first describe the neurobiological mechanisms of the fear response in healthy individuals and their aberrations in PTSD patients. Next, the mechanisms by which oxytocin is hypothesized to reduce the fear response in PTSD are discussed. It should be noted, however, that although symptoms of fear and anxiety often appear to be predominant, affect dysregulation in PTSD frequently includes excessive anger, guilt, and shame or emotional numbing, which may need to be addressed in psychotherapy as well (Cloitre, 2009).

2. SALIENCE PROCESSING AND FEAR INHIBITION

The ability to identify the most relevant information from a multitude of external and internal stimuli is essential in guiding behavior and promoting survival. The salience network, with the amygdala, dorsal anterior cingulate cortex (dACC) and anterior insula (AI) as its core nodes, is important in detecting biologically important information from the context and changing subsequent behavior (Seeley et al, 2007). Upon threat detection, amygdala responses initiate a range of autonomic and endocrine changes to enhance survival. Crucially, the detection of threat and subsequent expression of fear can be top-down controlled by various brain structures, especially the hippocampus and ventromedial prefrontal cortex (vmPFC).

2.1. Adaptive salience processing

The amygdala, dACC and AI are key nodes of the salience network, enhancing vigilance to biologically salient information (including threat) and subsequently adapting behavioral responses (Seeley et al, 2007). Neuroimaging studies have shown that these areas are involved in the detection of threat, acquisition of fear and the subjective experience of anxiety (Milad et al, 2007a; Pejic et al, 2013; Seeley et al, 2007; Simmons et al, 2006). Within this network, the amygdala is the crucial node in the detection of emotional salient stimuli (especially threat), as well as in acquisition and expression of fear responses. The AI is associated with detection of emotional salience and subsequent initiation of executive processes (e.g., directing attention and initiation of goal-directed behavior) (Menon, 2011). The dACC, on the other hand, seems to be especially important in the cognitive aspects of fear learning (Milad et al, 2007a) and performing goal-directed behavior upon salience detection (Menon, 2011).

In line with the involvement of the salience network in detection and acquisition of fear, activation of the amygdala, dACC and the AI has been found during fear conditioning ex-
perimts (Milad et al., 2007a; Phelps et al., 2004). In a typical fear conditioning paradigm, an emotionally neutral stimulus (conditioned stimulus, CS+), such as an auditory tone, is repeatedly paired with an aversive stimulus (unconditioned stimulus, US), such as an electric shock. After several pairings, presentation of the CS+ alone will elicit a fear response, the conditioned response (CR). During fear conditioning, the basolateral part of the amygdala (BLA) receives sensory information from the (somato)sensory cortex and thalamus and associates the CS+ with the US (see Rodrigues et al. (2009) for a review). The central nucleus of the amygdala (CeM) is responsible for the expression of fear, by projecting to the hypothalamus and brainstem to initiate the stress response upon threat detection (Rodrigues et al., 2009). This state of alarm involves various autonomic and endocrine changes originally aimed at enhancing survival in the face of threat (Rodrigues et al., 2009). To this end, blood pressure and heart rate are increased, digestion is inhibited and metabolic supply to the muscles is increased, by activating the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis (Rodrigues et al., 2009). Furthermore, the CeM projects to monoaminergic systems in the forebrain and brainstem, resulting in increased vigilance, arousal and alertness to salient information (Rodrigues et al., 2009).

As already stated, the amygdala, dACC and AI are also involved in the subjective experience of (state and trait) anxiety. For example, self-reported social trait anxiety is positively associated with amygdala responsiveness to the CS+ during fear acquisition (Pejic et al., 2013). In addition, the AI was found to be hyperresponsive in individuals with high self-reported trait anxiety compared to non-anxious individuals when anticipating aversive pictures of spiders and snakes (Simmons et al., 2006). Furthermore, functional connectivity of the dACC with other regions in the salience network was positively related to pre-scanning subjective anticipatory anxiety (Seeley et al., 2007).

2.2. Salience processing in PTSD

PTSD is characterized by an exaggerated fear response, in which previously neutral stimuli present at the time of trauma gain the ability to elicit fear responses in the aftermath of the traumatic event (Rothbaum and Davis, 2003). Hence, the original fear response to the traumatic event does not extinguish over time but becomes generalized to safe, unthreatening contexts (Rothbaum and Davis, 2003). Not surprisingly, in fear conditioning experiments increased fear acquisition (i.e. increased startle response to CS+) has been found in PTSD patients compared to healthy controls, although not consistently (see Jovanovic and Ressler (2010) for a review).

2.2.1. Anatomy

In line with the exaggerated fear response observed at the behavioral level, neuroimaging studies have found structural alterations and aberrant activations in nodes of the salience
network in PTSD patients. Structurally, it appears that the amygdala is essential for development of PTSD, as Koenigs and colleagues (2008) found that war veterans with amygdalar lesions did not develop PTSD. Several meta-analyses have investigated the association between amygdalar volume and PTSD (Karl et al., 2006; Kühn and Gallinat, 2013a; Woon and Hedges, 2009). One meta-analysis found consistently smaller left amygdala volume in PTSD patients compared to trauma-exposed (TC) and non-trauma-exposed healthy controls (NTC) (Karl et al., 2006). In line with these findings, a recent study showed smaller bilateral amygdala volume in PTSD patients compared to trauma-exposed controls, which was independent of trauma load, duration of PTSD and depressive symptoms (Morey et al., 2012). However, two other meta-analyses did not find altered amygdala volume in PTSD patients compared to trauma-exposed controls (Kühn and Gallinat, 2013a; Woon and Hedges, 2009) and to non-trauma-exposed controls (Woon and Hedges, 2009). Moreover, a recent study of Kuo and colleagues (2012) showed larger amygdala volume in combat-exposed veterans with PTSD compared to healthy combat veterans. Possibly, these discrepancies could be explained by complex interactions between adulthood and childhood trauma exposure with regard to amygdala volume (Kuo et al., 2012).

Although the other nodes of the salience network have been less frequently investigated, one recent meta-analysis of whole-brain findings indicated grey matter reduction in the dACC in PTSD patients compared to trauma-exposed controls (Kühn and Gallinat, 2013a). Furthermore, left dACC volume was found to be reduced in recent onset PTSD patients compared to non-trauma-exposed controls (Chen et al., 2012). Karl and colleagues (2006) also found grey matter reduction in the ACC of PTSD patients compared to trauma-exposed controls, however, no distinction between the ventral and dorsal subregions of the ACC was made. Concerning the AI, the two whole-brain meta-analyses did not find altered AI volumes in PTSD patients (Karl et al., 2006; Kühn and Gallinat, 2013a).

2.2.2. Functional neuroimaging studies
Recently, three meta-analyses summarized functional neuroimaging results on task-related activation in PTSD patients (Hayes et al., 2012; Patel et al., 2012; Sartory et al., 2013). One meta-analysis of 19 functional neuroimaging studies with 245 PTSD patients found bilateral amygdala hyperactivation during the presentation of trauma-related stimuli, compared to neutral stimuli (Sartory et al., 2013). Furthermore, a whole-brain meta-analysis of 36 functional imaging studies (PET and fMRI) in PTSD across different experimental paradigms found increased left amygdala and bilateral AI activity in PTSD patients compared to non-trauma-exposed controls, but not to trauma-exposed controls (Patel et al., 2012). The third meta-analysis of 26 functional PET and fMRI imaging studies in PTSD patients found bilateral amygdalar hyperactivation for mixed cognitive-emotional paradigms (e.g., emotional face-matching task), but not for symptom provocation studies (e.g., auditory trauma script) (Hayes et al., 2012). How-
ever, this amygdala hyperactivity in cognitive-emotion studies was only found when region of interest (ROI) analyses were included or at a more lenient spatial extent threshold in the whole-brain analysis (Hayes et al., 2012). Moreover, the meta-analysis of Saratory et al. (2013) did also fail to find amygdala hyperactivity in PTSD patients compared to trauma-exposed controls. Possibly this lack of amygdala hyperactivity at the whole-brain level resulted from the comparison group used (trauma-exposed and non-trauma-exposed controls together in Hayes et al. (2012) and trauma-exposed only in Sartory et al. (2013), since the meta-analysis of Patel and colleagues (2012) found amygdala hyperactivity compared to non-trauma-exposed controls, but not to trauma-exposed controls.

At the whole-brain level, the three recent meta-analyses on functional neuroimaging studies also showed hyperresponsiveness in the dACC in PTSD patients compared to trauma-exposed controls (Patel et al., 2012; Sartory et al., 2013), and non-trauma-exposed controls (Hayes et al., 2012). Moreover, a recent study not included in the meta-analysis showed that dACC hyperactivity was associated with PTSD symptom severity (Shin et al., 2011). Concerning the AI, the meta-analysis of Patel and colleagues (2012) found increased bilateral AI activity in PTSD patients compared to trauma-exposed controls, though not to non-trauma-exposed controls. Moreover, it has been found that enhanced AI activity to a US (i.e. electric shock) was positively associated with PTSD symptom severity (Linnman et al., 2011). Taken together, both dACC and bilateral AI hyperactivity seem to be related to (severity of) PTSD, whereas meta-analyses indicate that amygdala hyperactivity may also result from trauma exposure, irrespective of PTSD development.

2.3. Adaptive fear inhibition

Acquisition and expression of the fear response can be top-down controlled in safe environments. The vmPFC, consisting of the ventral ACC (vACC) and orbitofrontal cortex (OFC) can inhibit the fear response via inhibitory projections to the amygdala. Notably, these inhibitory projections are modulated by the hippocampus (Kalisch et al., 2006). See Figure 2.1 for a schematic illustration of medial and lateral prefrontal cortical structures.

An example of top-down fear inhibition is extinction, during which the CS+ is no longer paired with the US. Usually, the individual learns that the CS+ no longer represents danger (i.e. the extinction memory) and the CR is inhibited. The vmPFC has been found to be crucial in this process of fear extinction, by inhibiting the amygdala via efferent pathways running from the vmPFC to the BLA (Rodrigues et al., 2009). In this way, the output of the CeM is modulated, resulting in lower fear responses. In addition, it was found that greater thickness of the vmPFC is associated with better extinction memory and lower skin conductance response to the CS+ (Milad et al., 2005).
Another instance of top-down control over the amygdala is emotion regulation, in which the emotional meaning of an aversive stimulus is diminished by (un)conscious cognitive re-evaluation (e.g. reappraisal) (Ochsner et al., 2002). During a typical emotion regulation paradigm, the participant is presented with aversive stimuli (e.g. pictures depicting a mutilated body) and is asked to cognitively reinterpret the meaning of the picture. Previous research has shown that cognitively reappraising an aversive stimulus results in decreased negative affect towards the stimulus (McRae et al., 2010; Ochsner et al., 2002). Furthermore, it has been shown that distraction (e.g. working memory) during aversive stimulus presentation can also be used as an effective (implicit) emotion regulation strategy, in which negative affect elicited by the aversive stimulus is reduced (McRae et al., 2010). Human neuroimaging studies have shown decreased amygdala and increased vmPFC, dorsolateral PFC (dlPFC) and inferior

**FIGURE 2.1 Schematic illustration of medial and lateral prefrontal cortical structures**

Throughout this review, the ventromedial prefrontal structure is defined by all medial cortices below the black line in the figure, including ventral anterior cingulate cortex (vACC, pre-and perigenual ACC, Brodmann area (BA) 32, 24, and 25), the medial orbitofrontal cortex (mOFC, BA 11 and 12) and the ventromedial prefrontal cortex (vmPFC, BA 10 and 11). The dorsomedial PFC (dmPFC) roughly corresponds to BA 8 and 9. The (pre-)supplementary motor area (SMA) corresponds to the medial part of BA 6. The dorsal ACC includes the supragenual part of BA’s 24 and 32. Dorsolateral PFC (dlPFC) roughly corresponds to BA 46 and lateral parts of BA 9. The inferior frontal gyrus (IFG, also known as ventrolateral PFC) includes BA 44 and 45. The lateral OFC corresponds to BA 11 and 47. Note that the borders between the prefrontal areas are arbitrary and not clearly anatomically defined.
frontal gyrus (IFG) activity during explicit and implicit emotion regulation tasks (McRae et al., 2010; Ochsner et al., 2002). It has been suggested that the dPFC, although not extensively connected to the amygdala, exerts control over the amygdala via projections to the vmPFC (Delgado et al., 2008).

Since both extinction learning and emotion regulation processes involve top-down control over instinctive fear reactions, it is not surprising that these two processes share overlapping neurocircuitry (Diekhof et al., 2011). A recent meta-analysis of Diekhof and colleagues (2011) found one cluster in the vmPFC which was activated during extinction recall, emotion regulation and experiments in which reduction of negative emotions was induced by *a priori* misleading information. Furthermore, activation of the vmPFC was accompanied by a reduction of left amygdala activity.

Recently, it was suggested that the intensity of fear expression and subjective feelings of anxiety depends on the strength of functional and structural connectivity between the amygdala and mPFC, rather than activity of either brain region alone (Kim et al., 2011b). This hypothesis is based on the finding that stronger structural and functional coupling between the amygdala and mPFC is associated with reduced trait anxiety (see Kim et al. (2011b) for a review). In addition, it was found that individuals with a highly anxious temperament show weaker functional amygdala – perigenual ACC coupling during the processing of fearful and threatening facial expressions (Pezawas et al., 2005). Notably, 30% of the variation in self-reported anxiety temperament was accounted for by the strength of this coupling. Furthermore, stronger resting state connectivity between the amygdala and vmPFC was associated with lower self-reported levels of trait anxiety in healthy subjects (Kim et al., 2011a). In addition, the strength of the amygdala – vmPFC pathway, as expressed by structural integrity (measured with diffusion tensor imaging, DTI) is lower in individuals with high trait anxiety (Kim and Whalen, 2009). Taken together, stronger cross-talk between the amygdala and vmPFC seems to be crucial in top-down regulatory control over anxiety.

As mentioned above, the hippocampus has been implicated in top-down control over the fear response as well. Hippocampal involvement in fear conditioning and extinction was shown using a contextual fear conditioning paradigm, in which one context (CS+) is repeatedly paired with a US during the acquisition phase, whereas another context (CS-) is not. Notably, the ability to discriminate between contexts in which a US is presented or not appears to be dependent on hippocampal integrity (Pohlack et al., 2012). Furthermore, the hippocampus is involved in accurate recall of the extinction context as well. For example, increased activation in both the vmPFC and hippocampus was found during extinction recall when this occurred in the same context as the extinction learning (e.g. Kalisch et al., 2006), but not in the context in which conditioning took place (Kalisch et al., 2006). Possibly, the hippocampus is able to
inhibit the vmPFC unless contextual information signals that inhibition of the fear response is appropriate (Kalisch et al., 2006).

2.4. Fear inhibition in PTSD

Several studies have found deficient fear extinction (see Jovanovic and Ressler, 2010 for a review), as well as decreased extinction recall one day after extinction learning in PTSD patients compared to trauma-exposed controls (Milad et al., 2008). Furthermore, impaired safety learning has been observed in PTSD patients: combat-related PTSD patients with high current symptoms were cognitively aware of safety, but nevertheless showed increased startle responses to safety cues (CS-) in the presence of danger cues (CS+) compared to non-trauma-exposed controls and participants with remitted PTSD (Jovanovic et al., 2009). This impaired safety learning appears to be specific for PTSD, since participants with major depressive disorder (MDD) did not show such impairments (Jovanovic et al., 2010). Moreover, a recent study in Dutch soldiers found that reduced extinction learning prior to military deployment was associated with higher PTSD symptom severity in response to combat exposure (Lommen et al., 2013). Not surprisingly, the neurocircuitry model of PTSD emphasizes a crucial role of vmPFC hypoactivity and diminished structural and functional connectivity between the vmPFC and amygdala, resulting in reduced top-down control over fear responses. Furthermore, abnormal hippocampal functioning may underlie the inability to extinguish the fear response in safe contexts (Patel et al., 2012).

2.4.1. Anatomy

The recent meta-analysis of Kühn and Gallinat (2013) on nine whole-brain morphometry studies identified consistent grey matter reductions in the vmPFC and left hippocampus in PTSD patients compared to trauma-exposed controls. In addition, lower neuronal integrity (i.e. N-acetyl aspartate (NAA) as measured with magnetic resonance spectroscopy (MRS)) was found in the vACC and bilateral hippocampus of PTSD patients compared to non-trauma-exposed controls (Ham et al., 2007). Corroborating the evidence of the meta-analysis, a recent study showed both smaller volume and lower neuronal integrity particularly in the left hippocampus in PTSD patients compared to non-trauma-exposed controls (Shu et al., 2013). Smaller volumes were also associated with higher PTSD symptoms in the PTSD group (Shu et al., 2013). Furthermore, when looking at hippocampal subregions, a recent high resolution structural MRI study found grey matter reductions in the dentate gyrus and CA3 region of the hippocampus of PTSD patients compared to trauma-exposed controls (Wang et al., 2010). Notably, these hippocampal subregions have previously been associated with stress-induced structural remodelling (i.e. suppression of neurogenesis and dendritic branching) in rodents (Wang et al., 2010).
2.4.2. **Functional neuroimaging studies**

The whole-brain meta-analyses on functional imaging studies of Hayes and colleagues (2012) and of Patel and colleagues (2012) found vmPFC hypoactivation in PTSD patients compared to trauma-exposed and non-trauma-exposed controls, whereas the study of Sartory et al. (2013) did not show this hypoactivation. Instead, they found vACC hyperactivity during trauma-related stimuli in PTSD patients compared to trauma-exposed controls (Sartory et al., 2013). Patel and colleagues (2012) found vACC hypoactivity in PTSD patients compared to non-trauma-exposed controls and OFC hypoactivity compared to trauma-exposed controls (both regions are part of the vmPFC, see Figure 2.1). In addition, Hayes et al. (2012) found reduced activation in the vmPFC and in the IFG in PTSD patients compared to non-trauma-exposed and trauma-exposed controls together during symptom provocation studies (Hayes et al., 2012). Moreover, a recent study not included in the meta-analyses found vmPFC hypoactivation during an inhibition task in PTSD patients compared to trauma-exposed controls (Jovanovic et al., 2013). Notably, vmPFC hypoactivation was associated with greater fear potentiated startle during safety learning and extinction (Jovanovic et al., 2013), indicating diminished prefrontal top-down control over the fear response.

Concerning the hippocampus, the meta-analysis by Patel and colleagues (2012) showed decreased left parahippocampal activity in PTSD patients compared to trauma-exposed controls, but increased right hippocampal activation in PTSD patients compared to non-trauma-exposed controls. Furthermore, a recent study showed increased left hippocampal activation during successful encoding of negative words in complex PTSD patients compared to non-trauma-exposed controls, which was associated with co-morbid depressive symptom severity (Thomaes et al., 2013). Thus, distinct hippocampal subregions seem to be differentially associated with trauma exposure, PTSD symptomatology and co-morbidity, and experimental paradigm used. Although inconsistent findings have been reported, it has been suggested that this hippocampal hyperactivity could underlie enhanced fear conditioning and decreased inhibition learning seen in PTSD (Patel et al., 2012).

2.5. **Connectivity within and between networks**

As mentioned above, the strength of functional and structural connectivity between the amygdala and mPFC appears to be associated with (the subjective feeling of) anxiety. In PTSD patients, DTI studies have shown lower white matter tract integrity (i.e. lower fractional anisotropy values) in the cingulum bundle, compared to trauma-exposed controls (e.g., Fani et al., 2012b). Of note, the cingulum bundle is the main white matter tract connecting the ACC and hippocampus and projecting to the amygdala. In addition, increased PTSD symptom severity in the PTSD group was related to decreased cingulum integrity. Together, these results suggest that degraded communication between the ACC and hippocampus is involved in the pathophysiology of PTSD (Fani et al., 2012b).
Recently, it has been suggested that investigating connection strengths within and between intrinsic connectivity networks (i.e. brain areas that are structurally and functionally connected, as identified during rest) may provide new insights into the neural correlates underlying psychopathology (Menon, 2011). Concerning the salience network, enhanced functional connectivity between the amygdala and AI was recently found in PTSD patients compared to trauma-exposed controls (e.g., Sripada et al, 2012b), most likely indicating enhanced vigilance to threat. Moreover, the recent meta-analysis of Hayes et al. (2012) showed that this amygdala hyperactivity was associated with decreased vmPFC activity in PTSD patients compared to both non-trauma-exposed and trauma-exposed controls (Hayes et al, 2012). In addition, decreased inhibitory control of the ACC over the amygdala in PTSD patients compared to trauma-exposed has been shown, as well as reduced functional connectivity between the amygdala and left hippocampus (Sripada et al, 2012b). Furthermore, weaker connectivity between the posterior cingulate cortex (PCC) and amygdala, indicating less prefrontal inhibitory control, was associated with higher PTSD symptom scores within a PTSD group (Zhou et al, 2012).

Of note, the vmPFC, hippocampus and PCC are core nodes of the default mode network (DMN) which is typically engaged during rest and which is associated with internally focused thoughts and autobiographical memory. Recently, decreased DMN connectivity has been found in PTSD patients compared to trauma-exposed controls, as indicated by reduced coupling between the PCC, vmPFC and hippocampus (Sripada et al, 2012b). During rest, activity in the DMN is associated with deactivation or suppression of attentional and executive processes (i.e. decreased functional coupling of the DMN with nodes of the salience and executive networks) (Menon, 2011). In PTSD patients, however, co-activation between nodes of the DMN and salience network (e.g. increased connectivity between the hippocampus and both the AI and amygdala) has been found, indicating enhanced vigilance to salient stimuli, even during periods of rest (Sripada et al, 2012b). Notably, enhanced connectivity between the amygdala and DMN 6-12 weeks post-trauma was also found to predict the development of PTSD symptoms in acutely traumatized individuals (Lanius et al, 2010) indicating that aberrant connectivity between the DMN and salience network may represent post-trauma vulnerability for the development of PTSD.

In summary, the literature reviewed above suggests that the neurobiological correlates of PTSD involve enhanced salience processing (i.e. hyperactivity of the amygdala, AI and dACC) as well as diminished emotion regulation abilities and top-down control over the fear response (i.e. vmPFC hypoactivity and diminished structural and functional connectivity between the amygdala, vmPFC and hippocampus) (Fani et al, 2012b; Hayes et al, 2012; Patel et al, 2012). This enhanced salience processing and diminished top-down prefrontal control may result
in increased subjective anxiety and dysregulated autonomic and endocrine fear responses in PTSD patients compared to traumatized and non-traumatized controls. In Figure 2.2 we provide a graphic representation of the findings from the reviewed literature regarding the functional abnormalities in the fear circuitry previously observed in PTSD patients compared to traumatized and non-traumatized healthy controls.

2.6. Treatment effects on neurobiology

To date, it is unknown whether the aberrant activity and volumetric abnormalities of the brain regions described above represent pre-trauma vulnerability factors for PTSD or develop as a consequence of the index trauma or PTSD. Results of several studies indicate that abnormal activity and/or volume alterations in several of these brain regions may represent

**FIGURE 2.2 Neurobiological correlates of PTSD**

Schematic overview illustrating the previously observed functional abnormalities in the neurobiological fear circuitry in PTSD patients compared to traumatized and non-traumatized controls, based on the reviewed literature. PTSD is characterized by increased activity in key regions of the salience network (i.e. amygdala, anterior insula and dACC), which results in increased subjective anxiety and dysregulated autonomic and endocrine fear responses. Emotion regulation abilities and top-down control of prefrontal areas over the fear response are decreased. Red arrows indicate excitatory connections, blue arrows indicate inhibitory connections. Thicker arrows indicate increased connectivity, whereas thinner arrows indicate decreased connectivity between both areas. + indicates increased activation, - indicates decreased activation, ± indicates hypo- and hyperactivation both observed, - indicates dysregulation, and = indicates adaptive regulation.
pre-trauma vulnerabilities (e.g. smaller hippocampal volume; Gilbertson et al., 2002), whereas others have shown volumetric and functional changes as a consequence of trauma exposure (e.g. amygdala hyperactivity) or PTSD (e.g. Al and dACC hyperactivity) (Patel et al., 2012). Regardless of this unresolved issue, evidence suggests that successful pharmacological and psychological treatment can reverse at least some of these neurobiological abnormalities (see Quidé et al., 2012 for a review). Concerning the diminished top-down prefrontal control over the amygdala, treatment response to CBT (resulting in fewer PTSD symptoms) has been associated with decreased amygdala and increased PFC (including the ACC, OFC and dlPFC) activity compared to pre-treatment assessment (Quidé et al., 2012). These results support the notion that dampening amygdala hyperresponsiveness and increasing vmPFC top-down inhibitory control of the amygdala could be an important pharmacological target for MEP.

3. **OXYTOCIN: A POSSIBLE STRATEGY FOR MEDICATION-ENHANCED PSYCHOTHERAPY**

As the aforementioned literature suggests, dampening exaggerated fear responses by decreasing amygdala hyperactivity and enhancing prefrontal inhibitory control of the vmPFC over the amygdala appears to be a promising target for MEP in PTSD patients (Dunlop et al., 2012; Olff et al., 2010). Crucially, the neuropeptide oxytocin (OT) has been found to act on these mechanisms: oxytocin has anxiolytic properties, resulting in attenuated amygdala reactivity (Domes et al., 2007; Kirsch et al., 2005) and enhanced amygdala – vmPFC coupling (Sripada et al., 2013). Furthermore, oxytocin facilitates social bonding, which could promote engagement in the therapeutic alliance (Olff et al., 2010). In addition, oxytocin has been associated with increased regulation of HPA-axis and autonomic nervous system activity (ANS). Therefore, we propose that oxytocin is a promising pharmacological agent for treatment-augmentation in PTSD (Olff et al., 2010). Below, we describe the neurobiological mechanisms which may underlie the effects of oxytocin on exaggerated fear responses, possibly resulting in enhanced symptom reduction when used as additive to psychotherapy in PTSD.

Oxytocin is an old neuropeptide with highly preserved structure and function during evolution. Serving both as a hormone and neuropeptide, oxytocin is synthesized in the paraventricular nucleus of the hypothalamus (see for a review Meyer-Lindenberg et al., 2011). As a hormone, oxytocin is released into the bloodstream via the posterior pituitary gland and has an important role in lactation and parturition. Centrally, the neuropeptide oxytocin acts on various midbrain and frontal regions, such as the amygdala, PFC, brainstem and hippocampus (Meyer-Lindenberg et al., 2011). In humans, the effects of oxytocin on brain activity and behavior have been studied using intranasal oxytocin administration, which is based on evidence that small neuropeptides can bypass the blood brain barrier to enter the central
nervous system (Born et al., 2002; Neumann et al., 2013, but see also Guastella et al., 2013 for a critical evaluation). Oxytocin plasma levels were found to be elevated 30-40 minutes after intranasal oxytocin administration and returned to baseline 80-90 minutes post-administration (Gossen et al., 2012). However, since this study was conducted in a small sample size (i.e. 8 males) and no placebo control group was used, these findings should be interpreted with caution. Salivary oxytocin levels did not return to baseline for 7 hours post-administration (van Ijzendoorn et al., 2012). Since oxytocin has a half-life of 3-20 minutes, these prolonged effects are thought to be due to a feed-forward mechanism stimulating the release of endogenous oxytocin after the first exogenous oxytocin boost (van Ijzendoorn et al., 2012). Therefore, studies on behavioral and neural effects of intranasal oxytocin administration typically start measuring 40-45 minutes after oxytocin administration and last up to 50 minutes. Besides its easy application, a recent review showed that short-term use of intranasal oxytocin up to doses of 40IU results in minimal side effects and is equally safe to use in psychiatric patients as in healthy individuals (MacDonald et al., 2011). Hence, intranasal oxytocin appears to be a safe pharmacological intervention, except for pregnant or breastfeeding women, in which it can cause uterine contractions.

3.1. Oxytocin and anxiety

The anxiolytic properties of oxytocin may become apparent at both the behavioral and neural level. Behaviorally, reduced subjective anxiety during public speaking stress has been reported after a single dose of intranasal oxytocin (de Oliveira et al., 2012). However, a recent study showed an anxiogenic effect of intranasal oxytocin administration: the startle response to unpredictable threat (i.e. electric shocks) was increased after oxytocin administration compared to AVP and placebo administration (Grillon et al., 2013). Possibly, oxytocin has anxiolytic effects towards explicit fear cues but anxiogenic effects to unpredictable threat (Grillon et al., 2013). Moreover, in patients diagnosed with MDD, increased subjective state anxiety was found over the course of one psychotherapy session when oxytocin was administered before this session (Macdonald et al., 2013). Notably, a pilot study showed that a single intranasal oxytocin administration (24IU) resulted in decreased acute anxiety and severity of PTSD symptoms, as well as increased well-being, mood and desire for positive social interactions (Yatzkar and Klein, 2010). In addition, a pilot study of Pitman and colleagues (1993) suggested decreased physiological responding during combat imagery after one dose of intranasal oxytocin compared to placebo and AVP in veterans diagnosed with PTSD.

Animal studies have demonstrated that central oxytocin administration dampens amygdala reactivity via oxytocin receptors located in the lateral part of the CeM, thereby reducing output of the CeM to brainstem areas and the hypothalamus (Huber et al., 2005; Viviani et al., 2011). More specifically, upon activation of oxytocin receptors in the lateral part of the CeM, GABA-ergic interneurons are activated which inhibit neurons in the medial CeM. This
inhibitory effect of oxytocin results in decreased coupling of the medial part of the CeM to the periaqueductal grey (PAG), leading to decreased freezing responses in fear-conditioned rats (Viviani et al, 2011). This indicates that oxytocin administration in the CeM attenuates expression of the fear response. Moreover, a rodent study found that oxytocin microinjection into the hippocampus of male rats 1 hour and 7 days after stress exposure (i.e. predator scent) reduced subsequent freezing responses to trauma cues, increased glucocorticoid (GC) levels immediately after stress exposure and promoted faster recovery of the stress response (Cohen et al, 2010).

3.2. Oxytocin and salience processing

Various human neuroimaging studies have demonstrated altered oxytocin-induced activity in nodes of the salience network. Intranasal oxytocin administration in healthy individuals resulted in decreased amygdala reactivity (e.g. Domes et al, 2007; Kirsch et al, 2005, but see Domes et al, 2010; Lischke et al, 2012; Rilling et al, 2012). For example, neuroimaging studies in healthy males found that oxytocin reduced amygdala activity towards biologically salient stimuli such as emotional faces in an implicit (Domes et al, 2007) and explicit emotion recognition paradigm (Kirsch et al, 2005). Moreover, oxytocin was found to decrease amygdala activity in response to aversive and neutral pictures (Striepens et al, 2012) and infant crying (Riem et al, 2011). Of note, in a fear conditioning paradigm, intranasal oxytocin administration in healthy individuals attenuated negative ratings and amygdala reactivity towards faces previously associated with an electric shock (Petrovic et al, 2008). Besides dampening amygdala responsiveness, oxytocin administration in healthy individuals has been found to decrease the coupling between the amygdala and brainstem regions implicated in the behavioral and autonomic expression of fear (i.e. the PAG and reticular formation) (Domes et al, 2007; Kirsch et al, 2005; Rilling et al, 2012). Crucially, in male patients with generalized social anxiety disorder (GSAD) it was found that a single administration of intranasal oxytocin normalized bilateral amygdala hyperactivity towards fearful faces to the same level as healthy controls (Labuschagne et al, 2010). Notably, in this study no effect of oxytocin on amygdala reactivity was found in healthy controls, suggesting that oxytocin might be most effective in dampening amygdala responsiveness in pathological states, for example when the amygdala is hyperresponsive (Labuschagne et al, 2010), as in PTSD. In line with this, a recent study found that amygdala hyperactivity towards angry faces (vs. happy faces) in female borderline patients decreased after intranasal oxytocin administration (Bertsch et al, 2013). In contrast, a reversed effect (i.e. increased amygdala activity towards angry vs. happy faces after oxytocin administration compared to placebo) was found in healthy females, indicating that oxytocin may modulate social salience depending on individual personality characteristics (Bertsch et al, 2013).
Regarding the AI, oxytocin administration in healthy individuals was found to increase AI activity during successful encoding of aversive stimuli depicting human suffering (Striepens et al., 2012) and infant crying (Riem et al., 2011). The AI has been suggested to be important in switching between connectivity networks, thereby facilitating attentional and working memory abilities upon salience detection (Menon, 2011). Functional connectivity between the left AI and right amygdala was found to be reduced by oxytocin in healthy individuals while processing aversive pictures (Striepens et al., 2012). In contrast, oxytocin administration in healthy individuals was found to increase functional coupling between the left AI and left amygdala during successful encoding of aversive stimuli (Striepens et al., 2012), indicating that oxytocin might differentially alter the coupling of the AI with the left and right amygdala. Oxytocin has also been found to increase coupling between the AI and IFG during infant crying (Riem et al., 2011) and during the successful encoding of aversive stimuli (Striepens et al., 2012). It has been suggested that this enhanced AI-IFG connectivity, in accordance with decreased amygdala activity and reduced amygdala-AI coupling enables an individual to approach a person in need, even in the face of negative emotional cues (Striepens et al., 2012). Regarding the dACC, oxytocin administration resulted in attenuated dACC activity to fear-conditioned faces in healthy participants (Petrovic et al., 2008) and increased oxytocin-induced functional connectivity between the amygdala and dACC (Riem et al., 2012).

### 3.3. Oxytocin and fear inhibition

Oxytocin administration studies have found altered activity in prefrontal control areas and enhanced connectivity between the vmPFC and the amygdala in healthy participants and in GSAD patients (Labuschagne et al., 2010; Riem et al., 2012; Sripada et al., 2013). In GSAD patients, intranasal oxytocin administration attenuated vmPFC hyperactivity in response to sad faces to levels comparable to that of healthy controls (Labuschagne et al., 2011). Concerning prefrontal-amygdala connectivity, enhanced resting state connectivity between the amygdala and vmPFC was found in healthy males, possibly indicating enhanced top-down control of prefrontal areas over the amygdala (Sripada et al., 2013). Moreover, Riem et al. (2012) found increased oxytocin-induced functional connectivity between the amygdala, dACC, hippocampus and OFC while listening to infant laughter, suggesting enhanced top-down control over salient stimuli (Riem et al., 2012). In contrast, however, reduced functional connectivity between the left amygdala and left vACC was found while viewing aversive and neutral stimuli (Striepens et al., 2012). Presumably, differences in directionality of the oxytocin-induced effect on functional connectivity between the amygdala and prefrontal areas result from differences in experimental design used, in location of the effect (e.g. vACC vs. dACC, OFC and vmPFC) and gender of the participants.

Taken together, intranasal oxytocin administration may result in dampened amygdala and dACC activity, increased AI activity and changed functional connectivity between nodes of
the salience network. Decreased oxytocin-induced functional coupling between the amygdala and brainstem regions suggests attenuated fear expression, whereas enhanced vmPFC-amygdala connectivity indicates increased prefrontal top-down control over the amygdala. See Figure 2.3 for a graphic representation of the literature reviewed above regarding the previously observed effects of oxytocin on the neurobiological fear circuitry in healthy individuals.

3.4. Clinical application of oxytocin

When considering intranasal oxytocin in MEP for PTSD, it is important to note that top-down inhibition of prefrontal areas over the amygdala is crucial in extinction learning, which is reasoned to be the underlying mechanism of exposure therapy in PTSD (Rothbaum and Davis, 2003). Furthermore, it appears that the stress response, needed for effective extinction learning (Blundell et al, 2011), is not abolished after oxytocin administration, but may become more efficient, with a higher initial stress response and a faster return to baseline levels after termination of the stressor (Cohen et al, 2010). Several rodent studies showed that both peripheral (Cohen et al, 2010; Petersson et al, 1999) and central (Cohen et al, 2010) administration of oxytocin resulted in increased initial HPA-axis responsiveness under baseline conditions (Petersson et al, 1999) and after stress exposure (Cohen et al, 2010), which could indicate enhanced behavioral adaptation to stress (Cohen et al, 2010). Importantly, oxytocin administration (compared to placebo) resulted in faster return to baseline levels after termination of the stressor, implying increased suppression of the HPA-axis due to oxytocin (Cohen et al, 2010). In addition, human studies have also found that oxytocin is associated with faster recovery of both the neuroendocrine (Heinrichs et al, 2003; Taylor et al, 2000) and autonomic (Kubzansky et al, 2012) stress response. For example, the study of Heinrichs and colleagues (2003) investigated the influence of intranasal oxytocin and the presence of social support on subjective and neuroendocrine responses to public speaking stress in healthy males. Oxytocin administration without social support did not affect the increase in salivary cortisol levels in response to the stressor. However, these cortisol levels had returned to baseline 30 minutes after stress exposure, whereas the placebo group without social support still showed elevated cortisol levels 60 minutes after stress exposure (Heinrichs et al, 2003). Moreover, this effect has also been found in healthy women, in which higher oxytocin levels were associated with faster recovery of the cortisol response after a laboratory stress challenge (Taylor et al, 2000). In addition, intranasal oxytocin was found to both increase sympathetic activation during public speaking stress and accelerate stress recovery (i.e. greater vagal rebound) in both healthy male and female participants (Kubzansky et al, 2012).

Animal studies have indicated that oxytocin may facilitate extinction learning and decrease fear expression, although not consistently (e.g., Bohus et al, 1978; Toth et al, 2012). Several rodent studies have shown that intraventricular administration of oxytocin facilitated the
extinction of passive avoidance behavior, measured as the latency to enter a compartment previously associated with an aversive stimulus (e.g., Bohus et al., 1978). Importantly, Toth and colleagues (2012) recently showed that the effect of intracerebral oxytocin administration on extinction learning may be highly time-dependent. That is, oxytocin administered in the lateral ventricle of rodents prior to fear conditioning resulted in decreased fear expression during extinction training and facilitated extinction learning, whereas blockade of oxytocin neurotransmission by an oxytocin receptor antagonist resulted in impaired fear extinction learning. However, impaired extinction learning was found when oxytocin was administered before extinction learning (i.e. after fear conditioning) (Toth et al., 2012). Interestingly, oxytocin administration was shown to reverse the effects of social defeat on subsequent social avoidance in rodents (Lukas et al., 2011). That is, animals previously defeated by another male.
rat showed no social avoidance towards their defeater when oxytocin was administered 20 minutes before testing, whereas oxytocin administration had no effect on non-social anxiety, indicating that that oxytocin dampens learned social fear. Regarding extinction, a recent human study found increased extinction recall when oxytocin was administered before extinction training (Acheson et al., 2013). In addition, oxytocin administered after fear conditioning was found to attenuate amygdala and dACC activity to pre-conditioned faces as well as affective ratings of these faces (Petrovic et al., 2008).

To date, only one study has been published on the effects of intranasal oxytocin administration as a treatment strategy in medication-enhanced psychotherapy. In patients with social anxiety disorder, oxytocin was administered prior to 4 sessions of exposure therapy (Guastella et al., 2009). Although addition of oxytocin did not enhance overall symptom improvement compared to placebo, oxytocin did improve mental representation of the self as well as ratings of speech performance and appearance during a public speaking task (Guastella et al., 2009). This is probably the maximum effect that could be expected from the limited number of exposure sessions.

Notably, the therapeutic potential of intranasal oxytocin administration has been investigated in patients with other types of psychiatric disorders as well. For example, a study in schizophrenic patients showed that both three weeks of 40IU oxytocin administration twice a day resulted in a reduction of schizophrenic symptoms (including anxiety) and improved social cognition skills (Pedersen et al., 2011). In addition, a recent randomized-controlled trial in schizophrenic patients on antipsychotic medication (i.e. risperidone) showed that adjunct oxytocin administration of 20IU for 8 weeks resulted in a clinically significant reduction of positive symptoms (Modabbernia et al., 2013). However, caution must be applied when interpreting these results because both males and females were included into one patient group (this effect could not be further investigated due to the small number of female participants), menstrual cycle was not controlled for (Modabbernia et al., 2013; Pedersen et al., 2011) and the sample size was small (Pedersen et al., 2011). In young male patients with autism spectrum disorder, a single dose of 24IU (18IU for male patients aged 12 to 15) intranasal oxytocin resulted in better emotion recognition (Guastella et al., 2010), improved interaction with the most cooperative player in a ball game, and increased feelings of trust, compared to placebo (Andari et al., 2010). Moreover, a recent randomized controlled pilot study showed that intranasal oxytocin administration of 24IU for 6 weeks twice daily improved social cognition and quality of life of autistic adults (Anagnostotou et al., 2012). However, this study was also limited by the small sample size, the inclusion of both males and females in one patient group and the fact that menstrual cycle was not controlled for.
Despite the promising results of the abovementioned randomized clinical trials, prior to oxytocin application in routine clinical practice, more translational animal and human studies should be conducted to provide answers to basic questions regarding the effects of oxytocin administration (i.e. long-term consequences of oxytocin administration, difference in effects between acute and chronic oxytocin administration and whether intranasal oxytocin does enter the brain) (Macdonald and Feifel, 2013; Miller, 2013). Furthermore, more knowledge about the effects of oxytocin administration on anxiety and PTSD should be gained using both translational animal and (single and repeated dose) human studies, before oxytocin can be recommended in MEP for PTSD. Subsequently, randomized clinical trials need to be conducted in order to investigate whether intranasal oxytocin in MEP indeed results in improved treatment outcome.

3.5. Factors modulating oxytocin-induced effects

Recent literature has shown that the effects of oxytocin administration depend on inter-individual differences (Bartz et al, 2011b). Therefore, several factors potentially influencing the effectiveness of oxytocin in MEP need to be taken into account when investigating the therapeutic potential of oxytocin. First of all, some of the oxytocin-induced effects appear to be sex-specific. Several human neuroimaging studies have shown that oxytocin administration in healthy females resulted in enhanced, rather than attenuated, amygdala reactivity towards threatening scenes (Lischke et al, 2012) and fearful faces (Bertsch et al, 2013; Domes et al, 2010). Other studies in healthy women, however, found that oxytocin reduced amygdala reactivity towards infant crying (Riem et al, 2011) and negatively arousing pictures (Rupp et al, 2012). The contradictory results could possibly be explained by the different stages of the menstrual cycle during which the women were tested, as progesterone and estrogen levels are known to affect functioning of the oxytocin-system (de Vries, 2008). In addition, Rupp and colleagues (2012) found that intranasal oxytocin (24 IU) in breastfeeding women did not influence amygdala activity, possibly because of desensitization or saturation of the oxytocin receptors. These findings in healthy participants seem especially important given the fact that PTSD is two to three times more prevalent in females than in males (Olff et al, 2007). Therefore, sex-specific effects should be thoroughly investigated prior to routine clinical application of oxytocin.

Other inter-individual differences moderating the effects of oxytocin administration include emotion regulation and emotion recognition abilities, as well as attachment style and early caregiver experiences (for review see Olff et al, 2013). More specifically, it has been suggested that the pro-social effects of oxytocin are more pronounced in individuals who have something to gain with regard to social functioning (Olff et al, 2013). For instance, oxytocin-induced dampening of amygdala responsiveness towards facial stimuli was observed in individuals with low emotion regulation abilities only (Quirin et al, 2011). This is in line with the study
of Labuschagne and colleagues (2010), which showed that oxytocin attenuated amygdala hyperactivity in GAD patients, but not in healthy controls. This indicates that oxytocin might be most effective in dampening amygdala responsiveness under pathological states.

In addition, it seems that oxytocin enhances sensitivity to the salience of social cues (both positive and negative). This may result in enhanced pro-social behavior when the social cues are interpreted as ‘safe’ and in defensive or anti-social behavior when they are interpreted as ‘unsafe’ (for review see Olff et al., 2013). Furthermore, in the absence of unsafe cues from the social environment, inter-individual factors can determine whether the social environment is interpreted as safe or not. For example, it was shown that less anxiously attached individuals remembered their mother as more caring and close after oxytocin administration, whereas more anxiously attached individuals remembered their mother as less caring and more distant after oxytocin compared to placebo (Bartz et al., 2010). Moreover, Bartz and colleagues (2011a) found that intranasal oxytocin resulted in decreased trust and cooperation during a social dilemma game in patients with borderline personality disorder, a disorder characterized by fears of abandonment. However, the results of this last study should be interpreted with caution due to the small sample size, the inclusion of both males and females within one group and the fact that menstrual cycle was not controlled for (Bartz et al., 2011a).

As mentioned previously, the effects of oxytocin might be highly time-dependent (Toth et al., 2012). That is, intraventricular administration of oxytocin in rodents prior to fear conditioning was found to facilitate extinction learning, whereas it impaired extinction learning when applied after fear conditioning, but before fear extinction learning (Toth et al., 2012). This seems especially important since, if oxytocin would be administrated in MEP, it should typically be applied after trauma exposure, just before exposure therapy (i.e. the period of extinction learning). However, these results could also (partially) be explained by the high oxytocin doses used (i.e. 1 µg per animal), which could result in binding of oxytocin to AVP-receptors (Chini et al., 2014). Moreover, one can think of other variations in the timing of administration (i.e. administration of oxytocin after psychotherapy to facilitate stress recovery and extinction learning). Regardless of these issues, the results of the study of Toth and colleagues (2012) stress the importance of further detailed investigation of the time-dependent effects of oxytocin before clinical application. Furthermore, a recent study showed that the stress-dampening properties of intranasal oxytocin (i.e. attenuation of cortisol response after physical exercise), were stronger after 24IU compared to 48IU of oxytocin (Cardoso et al., 2013). This suggests that the effects of oxytocin may follow a non-linear dose-response curve, possibly due to binding of oxytocin to AVP-receptors at higher doses (Cardoso et al., 2013). This warrants further investigation into the optimal dose of intranasal oxytocin in MEP in PTSD.
4. CONCLUSION

Although there are several evidence-based treatments for PTSD, approximately one third of PTSD patients fail to fully recover after psychotherapy (Bradley et al, 2005). Therefore, focusing on new pharmacological interventions to augment treatment response in PTSD is highly relevant. PTSD is associated with an exaggerated fear response and diminished inhibitory top-down control over the fear response, which is characterized by amygdala hyperactivity, vmPFC hypoactivity and diminished structural and functional connectivity between both areas (Fani et al, 2012b; Hayes et al, 2012; Patel et al, 2012). Moreover, AI and dACC hyperactivity and smaller hippocampal volumes have been consistently found in PTSD patients (Kühn and Gallinat, 2013a; Patel et al, 2012). Pharmacological agents that inhibit activation of amygdala, AI and dACC and/or promote inhibitory control of the vmPFC over the amygdala, thereby enhancing fear inhibition, are important targets for MEP in PTSD patients (Dunlop et al, 2012; Olff et al, 2010). This seems especially important since successful response to CBT treatment has been associated with decreased amygdala and increased PFC activity compared to pre-treatment assessment (Quidé et al, 2012).

Given oxytocin’s anxiolytic properties, as well as its capacity to regulate HPA-axis functioning, physiological responsiveness and social bonding/attachment formation (Meyer-Lindenberg et al, 2011), we propose that intranasal oxytocin given prior to treatment may be effective in boosting response to psychotherapy in PTSD. Currently, the efficacy of oxytocin as strategy for MEP has not been investigated yet. Randomized clinical trials need to be conducted in order to investigate whether intranasal oxytocin given during trauma-focused psychotherapy results in a better treatment outcome. However, additional knowledge about the effects of oxytocin administration on anxiety and PTSD needs to be obtained via translational animal and single- and repeated-dose human studies, before conclusions on the use of oxytocin as MEP-strategy for PTSD can be drawn. Subsequently, randomized clinical trials need to be conducted in order to investigate whether combining intranasal oxytocin and trauma-focused psychotherapy results in improved treatment outcome. Importantly, given the fact that PTSD is more prevalent in females (Olff et al, 2007) and that effects of oxytocin are likely to be sex-specific (Domes et al, 2007) future research should be aimed at elucidating these sex-differences before oxytocin can be applied routinely in a clinical setting. In addition, other mediating variables should be investigated. Taken together, although extensive research into oxytocin is still needed, we suggest that oxytocin is a promising agent in MEP of PTSD and therefore worthwhile investigating.
ROLE OF THE FUNDING SOURCES & CONFLICT OF INTEREST

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CHAPTER 3

Intranasal oxytocin administration dampens amygdala reactivity towards emotional faces in male and female PTSD patients

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Chapter 3

ABSTRACT

Post-traumatic stress disorder (PTSD) is a disabling psychiatric disorder. As a substantial part of PTSD patients responds poorly to currently available psychotherapies, pharmacological interventions boosting treatment response are needed. Because of its anxiolytic and pro-social properties, the neuropeptide oxytocin (OT) has been proposed as promising strategy for treatment augmentation in PTSD. As a first step to investigate the therapeutic potential of oxytocin in PTSD, we conducted a double-blind, placebo-controlled, cross-over fMRI study examining oxytocin administration effects (40IU) on amygdala reactivity towards emotional faces in unmedicated male and female police officers with (n=37, 21 males) and without (n=40, 20 males) PTSD. Trauma-exposed controls were matched to PTSD patients based on age, sex, years of service and educational level. Under placebo, the expected valence-dependent amygdala reactivity (i.e. greater activity towards fearful-angry faces compared to happy-neutral faces) was absent in PTSD patients. Oxytocin administration dampened amygdala reactivity towards all emotional faces in male and female PTSD patients, but enhanced amygdala reactivity in healthy male and female trauma-exposed controls, independent of sex and stimulus valence. In PTSD patients, greater anxiety prior to scanning and amygdala reactivity during the placebo session were associated with greater reduction of amygdala reactivity after oxytocin administration. Taken together, our results indicate presumably beneficial neurobiological effects of oxytocin administration in male and female PTSD patients. Future studies should investigate oxytocin administration in clinical settings to fully appreciate its therapeutic potential.
INTRODUCTION

Post-traumatic stress disorder (PTSD) is a highly disabling psychiatric disorder, which develops in approximately ten percent of trauma-exposed individuals (de Vries and Olff, 2009). PTSD has an estimated lifetime prevalence of about eight percent in the general population (de Vries and Olff, 2009) and is characterized by symptoms of intrusions and avoidance, negative alterations in cognition and mood (e.g. diminished interest in significant activities), and hyperarousal (American Psychiatric Association, 2013). Exposure-based therapy, aimed at reducing excessive fear by repeatedly exposing the patient to (in vivo) trauma reminders (Foa et al, 2009), is the treatment of choice for PTSD. Unfortunately, a third of patients responds poorly to currently available psychotherapies (Bradley et al, 2005), stressing the need for novel pharmacological interventions to enhance treatment response (i.e. medication-enhanced psychotherapy (Dunlop et al, 2012)). One promising strategy to improve treatment efficacy in anxiety-related disorders is to target neurobiological correlates of fear and fear extinction (de Kleine et al, 2013), the underlying mechanism of exposure-based therapies (Rothbaum and Davis, 2003).

In PTSD, pharmacological agents aimed at dampening (excessive) fear responses and facilitating the therapeutic alliance seem especially appropriate. The prevailing neurocircuitry model of PTSD postulates amygdala hyperactivity and ventromedial prefrontal cortex (vmPFC) hypoactivity towards both trauma-related and non-trauma-related stimuli (Pitman et al, 2012). According to a meta-analysis of functional connectivity studies in PTSD, amygdala hyperactivity in PTSD patients was predominantly observed in response to negative, non-trauma-related stimuli (Hayes et al, 2012). This may result in decreased prefrontal control over the fear response and hence excessive fear in PTSD patients (Rauch et al, 2006). Notably, greater amygdala reactivity during extinction learning in PTSD patients has been associated with impaired extinction recall the next day (Milad et al, 2009). In PTSD, greater amygdala reactivity to fearful faces (Bryant et al, 2008a) and negative pictures (van Rooij et al, 2015) before treatment predicted worse treatment outcome. In addition, effective exposure therapy was associated with decreased amygdala and enhanced vmPFC activity towards emotional faces over the course of treatment (Felmingham et al, 2010).

Since the neuropeptide oxytocin (OT) has both anxiolytic (Heinrichs et al, 2003) and pro-social (Olff, 2012) properties, oxytocin has been suggested as a promising pharmacological agent to enhance treatment response in PTSD (Koch et al, 2014; Olff et al, 2010). Oxytocin has anxiolytic properties both at the neurobiological and behavioral level. Behaviorally, oxytocin administration resulted in decreased subjective anxiety during a public speaking stressor in healthy individuals (de Oliveira et al, 2012) and increased (recall of) extinction learning in both rodents (Zoicas et al, 2014) and healthy males (Acheson et al, 2013). Additionally, functional
MRI (fMRI) studies have shown that oxytocin administration dampened amygdala reactivity towards emotional stimuli in healthy males (Kirsch et al., 2005), males with generalized social anxiety disorder (GSAD) (Labuschagne et al., 2010) and females with borderline personality disorder (BPD) (Bertsch et al., 2013), although findings for females have been mixed (Domes et al., 2010). Furthermore, oxytocin administration resulted in increased resting-state functional connectivity between the amygdala and vmPFC in healthy males (Sripada et al., 2013) and in patients with GSAD (Dodhia et al., 2014), possibly enhancing top-down control over the fear response.

As a first step to examine the therapeutic potential of oxytocin in PTSD patients, we conducted an fMRI study investigating intranasal oxytocin administration effects on amygdala reactivity towards emotional faces in trauma-exposed police officers with and without PTSD. We included a highly trauma-exposed control group to account for potential confounding effects of trauma exposure on neurobiology. Both male and female participants were included to investigate possible sex-differential effects of oxytocin administration. For both PTSD patients and trauma-exposed controls, we hypothesized that oxytocin would dampen amygdala reactivity. We expected that oxytocin administration would have greater effects in PTSD patients than in trauma-exposed controls, as oxytocin administration effects may be more beneficial in those who have something to gain regarding fear regulation (Labuschagne et al., 2010) or social functioning (Olff et al., 2013).

**MATERIALS AND METHODS**

**Participants**

Male (n=21) and female (n=16) PTSD patients were recruited via a psychotrauma diagnostic outpatient clinic for police personnel (Diemen, the Netherlands) and via advertisements on websites and in journals of the Dutch police. PTSD patients had to fulfill the DSM-IV diagnostic criteria for PTSD, with a score of ≥ 45 on the Clinician-Administered PTSD scale (CAPS) (Blake et al., 1995). PTSD patients were excluded if they met DSM-IV criteria for current psychotic disorder, substance-related disorder, severe personality disorder, severe major depressive disorder (MDD) (i.e. involving high suicidal risk and/or psychotic symptoms) or current suicidal risk, according to the Dutch version of the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998; van Vliet et al., 2000) or the Structured Clinical Interview for DSM–IV (SCID) (First et al., 2012; van Groenestijn et al., 1999) (for patients recruited via the police outpatient clinic).

Trauma-exposed male (n=20) and female (n=20) police officers were recruited via advertisements on websites and in journals of the Dutch police force and were matched to PTSD pa-
tients based on age, sex, number of years of service and educational level. Trauma-exposed controls had to be exposed to at least one potentially traumatic event according to the DSM-IV PTSD A1 criterion, with a CAPS score of < 15 and no lifetime history of PTSD or MDD, or any current DSM-IV axis 1 disorder, according to the MINI.

All participants had to be between 18 and 65 years of age and eligible for MRI (i.e. no metals, pacemakers or claustrophobia). Participants had no history of neurological disorders (i.e. seizure history) or any severe or chronic systemic disease or unstable medical condition, including endocrinological disorders. In addition, participants did not use psychotropic medications and female participants could not be pregnant or breastfeeding. The study was conducted in accordance with the declaration of Helsinki and was approved by the Institutional Review Board of the Academic Medical Center in Amsterdam, the Netherlands. All participants received oral and written study information and provided written informed consent before participation.

**Experimental procedure**

This randomized, double-blind, placebo-controlled, cross-over study consisted of three appointments: one intake session (T0) and two fMRI sessions (T1 and T2). During the intake session, in-and exclusion criteria were checked with the MINI and CAPS diagnostic interviews. Between the intake sessions and first fMRI session (which were on average 21.25 (±17.96) days apart), participants filled out a questionnaire about demographical characteristics and trauma history. The number of different types of police-related traumatic events was assessed with the police life events checklist (PLES) (Carlier and Gersons, 1992) and the number of different types of childhood traumatic events was assessed with the self-report version of the Early Trauma Inventory short form (ETI-SF) (Bremner et al, 2007). The two fMRI sessions were scheduled on average 11.5 (±9.90) days apart. Participants were asked to abstain from alcohol and drugs the day before scanning and from food, beverages, nicotine and exercise 2.5 hours prior to scanning. At the beginning of each scanning session, state anxiety was measured with the State-Trait Anxiety Inventory (STAI-state) (Spielberger, 1983). Hereafter, the participants self-administered intranasal placebo (saline, NaCL0.9%, five puffs per nostril) or oxytocin (40IU Syntocininon, produced by Delpharm Huningue France, five puffs of 4IU per nostril), under experimenter supervision. Medication order was counterbalanced between sessions. An anatomical scan was first acquired, followed by an emotional face-matching task, which began on average 44.68 (±3.74) minutes after intranasal spray application. This is in line with previous intranasal oxytocin fMRI studies (Kirsch et al, 2005; Labuschagne et al, 2010), and coincides with the pharmacodynamic peak response of intranasal oxytocin in healthy individuals (Paloyelis et al, 2014).
The emotional face-matching task (Hariri et al., 2002) consisted of three conditions: one visuomotor control condition (elliptical scrambled faces) and two emotional faces conditions. The first emotion condition consisted of fearful and angry faces and the second emotion condition consisted of happy and neutral faces. Four blocks of each emotion condition were presented, two with fearful-angry faces and two with happy-neutral faces. The duration of each emotion block was 30 seconds, consisting of six trials of five seconds. The emotion blocks were interleaved with visuomotor control blocks. In total, four visuomotor control blocks were presented, each with a duration of 25 seconds, consisting of five trials of five seconds. No inter-stimulus or inter-block interval was used, resulting in a total task duration of 220 seconds. Two versions of the task were counterbalanced between the fMRI sessions, one version starting with a fearful-angry emotion block and the other version starting with a happy-neutral emotion block. Each trial consisted of three stimuli, with a cue stimulus presented on top and two target stimuli presented below. Participants were instructed to match the orientation (control blocks) or the emotional expression (emotion blocks) of the cue stimulus with one of the target stimuli. We used face stimuli from the NimStim face stimuli set (https://www.macbrain.org), presented with Presentation software version 16.0 (www.neurobs.com). See Supplementary Figure S3.1 for sample stimuli and the task design.

**fMRI acquisition**

Images were acquired on a 3T Philips Achieva MR system (Philips Medical Systems, Best, the Netherlands) with a 32-channel head coil. A high-resolution anatomical scan was obtained with a FAST MPRage sequence (220 slices; voxel size = 1 mm³; repetition time = 8.2s; echo time = 3.8s; flip angle = 8°). The functional scans were obtained using an echo planar sequence sensitive to the blood-oxygen-level-dependent (BOLD) contrast (110 volumes; voxel size = 3 mm³; TR = 2s, echo time = 28ms; flip angle = 76°).

**Data analysis**

*Demographics*

Differences between PTSD patients and trauma-exposed controls on demographical, clinical and trauma-related characteristics were tested with SPSS version 20.0 (IBM statistics, Armonk, NY, USA). Variables were first checked for outliers and normality, and log-transformed when necessary. Independent sample t-tests or repeated measures ANOVA’s were used for continuous variables and chi-square tests for categorical variables. A p-value of <.05 (two-tailed) was considered significant.

*fmRI data-analysis*

fmRI images were analyzed using SPM8 (http://fil.ion.ucl.ac.uk/spm/software/spm8). Preprocessing included realignment, slice-time correction, co-registration to the anatomical scan, spatial normalization to the Montreal Neurological Institute (MNI) template, resampling to
2mm³ voxels and spatial smoothing with a 5mm full-width half maximum (FWHM) Gaussian kernel to optimize amygdala spatial resolution. For each participant, the two emotional conditions were modelled as box-car regressors, convolved with a canonical hemodynamic response function. The six realignment parameters were included in the model to control for movement artifacts. To remove slow drifts of the signal, a high-pass filter (cut-off 1/200 Hz) was used and we removed temporal autocorrelation using the AR(1) process. Contrast images were obtained for the fearful-angry condition and the happy-neutral condition versus the visuomotor control condition. These images were subsequently entered into a second-level ANOVA with task condition (fearful-angry – happy-neutral) and medication (placebo – oxytocin) as within-subjects factors and group (PTSD – controls) and sex (male – female) as between-subject factors. We included medication order as covariate in the model, controlling for possible confounding effects of administration order. To assess baseline differences between PTSD patients and trauma-exposed controls, a second model was estimated using only contrast images acquired after placebo application. Regions of interest (ROIs) of the left and right amygdala were anatomically defined using the Harvard-Oxford probabilistic atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases, 50% probability. P-values were family-wise error (FWE) corrected for multiple comparisons in the predefined amygdala ROIs using small volume correction. In the placebo model, all main effects and interaction effects were tested for each amygdala ROI. In the oxytocin model, the main effect of medication and interaction effects with medication were additionally tested for each amygdala ROI. P FWE-values <.05 for the omnibus F-tests were considered significant and are reported in Montreal Neurological Institute (MNI) stereotactic coordinates. In case of significant interaction effects, post-hoc t-tests were performed within the second-level models to test the direction of observed interaction effects. Four participants had to be excluded from the analyses, because of scanner artifacts (n=2) and significant signal drop-out in the temporal cortex (n=2), leaving 37 trauma-exposed controls (18 males) and 36 PTSD patients (21 males) for analysis.

Correlation analysis
To investigate associations between (oxytocin administration effects on) amygdala reactivity and clinical characteristics (i.e. PTSD symptom severity and anxiety before scanning) in PTSD patients, we extracted contrast estimates from the peak coordinate of the oxytocin administration effect in PTSD patients (peak voxel xyz=−30 -4 -24, 5mm sphere). Partial correlation analyses between extracted contrast estimates and clinical characteristics in PTSD patients, controlling for medication order, were conducted in SPSS. Additionally, a mediation analysis was conducted in Process (Hayes, 2013), an add-on in SPSS. One female PTSD patient was a significant outlier on left amygdala reactivity towards fearful-angry faces (Z>3.29) and had to be excluded, leaving 35 PTSD patients (21 males) for this analysis.
TABLE 3.1 Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>PTSD patients (n=37)</th>
<th>Healthy controls (n=40)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=21)</td>
<td>Females (n=16)</td>
<td>Males (n=20)</td>
</tr>
<tr>
<td>Age, in years</td>
<td>42.29 (9.83)</td>
<td>37.56 (9.78)</td>
<td>41.35 (10.62)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Years of service</td>
<td>16.29 (10.82)</td>
<td>14.53 (10.74)</td>
<td>18.42 (10.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Middle</td>
<td>14 (67%)</td>
<td>15 (93%)</td>
<td>16 (85%)</td>
</tr>
<tr>
<td>High</td>
<td>7 (33%)</td>
<td>1 (7%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>CAPS total score</td>
<td>68.05 (15.62)</td>
<td>67.56 (11.83)</td>
<td>4.7 (4.79)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety (STAI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo session</td>
<td>47.14 (9.52)</td>
<td>47.37 (6.89)</td>
<td>28.77 (4.12)</td>
</tr>
<tr>
<td>Oxytocin session</td>
<td>47.52 (10.32)</td>
<td>48.32 (11.34)</td>
<td>29.33 (2.58)</td>
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<tr>
<td>Current comorbidity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MDD</td>
<td>4 (19%)</td>
<td>4 (25%)</td>
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<tr>
<td>Dysthymia</td>
<td>2 (9.5%)</td>
<td>1 (6.3%)</td>
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<td>Panic Disorder</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>n/a</td>
</tr>
<tr>
<td>Specific phobia</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Work-related traumatic events (PLES)</td>
<td>22.50 (5.95)</td>
<td>13.50 (4.49)</td>
<td>20.45 (6.42)</td>
</tr>
<tr>
<td>Childhood traumatic events (ETI)</td>
<td>6.09 (4.55)</td>
<td>5.25 (5.18)</td>
<td>3.65 (2.35)</td>
</tr>
<tr>
<td>Hormonal contraceptive use</td>
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<td></td>
<td></td>
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<tr>
<td>None</td>
<td>7 (44%)</td>
<td>8 (40%)</td>
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<tr>
<td>Hormonal</td>
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<td>8 (50%)</td>
<td>n/a</td>
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<tr>
<td>Menopause</td>
<td>1 (6%)</td>
<td>3 (15%)</td>
<td></td>
</tr>
<tr>
<td>Time medication –task, in minutes</td>
<td>44.44 (5.44)</td>
<td>45.61 (4.50)</td>
<td>44.88 (4.15)</td>
</tr>
</tbody>
</table>

a: session x group b: session x group
Mean (± standard deviation) or N (percentage) of demographic variables are shown for male and female PTSD patients and trauma-exposed controls. Differences between PTSD patients and trauma-exposed controls were tested for males and females separately. MDD = major depressive disorder, PLES = Police life events scale, ETI = early trauma inventory, CAPS = Clinician-Administered PTSD Scale, STAI = State-Trait Anxiety Inventory (State-version, administered before intranasal spray administration).

a) Main effect of session: $F(1,39) = .656, p = .423$; Main effect of group: $F(1,39) = .007 p = .935$;

b) Main effect of session: $F(1,34) = .998, p = .325$; Main effect of group: $F(1,34) = .261, p = .613$;  

* Significant at $p<.05$ level (two-tailed)

### RESULTS

#### Demographics and questionnaires

See Table 3.1 for participant characteristics. PTSD patients and trauma-exposed controls did not differ significantly in age, years of service, educational level and time between oxytocin administration and task performance (Table 3.1; all $p>.05$). In addition, female PTSD patients and female trauma-exposed controls did not differ significantly on hormonal contraceptive use ($X^2 = .690, p=.708$). As expected, PTSD patients showed higher total CAPS scores and state anxiety prior to scanning compared to trauma-exposed controls (all $p<.0001$). Male PTSD patients experienced more different types of early life traumatic events compared to male trauma-exposed controls ($t(39) = -2.18, p=.037$), whereas female trauma-exposed controls experienced more different types of work-related traumatic events compared to female PTSD patients ($t(34) = 2.114, p=.042$).

#### Amygdala reactivity under placebo

**Main effects**

As expected, the emotional face-matching task reliably activated the bilateral amygdala ($P_{FWE}.05$, main effect of condition, collapsed across all participants and all emotional faces). Additionally, a main effect of task condition was found in which the fearful-angry faces elicited more bilateral amygdala reactivity than happy-neutral faces ($P_{FWE}.001$). No other significant main effects were found for both the left and right amygdala (all $P_{FWE}.05$).

**Interaction effects**

A positive group by task condition interaction effect was found for the left amygdala ($P_{FWE}=.046$), but not for the right amygdala ($P_{FWE}=.187$) (See Figure 3.1; Table 3.2). Post-hoc t-tests showed that left amygdala was significantly more activated in trauma-exposed controls in response to fearful-angry faces compared to happy-neutral faces ($P_{FWE}.001$). In PTSD patients, however, this valence-dependent amygdala reactivity was absent for the left amygdala ($P_{FWE}=.359$). Right amygdala activity was significantly higher during fearful-angry compared to happy-neutral faces both in trauma-exposed controls ($P_{FWE}.001$) and PTSD patients ($P_{FWE}=.005$). Direct post-hoc comparison of amygdala reactivity towards fearful-angry
faces and happy-neutral faces between PTSD patients and trauma-exposed controls revealed no significant differences (all $P_{\text{FWE}}>.05$). No other significant interaction effects were found for both the left and right amygdala (all $P_{\text{FWE}}>.05$).

**Oxytocin effects on amygdala reactivity**

*Main effect of medication*

No significant main effect of oxytocin administration on left and right amygdala reactivity towards emotional faces was found (all $P_{\text{FWE}}>.05$).

*Interaction effects*

A significant group by medication interaction effect was found for the left amygdala ($P_{\text{FWE}}=.047$, See Figure 3.2; Table 3.2), but not for the right amygdala ($P_{\text{FWE}}=.188$). Further testing using post-hoc t-tests revealed enhanced left amygdala reactivity in trauma-exposed controls ($P_{\text{FWE}}=.016$) and dampened left amygdala reactivity ($P_{\text{FWE}}=.034$) in PTSD patients after oxytocin administration compared to placebo. These effects of oxytocin were independent of sex and stimulus valence (all $P_{\text{FWE}}>.05$). No other significant interaction effects of oxytocin administration on bilateral amygdala reactivity in PTSD patients or control participants were found (all $P_{\text{FWE}}>.05$).

**FIGURE 3.1 Difference in amygdala reactivity between PTSD patients and controls**

(A) Mean contrast estimates (± standard errors) in arbitrary units (a.u.) of the left amygdala cluster (peak xyz =-26 -2 -22) during the processing of fearful-angry and happy-neutral faces (versus the visuomotor control condition) in PTSD patients and trauma-exposed controls (males and females combined). Under placebo, trauma-exposed controls showed differential activation towards fearful-angry faces compared to happy-neutral faces, whereas this effect was absent in PTSD patients. (B) Statistical map of the positive group by emotion interaction effect under placebo, overlaid on single-subject template using a statistical threshold of $p<.01$ (uncorrected) (unmasked) for display purposes. * $p<.05$; ** $p<.001$
Subjective anxiety of patients prior to intranasal placebo administration was positively related to left amygdala reactivity towards emotional faces under placebo (r=.393, p=.021, Figure 3.3). The association between state anxiety and left amygdala reactivity was significant for reactivity towards happy-neutral faces (r=.412, p=.014), but not for reactivity towards fearful-angry faces (r=.274, p=.117). State anxiety prior to oxytocin administration was not related to left amygdala reactivity towards emotional faces after oxytocin administration (r=.064, p=.731), indicating that oxytocin administration abolished the association between left amygdala reactivity and anxiety prior to scanning. Additionally, the difference in contrast estimates between oxytocin and placebo administration was trend significant positively related to state anxiety prior to placebo administration (r=.298, p=.089, Figure 3.3). Notably, this effect was mediated by left amygdala reactivity after placebo administration (indirect effect: 95% confidence interval=.0018-.0248, effect=.0114, Boot SE=.057), indicating that PTSD patients with higher state anxiety and hence higher left amygdala reactivity during the placebo session showed stronger dampening of amygdala reactivity after oxytocin administration. No significant associations between left amygdala reactivity towards emotional faces and CAPS total score or subscales were found (all p>.05).

### TABLE 3.2 Significant main and interaction effects for the placebo and oxytocin effects

<table>
<thead>
<tr>
<th></th>
<th>Amygdala ROI</th>
<th>Coordinates X</th>
<th>Y</th>
<th>Z</th>
<th>Z-value</th>
<th>Cluster size</th>
<th>P_{FWE}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Main effect of task</em></td>
<td>Left</td>
<td>-28</td>
<td>-4</td>
<td>-18</td>
<td>4.35</td>
<td>168</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>20</td>
<td>-4</td>
<td>-14</td>
<td>5.61</td>
<td>205</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Task x Group interaction</strong></td>
<td>Left</td>
<td>-26</td>
<td>-2</td>
<td>-22</td>
<td>3.15</td>
<td>47</td>
<td>.046</td>
</tr>
<tr>
<td>Controls fearful-angry &gt; happy-neutral</td>
<td>Left</td>
<td>-28</td>
<td>-2</td>
<td>-18</td>
<td>5.05</td>
<td>189</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PTSD fearful-angry &gt; happy-neutral</td>
<td>Left</td>
<td>-20</td>
<td>4</td>
<td>-14</td>
<td>2.16</td>
<td>30</td>
<td>.359</td>
</tr>
<tr>
<td><strong>Oxytocin model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication x Group interaction</td>
<td>Left</td>
<td>-30</td>
<td>-4</td>
<td>-24</td>
<td>3.19</td>
<td>29</td>
<td>.047</td>
</tr>
<tr>
<td>Controls oxytocin &gt; placebo</td>
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<td>-30</td>
<td>0</td>
<td>-22</td>
<td>3.56</td>
<td>29</td>
<td>.016</td>
</tr>
<tr>
<td>PTSD placebo &gt; oxytocin</td>
<td>Left</td>
<td>-30</td>
<td>-4</td>
<td>-24</td>
<td>3.26</td>
<td>20</td>
<td>.034</td>
</tr>
</tbody>
</table>

Statistics of the significant main effect of task and group by task interaction under placebo (placebo model) and group by medication interaction (oxytocin model). Coordinates are given in MNI space. Significant at p<.05 level (two-tailed)
* Main effect of task: fearful-angry faces elicited more amygdala activity compared to happy-neutral faces.

**Association between amygdala reactivity and clinical characteristics in PTSD patients**

Subjective anxiety of patients prior to intranasal placebo administration was positively related to left amygdala reactivity towards emotional faces under placebo (r=.393, p=.021, Figure 3.3). The association between state anxiety and left amygdala reactivity was significant for reactivity towards happy-neutral faces (r=.412, p=.014), but not for reactivity towards fearful-angry faces (r=.274, p=.117). State anxiety prior to oxytocin administration was not related to left amygdala reactivity towards emotional faces after oxytocin administration (r=.064, p=.731), indicating that oxytocin administration abolished the association between left amygdala reactivity and anxiety prior to scanning. Additionally, the difference in contrast estimates between oxytocin and placebo administration was trend significant positively related to state anxiety prior to placebo administration (r=.298, p=.089, Figure 3.3). Notably, this effect was mediated by left amygdala reactivity after placebo administration (indirect effect: 95% confidence interval=.0018-.0248, effect=.0114, Boot SE=.057), indicating that PTSD patients with higher state anxiety and hence higher left amygdala reactivity during the placebo session showed stronger dampening of amygdala reactivity after oxytocin administration. No significant associations between left amygdala reactivity towards emotional faces and CAPS total score or subscales were found (all p>.05).
FIGURE 3.2 Effects of oxytocin on amygdala reactivity

(A) Mean contrast estimates (± standard errors) in arbitrary units (a.u.) of the left amygdala cluster (peak xyz = -30 -4 -24) during the processing of emotional faces (all emotional faces combined versus the visuomotor control condition) in PTSD patients and trauma-exposed controls (males and females combined) after placebo and oxytocin administration. Oxytocin administration resulted in dampened amygdala reactivity in PTSD patients and in enhanced amygdala reactivity in trauma-exposed controls. (B) Statistical map of the medication by group interaction effect, overlaid on single-subject template using a statistical threshold of p<.01 (uncorrected) (unmasked) for display purposes. * p<.05

FIGURE 3.3 Relationship between state anxiety and amygdala reactivity in PTSD patients

Scatterplots of correlations between: (A) state anxiety before placebo and oxytocin administration and left amygdala reactivity towards all emotional faces (compared to the visuomotor control condition) after placebo and oxytocin administration; (B) state anxiety before placebo administration and difference in left amygdala reactivity (placebo – oxytocin). Contrast estimates of left amygdala reactivity in arbitrary units (a.u.) were extracted using a 5mm sphere surrounding the peak voxel (xyz = -30 -4 -24) of the oxytocin administration effect in PTSD patients. STAI-state = State-Trait Anxiety Inventory (State- version)
DISCUSSION

This is the first study showing intranasal oxytocin administration effects on amygdala reactivity in male and female PTSD patients and trauma-exposed controls. Under placebo, the trauma-exposed controls showed greater amygdala reactivity towards fearful-angry faces compared to happy-neutral faces, whereas this differential amygdala reactivity was absent in PTSD patients (i.e. equivalent amygdala reactivity towards happy-neutral and fearful-angry faces). When collapsing across all emotional faces, oxytocin administration enhanced left amygdala reactivity in trauma-exposed controls, but dampened left amygdala reactivity in PTSD patients, independent of participants’ sex and valence of the stimuli. Notably, the amygdala dampening effect of oxytocin in PTSD patients was greatest in those patients with high state anxiety and hence high amygdala reactivity under placebo.

The valence-dependent amygdala reactivity (i.e. greater amygdala reactivity towards fearful-angry faces compared to happy-neutral faces) followed the expected pattern in trauma-exposed controls (Morris et al., 1996), but was absent in PTSD patients. Possibly, PTSD patients may be specifically sensitive to show greater amygdala responsiveness to the neutral, socially ambiguous faces in the happy-neutral condition. Indeed, a previous study showed elevated amygdala responses towards neutral pictures in PTSD patients compared to trauma-exposed controls (Brunetti et al., 2010). Additionally, we found that PTSD patients with higher state anxiety showed more amygdala reactivity especially towards happy-neutral faces under placebo. Notably, oxytocin administration dampened left amygdala reactivity towards all emotional faces in PTSD patients, independent of stimulus valence and participants’ sex. This finding corresponds with previous intranasal oxytocin administration studies in healthy individuals (Kirsch et al., 2005) and psychiatric patients with GSAD and BPD (Bertsch et al., 2013; Labuschagne et al., 2010). Our findings extend accumulating evidence that oxytocin may have anxiolytic properties, possibly by inhibiting fear-associated output of the central amygdala to the brainstem and hypothalamus (Huber et al., 2005).

We did not find amygdala hyperresponsiveness in PTSD patients compared to trauma-exposed controls under placebo. This findings is in line with the meta-analysis of Patel and colleagues (2012), who found amygdala hyperactivity in PTSD patients compared to non-trauma-exposed, but not compared to trauma-exposed controls. Thus, amygdala hyperresponsiveness may be a consequence of trauma exposure rather than of PTSD. Alternatively, amygdala hyperactivity in PTSD may only become apparent during presentation of trauma-related stimuli and not during processing of non-trauma related-related stimuli (van Rooij et al., 2014), such as the emotional faces used in the present study.
Contrary to our expectations, oxytocin administration enhanced left amygdala reactivity towards emotional faces in healthy male and female trauma-exposed controls. Notably, we found an overall amygdala enhancing effect, for all emotional faces, which may indicate generally enhanced processing of socially salient environmental cues (i.e. salience processing, Seeley et al, 2007). Although oxytocin administration has predominantly been found to dampen amygdala reactivity (e.g. Domes et al, 2007; Kirsch et al, 2005; Petrovic et al, 2008), increased amygdala reactivity has also been observed after oxytocin administration. Oxytocin administration resulted in enhanced amygdala reactivity towards happy faces in healthy males (Gamer et al, 2010), and towards fearful (Domes et al, 2010) and angry faces (Bertsch et al, 2013) and threatening scenes (Lischke et al, 2012) in healthy females. In addition, increased amygdala reactivity towards neutral faces was observed after oxytocin administration in male patients with Asperger’s syndrome (Domes et al, 2013, 2014). Notably, we found that oxytocin enhanced amygdala reactivity in both male and female trauma-exposed controls. This is in line with previous observations in healthy females, but at odds with previous findings in healthy males, which generally showed dampened amygdala reactivity towards negative stimuli after oxytocin administration (e.g. Domes et al, 2007; Kirsch et al, 2005; Petrovic et al, 2008). Our findings underline previous observations that oxytocin administration effects depend on inter-individual factors (e.g. psychopathology and/ or trauma-exposure).

Notably, we found opposite oxytocin administration effects on amygdala reactivity in PTSD patients versus trauma-exposed controls (i.e. dampening vs. enhancing effects). This fits with the hypothesis that oxytocin administration effects may be especially beneficial in those who lack proficiency in fear regulation (Labuschagne et al, 2010) or social functioning (Olff et al, 2013; Weisman and Feldman, 2013). We included highly trauma-exposed, but apparently resilient control participants, who did not have any current psychopathology or history of PTSD and MDD, despite having experienced numerous traumatic events in their lives. Presumably, our trauma-exposed controls did have little to gain regarding fear regulation, whereas PTSD patients may benefit from improved fear regulation. In support of this hypothesis, we found that PTSD patients with higher state-anxiety under placebo, showed more dampening of amygdala reactivity after oxytocin administration. This indicates that PTSD patients with high anxiety prior to scanning and high baseline amygdala reactivity may especially benefit from oxytocin administration. Alternatively, the observed difference in oxytocin administration effects may be explained by differences in sensitivity to effects of oxytocin administration between PTSD patients and (resilient) trauma-exposed controls. In healthy individuals, oxytocin effects seem to follow an inverted u-shape (Rilling et al, 2014), with doses around 24IU resulting in more anxiolytic effects than higher doses of oxytocin (Cardoso et al, 2013). Moreover, oxytocin applied at higher doses (e.g. 40IU used in this study) may result in anxiogenic effects in healthy individuals, due to competitive binding to arginine-vasopressin (AVP) receptors (Cardoso et al, 2013). Our observation of increased amygdala reactivity upon
Oxytocin administration in our control group fits with this notion. PTSD patients, on the other hand, may be less sensitive to oxytocin administration, due to alterations at the level of AVP and/or oxytocin receptors, and therefore show dampened amygdala reactivity upon administration of this relatively high dose. The notion that oxytocin system functioning may be affected in PTSD patients is supported by our observation of lower salivary oxytocin levels in male PTSD patients compared to male trauma-exposed controls (Frijling et al., 2015), in the same sample reported in this manuscript.

Notably, oxytocin administration effects were lateralized to the left amygdala, although the same (albeit non-significant) directionality of effects was observed in the right amygdala. In line with our findings, oxytocin administration effects in left amygdala reactivity towards emotional faces have been found in healthy males (Gamer et al., 2010; Kirsch et al., 2005), healthy females (Domes et al., 2010) and male patients with Asperger’s syndrome (Domes et al., 2014). On the other hand, oxytocin administration effects on right-sided amygdala reactivity towards emotional faces has also been observed in healthy males (Domes et al., 2007; Petrovic et al., 2008), females with and without borderline personality disorder (Bertsch et al., 2013) and males with Asperger’s syndrome (Domes et al., 2013). Only one study (in male GSAD patients) found oxytocin administration effects on bilateral amygdala reactivity (Labuschagne et al., 2010). Functional lateralization of amygdala reactivity has previously been found in healthy individuals: the right amygdala is associated with rapid, automatic detection and general arousal towards emotional stimuli, whereas the left amygdala is better able to distinguish stimuli associated with different levels of arousal, resulting in a more subtle emotional reaction (Gläscher and Adolphs, 2003). To date, no studies have explicitly investigated lateralization of oxytocin administration effects on amygdala reactivity. Given the previous mixed findings of oxytocin administration on left vs. right amygdala reactivity, future studies are necessary to resolve this issue.

Dampening amygdala reactivity towards fearful stimuli in PTSD may be beneficial during exposure therapy. Greater amygdala reactivity during extinction learning has been associated with impaired extinction learning (Milad et al., 2009) and greater amygdala reactivity towards fearful faces before treatment resulted in worse treatment outcome (Bryant et al., 2008a). In addition, higher amygdala (and dACC and insula) reactivity towards negative emotional pictures prior to treatment predicted persistence of PTSD symptoms after treatment (van Rooij et al., 2015). It has been suggested that excessive (amygdala) fear processing during psychotherapy may result in impaired extinction learning and difficulties in regulating anxiety (Bryant et al., 2008a). By dampening excessive fear processing during exposure-based therapies, oxytocin administration could result in enhanced treatment response. Besides this anxiolytic mechanism, oxytocin is hypothesized to enhance treatment response via pro-social effects (Olff et al., 2010). Oxytocin has been found to increase in-group trust, pro-social
behavior and neural reward sensitivity to social stimuli in men and women (e.g. Groppe et al, 2013; Van IJzendoorn and Bakermans-Kranenburg, 2012; Preckel et al, 2014; Striepens et al, 2014). This way, oxytocin could potentially enhance motivation for treatment, as well as the therapeutic alliance (Olff et al, 2010). Strength of therapeutic alliance, defined as the affective bond between the therapist and patient, is an important and consistent predictor of treatment success (Ormhaug et al, 2014) and depends on the patient’s attachment security: more secure attachment is related to stronger therapeutic alliance and vice versa (see Diener and Monroe, 2011 for a meta-analysis). Attachment style may be particularly relevant in PTSD: insecure attachment, has been associated with higher PTSD symptom severity in trauma-exposed individuals (see Woodhouse et al, 2015 for a meta-analysis). Notably, perception of attachment security was improved after oxytocin administration in healthy male students (Buchheim et al, 2009). However, opposite effects of oxytocin administration on attachment have also been found: anxiously attached healthy males remembered their mother as less caring after oxytocin administration, compared to placebo (Bartz et al, 2010). In addition, oxytocin administration in anxiously attached BPD patients and healthy controls decreased cooperation and trust (Bartz et al, 2011a). This emphasizes the need to investigate the effects of oxytocin administration on social processes in PTSD, including the therapeutic alliance and attachment security.

To our knowledge, this is the first study investigating neurobiological effects of oxytocin administration in PTSD patients, as a first step to show the potential clinical importance of oxytocin administration in PTSD patients. Moreover, we investigated sex-differential oxytocin administration effects on amygdala reactivity towards emotional faces, including both male and female PTSD patients within one intranasal oxytocin fMRI study. However, several limitations should be mentioned. First, we included females using oral contraceptives. Also, we did not standardize the phase of menstrual cycle our female participants were scanned in, to minimize time and hence potential differences in symptom severity between the two scanning sessions. We cannot exclude the possibility that this may have influenced our results, although similar effects of oxytocin on amygdala reactivity were found previously in females tested in the follicular phase (Domes et al, 2010) and luteal phase (Bertsch et al, 2013). Finally, to control for the potential confounding effect of trauma exposure, we included a trauma-exposed sample of police officers without PTSD, limiting the generalizability of our findings.

Taken together, we showed that oxytocin administration dampened left amygdala reactivity in male and female PTSD patients, which could presumably enhance treatment response in PTSD. As a next step, effects of oxytocin administration should be thoroughly investigated in clinical settings before considering routine clinical application of oxytocin as medication-enhanced treatment in PTSD.
FUNDING AND DISCLOSURES

All authors declare that they have no biomedical financial interests and no potential conflicts of interest. The study is supported by grants from ZonMw, the Netherlands organization for Health Research and Development (ZonMw, grant no. 40-00812-98-10041) and the Academic Medical Center Research Council (110614).

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL - CHAPTER 3

SUPPLEMENTARY FIGURE S3.1 Design of the emotional face-matching task

A) Representative sample stimuli of one trial for each task condition (A) and block design (B) of the emotional face-matching task for both task versions. Each emotional block consisted of 6 trials of 5 seconds (total 30 seconds) and each visuomotor control block consisted of 5 trials of 5 seconds (total 25 seconds). Both task versions took 220 seconds in total. C = visuomotor control condition; F-A = fearful-angry faces condition; H-N = happy-neutral faces condition.
CHAPTER 4

Intranasal oxytocin normalizes amygdala functional connectivity in post-traumatic stress disorder

Saskia B.J. Koch, Mirjam van Zuiden, Laura Nawijn, Jessie L. Frijling, Dick J. Veltman & Miranda Olff

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ABSTRACT

The neuropeptide oxytocin (OT) has been suggested as a promising pharmacological agent for medication-enhanced psychotherapy in post-traumatic stress disorder (PTSD), because of its anxiolytic and pro-social properties. We therefore investigated the behavioral and neurobiological effects of a single intranasal oxytocin administration (40IU) in PTSD patients. We conducted a randomized, placebo-controlled, cross-over resting-state fMRI study in male and female police officers with (n=37, 21 males) and without PTSD (n=40, 20 males). We investigated oxytocin administration effects on subjective anxiety and functional connectivity of basolateral (BLA) and centromedial (CeM) amygdala subregions with prefrontal and salience processing areas. In PTSD patients, oxytocin administration resulted in decreased subjective anxiety and nervousness. Under placebo, male PTSD patients showed diminished right CeM to left ventromedial prefrontal cortex (vmPFC) connectivity compared to male trauma-exposed controls, which was reinstated after oxytocin administration. Additionally, female PTSD patients showed enhanced right BLA to bilateral dorsal anterior cingulate cortex (dACC) connectivity compared to female trauma-exposed controls, which was dampened after oxytocin administration. Although caution is warranted, our findings tentatively suggest that oxytocin has the potential to diminish anxiety and fear expression of the amygdala in PTSD, either via increased control of the vmPFC over the CeM (males) or via decreased salience processing of the dACC and BLA (females). Our findings add to accumulating evidence that oxytocin administration could potentially enhance treatment response in PTSD.
INTRODUCTION

Post-traumatic stress disorder (PTSD) develops in about ten percent of trauma-exposed individuals (de Vries and Olff, 2009). Its symptoms include intrusions; avoidance of reminders of the traumatic event; negative alterations in cognitions and mood; and hyperarousal (American Psychiatric Association, 2013). Neurobiologically, PTSD is associated with amygdala hyperactivity towards negative emotional stimuli (Hayes et al., 2012), and increased activity in and connectivity between nodes of the salience network (i.e. amygdala, insula and dorsal anterior cingulate cortex; dACC) (Patel et al., 2012; Sripada et al., 2012b). Additionally, PTSD is associated with ventromedial prefrontal cortex (vmPFC) hypoactivity, as well as with decreased functional and structural connectivity between the amygdala and vmPFC (Hayes et al., 2012; Sripada et al., 2012a), supposedly leading to decreased top-down prefrontal control over the fear response.

As approximately one-third of PTSD patients completing treatment still meet criteria for PTSD (Bradley et al., 2005), there is a need for new or improved interventions to boost treatment response (de Kleine et al., 2013). Because of its anxiolytic and pro-social properties, the neuropeptide oxytocin (OT) may be a promising agent for medication-enhanced psychotherapy in PTSD (Koch et al., 2014; Olff et al., 2010). Previously, decreased amygdala reactivity towards emotional stimuli was found after intranasal oxytocin administration in healthy males (Kirsch et al., 2005), females with borderline personality disorder (BPD) (Bertsch et al., 2013), males with generalized social anxiety disorder (GSAD) (Labuschagne et al., 2010) and male and female PTSD patients (Koch et al., 2016a) (Chapter 3). In addition, oxytocin administration increased resting-state functional connectivity between the amygdala and vmPFC in healthy males (Sripada et al., 2013) and in males with GSAD, normalizing the diminished functional connectivity observed under placebo in GSAD patients (Dodhia et al., 2014). Notably, higher amygdala reactivity in PTSD patients before treatment predicted worse treatment outcome, possibly due to (more) impaired extinction learning and fear regulation (Bryant et al., 2008b). Additionally, vmPFC-amygdala connectivity is important in fear extinction learning (Milad et al., 2007b), assumed to be the underlying mechanism of exposure therapy in PTSD (Rothbaum and Davis, 2003). Therefore, by dampening excessive fear processing of the amygdala and increasing vmPFC to amygdala connectivity, oxytocin is hypothesized to enhance treatment response in PTSD.

Although most neuroimaging studies conceptualize the amygdala as a single brain region, neuroanatomical studies in primates (Stefanacci and Amaral, 2002) and humans (Amunts et al., 2005) revealed that the amygdala consists of structurally and functionally heterogeneous subregions, two of which are the basolateral nucleus (BLA) and the centromedial nucleus (CeM). The BLA is involved in fear learning, by integrating information from the (somato}
sensory cortex, thalamus and dACC (Jovanovic and Ressler, 2010). The dACC has excitatory projections to the BLA, which may excite the CeM. The CeM is primarily involved in fear expression, via efferent projections to the brainstem and hypothalamus (LeDoux, 1998). In addition, the vmPFC exerts top-down inhibitory control over the CeM (Jovanovic and Ressler, 2010), via excitatory projections to GABA-ergic intercalated interneurons, which can inhibit CeM output. Functional imaging studies in healthy individuals have confirmed these distinct connectivity patterns (Brown et al, 2014; Roy et al, 2009).

Recently, the distinct functional connectivity patterns of the BLA and CeM were investigated in male PTSD patients, showing increased connectivity of the BLA with the pregenual anterior cingulate cortex, dACC and dorsomedial prefrontal cortex (dmPFC), and decreased connectivity of the BLA with the inferior frontal gyrus (IFG), compared to trauma-exposed controls (Brown et al, 2014). However, when the two subregions were combined into one amygdala seed, only differential amygdala connectivity with the anterior medial PFC was found in PTSD patients (Brown et al, 2014). These findings indicate increased BLA connectivity with fear processing areas in PTSD, which would not have been identified using the entire amygdala as one seed region.

To investigate the neurobiological effects of oxytocin administration in PTSD patients, we conducted a randomized, placebo-controlled, cross-over resting-state fMRI study in trauma-exposed police officers with and without PTSD. We specifically investigated the effects of a single intranasal oxytocin administration on subjective anxiety and functional connectivity of BLA and CeM amygdala subregions with prefrontal areas (vmPFC) and salience processing areas (insula and dACC). We expected to find decreased connectivity between these amygdala subregions and prefrontal cortices, but increased connectivity with salience network nodes in PTSD patients compared to trauma-exposed controls. Additionally, we hypothesized that oxytocin would normalize aberrant amygdala connectivity patterns in PTSD patients. We included both sexes to investigate possible sex differential effects of oxytocin administration. This may be relevant since amygdala reactivity towards socially and non-socially threatening scenes was enhanced after oxytocin administration in healthy females (Lischke et al, 2012), in contrast to the amygdala dampening effects of oxytocin administration observed in healthy males in response to comparable stimuli (Kirsch et al, 2005).

**METHODS**

**Participants**

Participants (n=77) were recruited via advertisements in journals and on websites of the Dutch police, and via a psychotrauma diagnostic outpatient clinic for police personnel (PDC
Male (n=21) and female (n=16) PTSD patients fulfilled DSM-IV criteria for PTSD, with a score of > 45 on the Clinician Administered PTSD Scale (CAPS) (Blake et al., 1995). Current comorbidity was assessed with the Mini International Neuropsychiatric Interview (MINI-plus) (Sheehan et al., 1998) or the Structured Clinical Interview for DSM–IV (First et al., 2012) (for patients recruited via the police outpatient clinic, n=15). PTSD patients were excluded if they met diagnostic criteria for current substance-related disorder, severe major depressive disorder (MDD), psychotic disorder, personality disorder or current suicidal risk.

Forty trauma-exposed male (n=20) and female (n=20) controls were matched to the PTSD patients on sex, age, years of service and educational level. Control participants were exposed to at least one potentially traumatic event, according to the DSM-IV A1 PTSD criterion, and had a CAPS score of ≤15. Trauma-exposed controls did not have any current DSM-IV axis-1 psychopathology and no history of PTSD or MDD, as assessed with the MINI-plus.

The study was conducted in accordance with the declaration of Helsinki and approved by the Institutional Review Board of the Academic Medical Center in Amsterdam, the Netherlands. All participants provided written informed consent before study participation.

**Experimental procedure**

This within-subjects study consisted of three appointments: one baseline session (T0) and two fMRI sessions (T1 and T2). During T0, in- and exclusion criteria were assessed. T1 and T2 were scheduled on average 11.5 (±9.90) days apart and preferably took place in the afternoon or evening. Participants were asked to abstain from alcohol and drugs 24 hours before scanning and from food, nicotine, beverages (except water) and rigorous exercise 2.5 hours prior to scanning. Before scanning, participants self-administered intranasal oxytocin (40IU, Syntocinon, five puffs of 4IU per nostril) during one fMRI session and placebo (saline, NaCl0.9%, five puffs per nostril) during the other fMRI session, under experimenter supervision. The dose of 40IU was based on a previous study in which therapeutic effects of oxytocin administration in psychiatric patients were observed (Feifel et al., 2010). The order of nasal spray administration was randomized, double-blind, and counter-balanced between sessions. The resting-state scan was part of a larger scanning protocol. First, two task-based fMRI scans were conducted: an emotional face-matching task (Koch et al., 2016a) (Chapter 3) and a monetary (Nawijn et al., 2016) and social (Nawijn et al., in revision) incentive delay reward task. Hereafter, the resting-state scan was conducted, which began on average 72.51
(±4.03) minutes after intranasal spray administration, when neuropharmacological oxytocin administration effects on cerebral blood flow (Paloyelis et al, 2014) and elevated oxytocin levels in cerebrospinal fluid (CSF) (Striepens et al, 2013) are still observed. During resting-state scanning, participants were instructed to relax and let their mind wander, with open-eyes. At the end of each scanning session, we assessed whether participants had let their mind wander during resting-state scanning (yes-no question). In case specific thoughts were reported, content of thought was assessed with an open question. To investigate behavioral effects of oxytocin administration, we assessed current levels of anxiety, nervousness, happiness and sadness on visual analogue scales (VAS) from 0 (not at all) to 100 (entirely). During each fMRI session, these VAS questionnaires were administered before intranasal spray administration and after functional scanning was completed. Finally, after each scanning session, participants were asked to guess which intranasal spray they received, and the primary reason for their guess.

**fMRI acquisition**

Scanning was performed with a 3T Philips Achieva MR system (Philips Medical Systems, Best, the Netherlands), using a 32-channel head coil. A FAST MPRage sequence was used to obtain a high-resolution anatomical scan (220 slices; voxel size=1mm$^3$; repetition time=8.2s; echo time=3.8s; flip angle=8°). Resting-state scanning was performed using an echo planar sequence sensitive to BOLD contrast (7.9 minutes; 238 volumes; 37 slices; voxel size=3mm$^3$; repetition time=2s, echo time=28ms; flip angle=76°).

**Data-analysis**

*Demographics and questionnaires*

SPSS version 20 (IBM Statistics, Armonk, NY, USA) was used to assess differences in demographical characteristics between PTSD patients and trauma-exposed controls, for male and female participants separately. After checking for outliers and normality and (log)transforming when necessary, independent t-tests and repeated measures ANOVA’s were performed for continuous variables and chi-square tests for categorical variables. Additionally, we used two repeated-measures ANOVA’s to compare subjective anxiety and mood ratings during the oxytocin and placebo sessions: one for ratings acquired before drug administration and one for ratings acquired after scanning. Drug-order was included as covariate. VAS ratings were missing for two male PTSD patients. A p-value <.05 (two-tailed) was considered significant.

*fMRI data-analysis*

SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was used for fMRI analysis. Amygdala seeds for left and right BLA and CeM subregions were defined using the Juelich histological atlas (Amunts et al, 2005; Eickhoff et al, 2005) as implemented in FSL (FMRIB Software Library, http://fsl.fmrib.ox.ac.uk/fsl) (See Figure 4.1). For the CeM, voxels were included if they had a 50% prob-
ability or higher of belonging to the CeM amygdala subregion according to the Juelich atlas. Due to signal drop-out in the temporal cortex, a more stringent probability threshold of 80% (and hence a smaller seed) was used for the BLA subregion. For each participant, the first eigenvariates of left and right BLA and CeM amygdala time-courses were extracted using the Volume of Interest module in SPM8, which were subsequently used as regressor of interest in the first-level models (See the supplementary methods for details regarding preprocessing and first-level analyses).

**FIGURE 4.1 BLA and CeM subregion connectivity**

(A) Seeds for the basolateral (BLA) and centromedial (CeM) amygdala subregions used in the functional connectivity analyses, defined using the Juelich probabilistic atlas (Amunts et al., 2005; Eickhoff et al., 2005). Right-sided BLA and CeM connectivity under placebo in male trauma-exposed controls (B), female trauma-exposed controls (C), male PTSD patients (D) and female PTSD patients (E). Blue overlay represent the right BLA functional connectivity pattern, red overlay represent the right CeM functional connectivity pattern (MNI xyz = 6 -5 5). Left and right-sided connectivity patterns were comparable.

Contrast estimates of positive correlations with the amygdala seeds were used in second-level repeated measures ANOVA's, for each left and right-sided amygdala seed separately. Between-subjects factors included group (PTSD – control) and sex (male – female) and the within-subjects factor included drug (oxytocin – placebo). Drug-order was added as covariate. Additionally, we calculated the mean frame-wise displacement (FD) for each individual, for each session, using the Data Processing Assistant Resting-State fMRI toolbox (Chao-Gan and Yu-Feng, 2010). Mean FD values were added as a covariate to all second-level models, to account for variance in relative displacement between scans (Yan et al., 2013).

We tested within predefined regions of interest (ROIs) created with the anatomical 50% Harvard-Oxford probabilistic atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) corresponding to these regions: insula, vmPFC, (i.e. subcallosal ACC mask), dMPFC (i.e. superior frontal gyrus mask), middle frontal gyrus, orbital frontal cortex (OFC) and ACC (i.e. ACC and paracingulate cortex masks). P-values were family-wise error (FWE) corrected for multiple comparisons.
### TABLE 4.1 Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>PTSD patients (n=37)</th>
<th>Healthy controls (n=40)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=21)</td>
<td>Females (n=16)</td>
<td>Males (n=20)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.29 (9.83)</td>
<td>37.56 (9.78)</td>
<td>41.35 (10.62)</td>
</tr>
<tr>
<td>Years of service</td>
<td>16.29 (10.82)</td>
<td>14.53 (10.74)</td>
<td>18.42 (10.05)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
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<td></td>
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<tr>
<td>Low</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Middle</td>
<td>14 (67%)</td>
<td>15 (93%)</td>
<td>16 (85%)</td>
</tr>
<tr>
<td>High</td>
<td>7 (33%)</td>
<td>1 (7%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>CAPS total score</td>
<td>68.05 (15.62)</td>
<td>67.56 (11.83)</td>
<td>4.7 (4.79)</td>
</tr>
<tr>
<td>Current comorbidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDD</td>
<td>4 (19%)</td>
<td>4 (25%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>2 (9.5%)</td>
<td>1 (6.3%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Panic Disorder</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Specific phobia</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Work-related traumatic events (PLES)</td>
<td>22.50 (5.95)</td>
<td>13.50 (4.49)</td>
<td>20.45 (6.42)</td>
</tr>
<tr>
<td>Childhood traumatic events (ETI)</td>
<td>6.09 (4.55)</td>
<td>5.25 (5.18)</td>
<td>3.65 (2.35)</td>
</tr>
<tr>
<td>Hormonal contraceptive use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>n/a</td>
<td>7 (44%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Hormonal</td>
<td>8 (50%)</td>
<td>9 (45%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Menopause</td>
<td>1 (6%)</td>
<td>3 (15%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Time medication – resting-state (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>73.24 (5.91)</td>
<td>72.38 (5.90)</td>
<td>73.88 (4.31)</td>
</tr>
<tr>
<td>T2</td>
<td>72.10 (4.95)</td>
<td>72.75 (7.30)</td>
<td>73.20 (8.46)</td>
</tr>
</tbody>
</table>
within predefined ROIs using small volume correction. For significant interaction effects ($P_{FWE} < .05$), contrast estimates were extracted using Marsbar (http://marsbar.sourceforge.net) with a 5mm sphere surrounding the peak voxel of the interaction effects and post-hoc tested in SPSS. Alpha-values were Bonferroni corrected for the number of post-hoc t-tests conducted for each ROI (e.g. 0.05/8 = 0.0063).

Two trauma-exposed controls were excluded from the analyses due to scanner artifacts. Additionally, two male and two female PTSD patient were excluded due to excessive movement ($>6\text{mm/degrees in any direction}$). After exclusion of these participants, average translation and rotation in any direction were not significantly different between PTSD patients and trauma-exposed controls (all $p > .05$). In total, 33 PTSD patients (19 males) and 38 trauma-exposed controls (19 males) were included in CeM subregion analyses. For BLA amygdala subregion analyses, additional participants had to be excluded because of signal drop-out in voxels belonging to these seeds (one participant for left and two participants for right BLA, see supplementary methods). To ensure that our analyses were not driven by excessive head motion, we reran the analyses with a more conservative threshold of 3mm/degrees in any direction, additionally excluding one female participant, two male and two female PTSD patients (see supplementary methods).

**RESULTS**

**Demographics**

PTSD patients did not differ from trauma-exposed controls regarding age, years of service, educational level, hormonal contraceptive use (females only) and time between intranasal spray administration and resting-state scanning (all $p > .05$; see Table 4.1). Female trauma-exposed controls experienced more types of work-related traumatic events compared to female PTSD patients ($p = .042$), whereas male PTSD patients experienced more types of
childhood traumatic events than male trauma-exposed controls (p=.037). Of all participants, 85.91% reported alternating thoughts during resting-state scanning, as opposed to thoughts about a specific topic (i.e. 14.09%). Both PTSD patients and trauma-exposed controls were equally successful at letting their mind wander during resting-state scanning (T1 controls: 89.74% alternating thoughts, PTSD: 80.56% alternating thoughts, p=.264; T2 controls: 89.74% alternating thoughts, PTSD: 82.86% alternating thoughts, p=.390). During each scanning session, three PTSD patients reported symptom-related thoughts during resting-state scanning, whereas three trauma-exposed controls reported thoughts about leisure activities, family and friends (See Supplementary Table S4.1 for thought content in PTSD patients and trauma-exposed controls during the first and second fMRI session).

**Functional connectivity – CeM amygdala**

See Figure 4.1B for CeM and BLA functional connectivity patterns in male and female trauma-exposed controls and PTSD patients under placebo. A significant group by sex by drug interaction effect was found regarding functional connectivity of the right CeM with the left vmPFC (peak voxel xyz=-2 30 -8, Z=2.86, K=16, $P_{FWE}=.032$; See Figure 4.2). We therefore investigated oxytocin administration effects on right CeM to left vmPFC functional connectivity separately for male and female PTSD patients and trauma-exposed controls. Under placebo, male PTSD patients showed significantly less right CeM to left vmPFC connectivity compared to male trauma-exposed controls ($t(35)=3.93; p<.001$), whereas no connectivity differences were found for female participants ($p=.890$). In addition, we found a trend significant group by sex interaction effect regarding right CeM to right vmPFC connectivity (peak voxel xyz=2 30 -8, $Z=2.39$, $K=5$, $P_{FWE}=.077$), in which the male trauma-exposed controls showed stronger connectivity between the right-sided CeM and vmPFC compared to male PTSD patients ($t(35)=3.18, p=.003$). Oxytocin administration resulted in enhanced connectivity between the right CeM and left vmPFC in male PTSD patients ($t(17)=-3.32, p=.004$) but not in female PTSD patients or trauma-exposed controls (all $p>.05$). The under placebo observed difference in right CeM to left vmPFC connectivity between male PTSD patients and male trauma-exposed controls was no longer significant after oxytocin administration ($p=.570$). These results remained significant after additionally excluding participants moving more than 3mm/degrees in any direction (see Supplementary Table S4.2 and S4.3).

Controlling for differences in number of different types of work-related and childhood traumatic events did not alter our results. No main effects of drug, group by drug or sex by drug interactions were observed for left and right CeM functional connectivity (all $P_{FWE}>.05$). Under placebo, no main effects of sex or group or sex by group interactions were found regarding left and right CeM functional connectivity.
Oxytocin effects on amygdala connectivity in PTSD

When investigating oxytocin administration effects on left and right BLA functional connectivity, we found a (trend) significant group by sex by drug interaction effect regarding connectivity of the right BLA with the bilateral dACC (left: peak voxel xyz = -8 50 16, Z = 3.27, K = 26, P_{FWE} = 0.070; right: peak voxel xyz = 6 38 34, Z = 3.70, K = 99, P_{FWE} = 0.019). Therefore, oxytocin administration effects on right BLA connectivity with the bilateral dACC were investigated separately for male and female PTSD patients and trauma-exposed controls. Further testing showed that oxytocin administration decreased connectivity between the right BLA and right dACC in female PTSD patients (t(12) = 4.45, p = 0.001) See Figure 4.3) and tended to decrease right BLA to left dACC connectivity in female PTSD patients (t(12) = 2.61, p = 0.023) and in male trauma-exposed controls (t(18) = 2.49, p = 0.023). Under placebo, we found significantly greater right BLA to right dACC connectivity in female PTSD patients compared to female trauma-exposed (t(30) = -3.19, p = 0.001). This difference between female PTSD patients and trauma-exposed controls was absent after oxytocin administration (p = 0.902). These results remained significant after more stringently excluding participants with excessive head movement (i.e. 3mm/degrees in any direction) (see Supplementary Table S4.2 and S4.3). Regarding left BLA connectivity, no significant oxytocin administration effects were found for PTSD patients and trauma-exposed controls (all P_{FWE} > 0.05). Controlling for differences in number of different types of work-related and childhood traumatic events did not alter our results. No main effects of drug, group by drug or sex by drug interactions were observed for left and right BLA functional connectivity (all P_{FWE} > 0.05). No main effects of drug or other group by drug interactions were observed for left and right BLA functional connectivity (all P_{FWE} > 0.05).

**FIGURE 4.2 Right CeM amygdala – left vmPFC connectivity**

(A) Statistical map of the drug by group by sex interaction effect regarding functional connectivity of the right centromedial amygdala (CeM) with the left ventromedial prefrontal cortex (vmPFC). Results are overlaid on a single-subjects template and statistically thresholded at p<.005 (uncorrected) for display purposes. (B) Contrast estimates in arbitrary units (a.u.) of right CeM connectivity with the vmPFC (5mm sphere, peak voxel xyz = -2 30 -8) in male trauma-exposed controls and male PTSD patients. Error-bars represent standard errors of the mean (SEM). ** p<.005

**Functional connectivity – BLA amygdala**

When investigating oxytocin administration effects on left and right BLA functional connectivity, we found a (trend) significant group by sex by drug interaction effect regarding connectivity of the right BLA with the bilateral dACC (left: peak voxel xyz = -8 50 16, Z = 3.27, K = 26, P_{FWE} = 0.070; right: peak voxel xyz = 6 38 34, Z = 3.70, K = 99, P_{FWE} = 0.019). Therefore, oxytocin administration effects on right BLA connectivity with the bilateral dACC were investigated separately for male and female PTSD patients and trauma-exposed controls. Further testing showed that oxytocin administration decreased connectivity between the right BLA and right dACC in female PTSD patients (t(12) = 4.45, p = 0.001) See Figure 4.3) and tended to decrease right BLA to left dACC connectivity in female PTSD patients (t(12) = 2.61, p = 0.023) and in male trauma-exposed controls (t(18) = 2.49, p = 0.023). Under placebo, we found significantly greater right BLA to right dACC connectivity in female PTSD patients compared to female trauma-exposed (t(30) = -3.19, p = 0.001). This difference between female PTSD patients and trauma-exposed controls was absent after oxytocin administration (p = 0.902). These results remained significant after more stringently excluding participants with excessive head movement (i.e. 3mm/degrees in any direction) (see Supplementary Table S4.2 and S4.3). Regarding left BLA connectivity, no significant oxytocin administration effects were found for PTSD patients and trauma-exposed controls (all P_{FWE} > 0.05). Controlling for differences in number of different types of work-related and childhood traumatic events did not alter our results. No main effects of drug, group by drug or sex by drug interactions were observed for left and right BLA functional connectivity (all P_{FWE} > 0.05). No main effects of drug or other group by drug interactions were observed for left and right BLA functional connectivity (all P_{FWE} > 0.05).
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FIGURE 4.3 Right BLA amygdala – dACC connectivity

(A) Statistical map of the group by sex by drug interaction effect regarding functional connectivity of the right basolateral amygdala (BLA) with the dorsal anterior cingulate cortex (dACC). Results are overlaid on a single-subjects template and statistically thresholded at p<.005 (uncorrected) for display. (B) Contrast estimates in arbitrary units (a.u.) of right BLA - right dACC (5mm sphere, peak voxel xyz = 6 38 34) connectivity in female PTSD patients and trauma-exposed controls. Error-bars represent standard errors of the mean (SEM). ** p<.005

Under placebo, we observed a significant group by sex interaction effect for left BLA functional connectivity with the right insula (peak voxel xyz = 34 22 -4, Z=4.10, K=50, P_{FWE}=.003), in which male trauma-exposed controls showed enhanced functional connectivity between the left BLA and right insula compared to female trauma-exposed controls (p=.045). No sex differences were observed between male and female PTSD patients regarding left BLA – right insula connectivity (p=.152). Additionally, a significant group by sex interaction effect was observed for functional connectivity of the right BLA with the left IFG (peak voxel xyz = -50 18 4, Z=3.69, K=29, P_{FWE}=.006), in which male trauma-exposed controls showed increased functional connectivity between the right BLA and left IFG, compared to female trauma-exposed controls (p=.005). Again, no sex differences were found between male and female PTSD patients regarding right BLA to left IFG functional connectivity (p=.186) (see Supplementary Table S4.4 for test statistics of these group and sex differences under placebo).

Behavioral oxytocin effects in PTSD patients

We investigated whether oxytocin administration altered subjective experiences in PTSD patients. Compared to placebo, oxytocin administration in PTSD patients was associated with lower ratings of subjective anxiety (F(1,32)=4.42, p=.044) and nominally lower ratings of nervousness (F(1,32)=3.98 p=.055), but not happiness and sadness (all p>.05) (See Figure 4.4; See Supplementary Figure S4.1 for subjective ratings in male and female PTSD patients separately). Ratings of anxiety, nervousness, happiness and sadness prior to drug administra-
Oxytocin effects on amygdala connectivity in PTSD

Oxytocin-induced reductions in anxiety and nervousness were not significantly correlated with oxytocin-induced alterations in amygdala functional connectivity with the vmPFC (male PTSD patients) or right dACC (female PTSD patients) (all p>.05; Supplementary Table S4.5). CAPS total and subscale scores were not significantly correlated to functional connectivity measures under placebo (all p>.05; Supplementary Table S4.6).

After the first scanning session, 51.4% of trauma-exposed controls and 57.1% of PTSD patients correctly guessed which intranasal spray they received, which is not significantly different from chance levels (i.e. 50%, binominal test, controls: p=1.00, PTSD: p=.500). After the second scanning session, trauma-exposed controls still guessed at chance level (i.e. 62.2% of controls guessed correctly, 50% binominal test, p=.188), whereas PTSD patients were able to correctly guess intranasal treatment allocation (i.e. 77.1% of PTSD patients guessed correctly, 50% binominal test, p=.002). This seemed more pronounced in female PTSD patients (86.7% correct, 50% binominal test, p=.007) than in male PTSD patients (70% correct, 50% binominal test, p=.115). Of note, in those participants correctly guessing intranasal treatment allocation during the second scanning session, the most common reason for their correct guess was psychological effects, such as increased calmness, sleepiness and changes in concentration (i.e. 59.09% of controls and 55.56% of patients), whereas a minority reported mild side effects (e.g. dizziness, headache) (i.e. 4.54% of controls and 7.41% of patients) or differences in

FIGURE 4.4 Behavioral effects of oxytocin administration in PTSD patients

Average rating on visual analogue scales (VAS) assessing current subjective anxiety, nervousness, happiness and sadness on a scale of 0 (not at all) to 100 (entirely) after placebo and oxytocin administration PTSD patients (males and females combined). Error bars represent standard errors of the mean (SEM).

* p<.05; # p<.1
taste or smell between placebo and oxytocin (i.e. 22.7% of controls and 29.6% of patients) as primary reason.

**DISCUSSION**

We investigated oxytocin administration effects on amygdala subregion functional connectivity in male and female PTSD patients and trauma-exposed controls. We found diminished functional connectivity between the right CeM and left vmPFC in male PTSD patients under placebo, which was reinstated after oxytocin administration. Female PTSD patients, on the other hand, showed higher connectivity between the right BLA and right dACC under placebo, which was attenuated after oxytocin administration to levels similar to that of female trauma-exposed controls under placebo. These neural effects were paralleled by decreased subjective anxiety and nervousness in PTSD patients after oxytocin administration.

Under placebo, male PTSD patients showed lower CeM to vmPFC connectivity compared to male trauma-exposed controls. Previously, Sripada and colleagues (2012a) found decreased anti-correlations between amygdala and vmPFC activity in male PTSD patients compared to male traumatized controls, indicating decreased inhibitory connectivity between the vmPFC and amygdala. Decreased amygdala to prefrontal connectivity is thought to represent decreased top-down control over the fear response, via excitatory glutamatergic vmPFC projections to GABA-ergic interneurons of the amygdala, which in turn inhibit fear expression of the CeM (Jovanovic and Ressler, 2010). In line with the notion of top-down-control, vmPFC activity has been positively associated with extinction learning in healthy individuals, inhibiting the conditioned fear response of the amygdala when an extinction memory is retrieved (Milad et al, 2007b). Notably, we found oxytocin-induced decreased subjective anxiety, as well as increased connectivity between the vmPFC and CeM in male PTSD patients. Based on the prevailing amygdala neurocircuitry model, increased CeM to vmPFC connectivity may indicate increased top-down prefrontal control over the fear response, although caution should be warranted in assigning causal relations to correlational resting-state data.

Our finding of stronger BLA to dACC connectivity in female PTSD patients compared to female trauma-exposed controls replicates a previous finding on amygdala subregion connectivity, albeit in male veterans with PTSD (Brown et al, 2014). The dACC is implicated in the expression of fear via excitatory glutamatergic projections to the BLA, which in turn may increase fear-associated output of the CeM (Jovanovic and Ressler, 2010). Furthermore, the dACC and amygdala are key nodes of the salience network, which is important for directing attention to salient stimuli in the environment. This is particularly relevant in light of the commonly observed hypervigilance in PTSD. Indeed, dACC activity in female PTSD patients was previously
associated with attentional bias towards threatening facial expressions in a dot-probe task (Fani et al., 2012a). We found that oxytocin administration attenuated BLA-dACC connectivity in female PTSD patients to the same level as female trauma-exposed controls under placebo. Reducing (excessive) connectivity between the dACC and amygdala in PTSD patients may lead to decreased hypervigilance, thereby dampening fear responsiveness and decreasing subjective anxiety and nervousness.

Behaviorally, PTSD patients reported decreased subjective anxiety and nervousness after oxytocin administration, but no alterations in self-reported happiness or sadness. This is in line with a previous study, in which reduced subjective anxiety during public speaking stress in healthy males was observed after a single oxytocin administration (de Oliveira et al., 2012). PTSD patients, who are characterized by exaggerated fear responses, may be especially sensitive to the anxiolytic effects of oxytocin administration, resulting in decreased reported anxiety and nervousness in this group specifically. In addition, 77.1% of PTSD patients (but not trauma-exposed controls) were able to correctly guess treatment allocation at the end of the second scanning session, reporting noticeable oxytocin administration effects on psychological functioning as primary reason underlying their guess. These findings seem to be in line with the suggestion that oxytocin administration effects may be especially beneficial for those individuals who have something to gain regarding fear regulation (Labuschagne et al., 2010). As mentioned previously, our observed oxytocin-induced alterations in amygdala subregion connectivity may have resulted in decreased fear expression of the CeM, either via increased (inhibitory) top-down control of the vmPFC over the CeM (male PTSD patients) or via decreased excitation of the BLA by the dACC (female PTSD patients). This could be a potential underlying neural mechanism of the observed decreased subjective anxiety and nervousness. However, we did not find significant associations between oxytocin-related reductions in anxiety and nervousness and oxytocin-induced alterations in amygdala subregion functional connectivity. Several possible explanations can be put forward. First, anxiety and nervousness were assessed at the end of the fMRI session, approximately 25 minutes after resting-state scanning, and therefore we cannot exclude additional influences on these behavioral outcomes. Secondly, male and female PTSD patients showed differential neural, but not behavioral, correlates of oxytocin administration. Therefore, we conducted subgroup analyses on the association between oxytocin-induced alterations in subjective anxiety and amygdala subregion connectivity for male and female PTSD patients separately. Possibly, these analyses suffered from a lack of power due to the subgroup analysis. This may be especially true for male PTSD patients, in whom the correlation coefficients between oxytocin-induced subjective and neural alterations were of moderate effect size (i.e. anxiety: Pearson \( r = .333 \); nervousness: Pearson \( r = .336 \)).
In contrast to a previous intranasal fMRI resting-state study in which increased amygdala to vmPFC connectivity was found after oxytocin administration in healthy individuals (Sripada et al., 2013), we did not find oxytocin administration effects in our healthy trauma-exposed controls, except for the dampening of right BLA to right dACC connectivity in male controls. It has been suggested that the effects of oxytocin depend on inter-individual and contextual factors (Bartz et al., 2011b) and may be more beneficial in those who have something to gain regarding fear regulation (Labuschagne et al., 2010; Olff et al., 2013). Of note, we included a healthy sample of apparently highly resilient controls, with few PTSD symptoms and no current psychopathology, despite high trauma exposure. Presumably, they had little to gain regarding fear regulation, explaining the almost complete absence of oxytocin administration effects in this group.

Notably, our male and female PTSD patients showed distinct amygdala functional connectivity abnormalities compared to their same-sex traumatized controls, suggesting that the underlying neurobiological correlates of PTSD differs for males and females. To our knowledge, to date only one fMRI study investigated sex differences in functional connectivity of amygdala subregions, albeit in relation to the personality trait harm avoidance in healthy individuals (Li et al., 2012). Harm avoidance was more strongly associated with functional connectivity patterns of the BLA in females and of the CeM in males (Li et al., 2012). However, sex differences in functional connectivity patterns of amygdala subregions have not yet been studied in PTSD patients. Our findings underline the importance of investigating such possible neurobiological sex differences in PTSD.

Presumably, the effects of oxytocin administration on amygdala subregion connectivity in PTSD patients may be associated with dampened fear expression towards threat-related stimuli. Although causal relations cannot be inferred from these correlational data, the increased connectivity between the CeM and vmPFC after oxytocin administration observed in male PTSD patients may suggest increased prefrontal control over the amygdala. Additionally, the decreased BLA to dACC connectivity observed in female PTSD patients may represent decreased excitatory dACC projections to the BLA. However, this remains speculative. In line with the notion of dampened fear expression, PTSD patients showed decreased anxiety and nervousness after oxytocin, but no alterations in mood. In PTSD patients, higher amygdala reactivity towards masked fearful faces, measured before psychotherapy, has been associated with worse treatment outcome (Bryant et al., 2008a). In addition, increased amygdala and dACC activity towards pictures of negative emotional scenes before treatment predicted the persistence of PTSD symptoms post-treatment (van Rooij et al., 2015). It has been suggested that excessive fear during psychotherapy could impair extinction learning, resulting in less favorable therapy outcomes (Bryant et al., 2008a). Successful treatment has been associated with decreased amygdala activity and increased vmPFC activity towards emotional faces.
over the course of treatment (Felmingham et al., 2007). Notably, the vmPFC is important in extinction learning, the underlying mechanism of exposure therapy (Rothbaum and Davis, 2003) and the treatment of choice for PTSD (Foa et al., 2009). Previous animal (Zoicas et al., 2014) and human studies (e.g. Eckstein et al., 2014) have shown that oxytocin administration enhanced extinction learning (but see Acheson et al., 2015). In healthy individuals, oxytocin administration increased prefential involvement during the early phase of fear extinction, and attenuated general amygdala responsivity (Eckstein et al., 2014). Taken together, decreasing (excessive) fear response and subjective anxiety, and possibly enhancing fear extinction learning, may be beneficial during psychotherapy in PTSD. However, caution is warranted with regard to routine clinical oxytocin application in the context of medication-enhanced psychotherapy for PTSD. In a recent pilot study, oxytocin was administered prior to an exposure therapy session for arachnophobia (fear of spiders), investigating whether oxytocin could augment exposure-based treatment response (Acheson et al., 2015). Although behavioral measures of fear were not altered, oxytocin administration resulted in increased self-reported symptoms both at one week and one month follow-up, as well as decreased confidence in treatment and therapeutic alliance prior to the therapy session (Acheson et al., 2015).

To our knowledge, this is the first study investigating oxytocin administration effects on amygdala subregion functional connectivity. Additionally, oxytocin administration effects on resting-state functional connectivity have never been studied before in PTSD patients. Furthermore, male and female psychiatric patients have never been directly compared in one intranasal oxytocin fMRI study on amygdala functional connectivity. However, several limitations need to be addressed. First, no causal inferences can be drawn based on the correlational functional connectivity analyses, nor can we infer whether connections are inhibitory or excitatory. Although the vast majority of participants (i.e. > 85%) was able to let their mind wander during resting-state scanning, we cannot exclude that differences in content of thought between PTSD patients and trauma-exposed controls may have influenced our observed resting-state differences between PTSD patients and trauma-exposed controls. Future resting-state studies in PTSD patients should investigate the possible effects of content of thought on functional connectivity findings. Additionally, although our overall sample size was fairly large for a cross-over fMRI study in psychiatric patients, it was relatively small to investigate possible sex differential effects of oxytocin administration. Furthermore, we included a specific, highly traumatized sample of police officers, who experienced police-related traumatic events. This allowed us to control for the possible confounding effect of high trauma-exposure and differences in types of traumatic experiences, but also limits the generalizability of our findings to other PTSD patient samples and types of traumatic experiences. Additionally, we included female participants using hormonal contraceptives, which may have influenced basal oxytocin levels and oxytocin receptor affinity (Caldwell et
al, 1994). However, female PTSD patients did not differ from female trauma-exposed regarding hormonal contraceptive use, indicating that the observed differential oxytocin effects between these groups are unlikely to be explained by differences in hormonal contraceptive use. Finally, we were unable to control for the phase of menstrual cycle our female participants were scanned in, since we aimed to minimize the time and hence differences in PTSD symptom severity between both scanning sessions. As extinction recall (Milad et al, 2006) was previously found to be influenced by phase of menstrual cycle, future studies on medication-enhanced psychotherapy for PTSD should take phase of menstrual cycle into account.

In conclusion, we showed that oxytocin administration dampened subjective anxiety and nervousness in PTSD patients and that aberrant functional connectivity patterns in PTSD patients were normalized to similar levels as trauma-exposed controls. This suggests that oxytocin has the potential to diminish fear expression and enhance treatment response in PTSD patients. Our findings open the way for research investigating the therapeutic potential of oxytocin administration in PTSD patients in more clinical settings, for example during symptom provocation, and eventually during exposure-based therapy.

**FUNDING AND DISCLOSURE**

The study is supported by grants from ZonMw, the Netherlands organization for Health Research and Development (grant no. 91210041) and the Academic Medical Center Research Council (grant no. 110614). All authors declare that they have no biomedical financial interests and no potential conflicts of interest.

**ACKNOWLEDGEMENTS**

The authors thank all participants for their participation in this study. We thank Renée Hutter, Gré Westerveld, Marthe Hoofwijk and all other personnel of the PDC police outpatient clinic for their valuable help with recruitment of the PTSD patients.
Supplementary methods

Preprocessing and first-level model
After discarding the first five images, preprocessing consisted of realignment, slice-time correction, co-registration to the anatomical scan, spatial normalization to the Montreal Neurological Institute (MNI) template, resampling to 2mm\(^3\) voxels and spatial smoothing with a 8mm full-width half maximum (FWHM) Gaussian filter. For each participant, the first eigenvariate of left and right BLA and CeM amygdala time-courses were extracted using the Volume of Interest module in SPM8. For each amygdala seed (i.e. left & right CeM, left & right BLA) and each participant, a first-level model was created including the extracted amygdala time-course as regressor of interest and eight nuisance regressors: six realignment parameters to account for movement and the mean white matter and cerebrospinal fluid signals extracted from the tissue probabilities maps of SPM (thresholded at 0.5). In addition, we used a high-pass filter of 1/128Hz to remove slow drifts of the signal and the AR(1) process to remove temporal correlations. Due to signal drop-out in the temporal cortex, a more stringent probability threshold of 80% (and hence a smaller seed) was used for the BLA sub-region (signal drop-out in 50% probability BLA seed: n=7; signal drop-out in 80% probability BLA seed: n=3).

Exclusion of participants
Two trauma-exposed controls were excluded from the analyses due to scanner artifacts and two male and two female PTSD patient were excluded due to excessive movement (i.e. > 6mm/degrees in any direction). In total, 33 PTSD patients (19 males) and 38 trauma-exposed controls (19 males) were included in the CeM subregion analyses. For BLA amygdala subregion analyses, participants had to be excluded because of signal drop-out in voxels belonging to these seeds, resulting in 33 PTSD patients (19 males) and 37 trauma-exposed controls (19 males) for the left BLA and 31 patients (18 males) and 38 trauma-exposed controls (19 males) for the right BLA. To assure that our results were not driven by excessive head movement, we reran the analyses after excluding participants moving > 3mm/degrees in any direction. In total one female trauma-exposed control, four male PTSD patients and four female PTSD patients were removed for this analysis, resulting in 29 PTSD patients (17 males) and 37 trauma-exposed controls (19 males) for the CeM subregion analysis and 29 patients (17 males) and 36 controls (19 males) for the left BLA and 27 patients (16 males) and 37 controls (19 males) for the right BLA analysis.
SUPPLEMENTARY TABLE S4.1 Content of thought during resting-state scanning for both fMRI sessions

<table>
<thead>
<tr>
<th>A)</th>
<th>First session (T1)</th>
<th>Second session (T2)</th>
<th>Difference between T1 and T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>p-value</td>
</tr>
<tr>
<td>All participants</td>
<td>Total responses</td>
<td>75 (100%)</td>
<td>74 (100%)</td>
</tr>
<tr>
<td></td>
<td>Alternating thoughts</td>
<td>64 (85.33%)</td>
<td>64 (86.49%)</td>
</tr>
<tr>
<td></td>
<td>Specific thoughts</td>
<td>11 (14.67%)</td>
<td>10 (13.51%)</td>
</tr>
<tr>
<td>Trauma-exposed controls</td>
<td>Total responses</td>
<td>39 (100%)</td>
<td>39 (100%)</td>
</tr>
<tr>
<td></td>
<td>Alternating thoughts</td>
<td>35 (89.74%)</td>
<td>35 (89.74%)</td>
</tr>
<tr>
<td></td>
<td>Specific thoughts</td>
<td>4 (10.26%)</td>
<td>4 (10.26%)</td>
</tr>
<tr>
<td>PTSD patients</td>
<td>Total responses</td>
<td>36 (100%)</td>
<td>35 (100%)</td>
</tr>
<tr>
<td></td>
<td>Alternating thoughts</td>
<td>29 (80.56%)</td>
<td>29 (82.86%)</td>
</tr>
<tr>
<td></td>
<td>Specific thoughts</td>
<td>7 (19.44%)</td>
<td>6 (17.14%)</td>
</tr>
</tbody>
</table>

(B) | Leisure (N) | PTSD related (N) | Future (N) | Other (N) | Total (N) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>T1</td>
<td>3 (27.27%)</td>
<td>3 (27.27%)</td>
<td>3 (27.27%)</td>
<td>1 (9.09%)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>4 (40.00%)</td>
<td>3 (30.00%)</td>
<td>2 (20.00%)</td>
<td>1 (10.00%)</td>
</tr>
<tr>
<td>Trauma-exposed controls</td>
<td>T1</td>
<td>3 (75.00%)</td>
<td>0 (0.00%)</td>
<td>1 (25.00%)</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>3 (75.00%)</td>
<td>0 (0.00%)</td>
<td>1 (25.00%)</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>PTSD patients</td>
<td>T1</td>
<td>0 (0.00%)</td>
<td>3 (42.86%)</td>
<td>2 (28.57%)</td>
<td>2 (28.57%)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>1 (16.67%)</td>
<td>3 (50.00%)</td>
<td>1 (16.67%)</td>
<td>1 (16.67%)</td>
</tr>
</tbody>
</table>

(A) Number (percentage) of participants reporting thoughts about a specific topic (specific thoughts) or alternating thoughts (as instructed). (B) Number (percentage) of thought content categories for those participants reporting specific thoughts during scanning. Leisure: sports, activities, family or friends. Seven out of eleven participants reporting specific thoughts topic during the first resting-state scanning session (T1), also reported specific thoughts during the second resting-state session (T2). Number of PTSD patients and trauma-exposed controls reporting specific thoughts was not significantly different during T1 (Mann Whitney U=637.5; p=.264) and T2 (Mann Whitney U=635.50; p=.390).
SUPPLEMENTARY TABLE S4.2 Neuroimaging results after excluding extra participants based on movement (> 3mm/degree)

<table>
<thead>
<tr>
<th>Contrast Seed Region</th>
<th>Hemisphere</th>
<th>MNI</th>
<th>Z-score</th>
<th>P$_{FWE}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X  Y  Z</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Centromedial amygdala (CeM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group x Sex x Drug interaction (F-test)</td>
<td>Right CeM</td>
<td>vmPFC</td>
<td>L</td>
<td>-2 30 -8</td>
</tr>
<tr>
<td>Group x Sex interaction (F-test) – placebo</td>
<td>Right CeM</td>
<td>vmPFC</td>
<td>R</td>
<td>2 30 -8</td>
</tr>
<tr>
<td><strong>Basolateral amygdala (BLA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group x Sex x Drug interaction (F-test)</td>
<td>Right BLA</td>
<td>dACC</td>
<td>L</td>
<td>-8 50 16</td>
</tr>
<tr>
<td>Group x Sex x Drug interaction (F-test)</td>
<td>Right BLA</td>
<td>dACC</td>
<td>R</td>
<td>6 38 3</td>
</tr>
</tbody>
</table>

fMRI results after excluding additional participants based on head motion (i.e. > 3mm/degrees in any direction, see supplementary methods for excluded participants). Coordinates (xyz) are given in MNI stereotaxic space. P$_{FWE}$ corrected for multiple comparisons within the region of interest. L = left, R = right, vmPFC = ventromedial prefrontal cortex, dACC = dorsal anterior cingulate cortex.
### SUPPLEMENTARY TABLE S4.3 Results of post-hoc tests after excluding additional participants based on head movement (> 3mm/degree)

<table>
<thead>
<tr>
<th>Seed Region hemisphere</th>
<th>Hemisphere</th>
<th>t(df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group by Sex by Drug interaction effect - right CeM – left vmPFC connectivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo – male controls &gt; male PTSD</td>
<td>Right CeM vmPFC L</td>
<td>t(34) = 3.726</td>
<td>p=.001*</td>
</tr>
<tr>
<td>Placebo – female PTSD &gt; female controls</td>
<td>Right CeM vmPFC L</td>
<td>t(28) = -2.155</td>
<td>p=.040</td>
</tr>
<tr>
<td>Oxytocin – male controls &gt; male PTSD</td>
<td>Right CeM vmPFC L</td>
<td>t(34) = -.251</td>
<td>p=.803</td>
</tr>
<tr>
<td>Male control: placebo &gt; oxytocin</td>
<td>Right CeM vmPFC L</td>
<td>t(18) = 1.706</td>
<td>p=.105</td>
</tr>
<tr>
<td>Male PTSD: oxytocin &gt; placebo</td>
<td>Right CeM vmPFC L</td>
<td>t(16) = -3.014</td>
<td>p=.008#</td>
</tr>
<tr>
<td>Group by Sex interaction effect - right CeM – right vmPFC connectivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo – male controls &gt; male PTSD</td>
<td>Right CeM vmPFC R</td>
<td>t(33.53) = 2.928</td>
<td>p=.006*</td>
</tr>
<tr>
<td>Placebo – female PTSD &gt; female controls</td>
<td>Right CeM vmPFC R</td>
<td>t(28) = -2.249</td>
<td>p=.033</td>
</tr>
<tr>
<td>Group by Sex by Drug interaction – right BLA – right dACC connectivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo – male controls &gt; male PTSD</td>
<td>Right BLA dACC R</td>
<td>t(27) = 1.132</td>
<td>p=.266</td>
</tr>
<tr>
<td>Placebo – female PTSD &gt; female controls</td>
<td>Right BLA dACC R</td>
<td>t(27) = 3.080</td>
<td>p=.005*</td>
</tr>
<tr>
<td>Oxytocin – female controls &gt; female PTSD</td>
<td>Right BLA dACC R</td>
<td>t(27) = -3.96</td>
<td>p=.695</td>
</tr>
<tr>
<td>Female control: oxytocin &gt; placebo</td>
<td>Right BLA dACC R</td>
<td>t(17) = -1.480</td>
<td>p=.157</td>
</tr>
<tr>
<td>Female PTSD: placebo &gt; oxytocin</td>
<td>Right BLA dACC R</td>
<td>t(10) = 3.594</td>
<td>p=.005*</td>
</tr>
<tr>
<td>Group by Sex by Drug interaction – right BLA – left dACC connectivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo – male controls &gt; male PTSD</td>
<td>Right BLA dACC L</td>
<td>t(33) = 1.218</td>
<td>p=.232</td>
</tr>
<tr>
<td>Placebo – female PTSD &gt; female controls</td>
<td>Right BLA dACC L</td>
<td>t(27) = -2.209</td>
<td>p=.036</td>
</tr>
<tr>
<td>Oxytocin – female controls &gt; female PTSD</td>
<td>Right BLA dACC L</td>
<td>t(27) = -1.111</td>
<td>p=.913</td>
</tr>
<tr>
<td>Female control: oxytocin &gt; placebo</td>
<td>Right BLA dACC L</td>
<td>t(17) = -1.844</td>
<td>p=.410</td>
</tr>
<tr>
<td>Female PTSD: placebo &gt; oxytocin</td>
<td>Right BLA dACC L</td>
<td>t(10) = 2.557</td>
<td>p=.029</td>
</tr>
</tbody>
</table>

Results of the post-hoc independent or paired-sample t-tests testing the interaction effects displayed in Supplementary Table S2 after excluding additional participants based on excessive head movement (i.e. > 3mm/ degree in any direction).

L = left, R = right, CeM = centromedial nucleus, BLA = Basolateral nucleus, vmPFC = ventromedial prefrontal cortex, dACC = dorsal anterior cingulate cortex, df = degrees of freedom

* significant at p<.0063 level corrected for multiple comparisons
**SUPPLEMENTARY TABLE S4.4 Main effects of group, sex and group by sex interactions under placebo**

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Seed</th>
<th>Region</th>
<th>Hemisphere</th>
<th>MNI</th>
<th>Z-score</th>
<th>(P_{FWE})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BLA – left</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex x Group interaction (F-test)</td>
<td>BLA – left</td>
<td>Insula</td>
<td>R</td>
<td>34</td>
<td>22</td>
<td>-4</td>
</tr>
<tr>
<td>Controls: male &gt; female (t-test)</td>
<td>BLA – left</td>
<td>Insula</td>
<td>R</td>
<td>34</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Patients: female &gt; male (t-test)</td>
<td>BLA – left</td>
<td>Insula</td>
<td>R</td>
<td>34</td>
<td>22</td>
<td>-4</td>
</tr>
<tr>
<td><strong>BLA – right</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex x Group interaction (F-test)</td>
<td>BLA – right</td>
<td>IFG</td>
<td>L</td>
<td>-50</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>Controls: male &gt; female (t-test)</td>
<td>BLA – left</td>
<td>IFG</td>
<td>L</td>
<td>-48</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Patients: female &gt; male (t-test)</td>
<td>BLA – left</td>
<td>IFG</td>
<td>L</td>
<td>-50</td>
<td>18</td>
<td>4</td>
</tr>
</tbody>
</table>

Significant group by sex interaction effects are reported. No significant main effects of group and sex were found for bilateral CeM or BLA functional connectivity (all \(P_{FWE}>.05\)). MNI = Montreal Neurological Institute coordinates. \(P_{FWE}\) values were small volume corrected within the region of interest.
Chapter 4

SUPPLEMENTARY TABLE S4.5 Correlations between oxytocin-induced reductions in anxiety and nervousness and OT-induced alterations in amygdala functional connectivity

<table>
<thead>
<tr>
<th></th>
<th>Δ right CeM – left vmPFC FC (PL-OT) (male PTSD)</th>
<th>Δ right BLA – right dACC FC (PL-OT) (female PTSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ VAS anxiety (PL-OT) after scanning</td>
<td>r=.333; p=.192</td>
<td>r=.218; p=.496</td>
</tr>
<tr>
<td>Δ VAS nervousness (PL-OT) after scanning</td>
<td>r=.336; p=.188</td>
<td>r=-.194; p=.546</td>
</tr>
</tbody>
</table>

Correlations between oxytocin-induced reductions in anxiety and nervousness and oxytocin-induced alterations in amygdala functional connectivity, for right CeM connectivity in male PTSD patients and for right BLA connectivity in female PTSD patients. Contrast estimates were extracted using Marsbar, with a 5mm sphere around the peak voxel of the group by sex by drug interaction effects. Partial correlations were conducted, controlling for drug-order.

FC=functional connectivity, OT = oxytocin, PL = placebo, CeM = centromedial amygdala, vmPFC = ventromedial prefrontal cortex, BLA = basolateral amygdala, dACC = dorsal anterior cingulate cortex, VAS = visual analogue scale

SUPPLEMENTARY TABLE S4.6 Correlations between amygdala connectivity and PTSd symptom severity in male and female PTSd patients

<table>
<thead>
<tr>
<th></th>
<th>Right CeM – left vmPFC FC (male PTSD)</th>
<th>Right BLA – right dACC FC (female PTSd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PTSd symptom severity (CAPS-total)</td>
<td>r=.205; p=.432</td>
<td>r=-.152; p=.637</td>
</tr>
<tr>
<td>Re-experiencing symptoms (CAPS-B)</td>
<td>r=-.178; p=.493</td>
<td>r=.208; p=.516</td>
</tr>
<tr>
<td>Avoidance symptoms (CAPS-C)</td>
<td>r=.299; p=.244</td>
<td>r=-.295; p=.352</td>
</tr>
<tr>
<td>Hyperarousal symptoms (CAPS-D)</td>
<td>r=.292; p=.256</td>
<td>r=-.322; p=.307</td>
</tr>
</tbody>
</table>

Correlations between PTSD symptom severity and amygdala functional connectivity, for right CeM functional connectivity in male PTSD patients and for right BLA connectivity in female PTSD patients (after placebo administration only). Contrast estimates were extracted using Marsbar, with a 5mm sphere around the peak voxel of the group by sex by drug interaction effects. Partial correlations were conducted, controlling for drug-order.

FC = functional connectivity; CeM = centromedial nucleus, vmPFC = ventromedial prefrontal cortex, BLA = basolateral nucleus, dACC = dorsal anterior cingulate cortex, CAPS = clinician administered PTSD scale
SUPPLEMENTARY FIGURE S4.1 Subjective mood and anxiety ratings for male and female PTSD patients separately

Subjective mood and anxiety ratings after oxytocin and placebo administration on a Visual Analogue Scale (VAS) (0-100) for male and female PTSD patients separately. Drug x sex interaction effects were non-significant and differences between male and female PTSD patients were therefore not reported in the manuscript.
CHAPTER 5

Effects of intranasal oxytocin on distraction as emotion regulation strategy in male and female patients with post-traumatic stress disorder

Saskia B.J. Koch, Mirjam van Zuiden, Laura Nawijn, Jessie L. Frijling, Dick J. Veltman & Miranda Olff

Submitted for publication
Chapter 5

ABSTRACT

Post-traumatic stress disorder (PTSD) is characterized by difficulty down-regulating emotional and neural reactivity towards trauma-reminders. The neuropeptide oxytocin may enhance treatment response in PTSD, by targeting excessive fear and by improving (cognitive) emotion regulation abilities. However, oxytocin effects on cognitive emotion regulation in down-regulating negative affect and neural responding have never been investigated in healthy participants, or in PTSD patients. Therefore, we investigated behavioral and neural effects of intranasal oxytocin (40IU) administration on distraction as emotion regulation strategy during presentation of negative affective pictures in male and female police officers with and without PTSD (n=77), using a randomized placebo-controlled cross-over fMRI study. The distraction condition consisted of a working memory task. Under placebo, male PTSD patients showed no down-regulation of right-sided amygdala and posterior insula activity during distraction, which was positively correlated with overall PTSD symptom severity. After oxytocin administration, male PTSD patients showed posterior insula down-regulation during distraction. Compared to female trauma-exposed controls, female PTSD patients showed greater right amygdala down-regulation during distraction under placebo, which was positively associated with avoidance symptoms. After oxytocin administration, this group difference was absent. Additionally, all female participants showed up-regulation of left-sided amygdala activity during distraction after oxytocin. In conclusion, oxytocin effects were dependent on sex and (severity of) PTSD: whereas oxytocin in male PTSD patients reinstated impaired neural down-regulation towards negative stimuli during distraction, it diminished amygdala down-regulation during distraction in all females. Oxytocin administration may hold therapeutic promise for PTSD, although more research into inter-individual differences and contextual factors influencing oxytocin effects is warranted.
INTRODUCTION

Post-traumatic stress disorder (PTSD) can develop after trauma exposure (American Psychiatric Association, 2013; de Vries and Olff, 2009). PTSD has been conceptualized as a disorder of emotion regulation (Frewen and Lanius, 2006), with difficulties regulating emotional and physiological responses towards trauma reminders as important clinical characteristics. Corresponding with symptoms of emotional and physiological distress towards trauma reminders (American Psychiatric Association, 2013), PTSD has been associated with impaired down-regulation of emotional, physiological and neural responding towards (potential) threatening and trauma-related stimuli (Ehlers et al, 2010; Hayes et al, 2012; Orsillo et al, 2004). Neurobiological correlates of PTSD include hyperactivity and hyperconnectivity in salience processing areas, including the amygdala, anterior insula and dorsal anterior cingulate cortex (dACC) (Hayes et al, 2012; Patel et al, 2012; Sripada et al, 2012b), possibly representing the neural correlate of hypervigilance symptoms (Lopresto et al, 2016; Ronzoni et al, 2016). Additionally, ventromedial prefrontal cortex (vmPFC) hypoactivity and decreased connectivity between the vmPFC, amygdala and hippocampus observed in PTSD (Koch et al, 2016b (Chapter 6); Sripada et al, 2012a) suggest diminished top-down prefrontal control over the (amygdala-mediated) fear response, possibly underlying difficulties down-regulating emotional responses towards trauma-related stimuli (Rauch et al, 2006).

Emotion regulation refers to all conscious and non-conscious regulatory processes altering the experience or expression of emotions (Ochsner et al, 2002) and emotional actions (Roelofs et al, 2009). Cognitive emotion regulation strategies include reappraisal (changing the interpretation of the stimulus) (Ochsner et al, 2002) and distraction (diverting attention away from the emotional content, e.g. by performing a working memory (WM) task) (Kanske et al, 2011). Both strategies result in more positive evaluation of negative stimuli, dampened amygdala reactivity and activation of frontoparietal and dorsal midline cortices in healthy participants (Etkin et al, 2015; Kanske et al, 2011). Moreover, performing a WM task during recall of distressing memories results in decreased vividness and emotionality of the memory (Engelhard et al, 2010, 2011). PTSD has been associated with impaired down-regulation of negative affect (Rabinak et al, 2014; Xiong et al, 2013), and with dorsolateral (dLPFC) and dorsomedial prefrontal cortex (dmPFC) hypoactivity during cognitive reappraisal (New et al, 2009; Rabinak et al, 2014). However, to our knowledge, neural correlates of distraction as emotion regulation strategy have never been investigated in PTSD.

Treatments of choice for PTSD include exposure therapy and trauma-focused cognitive behavioral therapy (CBT), both involving exposure to reminders of the traumatic event (Foa et al, 2009). Additionally, CBT consists of cognitive restructuring, aimed at altering negative and distorted (trauma-related) beliefs and cognitions (Foa et al, 2009). Although generally
effective, approximately one-third of PTSD patients does not fully recover upon receiving psychotherapy (Bradley et al., 2005), underlining the need to investigate adjuvant interventions to enhance psychotherapy response. Improving cognitive emotion regulation skills in PTSD prior to or during psychotherapy may result in enhanced treatment response, possibly via increased tolerance to exposure-related distress (Bryant et al., 2013; Cloitre et al., 2010; de Kleine et al., 2013). For example, a designated training aimed at improving cognitive emotion regulation skills (e.g., mindfulness and distraction) prior to treatment resulted in improved response to CBT in PTSD (Bryant et al., 2013).

In addition to psychological interventions, cognitive emotion regulation skills may also be improved with psychopharmacological agents, such as the neuropeptide oxytocin. Oxytocin has been suggested as promising pharmacological agent to enhance treatment response in PTSD, by dampening fear responsiveness and improving fear regulation abilities (Koch et al., 2014 (Chapter 2); Olff et al., 2010). Intranasal oxytocin administration in healthy males resulted in decreased subjective anxiety during public speaking stress (Heinrichs et al., 2003). Additionally, blunted amygdala responsiveness towards emotional stimuli (Domes et al., 2007; Kirsch et al., 2005) and increased functional connectivity between the amygdala and vmPFC (Sripada et al., 2013) was found after oxytocin in healthy males, and in psychiatric patients with high anxiety (Bertsch et al., 2013; Dodhia et al., 2014; Labuschagne et al., 2010). Moreover, we recently observed that oxytocin administration in PTSD patients resulted in decreased subjective anxiety and nervousness (Koch et al., 2016c (Chapter 4), dampened amygdala reactivity towards emotional faces (Koch et al., 2016a (Chapter 3), and normalized functional connectivity of amygdala subregions with the prefrontal cortex to levels similar to healthy controls (Koch et al., 2016c (Chapter 4). Taken together, these findings indicate that oxytocin may have therapeutic potential for PTSD, by targeting neural correlates of exaggerated fear responsiveness, but also of deficient emotion regulation abilities. However, oxytocin effects on cognitive emotion regulation strategies, such as distraction, have never been studied in healthy individuals or psychiatric patients.

We therefore investigated oxytocin administration effects on behavioral and neural responses to distraction as cognitive emotion regulation strategy in male and female police officers with and without PTSD, using a randomized placebo-controlled cross-over functional magnetic resonance imaging (fMRI) study. We hypothesized to observe impaired down-regulation of negative affect and amygdala reactivity during distraction in PTSD patients, which would be improved after oxytocin administration. We included both male and female participants to investigate possible sex differences in PTSD neurobiology, emotion regulation and oxytocin effects.
METHODS AND MATERIALS

Participants and procedure
We included 77 police officers: 37 PTSD patients (21 males) and 40 trauma-exposed controls (20 males). Participants were between 18 and 65 years of age and eligible for MRI. Participants did not use psychotropic medication, had no history of neurological disorders, and females were not pregnant or breastfeeding. Participants were recruited via a psychodiagnostic center for police personnel (Diemen, the Netherlands, patients only) and via advertisements in journals and on websites of the Dutch police. PTSD patients met current DSM-IV diagnostic criteria for PTSD, with a Clinician-Administered PTSD Scale (CAPS) (Blake et al., 1995) score of ≥ 45. Current comorbidity was assessed with the Mini International Neuropsychiatric Interview (MINI-plus) (Sheehan et al., 1998) or the Structured Clinical Interview for DSM-IV (SCID) (First et al., 2012). PTSD patients were excluded if they met DSM-IV criteria for current psychotic disorder, severe major depressive disorder (MDD) (i.e. involving suicidal ideation and/or psychotic symptoms), substance abuse, suicidal ideation, or reported personality disorder.

Trauma-exposed controls were matched to PTSD patients based on sex, age, years of service and educational level. They were exposed to at least one potentially traumatic event (DSM-IV A1 criterion), with a current CAPS score of ≤15. Exclusion criteria were lifetime history of PTSD or MDD and any current DSM-IV axis-I or reported personality disorder.

This randomized, placebo-controlled cross-over fMRI study consisted of two scanning sessions (see Supplementary information for scanning acquisition details), which were on average 11.51 (±9.89) days apart. Participants abstained from alcohol and drugs 24 hours before scanning and from rigorous exercise, beverages (except for water) and nicotine 2.5 hours before scanning. Before each scanning session, participants self-administered either placebo (0.9% saline) or intranasal oxytocin (Syntocinon, 40IU, 5 puffs per nostril) under experimenter supervision. Drug-order was randomized, double-blind and counterbalanced between sessions. The study was approved by the Institutional Review Board of the Academic Medical Center (AMC) in Amsterdam and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before participation.

Distraction task
The distraction task (McRae et al., 2010) consisted of three conditions: passive viewing of neutral or negative pictures (attend-neutral & attend-negative) and distraction of negative pictures (distract-negative) (Figure 5.1). During both passive viewing conditions, participants were instructed to passively attend to the picture. In the distraction condition, participants were first presented with a six letter string, which had to be remembered during subsequent
FIGURE 5.1 Distraction task

(A) Design of the distraction task. The distraction task consisted of three conditions: 1) passive viewing of neutral pictures, 2) passive viewing of negative pictures and 3) working memory task (distraction) during the presentation of negative pictures. In the working memory condition, participants had to remember a six letter string. (B) After each trial, valence ratings were assessed using self-assessment manikins (SAM) on a 9-point scale, ranging from 1 (negative affect) to 9 (positive affect).

presentation of the negative picture. When the negative picture disappeared, a letter was shown and participants had to indicate with a button press whether that letter was part of the presented string. During both passive viewing conditions, participants were asked to press either the left or right button, to keep motor responses constant across task conditions. After each picture, emotional state was evaluated with self-assessment manikins (SAM), on a 9-point scale ranging from 1 (negative valence) to 9 (positive valence).

Neutral and negative pictures were selected from the International Affective Picture System (IAPS), based on normative valence and arousal ratings (Table 5.1) (Lang et al, 2008). Two task versions, each consisting of 20 neutral and 40 negative pictures, were used. Pictures in both tasks were matched for normative valence and arousal ratings, complexity and luminescence (all p>.05). The order of task versions was randomized and counterbalanced between sessions. After each scanning session, participants rated a random subset of presented pictures (10 per task condition) on valence and arousal using 9-point SAMs (valence: negative valence (1) - positive valence (9); arousal: aroused (1) - calm (9)) (Table 5.1).
Oxytocin effects on distraction as emotion regulation strategy in PTSD

Statistical analysis

Behavioral analysis

Significant outliers (standardized value>3.29) were removed (n=3 for fMRI valence ratings) and non-normally distributed variables were log-transformed to obtain normal distributions. Differences between PTSD patients and the controls on demographics, trauma history and PTSD symptom severity were tested within males and females separately, using independent sample t-tests (normally distributed) or Mann Whitney U-tests (non-normally distributed, i.e. when transformation did not result in normal distribution) for continuous and Chi-square tests for categorical variables.

Repeated measures analyses of covariance (ANCOVA’s) were performed on valence ratings, with the within-subject factors task condition (attend-neutral, attend-negative, distract-negative) and drug (placebo – oxytocin), the between subject-factors group (PTSD – control) and sex (male – female) and the covariate drug-order. Group differences in error-rates and reaction times were tested in the distraction condition only, because of high performance

TABLE 5.1 Mean valence and arousal ratings of presented pictures

<table>
<thead>
<tr>
<th></th>
<th>Norm IAPS</th>
<th>Controls (n=39)</th>
<th>PTSD (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valence (SD)</td>
<td>Arousal (SD)</td>
<td>Valence (SD)</td>
</tr>
<tr>
<td>Neutral pictures</td>
<td>4.99 (0.20)</td>
<td>2.70 (0.42)</td>
<td>5.65 (0.83)</td>
</tr>
<tr>
<td>(n=40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative pictures</td>
<td>2.25 (0.43)</td>
<td>6.15 (0.56)</td>
<td>4.35 (1.53)</td>
</tr>
<tr>
<td>(n=60)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post-scan mean (± standard deviations) valence and arousal ratings of pictures in the distraction task (both task versions combined). Valence and arousal ratings were assessed on a 9-point scale, ranging from 1 (negative affect/calm) to 9 (positive affect/ aroused). As valence and arousal ratings of negative pictures were not significantly different for the passive viewing and distraction task conditions (p=.451 and p=.178 respectively), all negative pictures were combined. Valence and arousal ratings were not significantly different for male and female participants for any of the conditions (all p>.05). We therefore report average valence and arousal ratings for both sexes combined. Across task versions, normative ratings of negative pictures were significantly lower on valence (p<.001), and higher on arousal (p<.001), compared to the neutral pictures. Multiple comparisons correction was performed using FDR correction (αFDR=.046).

U=Mann-Whitney U; a) Log-transformed; * for non-normally distributed variables, a one-sample Wilcoxon signed rank test instead of independent sample t-test was used (median neutral valence IAPS norm = 4.98; median negative valence IAPS norm = 2.21)
accuracy in the passive viewing conditions. Post-hoc tests were corrected for multiple comparisons using the Benjamini-Hochberg false-discovery rate (FDR) correction (Benjamin and Hochberg, 1995). An adjusted p-value of <.05 was considered significant.

fMRI analysis
Functional images were analyzed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) (See supplementary information for details regarding preprocessing and first-level analyses). First-level contrast images for attend-negative > attend-neutral and attend-negative > distraction were used in separate second-level repeated measures ANCOVAs, with between-subjects factors group (PTSD – controls), sex (males – females), within-subjects factor drug (placebo – oxytocin) and covariate drug-order. To investigate baseline differences between PTSD patients and trauma-exposed controls and overall task effects, the abovementioned models were estimated for images acquired under placebo only, without the factor drug.

Regions of interests (ROIs) for the bilateral amygdala, insula, anterior cingulate cortex (ACC) and middle frontal gyrus were based on the neural circuitry of distraction and emotional processing (Kanske et al, 2011; Rive et al, 2015) and anatomically defined using the 50% Harvard-Oxford probability atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). For all main and interaction effects, family-wise error (FWE) correction for multiple comparisons was conducted, both within ROIs and at whole brain level ($P_{FWE}$<.05 was regarded significant). Four participants were excluded due to scanning artifacts (n=2) and movement (n=2), leaving 34 PTSD patients (20 males) and 37 trauma-exposed controls (19 males) for the final analyses.

Correlation analysis
Within PTSD patients, we investigated associations between PTSD symptoms and (oxytocin effects on) observed neural group differences between PTSD patients and controls. Partial correlations were conducted between extracted contrast estimates (from a 5mm sphere surrounding the peak voxel of interaction effects) and CAPS total and subscale scores, while correcting for drug-order. The DSM-IV avoidance cluster (CAPS-C subscale) was divided into a C1 (avoidance) and C2 cluster (negative affect), to accommodate the DSM5 diagnostic criteria (American Psychiatric Association, 2013).

RESULTS
Demographics & trauma history
PTSD patients and trauma-exposed controls did not differ on demographic characteristics (Table 5.2). Male PTSD patients experienced significantly more types of childhood traumatic events than male trauma-exposed controls ($p$=.037), whereas female trauma-exposed con-
trols experienced significantly more types of work-related traumatic events than female PTSD patients (p=.042). Severity of PTSD, anxiety and depression symptoms did not differ across scanning sessions (all p>.05) (Supplementary Table S5.1).

**Behavioral results**

**Task performance**

A significant main effect of task condition was found (F(1.2,80.68)=7.85, p=.004): neutral pictures were rated more positive than negative pictures (attend-negative vs attend-neutral: F(1,67)=9.38, p=.003; distract-negative vs attend-neutral: F(1,67)=7.07, p=.010) (αFDR=.050).

A nominally significant task condition by group interaction effect (F(1.2,80.68)=3.56, p=.055) was found: PTSD patients rated negative (attend-negative: p=.003; distract-negative: p=.018), but not neutral (p=.5) pictures as significantly more negative than trauma-exposed controls (αFDR=.033). For all participants, distraction did not result in less negative ratings of negative pictures compared to passive viewing (p>.5) (Figure 5.2A).

Additionally, PTSD patients tended to make more errors during distraction compared to trauma-exposed controls (main effect of group: F(1,69)=3.17, p=.062), independent of drug or sex (See Supplementary Table S5.2 and supplementary information for additional results on error rates). See Table 5.2 and supplementary information for results on post-scan ratings.

A significant drug by group by sex interaction was found (F(1,67)=8.25, p=.005): male PTSD patients rated all pictures more positive after oxytocin compared to placebo (p=.003). No significant oxytocin effects on affective ratings were found for trauma-exposed controls (males: p=.325; females: p=.638) or female PTSD patients (p=.089) (αFDR=.0125) (See Figure 5.2B).

**fMRI results**

**Attend-negative vs. attend-neutral**

**Placebo**

Across all participants, passive viewing of negative (attend-negative) compared to neutral (attend-neutral) pictures significantly activated emotional processing areas, such the amygdala, hippocampus and ventral ACC (all P FWE<.05, whole-brain corrected) (Figure 5.3 and Supplementary Table S5.3). No significant effects of group, sex or group by sex interactions were found, both at the whole-brain level and for each ROI (all P FWE<.05).

**Oxytocin effects**

No significant oxytocin effects were found at the whole-brain corrected level, nor for ROIs (all P FWE<.05).
<table>
<thead>
<tr>
<th></th>
<th>PTSD patients (n=37)</th>
<th>Healthy controls (n=40)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=21)</td>
<td>Females (n=16)</td>
<td>Males (n=20)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.29 (9.83)</td>
<td>37.56 (9.78)</td>
<td>41.35 (10.62)</td>
</tr>
<tr>
<td>Years of service</td>
<td>16.29 (10.82)</td>
<td>14.53 (10.74)</td>
<td>18.42 (10.05)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Middle</td>
<td>14 (67%)</td>
<td>15 (93%)</td>
<td>16 (85%)</td>
</tr>
<tr>
<td>High</td>
<td>7 (33%)</td>
<td>1 (7%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>CAPS total score</td>
<td>68.05 (15.62)</td>
<td>67.56 (11.83)</td>
<td>4.7 (4.79)</td>
</tr>
<tr>
<td>Current comorbidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDD</td>
<td>4 (19%)</td>
<td>4 (25%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>2 (9.5%)</td>
<td>1 (6.3%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Panic Disorder</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>n/a</td>
</tr>
<tr>
<td>Specific phobia</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>n/a</td>
</tr>
<tr>
<td>Work-related traumatic events (PLES)</td>
<td>22.50 (5.95)</td>
<td>13.50 (4.49)</td>
<td>20.45 (6.42)</td>
</tr>
<tr>
<td>Childhood traumatic events (ETI)</td>
<td>6.09 (4.55)</td>
<td>5.25 (5.18)</td>
<td>3.65 (2.35)</td>
</tr>
<tr>
<td>Hormonal contraceptive use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>n/a</td>
<td>7 (44%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Hormonal</td>
<td>8 (50%)</td>
<td>9 (45%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Menopause</td>
<td>1 (6%)</td>
<td>3 (15%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use (AUDIT total score)</td>
<td>3.52 (3.40)</td>
<td>4.06 (4.79)</td>
<td>3.40 (1.67)</td>
</tr>
</tbody>
</table>

Mean (± standard deviation) of demographics, trauma history and PTSD symptom severity for male and female PTSD patients and trauma-exposed controls. Number of different types of police-related traumatic events was measured with the Police life events scale (PLES) (total score) (Carlier and Gersons, 1992). The number of different types of childhood traumatic events was assessed with the early trauma Inventory - Short Form (ETI-SF) (Bremner et al, 2007). PTSD = post-traumatic stress disorder; MDD = major depressive disorder; CAPS = clinician administered PTSD scale (Blake et al, 1995); AUDIT = alcohol use disorder identification test (Bush, 1998).
Oxytocin effects on distraction as emotion regulation strategy in PTSD

Distraction vs. attend-negative

Placebo

Across all participants, distraction (compared to attend-negative) activated emotional control areas, including the supplementary motor area, extending into the dorsal anterior cingulate cortex, anterior insula and the middle frontal gyrus (all $P_{	ext{FWE}}>.05$, whole-brain corrected, Figure 5.3 and Supplementary Table S5.3). The attend-negative condition was associated with significantly increased amygdala and hippocampus activity, compared to the distraction
Condition (all P FWE>.05, whole-brain corrected), indicating amygdala down-regulation during distraction (Figure 5.3 and Supplementary Table S5.3).

Females showed lower right anterior insula reactivity during distraction compared to males, irrespective of PTSD status (main effect sex: peak voxel xyz=34 10 -16, Z=3.41, P FWE=.047). Additionally, a group by sex interaction was found in the right amygdala (peak voxel xyz=26 0 -26, Z=3.14, P FWE=.043, Figure 5.4): distraction significantly dampened right amygdala reactivity in male (P FWE=.006) and female (P FWE=.003) trauma-exposed controls and female PTSD patients (P FWE<.001), but not in male PTSD patients (P FWE>.05). Right amygdala reactiv-
Oxytocin effects on distraction as emotion regulation strategy in PTSD

Oxytocin effects
We observed a significant group by sex by drug interaction in the right amygdala (peak voxel xyz=26 0 -28, Z=3.19, P_FWE=.034, Figure 5.4). The finding of greater amygdala down-regulation during distraction in female PTSD patients compared to female trauma-exposed controls under placebo was abolished after oxytocin (P_FWE>.05). After oxytocin, distraction still resulted in decreased amygdala reactivity compared to passive viewing of negative pictures in male (P_FWE<.001) and female (P_FWE<.001) controls and female PTSD patients (P_FWE=.030), whereas this effect was still absent in male PTSD patients (P_FWE=.136).

We found a significant sex by drug interaction in the left amygdala (peak voxel xyz=−26 0 -24, Z=3.16, P_FWE=.035, Figure 5.5). Distraction (compared to attend-negative) still resulted in dampened left amygdala reactivity after oxytocin administration, both in males (P_FWE=.004)
FIGURE 5.5 Sex by drug interaction in left amygdala

(A) Statistical map of the sex by drug interaction effect regarding left amygdala activity (attend-negative > distract). Results are overlaid on a single-subject template and statistically thresholded at $p<.05$ (uncorrected) for display purposes. (B) Contrast estimates in arbitrary units (a.u.) of left amygdala activity during attend-negative compared to distraction (5mm sphere, peak voxel $xyz = -26, 0, -24$) in male and female participants. Error-bars represent standard errors of the mean (SEM). $L =$ left; ** $P_{FWE}<.01$

FIGURE 5.6 Group by sex by drug interaction in right posterior insula

(A) Statistical map of the sex by drug interaction effect regarding right posterior insula activity (attend-negative > distract). Results are overlaid on a single-subjects template and statistically thresholded at $p<.05$ (uncorrected) for display purposes. (B) Contrast estimates in arbitrary units (a.u.) of right posterior insula activity during attend-negative compared to distraction (5mm sphere, peak voxel $xyz = 40, -14, 8$) in male and female trauma-exposed controls and PTSD patients. Error-bars represent standard errors of the mean (SEM). $L =$ left; * $P_{FWE}<.1$; * $P_{FWE}<.05$; ** $P_{FWE}<.01$
and females ($P_{FWE}=.009$). In females, however, left-sided amygdala reactivity during distraction was increased after oxytocin compared to placebo ($P_{FWE}=.005$).

We observed a significant group by sex by drug interaction in the right posterior insula (peak voxel $xyz=40 -14, 8$, $Z=3.18$, $P_{FWE}=.042$, Figure 5.6). Under placebo, right posterior insula reactivity was decreased during distraction (compared to attend-negative) in male trauma-exposed controls ($P_{FWE}=.015$), but not in other participants (all $P_{FWE}>.05$). Right posterior insula activity during distraction was nominally lower in male trauma-exposed controls compared to male PTSD patients ($P_{FWE}=.074$). After oxytocin, this group difference was absent: distraction (compared to attend-negative) now resulted in dampened right posterior insula reactivity in male PTSD patients ($P_{FWE}=.001$), but not in male trauma-exposed controls ($P_{FWE}=.499$).

**FIGURE 5.7 Associations between neural reactivity and symptom severity**

Correlations between right amygdala activity (attend-negative > distract) and avoidance symptoms in (A) male PTSD patients and (B) female PTSD patients under placebo and oxytocin. Correlations between right posterior insula activity (attend-negative > distract) and overall PTSD symptom severity in male PTSD patients (C) and female PTSD patients (D) under placebo and oxytocin. Contrast estimates in arbitrary units (a.u.) were extracted from a 5mm sphere surrounding the interaction effect in the right amygdala ($xyz=26 0 -28$) and right posterior insula ($xyz=40 -14, 8$). * $p<.05$; ** $p<.01$
pared to placebo, oxytocin resulted in nominally decreased right posterior insula reactivity during distraction in male PTSD patients ($P_{FWE}=.073$). No oxytocin effects on right posterior insula reactivity were found for female participants. For all analyses, comparable results were obtained after adjusting for baseline differences in childhood and work-related traumatic events.

**Associations with PTSD symptom severity**

Avoidance symptoms in female PTSD patients were positively associated with estimates for the attend-negative > distract-negative contrast in the right amygdala under placebo ($r=.580$, $p=.038$), but not under oxytocin ($r=-.141$, $p=.646$). The correlation coefficient magnitudes under placebo and oxytocin were significantly different (Steiger's Z=2.227, $p=.013$).

Total CAPS score, re-experiencing and hyperarousal symptoms in male PTSD patients were significantly correlated with estimates for the attend-negative > distract-negative contrast in the right posterior insula under placebo (total CAPS score: $r=-.671$, $p=.002$; re-experiencing: $r=-.580$, $p=.009$; hyperarousal: $r=-.713$, $p=.001$), but not under oxytocin (total CAPS score: $r=.109$, $p=.658$; re-experiencing: $r=.207$, $p=.395$; hyperarousal: $r=.156$, $p=.524$). The correlation coefficient magnitudes under oxytocin were all significantly different from the respective magnitudes under placebo (all $p<.05$) (Figure 5.7). No other significant correlations were found between bilateral amygdala and posterior insula activity and PTSD symptoms (all $p>.05$) (Supplementary Table S5.4).

**DISCUSSION**

We investigated neural and behavioral effects of oxytocin administration on distraction as emotion regulation strategy in male and female PTSD patients and trauma-exposed controls. The observed oxytocin effects were dependent on neural emotion regulation abilities under placebo, which were related to sex and (severity of) PTSD status.

Under placebo, male and female PTSD patients rated all negative pictures as more negative and arousing compared to trauma-exposed controls. Additionally, male and female PTSD patients made more errors during working memory (WM) performance in the distraction condition. These findings are in line with recent meta-analyses showing impaired neurocognitive functioning in PTSD, including decreased WM performance (Polak et al, 2012; Scott et al, 2015). Hyperarousal and bias towards threat in PTSD may switch prefrontal attentional resources towards external negative stimuli, at the cost of prefrontally-mediated neurocognitive functioning (Etkin et al, 2013; Scott et al, 2015). Therefore, WM impairments in PTSD may be especially apparent when confronted with threat- and trauma-related stimuli: in a
previous study, impaired WM performance was found in PTSD patients during the presentation of negative (but not neutral) emotional distracters (Zhang et al., 2013). Taken together, difficulty down-regulating emotional responses towards negative pictures may have resulted in decreased WM performance in PTSD patients during distraction. Crucially, lower (verbal) memory performance pre-treatment predicted worse response to psychotherapy for PTSD (Nijdam et al., 2015), indicating that treatment efficacy may depend on intact neurocognitive functioning.

Across all participants, distraction resulted in robust down-regulation of neural activity in emotion processing areas, including the amygdala and dACC. Contrary to our expectation, however, distraction did not result in more positive affective ratings. One possible explanation is that the negative pictures were likely not as emotionally evocative in our sample of police officers as in the general population: compared to the normative IAPS ratings, our participants rated the negative pictures as less negative and less arousing. This may have resulted in a ceiling effect regarding valence ratings during distraction: the valence ratings during passive viewing were not very negative, limiting room for improved affective ratings during distraction.

Under placebo, male PTSD patients showed no dampening of right-sided amygdala and posterior insula reactivity during distraction, whereas dampening was observed in healthy males. PTSD is associated with impaired inhibition of emotional, physiological and neural responsiveness towards negative stimuli (Ehlers et al., 2010; Hayes et al., 2012; Orsillo et al., 2004), possibly resulting from ineffective communication between the vmPFC and amygdala (Sripada et al., 2012a). That is, decreased prefrontal inhibitory control over the (amygdala-mediated) fear response may represent difficulties down-regulating emotional reactivity, presumably resulting in hypervigilance symptoms, and emotional and physiological distress towards trauma reminders (American Psychiatric Association, 2013). Furthermore, increased (neural) processing of negative stimuli may interfere with allocation of attentional resources to the cognitive WM task (Etkin et al., 2013), thereby diminishing the effectiveness of distraction to down-regulate emotion and impairing WM performance. Additionally, impaired down-regulation of posterior insula activity during distraction in male PTSD patients under placebo was related to greater re-experiencing and hyperarousal symptom severity. Whereas the anterior insula has been implicated in salience processing (Seeley et al., 2007) and distraction (Kanske et al., 2011), the posterior insula is involved in interoceptive awareness and empathic processing (Menon and Uddin, 2010). Compared to trauma-exposed controls, bilateral posterior insula hyperactivity towards trauma-related stimuli was previously observed in male veterans with PTSD (Shin et al., 2001). Interestingly, a recent meta-analysis on functional neuroimaging studies showed consistent right posterior insula reactivity towards emotional stimuli in healthy males, but not in females (Duerden et al., 2013). Our finding of greater
posterior insula activity towards negative pictures in PTSD males may therefore indicate (sex-specific) enhanced neural processing of emotionally evocative stimuli. Taken together, male PTSD patients under placebo presumably showed deficient down-regulation of neural responses towards negative stimuli.

Oxytocin administration in male PTSD patients resulted in more positive subjective ratings of all pictures, suggesting generally improved affect. Furthermore, in these patients, oxytocin resulted in decreased posterior insula reactivity upon distraction, suggesting that male PTSD patients were now better able to use distraction as emotion regulation strategy. In line with this finding, oxytocin administration in healthy males previously resulted in decreased posterior insula reactivity towards negative pictures inducing empathic pain (Bos et al., 2015).

Whereas in male PTSD patients we found impaired right-sided posterior insula and amygdala down-regulation during distraction, female PTSD patients showed robust down-regulation of bilateral amygdala activity during distraction under placebo. Right-sided amygdala down-regulation in female PTSD patients during distraction was even greater than in female trauma-exposed controls, and was positively related to avoidance symptom severity. Our findings suggest that distraction may be efficiently used in our sample of female PTSD patients to down-regulate amygdala reactivity towards negative stimuli, which was related to everyday use of avoidance strategies. After oxytocin administration, the group difference between female PTSD patients and trauma-exposed controls in right-amygdala down-regulation during distraction was abolished. Moreover, left-sided amygdala reactivity during distraction was increased after oxytocin in all female participants, irrespective of PTSD status.

Our oxytocin effects in male PTSD patients may hold promise for improving treatment efficacy. Excessive neural fear processing during psychotherapy may result in insufficient treatment outcome in PTSD (Bryant et al., 2008a): greater amygdala activity towards emotional stimuli before treatment predicted worse treatment outcome (Bryant et al., 2008a), and symptom persistence after treatment completion (van Rooij et al., 2015). Distraction may be used as emotion regulation strategy during psychotherapy, during which down-regulation of exposure-related distress could facilitate effective exposure (Bryant et al., 2013): greater reduction in PTSD symptom severity after CBT was found in PTSD patients who received emotion regulation training (e.g. improving emotion tolerance with distraction) pre-treatment compared to those who did receive supportive counseling (Bryant et al., 2013). Furthermore, during eye movement desensitization and reprocessing (EMDR) therapy for PTSD, traumatic memory retrieval is interrupted by a secondary task (e.g. eye movements) taxing WM (Andrade et al., 1997; Gunter and Bodner, 2008). By taxing WM capacity, retrieval of the traumatic memory is impaired, resulting in decreased vividness and emotionality of the traumatic memory (Andrade et al., 1997; Gunter and Bodner, 2008), which is crucial for reduction of
Oxytocin effects on distraction as emotion regulation strategy in PTSD symptoms (Brett and Ostroff, 1985). However, oxytocin did not normalize all neural deficits observed under placebo in male PTSD patients: amygdala down-regulation during distraction in male PTSD patients was still absent after oxytocin. Additionally, it remains to be investigated whether the oxytocin effects in female PTSD patients are beneficial to enhance psychotherapy efficacy. Therefore, our findings warrant further research into inter-individual differences in oxytocin effects on various emotion regulation strategies in PTSD, and its effects on treatment outcome.

Our findings in male PTSD patients contradict previous meta-analytic findings of increased insula activity after oxytocin in healthy individuals (Wigton et al, 2015). Additionally, our observations of enhanced amygdala reactivity after oxytocin are in line with previous findings in healthy females (Domes et al, 2010; Lischke et al, 2012), but contradict our previous findings of dampened amygdala activity after oxytocin in the current sample of male and female PTSD patients during an emotional face-matching task (Koch et al, 2016a) (Chapter 3) and in female patients with borderline personality disorder (BPD) (Bertsch et al, 2013). Additionally, it has been suggested that the anxiolytic oxytocin effects may be greater in individuals with suboptimal baseline emotion regulation (Labuschagne et al, 2010), which may depend on individual differences in sex and (severity of) psychopathology (Bartz et al, 2011b). For example, oxytocin resulted in decreased cortisol response towards public speaking stress in individuals with low emotion regulation abilities only (Quirin et al, 2011). Our findings under placebo suggested sex-differential effects on PTSD neurobiology: whereas male PTSD patients showed difficulties down-regulating neural processing during distraction, we observed robust bilateral amygdala down-regulation during distraction in female PTSD patients compared to controls. Amygdala down-regulation during reappraisal may be especially apparent in females: females (Rabinak et al, 2014), but not males (New et al, 2009) showed amygdala down-regulation during reappraisal as cognitive emotion regulation strategy, independent of PTSD status. In line with recent findings (Bertsch et al, 2013; Labuschagne et al, 2010; Quirin et al, 2011), our results suggest that both distraction and oxytocin effects are dependent on the abovementioned baseline (sex-and PTSD-related) differences in neural correlates of emotion regulation.

We are the first to investigate behavioral and neural oxytocin effects on distraction as emotion regulation strategy in healthy participants and psychiatric patients. We included a homogeneous sample of trauma-exposed police officers to control for potential confounding effects of trauma-exposure on neurobiology. Additionally, we investigated potential sex-differential effects on PTSD neurobiology, emotion regulation abilities and oxytocin administration effects. However, some limitations need to be mentioned. The inclusion of a homogeneous, but selective sample of highly traumatized police officers limits the generalizability of our findings. Additionally, we were unable to test our female participants during the same phase of
menstrual cycle since we aimed to minimize fluctuations in symptom severity and hence time between sessions. However, this could have confounded our findings in female participants (Caldwell et al, 1994). Additionally, we did not explicitly present distraction as cognitive emotion regulation strategy to our participants, to not influence their (behavioral) responding. However, this would presumably be different in a clinical setting, where distraction would be explained as emotion regulation strategy to consciously down-regulate exposure-related distress when needed.

To conclude, oxytocin resulted in down-regulation of neural responsiveness towards emotional stimuli during distraction in male PTSD patients, whereas it attenuated left amygdala down-regulation upon distraction in female participants. Although oxytocin may hold therapeutic promise in PTSD as potential enhancer of cognitive emotion regulation during psychotherapy, these findings emphasize the need for more research into oxytocin effects on various cognitive emotion regulation strategies in male and female PTSD patients, and its actual clinical effects on psychotherapy outcome.
SUPPLEMENTARY MATERIAL – CHAPTER 5

Supplementary methods

MRI acquisition
We acquired structural and functional MRI images with a 3T Philips Achieva MR system, using a 32-channel head coil. We used a FAST MPRage sequence to obtain a high-resolution T1-weighted structural image (220 slices; voxel size=1mm³; repetition time=8.2s; echo time=3.8s; flip angle=8°) and an echo planar sequence sensitive to the BOLD contrast for functional images (540 volumes; voxel size=3 mm³; T =2s, echo time=28ms; flip angle=76°).

fMRI analysis
Preprocessing involved realignment, slice-time-correction, co-registration, segmentation, normalization to the Montreal Neurological Institute (MNI) template, resampling to 2mm³ voxels and smoothing with a 6mm full-width half maximum Gaussian kernel. At first level, we included six realignment parameters and a high-pass filter (cut-off 1/128 Hz) and removed temporal autocorrelation using the AR(1) process.

Supplementary results

Online error rates and reaction time during distraction
A main effect of drug was found (F(1,69)=6.56, p=.013): all participants made more errors during distraction after oxytocin compared to placebo. These effects were driven by misses (i.e. no answer given within the allotted time: group main effect p=.012; drug main effect p=.001), and not by incorrect answers (all p>.05). No significant effects were found regarding reaction times in the distraction condition.

Post-scan ratings
Compared to the normative IAPS ratings, neutral and negative pictures were rated as significantly more positive and less arousing by all participants (all p<.05). Direct comparison between trauma-exposed controls and PTSD patients revealed higher arousal ratings of neutral and negative pictures and more negative valence ratings of negative pictures in PTSD (all p<.05; αFDR=.046).
### SUPPLEMENTARY TABLE S5.1 PTSD, anxiety and depression symptom severity in both drug conditions

#### Males

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=20)</th>
<th>PTSD patients (n=21)</th>
<th>Test statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PL OT</td>
<td>PL OT</td>
<td>Group Session</td>
</tr>
<tr>
<td>PTSD symptom severity (IES-R)</td>
<td>1.30 (2.1) 0.85 (1.3) &lt;br&gt; 47.6 (14.6) 47.3 (16.6)</td>
<td>F(1,39)=201.85 p&lt;.001 &lt;br&gt; F(1,39)=.068 p=.795</td>
<td>F(1,39)=.006 p=.936</td>
</tr>
<tr>
<td>HADS Anxiety</td>
<td>2.30 (2.1) 2.20 (2.1) &lt;br&gt; 11.3 (3.8) 11.2 (3.5)</td>
<td>F(1,39)=127.06 p&lt;.001</td>
<td>F(1,39)=.038 p=.846</td>
</tr>
<tr>
<td>HADS Depression</td>
<td>1.30 (1.9) 1.15 (1.5) &lt;br&gt; 11.1 (4.1) 10.9 (4.0)</td>
<td>F(1,39)=126.14 p&lt;.001</td>
<td>F(1,39)=.030 p=.864</td>
</tr>
</tbody>
</table>

#### Females

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=20)</th>
<th>PTSD patients (n=16)</th>
<th>Test statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PL OT</td>
<td>PL OT</td>
<td>Group Session</td>
</tr>
<tr>
<td>PTSD symptom severity (IES-R)</td>
<td>1.7 (3.3) 1.2 (1.9) &lt;br&gt; 41.0 (20.7) 42.8 (21.0)</td>
<td>F(1,33)=84.86 p&lt;.001</td>
<td>F(1,33)=.239 p=.628</td>
</tr>
<tr>
<td>HADS Anxiety</td>
<td>2.4 (2.5) 2.5 (1.9) &lt;br&gt; 11.1 (4.2) 11.0 (2.9)</td>
<td>F(1,33)=145.18 p&lt;.001</td>
<td>F(1,33)=.001 p=.976</td>
</tr>
<tr>
<td>HADS Depression</td>
<td>1.9 (2.2) 1.4 (1.5) &lt;br&gt; 10.9 (5.0) 10.9 (5.3)</td>
<td>F(1,34)=88.43 p&lt;.001</td>
<td>F(1,34)=.106 p=.747</td>
</tr>
</tbody>
</table>

Mean (± standard deviation) symptom severity scores and time between intranasal spray administration and task performance for the placebo and oxytocin sessions for trauma-exposed controls and PTSD patients. Repeated measures ANOVA’s were conducted within males and females separately to test for differences over sessions and between groups.

IES-R= Impact of Events Scales – Revised (total score); HADS = Hospital Anxiety and Depression Scale; PL= placebo; OT = oxytocin session
### SUPPLEMENTARY TABLE S5.2 Task performance of PTSD patients and trauma-exposed controls

<table>
<thead>
<tr>
<th></th>
<th>PTSD patients</th>
<th></th>
<th>Trauma-exposed controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=21)</td>
<td>Females (n=14)</td>
<td>Males (n=20)</td>
<td>Females (n=20)</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>OT</td>
<td>PL</td>
<td>OT</td>
</tr>
<tr>
<td>Neutral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valence (SAM)</td>
<td>5.86 (0.81)</td>
<td>6.19 (1.10)</td>
<td>5.40 (1.01)</td>
<td>5.41 (0.99)</td>
</tr>
<tr>
<td></td>
<td>5.94 (0.97)</td>
<td>5.79 (0.92)</td>
<td>5.51 (0.51)</td>
<td>5.51 (0.56)</td>
</tr>
<tr>
<td>Attend negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valence (SAM)</td>
<td>3.62 (0.84)</td>
<td>3.72 (0.77)</td>
<td>3.64 (0.78)</td>
<td>3.41 (1.06)</td>
</tr>
<tr>
<td></td>
<td>4.35 (0.80)</td>
<td>4.24 (0.86)</td>
<td>4.09 (0.72)</td>
<td>4.08 (0.54)</td>
</tr>
<tr>
<td>Distract negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valence (SAM)</td>
<td>3.76 (0.87)</td>
<td>3.92 (0.73)</td>
<td>3.81 (0.84)</td>
<td>3.65 (0.93)</td>
</tr>
<tr>
<td></td>
<td>4.43 (0.75)</td>
<td>4.39 (0.82)</td>
<td>4.15 (0.56)</td>
<td>4.17 (0.46)</td>
</tr>
<tr>
<td>Error rate (%)</td>
<td>13.15 (9.63)</td>
<td>11.90 (10.30)</td>
<td>19.64 (8.20)</td>
<td>20.36 (17.37)</td>
</tr>
<tr>
<td></td>
<td>14.50 (23.89)</td>
<td>11.50 (11.82)</td>
<td>12.50 (10.58)</td>
<td>12.25 (10.57)</td>
</tr>
<tr>
<td>Reaction time (ms)</td>
<td>1621.60 (230.42)</td>
<td>1655.45 (262.55)</td>
<td>1574.05 (130.89)</td>
<td>1625.56 (254.33)</td>
</tr>
<tr>
<td></td>
<td>1631.52 (236.75)</td>
<td>1645.52 (228.69)</td>
<td>1591.39 (178.22)</td>
<td>1603.9 (2314.00)</td>
</tr>
</tbody>
</table>

Mean (± standard deviations) valence rating for the three task conditions (i.e. attend neutral, attend negative and distract negative), and error rate and reaction times for the distraction condition for male and female PTSD patients and trauma-exposed controls. Reaction times were calculated for correct answers only.

SAM = self-assessment manikin. Valence rating on a 9-point scale, ranging from 1 (negative affect) to 9 (positive affect).
### SUPPLEMENTARY TABLE S5.3 Main task effects across groups

<table>
<thead>
<tr>
<th>Area</th>
<th>Side</th>
<th>Peak voxel (MNI)</th>
<th>T-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td><strong>Attend &gt; Neutral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior lateral occipital gyrus, extending to lateral occipital</td>
<td>Left</td>
<td>-48</td>
<td>-76</td>
</tr>
<tr>
<td>cortex and temporal occipital fusiform gyrus</td>
<td>Right</td>
<td>50</td>
<td>-66</td>
</tr>
<tr>
<td>Brainstem</td>
<td>Left</td>
<td>-6</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>6</td>
<td>-30</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>Right</td>
<td>46</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>-48</td>
<td>18</td>
</tr>
<tr>
<td>Brainstem</td>
<td>Left</td>
<td>-6</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>6</td>
<td>-30</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>Right</td>
<td>46</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>-48</td>
<td>18</td>
</tr>
<tr>
<td><strong>Distract &gt; Attend – negative pictures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplementary motor area, extending into dorsal anterior</td>
<td>Left/Right</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>cingulate cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td>Left</td>
<td>-32</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>34</td>
<td>20</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Left</td>
<td>-26</td>
<td>-62</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>34</td>
<td>-66</td>
</tr>
<tr>
<td>Superior lateral occipital cortex, extending into supramarginal</td>
<td>Left</td>
<td>-32</td>
<td>-62</td>
</tr>
<tr>
<td>gyrus</td>
<td>Right</td>
<td>34</td>
<td>-60</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>Left</td>
<td>-40</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td>Occipital pole</td>
<td>Left</td>
<td>-8</td>
<td>-100</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>28</td>
<td>-90</td>
</tr>
<tr>
<td>Inferior temporal gyrus</td>
<td>Left</td>
<td>-50</td>
<td>-56</td>
</tr>
<tr>
<td>Putamen</td>
<td>Left</td>
<td>-18</td>
<td>6</td>
</tr>
<tr>
<td>Precuneus cortex</td>
<td>Right</td>
<td>12</td>
<td>-68</td>
</tr>
<tr>
<td>Brainstem</td>
<td>Left/Right</td>
<td>-4</td>
<td>-34</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>Left</td>
<td>-62</td>
<td>-36</td>
</tr>
<tr>
<td>Precentral gyrus, extending into middle frontal gyrus</td>
<td>Left</td>
<td>-42</td>
<td>4</td>
</tr>
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</table>
### SUPPLEMENTARY TABLE S5.3 Main task effects across groups (continued)

<table>
<thead>
<tr>
<th>Area</th>
<th>Side</th>
<th>Peak voxel (MNI)</th>
<th>T-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td>Attend &gt; Distract – negative pictures</td>
<td></td>
<td>Left</td>
<td></td>
</tr>
<tr>
<td>Precuneus, extending into posterior cingulate gyrus</td>
<td>Left</td>
<td>-8</td>
<td>-52</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>8</td>
<td>52</td>
</tr>
<tr>
<td>Inferior lateral occipital cortex, extending into angular gyrus</td>
<td>Left</td>
<td>-48</td>
<td>-60</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>46</td>
<td>-64</td>
</tr>
<tr>
<td>Dorsal anterior cingulate cortex, extending into middle frontal gyrus</td>
<td>Left/Right</td>
<td>-6</td>
<td>52</td>
</tr>
<tr>
<td>Lingual gyrus/ temporal fusiform gyrus, extending into parahippocampal gyrus</td>
<td>Left</td>
<td>-30</td>
<td>-40</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>30</td>
<td>-38</td>
</tr>
<tr>
<td>Middle temporal gyrus, extending into temporal pole</td>
<td>Left</td>
<td>-58</td>
<td>-6</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>54</td>
<td>-10</td>
</tr>
<tr>
<td>Orbitofrontal cortex</td>
<td>Left</td>
<td>-42</td>
<td>30</td>
</tr>
<tr>
<td>Hippocampus, extending into amygdala</td>
<td>Left</td>
<td>-26</td>
<td>-14</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>28</td>
<td>-12</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>Right</td>
<td>54</td>
<td>30</td>
</tr>
</tbody>
</table>

Brain activity associated with 1) passive viewing of negative pictures compared to neutral pictures (attend > neutral); 2) distraction of negative pictures compared to passive viewing of negative pictures (distract > attend); 3) passive viewing of negative pictures compared to distraction of negative pictures (attend > distract). Main effects of task were assessed across all participants for the placebo session. 

P_{FWE} < .05 whole-brain corrected, minimal cluster-size = 20; MNI = Montreal Neurological Institute
### Chapter 5

**SUPPLEMENTARY TABLE S5.4 Correlations between amygdala and insula activity and symptom severity in PTSD patients**

<table>
<thead>
<tr>
<th>Contrasts estimates (distraction &gt; view negative)</th>
<th>Drug</th>
<th>CAPS total</th>
<th>CAPS - B</th>
<th>CAPS - C1</th>
<th>CAPS - C2</th>
<th>CAPS - D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male PTSD patients (n=20)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Amygdala</td>
<td>PL</td>
<td>r = -.41</td>
<td>r = -.287</td>
<td>r = .022</td>
<td>r = .149</td>
<td>r = .021</td>
</tr>
<tr>
<td></td>
<td>OT</td>
<td>r = .439#</td>
<td>r = .304</td>
<td>r = .290</td>
<td>r = .231</td>
<td>r = .415#</td>
</tr>
<tr>
<td>Left Amygdala</td>
<td>PL</td>
<td>r = .280</td>
<td>r = .348</td>
<td>r = .042</td>
<td>r = .049</td>
<td>r = .437#</td>
</tr>
<tr>
<td></td>
<td>OT</td>
<td>r = .102</td>
<td>r = .155</td>
<td>r = .144</td>
<td>r = .038</td>
<td>r = .231</td>
</tr>
<tr>
<td>Right posterior insula</td>
<td>PL</td>
<td>r = -.617**</td>
<td>r = -.580**</td>
<td>r = -.385</td>
<td>r = -.217</td>
<td>r = -.713**</td>
</tr>
<tr>
<td></td>
<td>OT</td>
<td>r = .109</td>
<td>r = .207</td>
<td>r = .136</td>
<td>r = -.145</td>
<td>r = .156</td>
</tr>
<tr>
<td><strong>Female PTSD patients (n=14)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Right Amygdala</td>
<td>PL</td>
<td>r = -.048</td>
<td>r = .016</td>
<td>r = .580*</td>
<td>r = -.298</td>
<td>r = .084</td>
</tr>
<tr>
<td></td>
<td>OT</td>
<td>r = -.118</td>
<td>r = -.020</td>
<td>r = -.141</td>
<td>r = -.012</td>
<td>r = -.017</td>
</tr>
<tr>
<td>Left Amygdala</td>
<td>PL</td>
<td>r = -.228</td>
<td>r = -.160</td>
<td>r = .249</td>
<td>r = -.331</td>
<td>r = .028</td>
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<tr>
<td></td>
<td>OT</td>
<td>r = -.163</td>
<td>r = -.014</td>
<td>r = -.281</td>
<td>r = -.033</td>
<td>r = .025</td>
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<tr>
<td>Right posterior insula</td>
<td>PL</td>
<td>r = .128</td>
<td>r = .389</td>
<td>r = .206</td>
<td>r = -.312</td>
<td>r = .028</td>
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<tr>
<td></td>
<td>OT</td>
<td>r = .230</td>
<td>r = .112</td>
<td>r = -.245</td>
<td>r = .367</td>
<td>r = .207</td>
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</tbody>
</table>

Partial correlations between extracted contrast estimates (for the distraction > view negative contrast) from a 5mm sphere surrounding the peak voxel of the interaction effects (right amygdala peak voxel xyz=26 0 -28; left amygdala peak voxel xyz=-26 0 -24; right posterior insula peak voxel xyz=40 -14 8) and clinician-rated PTSD symptom severity (CAPS), corrected for drug-order.

CAPS = clinician-administered PTSD scale. CAPS-B = re-experiencing cluster, CAPS-C1 = avoidance cluster, CAPS-C2 = negative affect cluster, CAPS-D = hyperarousal cluster.

# trend significant at p<.10 level, * significant at p<.05 level, ** significant at p<.01 level, r=pearson correlation coefficient
Aberrant resting-state brain activity in post-traumatic stress disorder (PTSD): a meta-analysis and systematic review

Saskia B.J. Koch, Mirjam van Zuiden, Laura Nawijn, Jessie L. Frijling, Dick J. Veltman & Miranda Olff

Depression and Anxiety (2016): 1-14
ABSTRACT

About ten percent of trauma-exposed individuals develop post-traumatic stress disorder (PTSD). Although a growing number of studies have investigated resting-state abnormalities in PTSD, inconsistent results suggest a need for a meta-analysis and a systematic review. Therefore, we conducted a systematic literature search in four online databases using keywords for PTSD, functional neuroimaging, and resting-state. In total, 23 studies matched our eligibility criteria. For the meta-analysis, we included 14 whole-brain resting-state studies, reporting data on 663 participants (298 PTSD patients and 365 controls). We used the activation likelihood estimation (ALE) approach to identify concurrence of whole-brain hypo- and hyperactivations in PTSD patients during rest. Seed-based studies could not be included in the quantitative meta-analysis. Therefore, a separate qualitative systematic review was conducted on nine seed-based functional connectivity studies. The meta-analysis showed consistent hyperactivity in the ventral anterior cingulate cortex (vACC) and the parahippocampus/amygdala, but hypoactivity in the (posterior) insula, cerebellar pyramis and middle frontal gyrus in PTSD patients, compared to healthy controls. Partly concordant with these findings, the systematic review on seed-based functional connectivity studies showed enhanced salience network (SN) connectivity, but decreased default mode network (DMN) connectivity in PTSD. Combined, these altered resting-state connectivity and activity patterns could represent neurobiological correlates of increased salience processing and hypervigilance (SN), at the cost of awareness of internal thoughts and autobiographical memory (DMN) in PTSD. However, several discrepancies between findings of the meta-analysis and systematic review were observed, stressing the need for future studies on resting-state abnormalities in PTSD patients.
INTRODUCTION

Approximately ten percent of trauma-exposed individuals develop post-traumatic stress disorder (PTSD), resulting in a lifetime prevalence of about eight percent (American Psychiatric Association, 2013; de Vries and Olff, 2009). PTSD is characterized by intrusions, including nightmares, flashbacks, and intrusive thoughts about the traumatic event, as well as avoidance of these thoughts, situations, and reminders of the traumatic event (American Psychiatric Association, 2013). In addition, cognitive impairments and lowered mood, and alterations in arousal and behaviour are core symptom clusters of PTSD (American Psychiatric Association, 2013). Investigating and identifying the neurobiological correlates of these PTSD symptom clusters may help establish novel therapeutic targets to improve therapy for PTSD. Recently, two meta-analyses on (predominantly) task-based functional brain imaging studies in PTSD patients (Hayes et al, 2012; Patel et al, 2012) have confirmed and extended the prevailing neurocircuitry model of PTSD (Rauch et al, 2006). This model involves enhanced salience processing, reflected by amygdala, anterior insula and dorsal anterior cingulate (dACC) hyperactivity, as well as ventromedial prefrontal (vmPFC) hypoactivity (Hayes et al, 2012; Patel et al, 2012). In addition, the model postulates that PTSD is associated with reduced functional connectivity between the vmPFC, hippocampus and amygdala, which may represent reduced top-down control over the fear response. Finally, hippocampal hyperactivity has been found, possibly underlying impaired extinction learning in PTSD patients (Rauch et al, 2006).

The abovementioned meta-analyses, however, predominantly included task-based studies, which mostly targeted specific brain areas (such as the amygdala) using symptom provocation designs or cognitive-emotional tasks. No functional magnetic resonance imaging (fMRI) resting-state studies were included in these meta-analyses, although the meta-analysis of Patel and colleagues (Patel et al, 2012) included several resting-state positron emission tomography (PET) and single-photon emission computed tomography (SPECT) studies. In resting-state studies, subjects are instructed to rest and let their mind wander while brain activity is measured using PET, SPECT, or more recently fMRI. In PET and SPECT, a radioactive tracer is used to measure either brain metabolism (e.g. glucose consumption) or cerebral blood flow, which are both related to neuronal activity. Two fMRI techniques are currently available to measure (neural correlates of) brain activity: arterial spin labelling (ASL) and amplitudes of low frequency fluctuations (ALFF). Whereas ASL provides a quantitative measure of cerebral blood flow using a magnetic tracer (Wolf and Detre, 2007), ALFF measures intrinsic regional activity in a low frequency band (i.e. 0.01-0.1 Hz) of blood-oxygenated level dependent (BOLD) signal intensity (Zang et al, 2007). Notably, whole-brain (but not seed-based) resting-state findings are unbiased by a priori targeted symptoms or predefined brain regions, and may offer novel neuroscientifically-informed targets for PTSD treatment (Lanius et al, 2015).
It has been suggested that three intrinsic connectivity networks (ICNs) may be especially important when studying psychiatric patients in task-free resting-state contexts. ICNs consist of structurally and functionally connected brain areas as identified during rest (Menon, 2011). First, the default mode network (DMN), with the medial PFC (including the vmPFC and vACC), hippocampus, and posterior cingulate cortex (PCC)/precuneus as its core nodes, has been associated with task-independent internally focused thought, and autobiographical memory (Greicius et al., 2003) The salience network (SN), with the dACC, anterior insula and amygdala as its core nodes, is involved in detection of and directing attention to biologically salient stimuli in the environment (Seeley et al., 2007). Finally, the central executive network (CEN) consists of the dorsolateral prefrontal cortex (dLPFC) and lateral parietal regions, and is associated with memory processes and goal-directed behaviour, such as decision-making and planning (Seeley et al., 2007). Typically, the DMN is activated during rest and disengaged during task performance (Greicius et al., 2003). It has been suggested that the SN (especially the anterior insula) mediates between the DMN and CEN, determining whether one should focus on external, biologically relevant information, or can engage in internally focused thought (Sridharan et al., 2008). See Figure 6.1 for a map of the DMN, SN and CEN.

In light of the triple network model, and given the hypervigilance towards potential threat in PTSD patients, increased connectivity and activity of SN nodes, decreased activity and connectivity of DMN nodes, and increased connectivity between nodes of the SN and DMN have been suggested as neural correlates of PTSD (Sripada et al., 2012b). Chronic PTSD patients showed decreased task-induced suppression of DMN activity, as well as impaired switching from the DMN to the CEN and SN during a working memory task, compared to healthy controls (Daniels et al., 2010). Additionally, PTSD patients showed increased coupling of SN nodes with the CEN and DMN network during subliminal presentation of threat-related stimuli (i.e. fearful faces and personal trauma words), indicating enhanced engagement of the SN in unconscious processing of trauma-related stimuli in PTSD (Rabellino et al., 2015). Although several resting-state studies in PTSD patients have been conducted, inconsistent results across these studies have been obtained. For example, both increased (Sripada et al., 2012b) and decreased (Bluhm et al., 2009) connectivity between nodes of the SN and DMN were associated with PTSD. Moreover, decreased connectivity between the amygdala and vmPFC, hypothesized to underlie decreased emotion regulation and extinction abilities in PTSD (Shin et al., 2006), was reported in some studies (Jin et al., 2013; Sripada et al., 2012a), whereas in another study no differences were found (Rabinak et al., 2011).

Because of the growing number of studies investigating resting-state abnormalities in PTSD, and the inconsistent findings across these studies, the aim of this study is to provide a comprehensive overview of all currently available resting-state studies in PTSD patients compared to healthy controls. We therefore conducted a quantitative meta-analysis on
Core nodes making up the salience network (SN), default mode network (DMN), and central executive network (CEN), based on independent component analysis of activation studies, included in the Brainmap database (http://brainmap.org/icns) (Laird et al., 2011) (for illustrative purposes only). The displayed core nodes of the DMN are the posterior cingulate cortex (PCC), ventromedial prefrontal cortex (vmPFC), and inferior parietal lobule (IPL). Displayed core nodes of the SN are the anterior insula (AI) and the dorsal anterior cingulate cortex (dACC). Displayed core nodes of the CEN are the dorsolateral prefrontal cortex (dlPFC) and posterior parietal cortex (PPC). Coordinates (xyz) are given in MNI space. R = right side.
whole-brain resting-state studies, using the activation likelihood estimation (ALE) approach to investigate the concurrence of whole-brain hypo- and hyperactivations in PTSD patients at rest across multiple imaging studies and modalities. Whole-brain resting-state modalities include PET, SPECT, and whole-brain fMRI. Seed-based functional connectivity, on the other hand, reflects temporal synchronization between brain areas, rather than brain activity of individual areas (Fox and Raichle, 2007). Due to variability in definition and positioning of the seeds in these studies, seed-based functional connectivity studies cannot be combined into one meta-analysis, and were therefore not included. However, to provide a complete overview of the current resting-state literature, we also performed a systematic review on seed-based functional connectivity studies in PTSD patients. Results of both the meta-analysis and systematic review are discussed within the context of the triple network model (i.e. SN, DMN and CEN).

**METHODS**

**Quantitative meta-analysis**

*Systematic literature search*

Using a systematic search process, the four online databases Pubmed, Embase, PsycINFO and PILOTS were searched for studies published before April 2015 using keywords for PTSD (e.g. post-traumatic stress disorder and PTSD), functional neuroimaging (e.g. fMRI, PET, SPECT) and resting-state (e.g. resting-state, connectivity, cerebral blood flow). In total, 513 publications were identified and each publication was independently screened for eligibility for the meta-analysis by two qualified researchers (SK, MvZ and/or LN). Discrepancies between researchers were discussed until agreement was reached. All types of trauma (adulthood and childhood trauma, including combat, accidents, physical and sexual abuse), comorbidity (e.g. major depressive disorder, substance abuse, mild traumatic brain injury) were included. Studies needed to be published in English, French, German or Dutch in peer-reviewed scientific journals.

*Study selection*

We included studies using various modalities (i.e. fMRI, PET and SPECT), in accordance with previous neuroimaging meta-analyses (Eickhoff et al., 2009; Kühn and Gallinat, 2013b). Studies needed to investigate whole-brain resting-state abnormalities in PTSD patients compared to (traumatized or non-traumatized) healthy controls. We included only PET or SPECT studies investigating brain (glucose) metabolism or cerebral blood flow, which are thought to reflect brain activity, thereby excluding studies using specific receptor ligands. Only fMRI studies investigating resting-state brain activity at the whole-brain level, such as arterial spin labeling (ASL; quantitative measure of cerebral blood flow), amplitudes of low frequency fluctuations
Brain activity in PTSD during rest

(Albert; a measure of spontaneous intrinsic brain activity), regional homogeneity (ReHo; local synchronized activity between neighboring voxels) and independent component analysis (ICA; functional connectivity in independent connectivity networks) were included. Hence, in line with a previous meta-analysis of resting-state brain activity in schizophrenia and major depression, fMRI studies using seed-based functional connectivity were excluded (Kühn and Gallinat, 2013b). Activation studies using a neutral resting-state baseline condition (i.e. script-driven imagery or treatment studies with pre-and post-treatment assessment) were selected if a comparison to the neutral baseline condition was made between PTSD patients and healthy controls. Furthermore, studies needed to report coordinates in standard stereotaxic space (i.e. Montreal Neurological Institute (MNI) or Talairach space).

This screening retrieved 35 articles, which were included if they reported coordinates for the whole-brain contrast comparing resting-state differences between PTSD patients and healthy controls. If a study used multiple imaging modalities, results of the modality with the largest sample size were used. If sample sizes were equal, we included results of the modality most frequently used by the other studies included in the meta-analysis, to minimize heterogeneity in modalities across included studies. If two studies used the same sample, results of the study with the largest sample size were included and, if sample sizes were equal, the study with the most frequently included modality was used. If PTSD patients were compared to both traumatized and non-traumatized controls, we included the contrast between PTSD patients and traumatized controls. Of the 35 initially selected articles, 14 studies were excluded because no baseline comparison between PTSD patients and healthy controls was reported, one study was excluded because of overlapping samples, one study was excluded because of no significant differences were found at the baseline level, one study was excluded since it was a case study describing only two PTSD patients, three studies were excluded because they did not report imaging results in standard stereotaxic space (i.e. no normalization to the MNI or Talairach template) and one study was excluded because results were not corrected for multiple comparisons (See Supplementary Table S6.1 for an overview of excluded studies). Backward reference searching on the bibliography of these 35 initially selected articles retrieved no additional studies. In total, 14 articles were included in the meta-analysis, reporting data on 663 participants (298 PTSD patients and 365 controls) (Table 6.1; Figure 6.2). In order to explore both the effects of increased and decreased resting-state activity, we conducted two separate analyses: 1) on the contrast PTSD > controls (61 foci from 14 papers) and 2) on the contrast controls > PTSD (43 foci from ten papers).

**ALE analysis**

The activation likelihood estimation (ALE) analysis technique has been widely used for coordinate-based quantitative meta-analyses of neuroimaging data (Eickhoff et al, 2009). Given the inherent uncertainty in localization of activation foci in a neuroimaging experiment (e.g.
<table>
<thead>
<tr>
<th>Study</th>
<th>N (PTSD/TC/NTC)</th>
<th>Gender (M/F)</th>
<th>Trauma type</th>
<th>Comorbidity (N)</th>
<th>Medication</th>
<th>Imaging modality - analysis</th>
<th>Resting-state protocol</th>
<th>Contrasts</th>
<th>Significant foci (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bing et al, 2013)</td>
<td>20/0/20</td>
<td>27/13</td>
<td>Motor vehicle accidents</td>
<td>MDD (3)</td>
<td>Unmedicated for at least 2 months</td>
<td>fMRI – ALFF</td>
<td>6 min, eyes closed</td>
<td>PTSD &gt; NTC</td>
<td>3</td>
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<tr>
<td>(Bonne et al, 2003)*</td>
<td>11/17/11</td>
<td>17/22</td>
<td>Civilian traumatic events</td>
<td>MDD (3)</td>
<td>Unmedicated</td>
<td>Tc-99m-HMPAO SPECT</td>
<td>25 min</td>
<td>PTSD &gt; TC</td>
<td>8</td>
</tr>
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<td>(Chung et al, 2006)</td>
<td>23/0/64</td>
<td>47/40</td>
<td>Civilian traumatic events</td>
<td>Not reported</td>
<td>Unmedicated for at least 2 weeks</td>
<td>Tc-88 ECD SPECT</td>
<td>Not reported</td>
<td>PTSD &gt; NTC</td>
<td>8</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>NTC &gt; PTSD</td>
<td>4</td>
</tr>
<tr>
<td>(Kim et al, 2007)</td>
<td>19/0/19</td>
<td>18/20</td>
<td>Taegu subway fire</td>
<td>MDD (7), somatoform disorder (2), panic disorder (1), social phobia (1), GAD (1)</td>
<td>no antidepressants and anxiolytics for at least 4 weeks</td>
<td>Tc-99m-HMPAO SPECT</td>
<td>40 min, eyes closed</td>
<td>PTSD &gt; NTC</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>NTC &gt; PTSD</td>
<td>1</td>
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<tr>
<td>(Kim et al, 2012)</td>
<td>12/0/15</td>
<td>0/27</td>
<td>Sexual assault</td>
<td>Not reported</td>
<td>flexible dose of venlafaxine (antidepressant, average 5.2 months)</td>
<td>18F-FDG PET</td>
<td>8 min per frame, nr of frames used unreported</td>
<td>PTSD &gt; NTC</td>
<td>2</td>
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<tr>
<td>(Lindauer et al, 2004)</td>
<td>15/15/0</td>
<td>18/12</td>
<td>Traumatic events during police service</td>
<td>Mild MDD (4)</td>
<td>Unmedicated</td>
<td>Tc-99m-HMPAO SPECT</td>
<td>60 min</td>
<td>PTSD &gt; TC</td>
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<td>2</td>
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<td>(Schuff et al, 2011)</td>
<td>17/12/3</td>
<td>32/0</td>
<td>Combat-related</td>
<td>Not reported</td>
<td>Not reported</td>
<td>ASL</td>
<td>~ 2 min</td>
<td>PTSD &gt; TC</td>
<td>4</td>
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<td>(Semple et al, 2000)</td>
<td>7/0/6</td>
<td>13/0</td>
<td>Combat related</td>
<td>Cocaine and alcohol abuse (7)</td>
<td>Unmedicated: No substance abuse for at least 38 days before scanning</td>
<td>15O butanol PET</td>
<td>Eyes closed, duration not reported</td>
<td>PTSD &gt; NTC</td>
<td>6</td>
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<td></td>
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<td>NTC &gt; PTSD</td>
<td>7</td>
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<td>Study</td>
<td>N (PTSD/TC/NTC)</td>
<td>Gender (M/F)</td>
<td>Trauma type</td>
<td>Comorbidity (N)</td>
<td>Medication</td>
<td>Imaging modality - analysis</td>
<td>Resting-state protocol</td>
<td>Contrasts</td>
<td>Significant foci (N)</td>
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<tr>
<td>(Shang et al, 2014)</td>
<td>18/20/0</td>
<td>15/23</td>
<td>Wenchuan earthquake</td>
<td>Not reported</td>
<td>Unmedicated</td>
<td>fMRI – ICA</td>
<td>6 min 40 sec, eyes closed</td>
<td>PTSD &gt; TC</td>
<td>3</td>
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<td></td>
<td>TC &gt; PTSD</td>
<td>6</td>
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<tr>
<td>(Yan et al, 2013)</td>
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<td>101/0</td>
<td>Combat-related</td>
<td>Not reported</td>
<td>Unmedicated</td>
<td>fMRI – ALFF</td>
<td>6 min 40 sec, eyes open</td>
<td>PTSD &gt; TC</td>
<td>10</td>
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<td></td>
<td></td>
<td></td>
<td>TC &gt; PTSD</td>
<td>8</td>
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<tr>
<td>(Yin et al, 2011a)</td>
<td>54/72/0</td>
<td>Not reported</td>
<td>Sichuan earthquake 2008</td>
<td>None</td>
<td>Unmedicated</td>
<td>fMRI – ALFF</td>
<td>6 min 40 sec, eyes closed</td>
<td>PTSD &gt; TC</td>
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<td>TC &gt; PTSD</td>
<td>5</td>
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<tr>
<td>(Zhong et al., 2014)</td>
<td>14/0/14</td>
<td>16-Dec</td>
<td>Civilian traumatic events</td>
<td>MDD (7)</td>
<td>n/a</td>
<td>fMRI – ReHo</td>
<td>6 min 50 sec, eyes closed</td>
<td>PTSD &gt; NTC</td>
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<td>NTC &gt; PTSD</td>
<td>2</td>
</tr>
<tr>
<td>(Zhu et al, 2014)</td>
<td>17/20/0</td>
<td>23/14</td>
<td>Wenchuan earthquake</td>
<td>None</td>
<td>Unmedicated</td>
<td>fMRI – ALFF</td>
<td>6 min 40 sec, eyes closed</td>
<td>PTSD &gt; TC</td>
<td>2</td>
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<td></td>
<td></td>
<td></td>
<td>TC &gt; PTSD</td>
<td>5</td>
</tr>
<tr>
<td>(Zhu et al, 2015)</td>
<td>21/17/0</td>
<td>29-Sep</td>
<td>Wenchuan earthquake</td>
<td>MDD (5), Dysthymia (1), GAD (1)</td>
<td>Unmedicated</td>
<td>fMRI – ALFF</td>
<td>6 min 40 sec, eyes closed</td>
<td>PTSD &gt; TC</td>
<td>1</td>
</tr>
</tbody>
</table>

* Contrast PTSD > TC was used

Note: PTSD = Post-traumatic stress disorder, NTC = non-traumatized controls, TC = traumatized controls, M = male, F = female, MDD = major depressive disorder, GAD = Generalized anxiety disorder, PET = positron emission tomography, SPECT = single-photon emission computed tomography, ASL = arterial spin labeling, ALFF = amplitudes of low frequency fluctuations, ICA = independent component analysis, ReHo = regional homogeneity
due to intersubject anatomical variability, small samples sizes, and differences in normalization algorithms), the activation foci are modeled as 3D Gaussian probability distributions centered at the reported coordinates (Turkeltaub et al., 2002). By taking the union of the probability distributions of all foci across all experiments, the ALE-statistic per voxel represents the likelihood that at least one experiment reported activation in the given voxel (Eickhoff et al., 2009). Significant results hence indicate concordance in the pattern of brain activity among independent experiments. We used the recently modified ALE approach implemented in the software Brainmap GingerALE 2.3.1 (http://brainmap.org/ale) as described by Eickhoff and colleagues (2009) and Turkeltaub and colleagues (2012). Reported Talairach coordinates were converted to MNI coordinates using tal2mni (Lancaster et al., 2007). To account for the number of subjects included in each experiment, a subject-specific full width half maximum (FWHM) threshold was used for each focus (median FWHM: 8.87, range: 8.55 – 9.66) (Eickhoff et al., 2009). The value of the FWHM depends on number of subjects included in the experiment, with smaller FWHM values and hence higher ALE values for studies with larger sample sizes. The method described by Turkeltaub and colleagues (2012) was employed to minimize the effects of an experiment with multiple activation foci reported close together. Multiple comparisons correction was performed using a cluster-level threshold of \( p < .05 \) with a cluster-forming threshold of \( p < .001 \) (uncorrected) (Eickhoff et al., 2012). When appropriate, ALE output brain regions were allocated to the SN, DMN, and CEN based on visual inspection.
of findings of previously published papers on the triple network model (Menon, 2011; Seeley et al, 2007).

**Systematic review**

*Literature search and study selection*

To provide a comprehensive overview of all currently available resting-state studies in PTSD patients, we also performed a systematic review on functional connectivity studies that could not be included in the quantitative meta-analysis. All publications retrieved with the systematic search process described above were screened for eligibility to include in the systematic review on seed-based functional connectivity studies in PTSD patients. Studies needed to make a comparison between PTSD patients and traumatized or non-traumatized controls. In order to look at the brain at rest, we included resting-state functional connectivity studies only; studies investigating task-related connectivity (i.e. using psychophysiological interactions or dynamic causal modeling) were excluded. One study was initially selected, but later withdrawn from publication upon authors’ request and therefore excluded from the systematic review (See Supplementary Table S6.1). In total, nine publications were included using this systematic search process, investigating functional connectivity of various seeds in PTSD patients compared to traumatized and non-traumatized controls (Table 6.2; Figure 6.2). Backward referencing on the bibliography of these articles retrieved no additional articles.

**RESULTS**

**Quantitative meta-analysis**

The results of the first analysis, on the PTSD > controls contrast, showed significant hyperactivity in the ventral anterior cingulate cortex (vACC; BA25), the fusiform gyrus (BA37), and the parahippocampus/amygdala in PTSD patients compared to healthy controls (Figure 6.3; Table 6.3). However, the fusiform gyrus cluster seemed to be located primarily in white matter (e.g. center of mass (MNI xyz = -48, -41,-13), is 65% white matter according to the 50% Harvard-Oxford atlas) and was therefore omitted from the results. The results from the second analysis, on the controls > PTSD contrast, showed significant hypoactivity in the pyramis of the cerebellum, the posterior insula (BA13), and the middle frontal gyrus (MFG; BA6) in PTSD patients compared to healthy controls (Figure 6.3; Table 6.3).

Of the fourteen studies included in the meta-analysis, twelve studies provided a measure of brain activity using various modalities and techniques (i.e. regional cerebral metabolism (PET), cerebral blood flow (SPECT & ASL), and spontaneous intrinsic brain activity (ALFF)). However, two studies did not measure resting-state brain activity per se: one study measured ReHo, which reflects local synchronized activity between neighboring voxels (Zhong et al,
<table>
<thead>
<tr>
<th>Study</th>
<th>N (PTSd/ TC/ NTC)</th>
<th>Gender (M/F)</th>
<th>Trauma type</th>
<th>Comorbidity (N)</th>
<th>Medication</th>
<th>Resting state protocol</th>
<th>Seed</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bluhm et al., 2009)</td>
<td>17/0/15 0/32</td>
<td>Childhood abuse</td>
<td>MDD (6), dysthymic disorder (1), depressive disorder noa (1), agoraphobia (3), GAD (2)</td>
<td>15 medicated patients</td>
<td>fMRI - 5.5 min, eyes closed</td>
<td>PCC/ precunes</td>
<td>PTSD: decreased connectivity with precuneus, mPFC, lateral parietal cortices, middle temporal gyrus, right amygdala and right insula</td>
<td></td>
</tr>
<tr>
<td>(Brown et al., 2014)</td>
<td>20/22/0 42/0</td>
<td>Combat-related</td>
<td>MDD (9)</td>
<td>12 medicated patients, 2 medicated TC</td>
<td>fMRI - 6 min 20 sec, eyes open</td>
<td>BLA</td>
<td>PTSD: increased connectivity in PTSD with pgACC and dACC. Decreased connectivity with IFG</td>
<td></td>
</tr>
<tr>
<td>(Chen and Etkin, 2013)</td>
<td>17/0/38 15/40</td>
<td>Civilian traumatic events (12), combat (3), childhood abuse (2)</td>
<td>MDD (12), panic disorder (3), bulimia (2)</td>
<td>4 medicated patients</td>
<td>fMRI - 8 min, eyes closed</td>
<td>Anterior hippocampus</td>
<td>No comparison PTSD vs healthy controls.</td>
<td></td>
</tr>
<tr>
<td>(Jin et al., 2013)</td>
<td>57/77/0 38/96</td>
<td>Sichuan earthquake 2008</td>
<td>None</td>
<td>n/a</td>
<td>6 min 40 sec, eyes closed</td>
<td>90 paired whole-brain ROIs</td>
<td>5 ACC seeds (caudal, dorsal, rostral, perigenual and subgenual)</td>
<td>PTSD: decreased connectivity between mPFC and amygdala, (para)hippocampus and rectus. Decreased connectivity between OFC and hippocampus. Increased negative connectivity between PCC and insula.</td>
</tr>
<tr>
<td>(Kennis et al., 2014)</td>
<td>31/25/25 81/0</td>
<td>Combat-related</td>
<td>MDD (9), MDD and anxiety disorder (6), anxiety disorder (2), MDD and somatoform disorder (2), somatoform disorder (1)</td>
<td>None</td>
<td>fMRI - 8 min, 44 sec, eyes open</td>
<td></td>
<td>No group difference PTSD patients versus traumatized and non-traumatized controls.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>N (PTSD/TC/NTC)</td>
<td>Gender (M/F)</td>
<td>Trauma type</td>
<td>Comorbidity (N)</td>
<td>Medication</td>
<td>Resting state protocol</td>
<td>Seed</td>
<td>Main findings</td>
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<tr>
<td>(Rabinak et al, 2011)</td>
<td>17/17/0</td>
<td>34/0</td>
<td>Combat-related</td>
<td>MDD (2), alcohol abuse (2)</td>
<td>None</td>
<td>fMRI – 8 min, eyes open</td>
<td>Right amygdala</td>
<td>PTSD: increased connectivity with right insula</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Left amygdala</td>
<td>No group differences</td>
</tr>
<tr>
<td>(Sripada et al, 2012a)</td>
<td>15/14/0</td>
<td>29/0</td>
<td>Combat-related</td>
<td>MDD (7), panic disorder (1)</td>
<td>Trazadone (2)</td>
<td>fMRI – 10 min, eyes open</td>
<td>Right amygdala</td>
<td>PTSD: increased connectivity with insula, reduced anticorrelation with dACC, decreased connectivity with hippocampus and OFC</td>
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<td></td>
<td></td>
<td>Left amygdala</td>
<td>PTSD: increased connectivity with insula, reduced anticorrelation with rACC</td>
</tr>
<tr>
<td>(Sripada et al, 2012b)</td>
<td>15/15/15</td>
<td>45/0</td>
<td>Combat-related</td>
<td>MDD (7), panic disorder (1)</td>
<td>Trazadone (2)</td>
<td>fMRI – 10 min, eyes open</td>
<td>vmPFC</td>
<td>PTSD: increased connectivity with right putamen and right insula. Decreased connectivity with left hippocampus</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>PCC</td>
<td>PTSD: Increased connectivity with supplementary motor area, right precental gyrus and superior temporal sulcus. Decreased connectivity with rACC</td>
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<td></td>
<td>Right Insula</td>
<td>PTSD: Increased connectivity with left peri-insula, right hippocampus and right amygdala</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left Insula</td>
<td>No group differences</td>
</tr>
</tbody>
</table>
| (Yin et al, 2011b)*                       | 54/72/0         | 37/89        | Sichuan earthquake 2008 | None | None | fMRI - 6 min 40 sec, eyes closed | Thalamus | PTSD: decreased connectivity with right medial frontal gyrus and ACC. Increased connectivity with IFG, middle frontal gyrus, precuneus and inferior parietal lobule | 131

PCC = posterior cingulated cortex; BLA = basolateral amygdala; CeM = centromedial amygdala; MDD = major depressive disorder; GAD = generalized anxiety disorder; pgACC = pregenual ACC; rACC = rostral ACC; OFC = orbitofrontal cortex; mPFC=medial prefrotal cortex * Same participant sample as study of Yin and colleagues (2011a), included in the meta-analysis
While another study used ICA, which represents functional connectivity in independent connectivity networks (Shang et al., 2014). Therefore, we performed both analyses again without the results of these two studies. The results of both meta-analyses did not change, except that the cerebellar pyramis cluster in the control > PTSD comparison no longer reached significance after exclusion of both studies.
The results of the nine seed-based functional connectivity studies are presented Table 6.2. The main findings are described in relation to the triple network model. As the functional connectivity studies specifically investigated seeds located in the SN and DMN, we will describe these findings in relation to SN, DMN, and between SN-DMN network connectivity.

**SN connectivity**

Four studies investigated the connectivity of SN nodes in PTSD patients, using (subregions of) the amygdala as a seed (Brown et al., 2014; Rabinak et al., 2011; Sripada et al., 2012a). Increased connectivity between the amygdala and insula, both core nodes of the SN, was consistently found in PTSD patients compared to both traumatized and non-traumatized controls (Rabinak et al., 2011; Sripada et al., 2012a). Regarding amygdala to dACC connectivity, another core node of the SN, findings were mixed, as both increased (Brown et al., 2014), decreased (negative) (Sripada et al., 2012a), and unaltered (Rabinak et al., 2011) amygdala to dACC connectivity patterns were found in PTSD patients in comparison to controls. It is possible that these discrepant findings can be explained by the varying definitions of the amygdala seeds (i.e. entire amygdala or subregions, either defined based on anatomical atlases or functional ROIs).

**DMN connectivity**

Three studies have investigated functional connectivity between seeds of the DMN, such as the posterior cingulate cortex (PCC), vmPFC, and the hippocampus (Bluhm et al., 2009; Chen and Etkin, 2013; Sripada et al., 2012b). Although a variety of DMN seeds were used (i.e. PCC, precuneus, vmPFC, and hippocampus), results of these studies consistently show decreased...
connectivity between nodes of the DMN in PTSD patients compared to both traumatized and non-traumatized controls (Bluhm et al, 2009; Chen and Etkin, 2013; Sripada et al, 2012b).

**Between-network connectivity**

In several studies, it was investigated whether connectivity between nodes of the DMN and SN was altered in PTSD patients compared to healthy controls. Both increased (positive and negative) (Jin et al, 2013; Sripada et al, 2012b) and decreased (Bluhm et al, 2009) connectivity between the PCC/precuneus and nodes of the SN were found. These contradictory findings may be due to differences in (childhood) trauma exposure: increased between-network connectivity was found in PTSD patients exposed to combat or natural disaster (Jin et al, 2013), whereas decreased between-network connectivity was found in female PTSD patients with chronic early life trauma (Bluhm et al, 2009). Additionally, decreased connectivity between the amygdala and prefrontal cortex (i.e. IFG, vmPFC, middle frontal cortex), was observed in PTSD patients in most amygdala connectivity studies (Brown et al, 2014; Jin et al, 2013; Sripada et al, 2012a). However, several other studies did not find connectivity differences in the amygdala to vmPFC pathway, using the amygdala (Rabinak et al, 2011) or ACC (Kennis et al, 2014) as seed.

**DISCUSSION**

In this study, we investigated resting-state activity in PTSD patients compared to healthy controls. The quantitative meta-analysis on whole-brain resting-state activations in PTSD showed consistent hyperactivity in the vACC, and parahippocampus/amygdala in PTSD, whereas the (posterior) insula, cerebellar pyramidis, and MFG showed consistent hypoactivity in PTSD. In addition, our systematic review of seed-based functional connectivity studies showed enhanced connectivity between nodes of the SN, decreased connectivity between nodes of the DMN, as well as altered connectivity between nodes of the SN and DMN in PTSD patients compared to healthy controls, partly concordant with findings of the meta-analysis.

**Salience Network**

According to our quantitative meta-analysis, PTSD patients showed increased resting-state activity in the amygdala, compared to healthy controls (both trauma-exposed and non-trauma-exposed combined). In accordance with our findings, a previous whole-brain meta-analysis of 36 functional imaging studies (PET, SPECT, and fMRI) also found enhanced amygdala activity in PTSD patients compared to non-traumatized controls, across different experimental paradigms (Patel et al, 2012). Amygdala hyperactivity was found during presentation of trauma-related stimuli in a visual working memory task in PTSD patients compared to healthy controls, possibly representing the neurobiological correlate of the prominent
symptom of hypervigilance in PTSD (Morey et al, 2009; Zhang et al, 2013). Our meta-analysis findings underscore the importance of amygdala hyperactivity, indicating that increased neural hypervigilance in PTSD patients is also present in task-free contexts, in the absence of symptom provocation or trauma-related stimuli. In support of this meta-analytic finding, the functional connectivity studies included in the systematic review also reported increased connectivity between areas of the SN (i.e. amygdala and insula), indicating enhanced salience processing during rest in PTSD patients or, alternatively, increased anxiety in PTSD patients during scanning. Either way, this finding is consistent with the clinical presentation of hypervigilance in PTSD.

**Default Mode Network**

The quantitative meta-analysis also showed consistent vACC hyperactivity in PTSD patients compared to healthy controls. The vACC is not only an important node in the DMN, but is also involved in top-down control over fear responses (Milad et al, 2007b). Notably, increased prefrontal cortex activity, and thus excessive inhibitory prefrontal control, has been associated with dissociation symptoms in PTSD, such as inhibition of (emotional content of) traumatic memories, depersonalization, and derealization (Lanius et al, 2012). Furthermore, compared to female non-trauma-exposed controls, left vACC hyperactivity was found during encoding of negative words in an emotional Stroop paradigm in female complex PTSD patients, characterized by symptoms of dissociation, somatization, and problems with affect-regulation and self-perception (Thomaes et al, 2013). Therefore, our meta-analytic finding of vACC hyperactivity in PTSD patients may be explained by symptoms of depersonalization and derealization in a subset of PTSD patients, underlining that neural resting-state abnormalities in PTSD may depend on specific PTSD symptom clusters. However, symptoms of depersonalization and derealization were not specifically reported in the included studies. Our findings on vACC hyperactivity in PTSD patients during rest is at odds with results from task-based studies, which generally show vACC (and vmPFC) hypoactivity (Patel et al, 2012). This discrepancy could indicate an imbalance in vACC activity, in which the vACC is (ineffectively) recruited during rest, possibly to compensate for SN hyperactivity.

In contrast to the finding of vACC hyperactivity in PTSD, functional connectivity studies suggest decreased connectivity between nodes of the DMN, using the PCC, posterior hippocampus, and vmPFC as seeds. Dysfunctional (DMN) task-independent resting-state activity in PTSD patients may indicate impaired internally focused thought and autobiographical memory processes during rest in PTSD patients. In accordance with this notion, depersonalization/derealization symptom severity in PTSD was related to decreased (resting-state) vmPFC connectivity within the ventral anterior DMN, which is involved in self-referential processing (Tursich et al, 2015).
In line with the previously hypothesized decrease in internally focused thought in PTSD patients (Sripada et al, 2012b), our meta-analysis showed significant hypoactivity of the posterior insula in PTSD patients compared to healthy controls. The posterior insula is associated with interoceptive awareness, homeostasis, and regulation of physiological reactivity (Menon and Uddin, 2010). This internal bodily information is subsequently relayed to the anterior insula (i.e. an important node of the SN), where both internal (bodily) and external (environmental) information are integrated and evaluated, and where saliency is attributed (Uddin, 2014). Decreased posterior insula activity therefore suggests diminished internally focused thoughts and somatosensory awareness in PTSD, which may coincide with enhanced salience processing of environmental cues by the anterior insula.

**Between-network connectivity – DMN & SN**

Our observation of consistently diminished DMN connectivity in our systematic review seems to contradict the meta-analytic finding of vACC hyperactivity in PTSD patients. The vACC is generally considered to be part of the medial PFC, a core node of the DMN (Greicius et al, 2003). However, vACC functional connectivity with nodes of other networks (e.g. SN) may be increased in PTSD (Sripada et al, 2012b), which could explain our observation of vACC hyperactivity during rest. Indeed, two studies in the systematic review observed increased DMN and SN connectivity (Jin et al, 2013; Sripada et al, 2012b). During rest, the DMN is usually engaged, whereas the SN and CEN are deactivated, enabling an individual to engage in internally focused thought (Sridharan et al, 2008). Therefore, increased communication between the DMN and SN during rest could indicate increased threat processing and hypervigilance (SN activity), at the cost of awareness of internal sensations and thoughts (DMN activity) (Sripada et al, 2012b). Notably, increased functional connectivity between the PCC/precuneus and right amygdala was observed within two days post-trauma in trauma-exposed individuals who subsequently developed PTSD, compared to those who did not develop PTSD (Qin et al, 2012). Additionally, functional connectivity between the PCC/precuneus and right amygdala, as measured five to six weeks post-trauma in recently trauma-exposed individuals, predicted PTSD symptom severity at 12 weeks post-trauma (Lanius et al, 2010). This suggests that increased DMN – SN connectivity shortly post-trauma may also represent a vulnerability factor for PTSD development. However, another study in the systematic review found decreased connectivity between the PCC and (right) insula in PTSD patients compared to healthy controls (Bluhm et al, 2009), indicating decreased connectivity between the SN and DMN. These discrepant findings could perhaps be explained by differences in childhood trauma exposure between these studies, suggesting that chronic early life trauma is associated with altered cross-talk between the SN and DMN (Bluhm et al, 2009). Interestingly, early life trauma has been associated with the dissociative PTSD subtype (Zlotnick et al, 1996), which is characterized by suppression of emotional responses to trauma reminders (including amygdala hypoactivity and hyperactivity in the ACC and medial PFC) and may therefore
be related to increased connectivity between the prefrontal cortex and the amygdala (Lanius et al., 2012). Future studies should elucidate the seemingly discrepant findings of decreased DMN connectivity and vACC hyperactivity during rest, as well as possible effects of early life trauma and dissociation symptoms on (between-network) connectivity.

Additionally, results of our systematic review of functional connectivity studies suggest that PTSD is associated with decreased prefrontal inhibitory control over the amygdala, as reflected by findings of decreased anticorrelation between the amygdala and rACC (Sripada et al., 2012a), decreased connectivity with the medial PFC (Jin et al., 2013), and decreased connectivity between the basolateral amygdala subregion (BLA) and IFG (Brown et al., 2014). Interestingly, Jin and colleagues (2013) found that decreased functional connectivity between the amygdala and medial PFC in PTSD patients was associated with greater PTSD symptom severity. It is possible that the decreased connectivity between the amygdala and prefrontal cortices results in diminished top-down inhibitory control over fear response and thus results in an exaggerated fear response in PTSD patients.

Central Executive Network and Emotion regulation

In our meta-analysis, we found resting-state hypoactivity in the middle frontal gyrus (MFG) during rest in PTSD patients compared to healthy controls, consistent with the findings from Patel and colleagues (Patel et al., 2012). The MFG is part of the CEN, which is specifically activated during executive functioning and cognitive performance. Previous functional connectivity studies in PTSD patients investigated CEN connectivity using various task-based paradigms (Cisler et al., 2013; Daniels et al., 2010), and not under resting conditions. Previously, impaired task-induced functional connectivity of the CEN in PTSD patients, compared to healthy controls, was observed during a working memory task (Daniels et al., 2010). PTSD patients showed increased DMN connectivity, indicating impaired switching of PTSD patients between task-irrelevant (DMN) and task-relevant (CEN) networks during cognitive performance (Daniels et al., 2010; Lanius et al., 2015). Moreover, decreased connectivity within the CEN (including diminished connectivity between the premotor cortex and right middle frontal gyrus) in assaulted adolescent girls (aged 12-16) with PTSD symptoms, compared to non-trauma-exposed adolescent girls, was found during the processing of fearful vs neutral stimuli (Cisler et al., 2013). Additionally, the MFG is important for the execution of emotion regulation strategies (Grecucci et al., 2013; Kohn et al., 2014). This seems especially important since diminished emotion regulation abilities (i.e. inability to down-regulate negative emotions) have been found in PTSD patients compared to non-traumatized controls (New et al., 2009; Xiong et al., 2013). In line with this, an emotion regulation study found that PTSD patients show MFG hypoactivity compared to non-traumatized controls when reappraising negative pictures (New et al., 2009). The present results suggest that MFG hypoactivity in
PTSD is not only present during explicit emotion regulation but also during rest, indicating a more general deficiency in this area.

Additionally, results of the meta-analysis indicate hypoactivity in the cerebellar pyramis in PTSD patients compared to healthy controls. Although cerebellar dysfunction is not often described in relation to PTSD, the cerebellum is connected to the limbic lobe and brainstem, and has been implicated in the experiencing and regulation of emotions (Schutter and van Honk, 2005) as well as in the pathophysiology of PTSD (Baldaçara et al, 2011; De Bellis and Kuchibhatla, 2006). Consistent cerebellar hypoactivity in PTSD compared to healthy controls has also been found in the meta-analysis of Hayes and colleagues (2012), and was demonstrated experimentally during trauma script imagery in female abuse-related PTSD patients (Bremner et al, 1999), although cerebellar hyperactivity during trauma imagery has also been found in adolescent PTSD patients (Yang et al, 2004). In line with our finding, smaller cerebellar volume has been found in adolescent (De Bellis and Kuchibhatla, 2006) and adult (Baldaçara et al, 2011) PTSD patients compared to healthy controls, which was associated with greater severity of PTSD symptoms (Baldaçara et al, 2011). Taken together, smaller cerebellar volume and hypoactivity of the cerebellum during rest may play a role in decreased emotion regulation abilities observed in PTSD.

**The triple network model and implications for treatment**

In sum, our quantitative meta-analysis finds consistent hypo- and hyperactivations in SN, DMN, and CEN areas in PTSD patients compared to healthy controls across the currently available whole-brain resting-state studies. These findings were partially confirmed by seed-based connectivity studies. Combined, our findings suggest that PTSD is associated with aberrant activity in specific brain areas in task-free contexts without symptom provocation or trauma reminders. Our results were described in the context of the triple network model. The triple network model postulates that dysfunctional within and between-network connectivity may underlie psychopathology (Menon, 2011), including the complex sets of PTSD symptoms (Patel et al, 2012). Regarding the triple network of PTSD, SN hyperactivity, DMN hypoactivity, aberrant cross-talk between these networks could underlie the development and maintenance of hyperarousal and re-experiencing symptoms, as well as impaired autobiographical memory in PTSD (Patel et al, 2012). The traditional neurocircuitry model of PTSD proposed by Rauch and colleagues (2006) emphasizes decreased prefrontal inhibitory control over hyperactive limbic areas, including the amygdala. Notably, core brain areas of this traditional model (e.g. amygdala, vmPFC, and insula) are also core nodes of the intrinsic connectivity networks. Thus, the different models are not mutually exclusive, but are instead complementary when investigating the underlying neural correlates of PTSD. The triple network model may specifically offer new insights into the neurocognitive function of brain areas involved in the pathophysiology of PTSD, reflected by their function in the specific network.
The current synthesis of resting-state findings underscores aberrant intrinsic network activity and connectivity in PTSD patients. The distinct resting-state abnormalities may underlie different PTSD symptom clusters (Lanius et al., 2015). With psychotherapy, these different PTSD symptom clusters (and underlying aberrant neural networks) can be targeted. For example, we found evidence for enhanced activity and connectivity in SN nodes, possibly reflecting hypervigilance symptoms in PTSD, which may be targeted by anxiety management skills training (Lanius et al., 2015) and/or pharmacotherapy (Quidé et al., 2012). Additionally, based on the current findings, PTSD treatment should address distorted self-referential processing and awareness (i.e. targeting altered DMN activity and connectivity), as well as diminished cognitive functioning (i.e. targeting CEN hypoactivity), as, for example, with cognitive remediation therapy (Lanius et al., 2015).

Furthermore, we found altered functioning in all three main intrinsic connectivity networks for PTSD patients, suggesting that neural resting-state abnormalities in PTSD may be a robust biomarker of PTSD. For example, resting-state fMRI may be able to differentiate between treatment responders and non-responders, thereby providing new insights into treatment (non)response. Evidence suggests that successful pharmacological treatment and psychotherapy can reverse at least some of the brain abnormalities observed in PTSD (Quidé et al., 2012). For example, increased resting-state brain activity (as measured with SPECT) has been found in the MFG after successful psychotherapy in PTSD patients (Lansing et al., 2005), possibly reflecting increased emotion regulation abilities after treatment. To date, however, neurobiological effects of PTSD treatment on resting-state functional connectivity have not been investigated. Our meta-analysis may provide new insights and directions for future studies investigating possible treatment effects on brain functioning in PTSD, and for studies investigating (resting-state) biomarkers for treatment (non)response.

Moreover, resting-state fMRI may offer the possibility of predicting treatment response prior to treatment by using machine-learning algorithms (Wolfers et al., 2015). Although, to our knowledge, this has never been conducted in PTSD, treatment response to electroconvulsive shock therapy in depressive patients could be predicted based on resting-state fMRI (but not structural MRI) prior to treatment with 84% sensitivity and 85% specificity (van Waarde et al., 2015). Predicting treatment outcome in psychiatric patients (including PTSD) prior to treatment is highly clinically relevant, as this allows for individual tailoring of psychotherapy and/or psychopharmacology, based on subject-specific biological and/or clinical manifestations of the psychiatric disorder, which could potentially decrease drop-out rates and accelerate treatment efficacy. Additionally, resting-state fMRI is easier to implement than task-based fMRI procedures, with less variability in testing procedures and lower burden for patients.
Limitations

There are several limitations in the present study. First, this meta-analysis includes results obtained from different neuroimaging modalities (fMRI, PET, and SPECT) and different analysis techniques. Although our meta-analysis adheres to current standards in neuroimaging meta-analyses (Eickhoff et al., 2009), different imaging modalities and analysis techniques may induce variability in the observed effects and reported coordinates, hence, variability in the meta-analytic results. Although the number of studies in our meta-analysis is above the median (N=12.5) of a recent overview article of neuroimaging meta-analyses (David et al., 2013), a second limitation is the small number of studies (N=14 for the meta-analysis, N=9 for the systematic review) and the small number of foci in the meta-analysis (N=61 for PTSD > healthy controls contrast; N=43 for the healthy controls > PTSD contrast), which could have induced false positives regarding the meta-analytic findings. Moreover, the small number of included studies precluded conducting subgroup analyses comparing PTSD patients to traumatized controls (N=8 studies) and to non-traumatized controls (N=7 studies) separately. This may be especially important since different results on e.g. amygdala (hyper)activity have been found depending on the control groups used (i.e. traumatized vs. non-traumatized control group) (Patel et al., 2012).

Additionally, PTSD patients with comorbid psychiatric disorders, most predominantly major depressive disorder, were frequently included in the resting-state studies. These and other potentially confounding factors – such as medication use, coexisting psychotherapy, and sex – could all potentially influence resting-state results. Due to the limited sample size of our meta-analysis and the inclusion of mixed PTSD samples within the same studies (i.e. male and female PTSD patients, PTSD patients with and without comorbidities, and PTSD patients with and without medication use and/or psychotherapy), we could not disentangle the effects of PTSD from effects of these potential confounders. In addition, investigating the effects of time since trauma and duration of PTSD symptoms on (resting-state) brain activity may provide important new insights into the neurobiology of PTSD. For example, some neurobiological alterations may be characteristic of PTSD at an early stage in the disorder, while others may become apparent with more chronic PTSD duration. Unfortunately, relatively few studies reported time since trauma or duration of PTSD symptoms, which precluded us from systematically investigating the effects of these important clinical factors. Future meta-analyses and systematic reviews with sufficient sample size and more available information on clinical characteristics should attempt to elucidate the effects of these potential confounders and important clinical factors. Due to variability in how seeds were positioned, we could not include the results of functional seed-based connectivity studies in the quantitative meta-analysis; thus, we included all functional connectivity studies in a systematic review. However, one limitation of the systematic review is a possible seed selection bias: the included functional connectivity studies predominantly investigated seeds located in
the DMN (e.g. PCC/precuneus, vmPFC) or SN (e.g. amygdala, insula), thereby likely biasing findings towards these networks and disregarding other networks, such as the CEN. A final limitation of the study pertains to the inherent limitations of the ALE approach, in which peak activations are used instead of statistics (i.e. mean, variance and effects sizes) which are used in traditional meta-analytic approaches (Borenstein et al, 2009). In ALE-based meta-analyses it is therefore impossible to control for the statistical inference used (i.e. more stringent or lenient statistical threshold) and to take null-results into account, which could bias results. Therefore, we excluded one study which did not control for multiple comparisons. Indeed, it has been suggested that image-based meta-analyses using the full statistical images give more accurate results (Salimi-Khorshidi et al, 2009). However, since the full statistical map of each study is usually not reported in published papers, coordinate-based meta-analysis (e.g. ALE) is still the current standard.

Conclusions
This is the first quantitative meta-analysis and systematic review on resting-state abnormalities in PTSD. Taken together, both our quantitative and qualitative results indicate that during rest, PTSD is associated with enhanced SN processing (i.e. increased SN connectivity and parahippocampus/amygdala hyperactivity), decreased DMN connectivity, posterior insula hypoactivity, and altered connectivity between the nodes of the SN and DMN. This could indicate enhanced threat and salience processing in task-free resting-state contexts in PTSD patients, at the cost of internally focused thought, interoceptive awareness, and autobiographical memory. In addition, hypoactivity of the cerebellar pyramidis and MFG may indicate decreased emotion regulation abilities (i.e. CEN hypoactivity). These findings may be important for clinical practice as they underscore the clinical observation that hypervigilance and difficulties regulating affect in PTSD are not only present in response to trauma reminders and symptom provocation, but also during periods of rest. It is possible that PTSD patients still experience unwanted thoughts or other symptoms during resting-state scans, reflecting the ecological validity and importance of resting-state scanning for psychiatric disorders. However, to our knowledge, no studies to date explicitly investigated the content of thoughts in PTSD patients during resting-state imaging. Therefore, future studies should try to elucidate possible effects of the occurrence of trauma-related PTSD symptoms – including unwanted thoughts, hypervigilance, and symptoms of depersonalization and derealization – on resting-state activity and functional connectivity. Finally, as mentioned before, resting-state fMRI may become a potential tool to evaluate and predict PTSD treatment response.
### SUPPLEMENTARY TABLE S6.1 List of excluded studies

<table>
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<tr>
<th>Excluded reference</th>
<th>Reason for exclusion</th>
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### SUPPLEMENTARY TABLE S6.1 List of excluded studies (continued)

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<tbody>
<tr>
<td>Shin LM, Orr SP, Carson MA, et al. Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. JAMA Arch Gen Psychiatr; 2004; 61:168–76.</td>
<td>No baseline comparison made between controls and PTSD patients</td>
</tr>
</tbody>
</table>

**Systematic review**

| Yan, X. et al., Precuneal and amygdala spontaneous activity and functional connectivity in war-zone-related PTSD. Psychiatry research. 2015 | Withdrawn from publication on authors’ request                                       |
CHAPTER 7

Decreased uncinate fasciculus tract integrity in male and female PTSD patients: a diffusion tensor imaging study

Saskia B.J. Koch, Mirjam van Zuiden, Laura Nawijn, Jessie L. Frijling, Dick J. Veltman & Miranda Olff

Submitted for publication
ABSTRACT

Post-traumatic stress disorder (PTSD) is a disabling psychiatric disorder with a lifetime prevalence of circa 8%. PTSD has been associated with altered white matter integrity in the cingulum and superior longitudinal fasciculus. However, findings in these previous diffusion tensor imaging (DTI) studies have been inconsistent, showing high variability in location and direction of effects. Therefore, we performed probabilistic tractography of the bilateral uncinate fasciculus, cingulum and superior longitudinal fasciculus (both temporal and parietal projections) in male and female police officers with (n=38, 21 males) and without (n=39, 20 males) PTSD. Compared to trauma-exposed controls, PTSD patients showed significantly higher mean diffusivity (MD) of the right uncinate fasciculus, the major white matter tract connecting the amygdala to the prefrontal cortex (p=.012). No other significant between-group or group by sex differences were observed. In PTSD patients, MD of the right uncinate fasciculus was positively associated with anxiety symptoms (r=.410, p=.013), and amygdala activity (r=.416, p=.014) and trend-wise with ventromedial prefrontal cortex (vmPFC) activity (r=.296, p=.084) towards happy and neutral faces. However, our specific sample of highly trauma-exposed police officers limits the generalizability of our findings to other PTSD patients groups (e.g. civilian trauma). In conclusion, PTSD patients showed diminished structural connectivity between the amygdala and vmPFC, which was correlated with higher anxiety symptoms and increased functional activity of these brain regions. These findings provide additional evidence for the prevailing neurocircuitry model of PTSD, postulating that ineffective communication between the amygdala and vmPFC underlies decreased top-down control over fear responses.
INTRODUCTION

Approximately 10% of trauma-exposed individuals develops a post-traumatic stress disorder (PTSD) (de Vries and Olff, 2009), characterized by intrusions and avoidance of reminders of the traumatic event, altered cognitions and mood and hyperarousal (American Psychiatric Association, 2013). The prevailing neurocircuitry model of PTSD postulates amygdala hyperactivity and vmPFC hypoactivity (Hayes et al., 2012; Rauch et al., 2006). The amygdala plays a pivotal role in fear expression and salience processing (Phelps and LeDoux, 2005), whereas the ventromedial prefrontal cortex (vmPFC) is important in top-down control over the fear response (Milad et al., 2007b). Additionally, decreased resting-state functional connectivity between the amygdala and vmPFC was observed in male PTSD patients, possibly underlying diminished top-down prefrontal control over the fear response (Sripada et al., 2012a).

White matter (WM) fibers, which make up over 40% of total brain volume (Morell and Norton, 1980), allow effective communication between (cortical and subcortical) brain areas. WM integrity can be investigated non-invasively with diffusion tensor imaging (DTI), measuring the direction and coherence of water diffusion within myelinated tracts (Hagmann et al., 2006). The DTI metrics fractional anisotropy (FA) and mean diffusivity (MD) are predominantly used to quantify WM integrity: FA provides a measure of diffusion direction and represents axonal density and coherence, whereas MD provides an overall measure of membrane integrity (Song et al., 2003). To date, several DTI studies investigated WM alterations in PTSD, showing that PTSD is associated with altered WM integrity of major WM tracts, including the cingulum and superior longitudinal fasciculus (Daniels et al., 2013). The cingulum connects the cingulate cortex to the entorhinal cortex and amygdala (Schmahmann and Pandya, 2006), and the superior longitudinal fasciculus connects the parietal, temporal and occipital lobes to the ipsilateral frontal cortex (Schmahmann et al., 2007). Additionally, decreased FA of the uncinate fasciculus (i.e. the major WM tract connecting the amygdala with the vmPFC and orbitofrontal cortex) was associated with greater subclinical PTSD symptoms severity, as well as with lower startle response during extinction in combat-exposed participants (Costanzo et al., 2016). However, unexpectedly, to date no structural alterations have been found in uncinate fasciculus in PTSD patients.

Although PTSD has been associated with altered WM integrity, previous findings have been inconsistent, showing high variability in the location and direction of observed effects. According to a quantitative meta-analysis of seven whole-brain DTI studies comparing PTSD patients to healthy controls, PTSD was associated with both increased and decreased FA at different locations in the left superior longitudinal fasciculus (Daniels et al., 2013). Additionally, both increased and decreased FA was found in PTSD patients at different locations in the bilateral cingulum (Daniels et al., 2013). These mixed findings may be due to different study
designs: PTSD patients were compared to trauma-exposed (Abe et al, 2006; Bierer et al, 2015; Fani et al, 2012b; Schuff et al, 2011) or non-trauma-exposed (Kim et al, 2005, 2006; Zhang et al, 2011) controls, although trauma-exposure has been found to alter tract integrity (Sekiguchi et al, 2014). Additionally, both males and females have been included in the same studies (Abe et al, 2006; Kim et al, 2005, 2006; Zhang et al, 2011), without specifically investigating or accounting for possible sex differential effects on tract integrity (Hsu et al, 2008).

Therefore, in this study, we investigated integrity of major WM tracts in PTSD patients compared to trauma-exposed controls, including both male and female participants to take sex differences into account. We reconstructed major WM pathways using an automated and unbiased reconstruction of a priori determined anatomical WM tracts using global probabilistic tractography (TRACULA: TRActs Constrained by UnderLying Anatomy) (Yendiki et al, 2011). Major WM pathways are reconstructed based on probability distributions of fiber orientations of all voxels along the entire tract, providing one DTI metric per tract (Yendiki et al, 2011). Compared to previously used whole-brain voxel-wise analysis techniques, the number of multiple comparisons is drastically reduced using TRACULA, thereby increasing statistical power to detect relatively subtle differences in tract integrity. Additionally, no inter-subject registration is needed, increasing sensitivity to inter-individual anatomical variability (Smith et al, 2006), which may be especially important since PTSD has been associated with grey matter alterations (Kühn and Gallinat, 2013a).

Given previous meta-analytic findings (Daniels et al, 2013) and the predominant neurocircuitry model of PTSD (Rauch et al, 2006), we investigated tract integrity of the bilateral uncinate fasciculus, cingulum bundle and superior longitudinal fasciculus (both temporal and parietal projections) in male and female police officers with and without PTSD. We investigated group differences in average FA and MD values of each WM tract. Additionally, we conducted a trajectory analysis, in which average FA and MD values were examined at different locations of the WM tracts. Moreover, we investigated clinical (i.e. PTSD symptom severity and subjective anxiety) and neural (amygdala and vmPFC activity towards emotional faces) correlates of altered WM integrity in PTSD.

METHODS

Participants
Male and female police officers with PTSD (n=38, 21 males) and without PTSD (n=39, 20 males) were recruited via advertisements on websites and in journals of the Dutch police and via a psychotrauma diagnostic center for police personnel (PDC politiepoli, Diemen, the
Netherlands, patients only). Control participants were matched to the PTSD patients based on age, sex, educational level and years of service.

Participants were between 18 and 65 years of age, eligible for MRI and did not use any psychotropic medication. PTSD patients met DSM-IV criteria for current PTSD, with a Clinician-Administered PTSD Scale (CAPS) (Blake et al, 1995) total score of ≥ 45. PTSD patients were excluded in case of previously diagnosed personality disorder, current substance-related disorder, severe major depressive disorder (MDD), psychotic disorder, or current suicidal risk. Trauma-exposed controls experienced at least one traumatic event, according to the DSM-IV PTSD A1 criterion (American Psychiatric Association, 2013) with a CAPS score of ≤ 15. They did not have any current DSM-IV axis-1 psychopathology or diagnosed personality disorder or lifetime history of PTSD or MDD. For all participants, we assessed psychopathology (other than PTSD) with the Dutch version of the Mini Neuropsychological Interview (MINI) (Sheehan et al, 1998) or the Structured Clinical Interview for DSM-IV (SCID) (First et al, 2012) (for patients of the outpatient clinic). One female PTSD patient started using benzodiazepines daily in the week prior to the second MRI scan. This participant was not excluded from the DTI analysis, as we did not expect short-term medication effects on WM integrity.

The Institutional Review Board of the AMC in Amsterdam the Netherlands approved this study. We conducted the study in accordance with the Declaration of Helsinki and obtained written informed consent before study participation.

**Procedure**

This study is part of a double-blind, randomized, placebo-controlled cross-over fMRI study on the neural correlates of oxytocin administration in PTSD patients (Koch et al, 2016a) (Chapter 3). The study consisted of three appointments: one intake session, during which in-and exclusion criteria were checked, and two MRI sessions. The second MRI session took place on average 12.77 (±14.87) days after the first fMRI session. Both MRI sessions started with the acquisition of a structural image: a structural T1-weighted image was obtained during the first session and a DTI scan was made during the second session. Subsequent functional imaging involved (among others) an emotional face-matching task (Hariri et al, 2002) to elicit robust amygdala activation (Koch et al, 2016a) (Chapter 3). The emotional face-matching task consisted of one visuomotor control condition (scrambled faces) (4 blocks) and two emotional conditions: one with fearful and angry faces (2 blocks) and one with happy and neutral faces (2 blocks) (see Supplementary Figure S7.1 for the task design). For the current study, we used functional images acquired under placebo only.

Prior to each scanning session, severity of current depressive and anxiety symptoms in the past week was assessed with the Hospital Anxiety and Depression Scale (HADS) (Zigmond
and Snaith, 1983), consisting of an anxiety (HADS-A) and depression (HADS-D) subscale. PTSD symptom severity in the past week was assessed with the revised Impact of Events Scale (IES-R) (Weiss, 2004). Additionally, participants filled out questionnaires on various demographic variables, alcohol use (Alcohol Use Disorders Identification Test, AUDIT) (Bush, 1998), childhood trauma exposure (the Early Trauma Inventory, ETI-SF) (Bremner et al., 2007) and police-related traumatic events (the Police Life Events Scale, PLES) (Carlier and Gersons, 1992).

**Image acquisition**

Participants were scanned with a 3T Philips Achieva MR system (Best, the Netherlands), using a 32-channel head coil. To acquire a high-resolution anatomical T1-weighted scan, a FAST MPRage sequence was used (220 slices; voxel size=1mm³; repetition time=8.2s; echo time=3.8s; flip angle=8°). Diffusion weighted images (DWIs) were acquired in 32 directions, with a low b (b=0) images and a b-value of 1000 sec/mm² (60 slices, voxel size=3mm³; repetition time=7542ms; echo time=86ms). The functional scans were obtained using an echo planar sequence sensitive to the BOLD contrast (110 volumes, voxel size=3mm³; repetition time=2s, echo time=28ms; flip angle=76°).

**DTI analysis**

Preprocessing of the DWIs was conducted with in-house developed software, programmed in Matlab (The Mathworks, Natick, MA) and executed on the Dutch e-science Grid (http://www.biggrid.nl), with a web interface to the e-Bioinfra gateway (Olabarriaga et al., 2010; Shahand et al., 2011). We corrected for head movement and deformations induced by eddy currents using affine registration of the DWIs to the non-diffusion weighted image. Hereafter, the gradient directions were corrected by the rotation component of the transformation. The DWIs were resampled isotropically. Finally, rician noise was reduced with an adaptive noise filtering method, resulting in higher precision of the diffusivity values (Caan et al., 2010).

Voxel-wise probability distributions of diffusion direction were estimated on the preprocessed DWIs using the ball-and-stick model, as implemented in BedPostX (Bayesian Estimation of Diffusion Parameters obtained using sampling techniques) (Behrens et al., 2007) of FSL (Functional Software Library). Automated cortical and subcortical segmentation of the structural T1-weighted scan was performed with FreeSurfer version 5.0, using default options (Fischl, 2012). Both BedPostX and FreeSurfer analyses were executed on the Dutch e-science Grid.

Global probabilistic tractography was performed in TRACULA (Yendiki et al., 2011), using the estimated diffusion probability distributions as provided by BedPostX and (sub)cortical segmentations as provided by FreeSurfer. The bilateral cingulum bundle, uncinate fasciculus, and the superior longitudinal fasciculus (both parietal and temporal projections) were recon-
structured (See Figure 7.1). Additional preprocessing in TRACULA included intra-subject registration of DWIs to the structural T1-weighted image, inter-subject registration of structural T1-weighted images to a common template space, creation of cortical and WM masks from FreeSurfer reconstructions and tensor fitting for extraction of tensor-based measures (i.e. FA & MD). Finally, anatomical priors for the WM pathways were computed from the TRACULA atlas, which consists of 30 manually labelled healthy individuals (Yendiki et al, 2011).

**FIGURE 7.1 Trajectories of the uncinate fasciculus, cingulum and superior longitudinal fasciculus**

Trajectories of the uncinate fasciculus, cingulum and superior longitudinal fasciculus (temporal and parietal projections) of a representative subject. Unc.Fas = uncinate fasciculus, SLFP = superior longitudinal fasciculus, parietal projections, SLFT = superior longitudinal fasciculus, temporal projections.

For each participant, WM tracts were reconstructed by simultaneously fitting the shape of each pathway to the individual diffusion probability distributions and to the anatomical priors based on the TRACULA atlas (number of control points: 7; number of permutations: 1000). Average weighted FA and MD values were extracted for each reconstructed tract (See Supplementary Table S7.1 for correlations between FA and MD values of each tract). For each tract, we additionally extracted weighted FA and MD values for each voxel. To minimize the number of multiple comparisons, FA and MD values were averaged over segments of five successive voxels for each tract. Given the varying length of the different tracts, this resulted in five segments for the uncinate fasciculus, six segments for the cingulum bundle, four segments for the parietal projections and seven segments for the temporal projections of the superior longitudinal fasciculus.

**Emotional face-matching task**

After preprocessing, the emotional face-matching conditions (i.e. fearful-angry and happy-neutral faces) were each contrasted with the visuomotor control condition (i.e. scrambled faces) for each participant at the first level (Koch et al, 2016a) (**Chapter 3**). These contrast images were fit into a second-level model with between-subject factors group (PTSD pa-
tient – control) and sex (male – female), within-subjects factor emotion (fearful-angry faces and happy-neutral faces) and covariate drug-order. Amygdala and vmPFC activity during emotional face-matching was assessed for the overall task effect across all participants (see Supplementary material for fMRI data analysis). Individual contrast estimates of amygdala and vmPFC activity towards fearful-angry and happy-neutral faces were extracted from a 5mm sphere surrounding the left (peak xyz=−20 -8 -16, P_{FWE}<.001) and right (peak xyz=24 -10 -14, P_{FWE}<.001) amygdala peak task activation and from a 10mm sphere surrounding the vmPFC peak task activation (peak xyz = 6 48 -2, P_{FWE}=.001) (all whole-brain corrected).

Statistical analysis
Statistical analyses were conducted with SPSS 20.0 (IBM, Armonk, NY, USA). All variables were first checked for outliers (i.e. standardized value > 2.58) within each group of participants and outliers were removed. Variables were checked for normality (standardized skewness and/or kurtosis > 2.58) and were transformed when necessary.

Differences between PTSD patients and trauma-exposed controls on demographic characteristics, alcohol use and trauma exposure history were analyzed for males and females separately, using independent t-tests for continuous and chi-square tests for categorical variables. To investigate tract integrity differences between PTSD patients and trauma-exposed controls, repeated-measures analyses of covariance (ANCOVA’s) were conducted for each tract separately, with the within-subject factor hemisphere (left - right) and between-subject factors group (PTSD patients – controls) and sex (males - females), while controlling for confounding variables. We included tract volume in number of voxels as covariate in all analyses to control for possible partial volume effects (Vos et al, 2011). Additionally, we included age as covariate because (prefrontal) WM tract integrity has previously been found to decrease with age (Sexton et al, 2014). For the trajectory analysis, the within-subjects factor segment was additionally included in the repeated measures ANCOVAs.

We adjusted the alpha for multiple comparisons (n=8) with a partial Bonferroni correction, taking the high correlation between all tract integrity measures into account and thereby balancing Type-I and Type-II errors. Since FA and MD values of all tracts were highly correlated, treating the variables as independent would have resulted in too stringent correction with a conventional Bonferroni correction. Therefore, the correlation between all tract measures (r=.484) was taken into account using the Simple Interactive Statistical Analysis Bonferroni tool (www.quantitativeskills.com/sisa/ calculations/bonfer.htm) (Li et al, 2014), rendering a p-value of <.017 significant. For trajectory analysis, a mean correlation coefficient of r=.280 was observed, resulting in a corrected alpha of <.011 for these analyses. In case of significant interaction effects, post-hoc tests were corrected for multiple comparisons using a Bonferroni correction (i.e. adjusted alpha=.05/number of post-hoc tests).
Partial correlation analyses between symptom severity and tract integrity were conducted within PTSD patients, while correcting for age and tract volume. Additionally, we computed partial correlations between extracted contrast estimates of amygdala and vmPFC activity towards fearful-angry and happy-neutral faces and uncinate fasciculus tract integrity within PTSD patients, while controlling for age and tract volume.

RESULTS

Demographics
PTSD patients and trauma-exposed controls did not differ in age, years of service, educational level and alcohol use (Table 7.1). Compared to male trauma-exposed controls, male PTSD patients experienced more types of childhood traumatic events ($t(39)=-2.18$, $p=.037$), whereas female trauma-exposed controls experienced more types of work-related traumatic events compared to female PTSD patients ($t(34)=2.27$ $p=.028$).

Group differences in tract integrity
A significant hemisphere by group interaction effect was found for MD of the uncinate fasciculus ($F(1,67)=7.575$, $p=.008$), in which PTSD patients showed higher MD of the right uncinate fasciculus ($F(1,68)=6.70$, $p=.012$, $\omega^2=.0704$), compared to trauma-exposed controls (See Table 7.2 and Figure 7.2). These findings remained significant after controlling for group differences in childhood and work-related traumatic events (group main effect right uncinate fasciculus MD: PLES as covariate: $F(1,67)=5.29$, $p=.025$, $\omega^2=.0546$; ETI as covariate: $F(1,67)=5.18$, $p=.026$, $\omega^2=.0520$). No significant differences between PTSD patients and trauma-exposed controls were found regarding MD values of left uncinate fasciculus, FA values of the bilateral uncinate fasciculus, nor for FA and MD values of the left and right cingulum bundle and the superior longitudinal fasciculus (both temporal and parietal projections) (all $p>.1$). We found significant effects of sex and hemisphere, showing differences in tract integrity between males and females and/or between the left and right hemisphere (See Supplementary Table S7.2).

Trajectory analysis
We investigated WM integrity differences between PTSD patients and trauma-exposed controls for different locations on the uncinate fasciculus, cingulum and the superior longitudinal fasciculus (both parietal and temporal projections). No significant (hemisphere by) group by segment interactions were found in any of the tracts (all $p>.01$), except for a nominally significant group effect for uncinate fasciculus MD (i.e. PTSD patients > trauma-exposed controls, $p=.022$). See supplementary Table S7.3 for significant main effects of segment and hemisphere by sex by segment interactions.
## TABLE 7.1 Participant characteristics and test statistics

<table>
<thead>
<tr>
<th></th>
<th>PTSD patients (n=37)</th>
<th>Healthy controls (n=39)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=21)</td>
<td>Females (n=17)</td>
<td>Males (n=20)</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.29 (9.83)</td>
<td>38.84 (9.70)</td>
<td>41.35 (10.62)</td>
</tr>
<tr>
<td>Years of police service</td>
<td>16.29 (10.82)</td>
<td>14.19 (10.47)</td>
<td>18.42 (10.05)</td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Middle</td>
<td>14 (67%)</td>
<td>14 (88%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>High</td>
<td>7 (33%)</td>
<td>2 (12%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td><strong>Symptom severity</strong></td>
<td></td>
<td></td>
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<tr>
<td>CAPS total score</td>
<td>68.05 (15.62)</td>
<td>67.35 (11.49)</td>
<td>4.70 (4.79)</td>
</tr>
<tr>
<td>CAPS – re-experiencing</td>
<td>20.95 (6.45)</td>
<td>21.00 (5.30)</td>
<td>0.70 (1.17)</td>
</tr>
<tr>
<td>CAPS – avoidance</td>
<td>21.57 (7.97)</td>
<td>22.65 (5.65)</td>
<td>1.20 (2.35)</td>
</tr>
<tr>
<td>CAPS – hyperarousal</td>
<td>25.71 (5.79)</td>
<td>23.59 (4.37)</td>
<td>2.80 (3.68)</td>
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<td>IES-R total score</td>
<td>44.57 (14.51)</td>
<td>39.82 (23.43)</td>
<td>1.10 (1.85)</td>
</tr>
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<td><strong>Current comorbidity</strong></td>
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<td>MDD</td>
<td>4 (19%)</td>
<td>4 (25%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>2 (9.5%)</td>
<td>1 (6.3%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Panic Disorder</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>n/a</td>
</tr>
<tr>
<td>Specific phobia</td>
<td>1 (4.8%)</td>
<td>-</td>
<td>n/a</td>
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<tr>
<td>HADS – Anxiety</td>
<td>11.52 (0.71)</td>
<td>12.47 (1.20)</td>
<td>1.60 (0.39)</td>
</tr>
<tr>
<td>HADS – Depression</td>
<td>10.40 (1.01)</td>
<td>10.01 (1.23)</td>
<td>0.95 (0.35)</td>
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<td><strong>Trauma history &amp; alcohol use</strong></td>
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<tr>
<td>Work-related traumatic events (PLES)</td>
<td>22.50 (5.95)</td>
<td>13.47 (9.17)</td>
<td>20.45 (6.42)</td>
</tr>
<tr>
<td>Violent incidents</td>
<td>9.65 (3.45)</td>
<td>5.06 (4.46)</td>
<td>8.30 (3.57)</td>
</tr>
</tbody>
</table>
### TABLE 7.1 Participant characteristics and test statistics (continued)

<table>
<thead>
<tr>
<th></th>
<th>PTSD patients (n=37)</th>
<th>Healthy controls (n=39)</th>
<th>Statistics</th>
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<tbody>
<tr>
<td></td>
<td>Males (n=21)</td>
<td>Females (n=17)</td>
<td>Males (n=20)</td>
</tr>
<tr>
<td>Confrontation with tragic incidents</td>
<td>12.85 (3.38)</td>
<td>8.41 (5.32)</td>
<td>12.15 (3.33)</td>
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<td>Childhood traumatic events (ETI)</td>
<td>6.09 (4.55)</td>
<td>5.12 (5.05)</td>
<td>3.65 (2.35)</td>
</tr>
<tr>
<td>AUDIT, alcohol use (total score)</td>
<td>3.52 (3.40)</td>
<td>4.06 (4.64)</td>
<td>3.40 (1.67)</td>
</tr>
</tbody>
</table>

Mean (± standard deviation) or N (%) of demographic variables, symptom severity, trauma history and alcohol use in PTSD patients and trauma-exposed controls. Differences between PTSD patients and trauma-exposed controls were tested separately for male and female participants.

CAPS = clinician administered PTSD scale, IES-R = impact of events scale – revised, MDD = major depressive disorder, HADS = hospital anxiety and depression scale, PLES = police life event scale, ETI = early trauma inventory, AUDIT = Alcohol Use Disorder Identification test. PLES: Violent incidents included physical aggression, confrontation with violent death, suicide, and shooting incidents. Confrontation with tragic incidents included witnessing (child) sexual or physical abuse, (deadly) traffic accidents and (natural) disasters.
Chapter 7

Correlations with symptom severity in PTSD patients

We explored whether right uncinate tract integrity was related to symptom severity in PTSD patients. HADS anxiety was positively correlated with MD (r=.410, p=.013) and negatively correlated with FA (r=-.292, p=.083) of the right uncinate fasciculus (See Figure 7.3). HADS depression and PTSD severity (CAPS total and subscale scores) were not significantly related to WM integrity of the right uncinate fasciculus (all p>.1) (See Figure 7.3 & Supplementary Table S7.4).

### TABLE 7.2 Results of the repeated-measures ANOVA’s on FA and MD values of each tract

<table>
<thead>
<tr>
<th>Tract</th>
<th>FA values</th>
<th>MD values</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F(df)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Uncinate Fasciculus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of group</td>
<td>F(1,66)=.130</td>
<td>.719</td>
</tr>
<tr>
<td>Group x sex interaction</td>
<td>F(1,66)=1.209</td>
<td>.276</td>
</tr>
<tr>
<td>Hemisphere x group interaction</td>
<td>F(1,66)=1.513</td>
<td>.223</td>
</tr>
<tr>
<td>Hemisphere x group x sex interaction</td>
<td>F(1,66)=.789</td>
<td>.378</td>
</tr>
<tr>
<td><strong>Cingulum Bundle</strong></td>
<td></td>
<td></td>
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<tr>
<td>Main effect of group</td>
<td>F(1,68)=.521</td>
<td>.473</td>
</tr>
<tr>
<td>Group x sex interaction</td>
<td>F(1,68)=.779</td>
<td>.380</td>
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<tr>
<td>Hemisphere x group interaction</td>
<td>F(1,68)=2.479</td>
<td>.120</td>
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<tr>
<td>Hemisphere x group x sex interaction</td>
<td>F(1,69)=.327</td>
<td>.569</td>
</tr>
<tr>
<td><strong>Superior Longitudinal Fasciculus – parietal projections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of group</td>
<td>F(1,68)=2.757</td>
<td>.101</td>
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<tr>
<td>Group x sex interaction</td>
<td>F(1,68)=.090</td>
<td>.765</td>
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<td>Hemisphere x group interaction</td>
<td>F(1,68)=.620</td>
<td>.434</td>
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<td>Hemisphere x group x sex interaction</td>
<td>F(1,68)=.017</td>
<td>.897</td>
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<tr>
<td><strong>Superior Longitudinal Fasciculus – temporal projections</strong></td>
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</tr>
<tr>
<td>Main effect of group</td>
<td>F(1,69)=2.668</td>
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<tr>
<td>Group x sex interaction</td>
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<td>.686</td>
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<tr>
<td>Hemisphere x group interaction</td>
<td>F(1,69)=.096</td>
<td>.758</td>
</tr>
<tr>
<td>Hemisphere x group x sex interaction</td>
<td>F(1,69)=.948</td>
<td>.334</td>
</tr>
</tbody>
</table>

Main and interaction effects of group (trauma-exposed controls and PTSD patients) of the repeated measures ANOVA’s on FA and MD values of the uncinate fasciculus, cingulum bundle and parietal and temporal projections of the superior longitudinal fasciculus, corrected for age and tract volume. A p-value <.017 was regarded significant for main and interaction effects. For the post-hoc test of the group x hemisphere interaction effect, a p-value <.025 was regarded significant.

df = degrees of freedom; FA = fractional anisotropy; MD = mean diffusivity; * significant at p-corrected <.05 level; # significant at p-corrected <.1 level
Task activation across participants

As expected, the bilateral amygdala was robustly activated, whereas the vmPFC was deactivated when looking at emotional faces (all \( P_{\text{FWE}} < 0.05 \) whole-brain corrected), across all participants and conditions (See Supplementary Figure S7.1). Exploratory analyses showed significantly less vmPFC deactivation towards happy-neutral faces in PTSD patients compared to healthy trauma-exposed controls (\( t(72) = -2.689, p = 0.009 \)), whereas no group differences were observed for amygdala activity (See Supplementary Figure S7.1).

FIGURE 7.2 Right uncinate fasciculus tract integrity in PTSD patients and trauma-exposed controls

Average (A) fractional anisotropy (FA) and (B) mean diffusivity (MD) for male and female PTSD patients and trauma-exposed controls. Error bars represent standard deviations of the mean. * \( p < 0.05 \)

FIGURE 7.3 Correlations between right uncinate fasciculus integrity and symptom severity in PTSD patients

Correlations between (A) fractional anisotropy (FA) and (B) mean diffusivity (MD) of the right uncinate fasciculus and anxiety (HADS anxiety subscale) and depression symptoms (HADS depression subscale). \( r = \) Pearson correlation coefficient; FA = fractional anisotropy, MD = mean diffusivity; HADS = hospital and anxiety and depression scale
Correlations with amygdala and vmPFC activity in PTSD patients

In order to characterize neural correlates of altered uncinate fasciculus tract integrity within PTSD patients, we investigated correlations between uncinate fasciculus tract integrity and amygdala and vmPFC activity towards emotional faces. Right-sided amygdala activity towards happy-neutral faces (and not towards fearful-angry faces) was significantly negatively correlated with FA ($r=-.348$, $p=.044$), but positively correlated with MD ($r=.416$, $p=.014$) of the right uncinate fasciculus (See Figure 7.4). Additionally, vmPFC deactivation towards happy-neutral faces was negatively correlated with FA ($r=-.505$, $p=.002$) and nominally positively correlated with MD ($r=.296$, $p=.084$) of the right uncinate fasciculus. Deactivation of the vmPFC towards fearful-angry faces was negatively correlated with FA ($r=-.413$, $p=.014$), but not with MD values of the right uncinate fasciculus ($r=.265$, $p=.123$).

**FIGURE 7.4 Correlations between right uncinate fasciculus integrity and amygdala and vmPFC activity**

Correlations between (A) fractional anisotropy (FA) and (B) mean diffusivity (MD) of the right uncinate fasciculus and right amygdala reactivity towards emotional faces compared to the control condition. Contrast estimates in arbitrary units (a.u.) were extracted from a 5mm sphere surrounding the peak activation effect in the right amygdala (xyz = 24 -10 -14). Correlations between (C) FA and (D) MD of the right uncinate fasciculus and ventromedial prefrontal cortex (vmPFC) reactivity towards emotional faces compared to the control condition. Contrast estimates in arbitrary units (a.u.) were extracted from a 10mm sphere surrounding the peak activation effect in the vmPFC (xyz = 6 48 -2).
We investigated integrity of major anatomical WM tracts in male and female police officers with and without PTSD using a global probabilistic tractography method. Compared to trauma-exposed controls, PTSD was associated with higher MD of the right uncinate fasciculus, which connects the vmPFC and amygdala. Within PTSD patients, right uncinate fasciculus MD values were positively associated with anxiety, as well as with amygdala and vmPFC activity towards emotional stimuli.

Our finding of lower WM integrity of the uncinate fasciculus in PTSD is in line with the prevailing neurocircuitry model (Rauch et al, 2006). This model postulates diminished communication between the amygdala and vmPFC in PTSD, resulting in decreased inhibitory prefrontal control over the fear response, and increased amygdala reactivity towards trauma-related or emotional stimuli (Hayes et al, 2012; Rauch et al, 2006). It has been suggested that the strength of structural and functional connectivity between the amygdala and vmPFC, rather than the activity of either brain region alone, may be especially important in terms of fear regulation and subjective anxiety (See Kim et al, 2011b for a review). For example, in healthy females, the frequency of cognitive emotion regulation strategies (i.e. reappraisal) during everyday life was related to greater left uncinate fasciculus tract integrity (Zuurbier et al, 2013). Notably, we found a positive association between right uncinate fasciculus MD and anxiety symptoms in PTSD patients.

Functionally, we found that MD of the right uncinate fasciculus was positively correlated with right amygdala and vmPFC activation towards happy and neutral faces in PTSD patients. Previously, lower uncinate fasciculus integrity was related to higher amygdala reactivity towards fearful faces in healthy participants (Kim and Whalen, 2009) and towards happy and sad faces in children and adolescents (aged 9 to 19) (Swartz et al, 2014). Moreover, lower structural uncinate fasciculus tract integrity in patients with generalized social anxiety disorder (compared to healthy controls) was associated with decreased functional amygdala to vmPFC connectivity (Tromp et al, 2012). In our study, we observed vmPFC deactivation towards emotional faces. The vmPFC is part of the default mode network (DMN), an intrinsic connectivity network which has been associated with task-independent internally focused thought and is usually disengaged during task performance (Greicius et al, 2003). PTSD has been associated with decreased suppression of DMN activity during task performance (Daniels et al, 2010). In line with this observation, we found significantly diminished vmPFC deactivation towards happy-neutral faces in PTSD patients compared to trauma-exposed controls. Taken together, our findings indicate that lower uncinate fasciculus tract integrity in PTSD patients is not only associated with higher anxiety, but also with greater amygdala activity and decreased vmPFC deactivation towards emotional stimuli. Notably, these observations are in the line with the
prevailing neurocircuitry model of PTSD, underlining that impaired communication between the vmPFC and amygdala may result in increased (amygdala-mediated) fear responses.

Of note, we observed significant group differences for MD but not FA of the right uncinate fasciculus. Although the exact neurobiological underpinnings of MD and FA are still under debate, FA and MD likely represent complementary aspects of WM microstructure: whereas FA represents diffusion orientation, MD is an overall measure of membrane integrity, independent of fiber orientation (Song et al, 2003). Our findings of increased uncinate fasciculus MD in PTSD therefore suggest (subtle) microstructural changes in membrane integrity, reflected by an altered diameter of the diffusion tensor (i.e. increased MD), without changes in directionality of diffusion (i.e. unaltered oval shape of the diffusion tensor: FA). It has been suggested that MD may be more sensitive to subtle WM alterations as a consequence of chronic stress exposure: a recent DTI study in elderly males showed a positive association between salivary cortisol before a mild cognitive stressor and MD (but not FA) of the uncinate fasciculus (Cox et al, 2015).

From our study it cannot be concluded whether lower right uncinate fasciculus tract integrity in PTSD may have developed as a consequence of the disorder, or may represent a trait and pre-existing (i.e. pre-trauma) vulnerability factor for PTSD development. In support of the suggestion that it may represent a pre-trauma vulnerability factor, lower uncinate fasciculus FA was found in healthy females with low expression of the serotonin transporter (i.e. 5HTTLPR) (Pacheco et al, 2009), associated with high anxiety and increased PTSD risk (Liu et al, 2015). Also, in a prospective imaging study, lower FA and higher MD of vmPFC WM (measured within two days post-trauma) predicted PTSD development at one and six months post-trauma (Sun et al, 2013). Alternatively, lower uncinate fasciculus tract integrity in PTSD may be an acquired characteristic. For example, soldiers with increased PTSD symptoms after military deployment showed decreased uncinate fasciculus tract integrity, compared to pre-deployment baseline (Admon et al, 2013). In addition, higher anxiety symptoms 3-4 months post-trauma were associated with an increase in FA of the uncinate fasciculus and left anterior cingulum from pre-trauma to 3-4 months post-trauma (Sekiguchi et al, 2014).

Contrary to previous DTI studies, we did not find significant WM alterations in PTSD in the cingulum bundle and superior parietal fasciculus. The cingulum connects the cingulate cortex to the hippocampus and amygdala (Schmahmann and Pandya, 2006) and decreased structural connectivity between these areas was hypothesized to impair fear extinction learning in PTSD (Fani et al, 2012b). Notably, WM alterations of the cingulum bundle may develop over time with persistent PTSD symptoms: higher FA values of the dorsal cingulum were found post-treatment in males with persistent PTSD (i.e. who did not recover after treatment), compared to pre-treatment baseline, whereas no baseline differences were observed.
White matter tract integrity in PTSD between PTSD patients and combat-exposed controls (Kennis et al, 2015). However, both increased and decreased FA values were previously found in the cingulum bundle and superior longitudinal fasciculus in PTSD patients (Daniels et al, 2013). Contradictory results may be explained by differences in study designs, such as control group used (i.e. non-traumatized or traumatized controls), the duration of PTSD and sex specific effects on tract integrity. We observed lower WM integrity of all investigated WM bundles in females, compared to males, independent of PTSD diagnosis. Previous DTI studies also showed lower FA, but higher MD and RD (i.e. generally indicating lower WM integrity) in various WM tracts in healthy females compared to males (Kranz et al, 2014). Moreover, although we did not find altered WM integrity between male and female PTSD patients, the neurobiology of PTSD may be different for males and females (Shvil et al, 2014). Therefore, it is important to investigate the neurobiological underpinnings of the sex-related WM alterations, both in healthy individuals and in PTSD.

Limitations
Some limitations of our study need to be mentioned. First, we included a specific sample of highly trauma-exposed police officers, who experienced high levels of work-related traumatic events. Although this provided a homogeneous sample and robust control for neurobiological effects of trauma, it may also have limited the generalizability of our findings to other PTSD patient groups (i.e. civilian trauma). Secondly, we included some PTSD patients with (mild to moderate) MDD which may have influenced our findings (Isaac et al, 2015). However, bilateral uncinate fasciculus tract integrity did not correlate with depression severity in PTSD, suggesting that our findings were not confounded by comorbid depressive symptoms.

Conclusion
We investigated WM integrity of major WM tracts in male and female PTSD patients and trauma-exposed controls using global probabilistic tractography for the first time. Decreased right uncinate fasciculus tract integrity was observed in male and female PTSD patients compared to trauma-exposed controls, which was associated with higher anxiety symptoms, as well as with higher amygdala activity and reduced vmPFC deactivation towards emotional stimuli. Although replication is warranted, these findings fit with the prevailing neurocircuitry model of PTSD, suggesting that ineffective communication between the vmPFC and amygdala results in reduced top-down control over fear-responses.
ACKNOWLEDGEMENTS

The authors thank all participants for their participation in this study. We thank Renée Hutter, Gré Westerveld, Marthe Hoofwijk and all other personnel of the PDC police outpatient clinic for their valuable help with recruitment of the PTSD patients. The study is supported by grants from ZonMw, the Netherlands organization for Health Research and Development (grant no. 40-00812-98-10041) and the Academic Medical Center Research Council (110614). All authors declare that they have no biomedical financial interests and no potential conflicts of interest.
SUPPLEMENTARY MATERIAL – CHAPTER 7

Supplementary Methods

Emotional face-matching task

The emotional face-matching task consisted of three conditions: one visuomotor control condition with scrambled faces (4 blocks), one emotional condition with fearful and angry faces (2 blocks), and one emotional condition with happy and neutral faces (2 blocks) (Koch et al., 2016a) (Chapter 3). During each trial (duration 5 seconds), three faces were presented: one face on top (cue) and two faces below (target). Participants had to match the emotion or orientation (control blocks) of the cue stimulus with one of the two targets below.

The fMRI images were analyzed with SPM8 (http://fil.ion.ucl.ac.uk/spm/software/spm8). Pre-processing of the fMRI images consisted of realignment, slice-time correction, co-registration to the anatomical scan, segmentation, spatial normalization to the Montreal Neurological Institute (MNI) template, resampling to 2mm³ voxels and spatial smoothing with a 5mm full-width half maximum (FWHM) Gaussian kernel. At the first-level, the two emotional conditions were modelled as box-car regressors, convolved with a canonical hemodynamic response function. We included the six realignment parameters to control for movement. A high-pass filter (cut-off 1/200 Hz) was used to remove slow drifts of the signal and the AR(1) process was used to remove temporal autocorrelation. We obtained contrast images for the fearful-angry condition and the happy-neutral condition versus the visuomotor control condition. The contrast images were subsequently entered in a second-level repeated-measures ANOVA with task condition (fearful-angry – happy-neutral) as within-subject factor and group (PTSD – controls) and sex (male – female) as between-subject factors. The covariate medication-order was included to control for possible confounding effects of order in which medication was administered. Only contrast images acquired after placebo administration were used for the current analysis. The female PTSD patient who started using benzodiazepines between the first and second scanning session was excluded from this analysis.
SUPPLEMENTARY FIGURE S7.1 Design of the emotional face-matching task and task activation

(A) The emotional face-matching task consisted of three conditions: a visuomotor control condition with scrambled faces, one emotion condition with fearful-angry faces (blue) and one emotion condition with happy-neutral faces (purple). (B) Bilateral amygdala activation and (D) vmPFC deactivation towards emotional faces (fearful-angry and happy-neutral condition combined) vs the visuomotor control condition on a single-subject template image (P_{FWE}<.05 whole-brain corrected). (C) Contrast estimates of amygdala activation and (E) vmPFC deactivation towards fearful-angry faces (blue) and happy-neutral faces (purple). PTSD patients showed significantly less vmPFC deactivation compared to controls. * p <.05.
**SUPPLEMENTARY TABLE S7.1 Correlation of FA and MD values of each tract**

<table>
<thead>
<tr>
<th></th>
<th>Left Hemisphere</th>
<th>Right Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncinate Fasciculus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Hemisphere</td>
<td>FA(^1) 1</td>
<td>FA (r = .685^{<strong>} (n=74)) (r = .430^{</strong>} (n=74)) 1</td>
</tr>
<tr>
<td></td>
<td>MD(^1) (r = -.605^{**} (n=73)) 1</td>
<td>MD (r = -.425^{<strong>} (n=74)) (r = .706^{</strong>} (n=74)) (r = -.543^{**} (n=75)) 1</td>
</tr>
<tr>
<td>Right Hemisphere</td>
<td>FA (r = .685^{<strong>} (n=74)) (r = -.425^{</strong>} (n=74)) 1</td>
<td>FA (r = .798^{**} (n=75)) (r = -.280^{*} (n=74)) 1</td>
</tr>
<tr>
<td></td>
<td>MD (r = -.381^{**} (n=74)) 1</td>
<td>MD (r = -.268^{*} (n=74)) (r = .535^{<strong>} (n=73)) (r = -.315^{</strong>} (n=74)) 1</td>
</tr>
<tr>
<td><strong>Cingulum Bundle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Hemisphere</td>
<td>FA (r = .798^{**} (n=75)) (r = -.280^{*} (n=74)) 1</td>
<td>FA (r = .740^{<strong>} (n=74)) (r = -.420^{</strong>} (n=74)) 1</td>
</tr>
<tr>
<td></td>
<td>MD (r = -.627^{**} (n=74)) 1</td>
<td>MD (r = -.691^{<strong>} (n=74)) (r = .728^{</strong>} (n=75)) (r = -.623^{**} (n=74)) 1</td>
</tr>
<tr>
<td>Right Hemisphere</td>
<td>FA (r = .761^{<strong>} (n=75)) (r = -.569^{</strong>} (n=75)) 1</td>
<td>FA (r = .656^{<strong>} (n=75)) (r = -.656^{</strong>} (n=75)) 1</td>
</tr>
<tr>
<td></td>
<td>MD (r = .705^{<strong>} (n=75)) (r = .841^{</strong>} (n=75)) (r = -.713^{**} (n=75)) 1</td>
<td></td>
</tr>
</tbody>
</table>

1) One outlier (Z>2.58) removed from analysis; FA = fractional anisotropy; MD = mean diffusivity; N = sample size; SLF = superior longitudinal fasciculus; r = Pearson correlation coefficient; # p<.1; * p<.05; **p<.01
### Chapter 7

#### SUPPLEMENTARY TABLE S7.2 Results of the repeated-measures ANOVA’s of each tract

<table>
<thead>
<tr>
<th>Tract</th>
<th>FA values</th>
<th>MD values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(df)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Uncinate Fasciculus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-subject effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of group</td>
<td>F(1,66) = .130</td>
<td>.719</td>
</tr>
<tr>
<td>Main effect of sex (FA males &gt; females)</td>
<td>F(1,66) = 5.357</td>
<td>.024*</td>
</tr>
<tr>
<td>Group x sex interaction</td>
<td>F(1,66) = 1.209</td>
<td>.276</td>
</tr>
<tr>
<td>Within-subject effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of hemisphere</td>
<td>F(1,66) = .022</td>
<td>.883</td>
</tr>
<tr>
<td>Hemisphere x group interaction</td>
<td>F(1,66) = 1.513</td>
<td>.223</td>
</tr>
<tr>
<td>Hemisphere x sex interaction</td>
<td>F(1,66) = .941</td>
<td>.343</td>
</tr>
<tr>
<td>Hemisphere x group x sex interaction</td>
<td>F(1,66) = .789</td>
<td>.378</td>
</tr>
<tr>
<td><strong>Cingulum Bundle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-subject effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of group</td>
<td>F(1,68) = .521</td>
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<tr>
<td>Main effect of sex (FA males &gt; females)</td>
<td>F(1,68) = 6.701</td>
<td>.012*</td>
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<tr>
<td>Group x sex interaction</td>
<td>F(1,68) = .779</td>
<td>.380</td>
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<tr>
<td>Within-subject effects</td>
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<tr>
<td>Main effect of hemisphere</td>
<td>F(1,68) = 2.387</td>
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<tr>
<td>Hemisphere x group interaction</td>
<td>F(1,68) = 2.479</td>
<td>.120</td>
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<td>Hemisphere x sex interaction</td>
<td>F(1,69) = 1.673</td>
<td>.200</td>
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<td>Hemisphere x group x sex interaction</td>
<td>F(1,69) = .327</td>
<td>.569</td>
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<td><strong>Superior longitudinal fasciculus – parietal projections</strong></td>
<td></td>
<td></td>
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<tr>
<td>Between-subject effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of group</td>
<td>F(1,68) = 2.757</td>
<td>.101</td>
</tr>
<tr>
<td>Main effect of sex (MD females &gt; males)</td>
<td>F(1,68) = 3.865</td>
<td>.053</td>
</tr>
<tr>
<td>Group x sex interaction</td>
<td>F(1,68) = .090</td>
<td>.765</td>
</tr>
<tr>
<td>Within-subject effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of hemisphere (MD left &gt; right)</td>
<td>F(1,68) = .897</td>
<td>.347</td>
</tr>
<tr>
<td>Hemisphere x group interaction</td>
<td>F(1,68) = .620</td>
<td>.434</td>
</tr>
<tr>
<td>Hemisphere x sex interaction</td>
<td>F(1,68) = 1.391</td>
<td>.242</td>
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<tr>
<td>Hemisphere x group x sex interaction</td>
<td>F(1,68) = .017</td>
<td>.897</td>
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<td><strong>Superior longitudinal fasciculus – temporal projections</strong></td>
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<tr>
<td>Between-subject effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of group</td>
<td>F(1,69) = 2.668</td>
<td>.107</td>
</tr>
<tr>
<td>Main effect of sex (MD females &gt; males)</td>
<td>F(1,69) = 1.568</td>
<td>.215</td>
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<tr>
<td>Group x sex interaction</td>
<td>F(1,69) = .165</td>
<td>.686</td>
</tr>
<tr>
<td>Within-subject effects</td>
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</tbody>
</table>
### SUPPLEMENTARY TABLE S7.2 Results of the repeated-measures ANOVA’s of each tract (continued)

<table>
<thead>
<tr>
<th></th>
<th>FA values</th>
<th></th>
<th>MD values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(df)</td>
<td>P-value</td>
<td>F(df)</td>
<td>P-value</td>
</tr>
<tr>
<td>Main effect of hemisphere (MD left &gt; right)</td>
<td>F(1,69) = .166</td>
<td>.685</td>
<td>F(1,69) = 6.874</td>
<td>.011*</td>
</tr>
<tr>
<td>Hemisphere x group interaction</td>
<td>F(1,69) = .096</td>
<td>.758</td>
<td>F(1,69) = .742</td>
<td>.392</td>
</tr>
<tr>
<td>Hemisphere x sex interaction</td>
<td>F(1,69) = 2.768</td>
<td>.101</td>
<td>F(1,69) = .885</td>
<td>.350</td>
</tr>
<tr>
<td>Hemisphere x group x sex interaction</td>
<td>F(1,69) = .948</td>
<td>.334</td>
<td>F(1,69) = .000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Results of the repeated measures ANOVA’s on FA and MD values of the uncinate fasciculus, cingulum bundle and parietal and temporal projections of the superior longitudinal fasciculus (SLF), corrected for age and tract volume. A p-value <.017 was regarded significant for main and interaction effects. For the post-hoc test of the group x hemisphere interaction effect, a p-value of <.025 was regarded significant.

df = degrees of freedom; FA = fractional anisotropy; MD = mean diffusivity; * significant at p-corrected <.05 level; # significant at p-corrected <.1 level;
### SUPPLEMENTARY TABLE S7.3 Significant results of trajectory analysis

<table>
<thead>
<tr>
<th>Tract</th>
<th>FA values</th>
<th>MD values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(df)</td>
<td>P</td>
</tr>
<tr>
<td>Uncinate Fasciculus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Between-subject effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of group (MD PTSD &gt; controls)</td>
<td>F(1,60) = .001</td>
<td>.976</td>
</tr>
<tr>
<td>Main effect of sex (FA males &gt; females)</td>
<td>F(1,60) = 7.575</td>
<td>.008*</td>
</tr>
<tr>
<td><strong>Within-subject effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of segment</td>
<td>F(2.625, 157.487) = 1.378</td>
<td></td>
</tr>
<tr>
<td>Cingulum Bundle</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Between-subject effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of sex (FA males &gt; females)</td>
<td>F(1,61) = 6.707</td>
<td>.012*</td>
</tr>
<tr>
<td><strong>Within-subject effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of segment</td>
<td>F(2.413, 147.186) = 8.911</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Superior longitudinal fasciculus – temporal projections</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Between-subject effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of sex (FA males &gt; females)</td>
<td>F(1,61) = 7.404</td>
<td>.008*</td>
</tr>
<tr>
<td><strong>Within-subject effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of hemisphere</td>
<td>F(3.745, 265.178) = .396</td>
<td>.963</td>
</tr>
<tr>
<td>Main effect of segment</td>
<td>F(3.745, 265.178) = 1.398</td>
<td>.739</td>
</tr>
<tr>
<td>Segment x group interaction</td>
<td>F(3.745, 265.178) = .479</td>
<td>.739</td>
</tr>
<tr>
<td>Segment x sex interaction</td>
<td>F(3.745, 265.178) = 1.398</td>
<td>.238</td>
</tr>
<tr>
<td>Hemisphere x segment x group</td>
<td>F(4.473, 272.824) = .871</td>
<td>.492</td>
</tr>
<tr>
<td>Hemisphere x segment x group</td>
<td>F(4.473, 272.824) = 1.195</td>
<td>.313</td>
</tr>
</tbody>
</table>

Significant results of the repeated measures ANOVA’s on FA and MD values of the uncinate fasciculus, cingulum bundle and parietal and temporal projections of the superior longitudinal fasciculus (SLF), corrected for age and tract volume. A p-value of <.011 was regarded significant for all main and interaction effects; a p-value of <.022 was regarded trend significant.

df = degrees of freedom; FA = fractional anisotropy; MD = mean diffusivity; * significant at p-corrected <.05 level; # significant at p-corrected <.1 level;
### SUPPLEMENTARY TABLE S7.4 Correlations between right uncinate fasciculus integrity and symptom severity in PTSD patients

<table>
<thead>
<tr>
<th></th>
<th>Right uncinate fasciculus</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FA</td>
<td>MD</td>
<td></td>
</tr>
<tr>
<td>CAPS total score</td>
<td>r = .055 (n=33)</td>
<td>r = .017 (n=33)</td>
<td></td>
</tr>
<tr>
<td>CAPS – re-experiencing (cluster B)</td>
<td>r = .103 (n=33)</td>
<td>r = .042 (n=33)</td>
<td></td>
</tr>
<tr>
<td>CAPS – avoidance (cluster C)</td>
<td>r = .133 (n=33)</td>
<td>r = .007 (n=33)</td>
<td></td>
</tr>
<tr>
<td>CAPS – hyperarousal (cluster D)</td>
<td>r = -.025 (n=33)</td>
<td>r = -.025 (n=33)</td>
<td></td>
</tr>
</tbody>
</table>

Correlations between right uncinate fasciculus tract integrity measures (FA and MD) and PTSD symptom severity (CAPS total score and subscales). Partial correlations were conducted, controlling for age. $r$ = Pearson correlation coefficient; FA = fractional anisotropy; MD = mean diffusivity; CAPS = clinician administered PTSD scale.
‘BOOSTING OXYTOCIN AFTER TRAUMA - Effects of oxytocin on fear neurocircuitry in patients with post-traumatic stress disorder’
After experiencing a traumatic event, about 10% of individuals develop a post-traumatic stress disorder (PTSD) (American Psychiatric Association, 2013; de Vries and Olff, 2009). PTSD is a disabling psychiatric disorder, characterized with exaggerated fear responses towards reminders of the traumatic event. Although generally effective, a substantial part of PTSD patients does not fully benefit from existing psychotherapies (Bradley et al., 2005), underlining the need to investigate new psychological or pharmacological interventions to enhance treatment response in PTSD. Oxytocin has been suggested as promising pharmacological adjuvant for medication-enhanced psychotherapy in PTSD, by dampening the exaggerated fear response, as well as enhancing motivation for therapy and improving the therapeutic alliance (Olff et al., 2010).

As a first step to investigate the therapeutic potential of oxytocin in PTSD, we conducted an fMRI study on the effects of oxytocin on fear- and reward-related neural processes in male and female police officers with (n=37, 21 males) and without (n=40, 20 males) PTSD (the BOOSTER study). The aim of this PhD thesis was to describe the acute effects of a single oxytocin administration on fear neurocircuitry in PTSD patients (Chapter 2-5). In the second part of this thesis, the neurobiology of PTSD was investigated, focusing on altered resting-state brain activity and connectivity, as well as structural connectivity of major white matter tracts (Chapter 6-7). This general discussion will commence with a summary of the main findings and conclusions of the previous chapters. Next, these findings will be integrated in the existing literature, the clinical implications of our findings and limitations will be discussed and a research agenda will be provided.

**SUMMARY OF FINDINGS**

**Part 1: Oxytocin effects on fear neurocircuitry in PTSD**

Chapter 2 (Koch et al., 2014) consisted of an in-depth literature review describing the fear neurocircuitry of healthy participants, and its alterations in PTSD. In addition, the hypothesized effects of oxytocin on the fear neurocircuitry in PTSD were discussed. According to the literature review, PTSD is associated with increased activity and connectivity in salience processing areas (i.e. amygdala, anterior insula and dorsal anterior cingulate cortex), as well as with decreased prefrontal control over amygdala-mediated fear responsiveness. Oxytocin was hypothesized to enhance treatment response in PTSD via a dual mechanism. First, oxytocin may reduce amygdala hyperactivity and enhance functional connectivity between the amygdala and vmPFC. Additionally, oxytocin may result in improved treatment response by enhancing the therapeutic alliance and motivation for treatment, via increased neural reward sensitivity.
In Chapter 3 (Koch et al, 2016a) we investigated the effects of a single oxytocin administration on amygdala reactivity towards emotional faces (i.e. fearful-angry and happy-neutral) in male and female police officers with and without PTSD. We observed potential anxiolytic properties of oxytocin administration in PTSD patients, as evidenced by dampened left amygdala reactivity towards salient stimuli after oxytocin administration.

Under placebo, the expected valence-dependent effect of greater amygdala activity towards fearful-angry compared to happy-neutral faces (Morris et al, 1996) was absent in male and female PTSD patients. Moreover, a positive correlation was found between state anxiety and amygdala reactivity towards happy-neutral (but not fearful-angry) faces in PTSD patients. These findings may indicate diminished differentiation between stimuli potentially signaling negative or threatening information (i.e. fearful-angry faces) and stimuli signaling positive and/or neutral, socially ambiguous and therefore potentially threatening information (i.e. happy-neutral faces) in PTSD patients.

After oxytocin administration, left amygdala reactivity towards all emotional faces was significantly dampened in PTSD patients, independent of stimulus valence and participant’s sex (Koch et al, 2016a). In male and female trauma-exposed controls, on the other hand, oxytocin administration enhanced amygdala reactivity towards all emotional faces. These findings underline recent suggestions that oxytocin effects depend on inter-individual factors, such as (severity of) psychopathology (Bartz et al, 2011b; Olff et al, 2013), with greater (anxiolytic) effects in individuals with suboptimal baseline fear regulation (Labuschagne et al, 2010) (see below for a discussion on this issue). In line with this hypothesis, dampening of amygdala reactivity after oxytocin administration was greater in PTSD patients reporting higher anxiety under placebo.

In Chapter 4 (Koch et al, 2016c), we investigated the effects of oxytocin administration on subjective anxiety and resting-state functional connectivity of the centromedial (CeM) and basolateral (BLA) amygdala subregions with prefrontal and salience processing areas in male and female PTSD patients and trauma-exposed controls. We found dampened subjective and amygdala-mediated fear processing after oxytocin administration in PTSD patients, and our findings again emphasized that oxytocin effects were dependent on individual differences (Bartz et al, 2011b; Olff et al, 2013).

Compared to male trauma-exposed controls, male PTSD patients showed decreased functional connectivity between the right CeM and left ventromedial prefrontal cortex (vmPFC) under placebo, possibly indicating decreased prefrontal inhibitory control over the amygdala-mediated fear response (Jovanovic and Ressler, 2010). After oxytocin, functional connectivity between the CeM and vmPFC was reinstated in male PTSD patients, abolishing the under
placebo observed group difference between male patients and trauma-exposed controls. Oxytocin presumably resulted in increased inhibitory prefrontal control over the amygdala in male PTSD patients, which may result in decreased amygdala fear processing. Compared to female trauma-exposed controls, female PTSD patients showed enhanced functional connectivity between the right BLA and right dACC under placebo (Koch et al., 2016c), which may represent the neural correlate of hypervigilance. After oxytocin administration in female PTSD patients, functional connectivity between the BLA and dACC was dampened to the same level as in female trauma-exposed controls, possibly decreasing excitatory projections of the dACC to the BLA and hence symptoms of hypervigilance.

Behaviorally, male and female PTSD patients reported decreased anxiety and nervousness (but no alterations in happiness and sadness) after oxytocin administration, compared to placebo. Moreover, the majority of PTSD patients (i.e. 77.1%) was able to correctly indicate treatment allocation after the second scanning session, primarily based on noticeable psychological oxytocin effects. In male and female trauma-exposed controls, on the other hand, no behavioral or neural oxytocin administration effects were found.

Finally, in Chapter 5, we investigated behavioral and neural oxytocin effects during distraction as cognitive emotion regulation strategy in male and female PTSD patients. The distraction condition consisted of working memory task (i.e. keeping a string of letters in mind) during simultaneous presentation of negative pictures. Oxytocin effects were dependent on baseline functioning under placebo, which was related to sex and (severity of) psychopathology: oxytocin reinstated the ability to down-regulate neural (i.e. posterior insula) processing during distraction in male PTSD patients, whereas it enhanced amygdala activity during distraction in female PTSD patients.

Under placebo, male and female PTSD patients rated all negative pictures (both in passive viewing and distraction condition) as significantly more negative compared to trauma-exposed controls. Contrary to our expectations and previous findings (Kanske et al., 2011; McRae et al., 2010), distraction in all participants did not result in more positive ratings of negative pictures, compared to passive viewing. This could have been due to a ceiling effect: our participants rated the negative pictures in the passive viewing condition as significantly more positive compared to normative ratings, and it may have been deemed socially inappropriate to positively evaluate pictures clearly depicting negative scenes during the distraction condition.

Under placebo, male PTSD patients showed no down-regulation of right-sided amygdala and posterior insula activity during distraction. Impaired dampening of posterior insula during distraction was positively correlated with hyper-arousal, intrusions and overall PTSD symp-
tom severity. These observations may indicate difficulty down-regulating neural processing towards negative evocative stimuli during distraction. After oxytocin administration, male PTSD patients rated all (neutral and negative) pictures as more positive, and showed right posterior insula (but not amygdala) down-regulation during distraction. Female PTSD patients, on the other hand, showed robust down-regulation of bilateral amygdala reactivity during distraction under placebo. Compared to female trauma-exposed controls, dampening of right-sided amygdala reactivity during distraction was even greater in female PTSD patients, which was positively related to severity of avoidance symptoms. After oxytocin administration, this group difference was abolished. Additionally, dampening of left-sided amygdala reactivity was diminished after oxytocin administration in all female participants, possibly indicating increased neural processing of negative stimuli during distraction in females.

Part 2: Neurobiological alterations in PTSD

In Chapter 6 (Koch et al., 2016b) we conducted a literature study on all currently available resting-state studies investigating brain activity and functional connectivity in PTSD patients, compared to healthy (trauma-exposed and non-trauma-exposed) controls. After a systematic literature search, we conducted a meta-analysis on whole-brain resting-state studies (n=14, including 298 PTSD patients and 365 controls) using the activation likelihood estimation (ALE) approach (Turkeltaub et al., 2002), to identify concurrence of hyper- and hypoactivations in PTSD during rest. Since seed-based functional connectivity studies could not be included in the quantitative meta-analysis, we additionally performed a separate systematic review on these studies (n=9). PTSD was associated with hypervigilance and enhanced salience processing during rest, at the cost of autobiographical memory and internal thoughts (i.e. posterior insula hypoactivity and decreased DMN connectivity) and emotion regulation abilities (i.e. cerebellar pyramis and middle frontal gyrus hypoactivity).

Results of the quantitative meta-analysis indicated consistent resting-state hyperactivity in the ventral anterior cingulate cortex (vACC) and parahippocampal gyrus/amygdala, as well as hypoactivity in the posterior insula, cerebellar pyramis and middle frontal gyrus in PTSD compared to healthy controls (Koch et al., 2016b). According to the systematic review on seed-based functional connectivity studies, PTSD was associated with enhanced functional connectivity between nodes of the salience network (SN), decreased functional connectivity between nodes of the default mode network (DMN) and altered between-network connectivity (Koch et al., 2016b; Lanius et al., 2015). Additionally, PTSD was associated with decreased functional connectivity between the prefrontal cortex and amygdala, possibly underlying diminished prefrontal inhibitory control over the fear response in PTSD (Rauch et al., 2006).
Finally, in Chapter 7 we investigated structural integrity of a priori defined major white matter tracts in PTSD patients, using diffusion tensor imaging (DTI). Based on previous DTI studies in PTSD patients (Daniels et al., 2013) and the predominant neurocircuitry model of PTSD (Rauch et al., 2006), we investigated white matter integrity of the bilateral uncinate fasciculus, cingulum and superior longitudinal fasciculus (both parietal and temporal projections) in PTSD patients using probabilistic tractography (Yendiki et al., 2011). We found anatomical evidence in line with the prevailing neurocircuitry model of PTSD, postulating that ineffective communication between the amygdala and vmPFC may result in decreased top-down control over the fear response and hence exaggerated fear.

We observed higher mean diffusivity (MD) of the right uncinate fasciculus (i.e. the major white matter tract connecting the amygdala to the prefrontal cortex) in male and female PTSD patients, compared to trauma-exposed controls. MD represents an overall measure of membrane integrity (Song et al., 2003). In PTSD patients, MD of the right uncinate fasciculus was positively correlated with anxiety symptoms, and amygdala and vmPFC activation towards happy-neutral faces. No other group differences were observed regarding left uncinate fasciculus tract integrity, or integrity of the other major white matter tracts.

INTEGRATION OF FINDINGS

In this section, the findings of the previous chapters will be integrated. First, the findings on the neurobiological correlates of PTSD will be combined, based on the literature review and meta-analysis of resting-state studies (Chapter 6), the structural connectivity study (Chapter 7) and functional differences in amygdala activity and connectivity between PTSD patients and trauma-exposed controls observed under placebo (Chapter 3-5). Secondly, the findings of oxytocin administration effects on amygdala activity and connectivity in PTSD (Chapter 3-5) will be integrated.

Neurobiology of PTSD

Based on previous neurobiological studies, the prevailing neurocircuitry model of PTSD postulates amygdala hyperactivity towards trauma-and threat-related stimuli, possibly resulting from deficient top-down prefrontal control over the fear response (Rauch et al., 2006). Contrary to this model, we did not find evidence for overall amygdala hyperactivity towards emotional stimuli (i.e. positive and negative faces and negative scenes) in PTSD patients compared to trauma-exposed controls. Possibly, PTSD is associated with amygdala hyperactivity towards trauma-related stimuli only, which does not generalize to the emotional, but trauma-unrelated stimuli used in our study. In line with this suggestion, a previous fMRI study found no amygdala hyperactivity towards trauma-unrelated positive and negative
pictures in male veterans with PTSD, compared to combat-exposed controls (van Rooij et al., 2014). Additionally, it has been suggested that amygdala hyperactivity may result as a consequence of trauma exposure, rather than PTSD per se. For example, enhanced amygdala activity towards emotional faces was observed post-deployment in healthy soldiers, compared to pre-deployment baseline (van Wingen et al., 2011). Furthermore, a meta-analysis on functional neuroimaging studies in PTSD found amygdala hyperactivity in PTSD when compared to non-trauma-exposed controls, but not when compared to trauma-exposed controls (Patel et al., 2012). These findings emphasize that neurobiological alterations of PTSD should be investigated relative to a trauma-exposed control group, thereby controlling for the potentially confounding effects of trauma exposure on neurobiology.

Our results, however, do suggest that PTSD is associated with impairments down-regulating amygdala activity in potentially safe, unthreatening contexts. That is, during the emotional face-matching task (Chapter 3), PTSD patients did not show the expected valence-dependent pattern of decreased amygdala activity towards happy-neutral faces compared to fearful-angry faces (Morris et al., 1996). Notably, amygdala activity towards happy-neutral faces (but not fearful-angry faces) in PTSD patients was associated with higher state anxiety (Chapter 3), and decreased structural white matter integrity of the uncinate fasciculus, the major white matter tract connecting the amygdala and vmPFC (Chapter 7). Additionally, during the distraction task (Chapter 5), male PTSD patients showed difficulties down-regulating right amygdala (and posterior insula) activity during distraction (i.e. working memory performance), compared to the presumably more distressing passive viewing condition. Finally, we found meta-analytic evidence for hyperactivity in the parahippocampal gyrus/amygdala in PTSD patients during rest, compared to healthy (trauma-exposed and non-trauma-exposed) controls, which may again indicate deficient down-regulation of amygdala activity in PTSD during a potentially safe resting-state context (Chapter 6). Interestingly, PTSD has been associated with generalization of fear experienced during the traumatic event to safe contexts, with stimuli resembling aspects of the traumatic event (Lissek et al., 2005), possibly explaining our findings of deficient amygdala down-regulation in safe conditions in PTSD. As amygdala hyperactivity was associated with hyperarousal symptoms in PTSD (Lopresto et al., 2016; Ronzoni et al., 2016), our findings may represent the neural correlate of hypervigilance in PTSD, even in trauma-unrelated, safe contexts.

It has been suggested that diminished top-down prefrontal inhibitory control over the amygdala may underlie deficits in down-regulating amygdala activity in PTSD (Rauch et al., 2006), and that connectivity strength of the pathway connecting the amygdala and vmPFC may be more informative in explaining fear and anxiety than activity of either brain region alone (Kim et al., 2011b). Structurally, we observed decreased white matter integrity of the uncinate fasciculus (i.e. the tract connecting the amygdala with the prefrontal cortex) in male
Summary and general discussion

and female PTSD patients, which was related to higher state anxiety, as well as with higher amygdala reactivity and decreased vmPFC suppression towards emotional stimuli within PTSD patients (Chapter 7). Functionally, PTSD was associated with decreased functional connectivity between the prefrontal cortex and amygdala, according to the systematic review of seed-based resting-state studies in PTSD patients (Chapter 6). Moreover, we found decreased functional connectivity between the centromedial nucleus of the amygdala (CeM) and the vmPFC in male PTSD patients compared to male trauma-exposed controls (Chapter 4), possibly indicating deficient inhibitory prefrontal control over the amygdala. In line with this finding, decreased connectivity between the amygdala and vmPFC was previously found in male veterans with PTSD (Sripada et al, 2012a). In female PTSD patients, on the other hand, we found enhanced connectivity between the basolateral nucleus of the amygdala (BLA) and dACC compared to female trauma-exposed controls. Previously, increased functional connectivity between the BLA and dACC was also found in male veterans with PTSD (Brown et al, 2014). As the amygdala and dACC are both core nodes of the salience network (i.e. involved in directing attention to salient biological information in the environment (Seeley et al, 2007)), we hypothesized that increased functional connectivity between the dACC and BLA may represent the neural signature of hypervigilance symptoms, especially in female PTSD patients. Supporting this suggestion of enhanced neural salience processing in PTSD, we found evidence in the existing PTSD literature for increased connectivity between core nodes of the salience network, but decreased connectivity between core nodes of the default mode network in PTSD patients during rest (Chapter 6).

Importantly, our observations strongly suggest that the neurobiology of PTSD is partly different for males and females: male and female PTSD patients showed distinct amygdala subregion functional connectivity alterations (Chapter 4), as well as distinct (in)abilities to down-regulate amygdala reactivity during distraction as emotion regulation strategy (Chapter 5). Male PTSD patients showed impaired down-regulation of neural processing towards negative stimuli during distraction, which was related to overall PTSD symptom severity. Female PTSD patients, on the other hand, showed robust dampening of amygdala reactivity during distraction, which was positively related to avoidance symptom severity (Chapter 5). Although sex differences in neurobiology of PTSD have scarcely been studied, evidence exists for sex-differential effects on neurobiology of PTSD (Felmingham et al, 2010; Shvil et al, 2014). According to a recent rodent study, high freezing behavior during extinction (i.e. indicating worse extinction learning) was associated with altered extinction learning in male animals, but altered fear acquisition in female animals (Gruene et al, 2015). Furthermore, in human studies, male PTSD patients showed greater skin conductance response and enhanced dACC activity during extinction recall compared to female PTSD patients (Shvil et al, 2014). Additionally, enhanced hippocampal activity towards masked fearful faces was observed in male PTSD patients, compared to female PTSD patients (Felmingham et al, 2010). However,
male and female PTSD patients may have experienced different types of traumatic events (i.e. more work-related traumatic events in males, more interpersonal traumatic events in females), potentially confounding the abovementioned sex-differential findings (Shvil et al, 2014) and underlining the importance to investigate the effects of both sex and type of trauma exposure on neurobiology of PTSD.

Taken together, our findings generally confirmed the traditional neurocircuity model of PTSD, emphasizing diminished structural and functional connectivity between the amygdala and prefrontal cortex, as well as increased functional connectivity between core nodes of the salience network in PTSD patients. Increased activity and connectivity between core nodes of the salience network may represent the neural correlate of hypervigilance symptoms in PTSD. However, our findings challenge the (generally accepted) notion of general amygdala hyperactivity as important neurobiological signature of PTSD (Rauch et al, 2006). Rather, amygdala responsivity may be altered in PTSD in a more subtle way: PTSD may be associated with difficulties down-regulating amygdala reactivity under safe conditions, possibly representing the neural correlate of hypervigilance symptoms. Additionally, amygdala hyperactivity in PTSD may become apparent towards trauma-related stimuli only, and not towards emotional, trauma-unrelated stimuli. Finally, our findings emphasize the importance to take sex differences into account when investigating neurobiological correlates of PTSD, which seems especially important since the prevalence of PTSD is two to three times higher in females than in males (Olff et al, 2007).

**Effects of oxytocin administration in PTSD patients**

In line with the previously observed anxiolytic effects of oxytocin (Heinrichs et al, 2003; de Oliveira et al, 2012), intranasal oxytocin administration in male and female PTSD patients resulted in decreased self-reported anxiety and nervousness, but did not affect mood ratings (Chapter 4). Corroborating with our findings, subjective anxiety during public speaking stress was found to be reduced after oxytocin administration in healthy individuals (de Oliveira et al, 2012). In addition, male (but not female) PTSD patients rated all neutral and negative pictures as more positive after oxytocin (Chapter 5). Moreover, PTSD patients (but not trauma-exposed controls) were successful at correctly indicating treatment allocation during the second session, which was primarily based on subjective psychological effects of oxytocin administration, such as changes in concentration, increased calmness and sleepiness (Chapter 4).

In accordance with these anxiolytic effects of oxytocin at the behavioral level, oxytocin administration dampened amygdala reactivity towards emotional faces in PTSD patients, independent of valence or participant’s sex (Chapter 3). These findings are in line with previous oxytocin administration studies in healthy males (Kirsch et al, 2005) and psychiatric
patients with high anxiety (Bertsch et al., 2013; Labuschagne et al., 2010), in which oxytocin diminished amygdala reactivity towards emotional stimuli. Moreover, findings of the amygdala functional connectivity study (Chapter 4) suggested that oxytocin may have resulted in decreased amygdala activity via different mechanisms in male and female PTSD patients, influencing aberrant sex-dependent functional connectivity patterns observed under placebo: oxytocin presumably resulted in increased inhibitory control of the vmPFC over the CeM in male PTSD patients and in decreased excitatory projections of the dACC to the BLA in female PTSD patients (Chapter 4). Decreased amygdala activity may have resulted in dampened subjective anxiety and nervousness in male and female PTSD patients. In line with previous observations in the oxytocin literature (Bertsch et al., 2013; Labuschagne et al., 2010), it seems that oxytocin especially acted on suboptimal amygdala-mediated neural fear processes observed under placebo. For instance, previous studies showed that oxytocin especially down-regulated amygdala hyperactivity observed under placebo in psychiatric patients compared to healthy controls, whereas no or opposite oxytocin effects were found in healthy controls (Bertsch et al., 2013; Labuschagne et al., 2010).

An increasing body of literature suggests that the effects of oxytocin administration depend on individual differences in baseline neural functioning, which may be related to individual characteristics such as psychopathology, sex and personality traits (Bartz et al., 2011b; Labuschagne et al., 2010; Olff et al., 2013; Rilling et al., 2014). For example, oxytocin administration was found to increase rather than decrease amygdala reactivity in healthy females (Domes et al., 2010; Lischke et al., 2012). It has been suggested that the effects of oxytocin follow an inverted U-shaped dose response curve, with more beneficial (anxiolytic) oxytocin effects in individuals with suboptimal fear regulation, but no or even anxiogenic effects in individuals with optimal fear regulation (Labuschagne et al., 2010; Rilling et al., 2014). Our findings generally corroborate this suggestion. For example, PTSD patients reporting higher anxiety before scanning and greater amygdala reactivity towards emotional faces under placebo (i.e. suboptimal fear regulation) showed greater reduction of amygdala activity after oxytocin administration (Chapter 3), indicating greater anxiolytic oxytocin effects in PTSD patients with higher baseline anxiety. Furthermore, the sex-dependent findings regarding oxytocin effects on distraction as emotion regulation strategy may be explained from this viewpoint: whereas oxytocin reinstated down-regulation of the posterior insula during distraction in male PTSD patients, oxytocin diminished amygdala down-regulation during distraction in female participants (both PTSD patients and controls), who already showed amygdala down-regulation during distraction under placebo (Chapter 5). Interestingly, fear regulation abilities may be dependent on functioning of the oxytocin system (Neumann and Landgraf, 2012), which may be altered in PTSD (Frijling et al., 2015). For example, a polymorphism of the oxytocin receptor gene (OXTR) in youth was associated with reduced amygdala volume and enhanced amygdala reactivity towards emotional faces, which was positively correlated with
early life stressors (Marusak et al, 2015). Moreover, within the same sample of participants described in this PhD thesis, we found decreased endogenous salivary oxytocin levels in male PTSD patients, compared to trauma-exposed controls (Frijling et al, 2015), indicating altered oxytocin system functioning in PTSD.

Finally, in our male and female trauma-exposed controls oxytocin administration resulted in increased amygdala reactivity towards emotional faces (Chapter 4). Additionally, oxytocin resulted in enhanced amygdala reactivity during distraction in female controls (Chapter 5). Whereas these findings are in line with previous oxytocin studies in healthy female participants (Bertsch et al, 2013; Domes et al, 2010; Lischke et al, 2012) and in recently trauma-exposed male and female emergency department patients (Frijling et al, 2016), they are at odds with previous observations in healthy males, generally showing dampened amygdala activity after oxytocin administration (Domes et al, 2007; Kirsch et al, 2005; Petrovic et al, 2008). Notably, our trauma-exposed control participants all experienced numerous (work-related) traumatic events during their lives, without developing significant (current and lifetime) PTSD symptoms or any other current psychiatric disorder, thus representing a resilient sample. Based on the dose-response-curve hypothesis of oxytocin outlined above, it may be reasoned that our trauma-exposed control participants did have little to gain regarding fear regulation, which may account for the opposite and absent oxytocin administration effects in this sample.

CLINICAL IMPLICATIONS, LIMITATIONS AND RESEARCH AGENDA

Clinical implications
As a first step to test the therapeutic potential of oxytocin administration in PTSD, we investigated the effects of a single oxytocin administration on fear neurocircuitry in male and female PTSD patients. Decreasing (amygdala-mediated) fear processing during treatment may have beneficial effects on outcome of trauma-focused psychotherapy. Previously, greater amygdala activity during extinction learning was found to impair extinction learning in healthy participants (Milad et al, 2009). Extinction learning is the hypothesized underlying mechanism of exposure therapy (Rothbaum and Davis, 2003). In PTSD patients, greater amygdala reactivity towards emotional stimuli prior to treatment was related to worse treatment outcome (Bryant et al, 2008a; van Rooij et al, 2015). Therefore, it has been suggested that dampening excessive (amygdala) fear processing during psychotherapy may enhance extinction learning and improve emotion regulation abilities (Bryant et al, 2008a), which may result in enhanced treatment outcome. In line with this suggestion, improving emotion regulation abilities (such as distraction) in PTSD patients prior to cognitive behavioral therapy (CBT) was found to enhance treatment response (i.e. decreased drop-out rate.
and PTSD symptoms at follow-up) compared to a supportive counseling group (Bryant et al., 2013). We found that oxytocin administration in PTSD patients resulted in decreased self-reported anxiety and nervousness (Chapter 4) and dampened amygdala reactivity towards emotional stimuli (Chapter 3), possibly via increased inhibitory prefrontal control over the amygdala in male PTSD patients, and via decreased connectivity between salience network nodes in female PTSD patients (Chapter 4). We did not find evidence for overall amygdala hyperactivity in PTSD patients under placebo (Chapter 3), but our results support the notion that PTSD is rather associated with impaired amygdala down-regulation in safe contexts. Our findings suggest that oxytocin could potentially enhance treatment response in PTSD, by dampening excessive fear processing during therapy. Notably, oxytocin effects were dependent on (deficits in) fear regulation abilities under placebo, normalizing sex-dependent aberrant amygdala functional connectivity patterns (Chapter 4) and showing sex-differential effects on neural processing during distraction: whereas oxytocin reinstated posterior insula down-regulation towards negative evocative stimuli during distraction in male PTSD patients, it increased amygdala processing during distraction in female PTSD patients who showed (normal) amygdala down-regulation under placebo (Chapter 5). The findings of overall improved affect and posterior insula down-regulation during distraction in male PTSD patients (Chapter 5) may hold promise for improving treatment response in PTSD, as improved emotion regulation abilities may enhance tolerance to exposure-related distress, thereby improving treatment response (Bryant et al., 2013). However, our observation of opposite oxytocin effects in female PTSD patients during distraction warrant more research into the effects of contextual and individual factors modulating oxytocin effects when investigating the clinical potential of oxytocin in psychiatric disorders, such as PTSD.

Recent rodent (Zoicas et al., 2014) and human (Acheson et al., 2013; Eckstein et al., 2014) oxytocin administration studies showed enhanced (recall of) extinction learning when oxytocin was administered after fear conditioning but prior to extinction learning. Additionally, oxytocin administration in healthy males resulted in dampened overall amygdala activity and increased prefrontal involvement during the early phase of extinction learning (Eckstein et al., 2014). As extinction is supposedly the underlying working mechanism of exposure therapy, oxytocin may hold promise to improve extinction learning in PTSD, thereby enhancing response to exposure therapy. However, when administered prior to fear conditioning in healthy males, oxytocin resulted in increased behavioral, physiological and cingulate cortex activity towards fear conditioned stimuli (Eckstein et al., 2016). Together with previous findings that oxytocin increased episodic memory for aversive social events (Striepens et al., 2012), these observations suggest time-dependent effects of oxytocin, with anxiogenic oxytocin effects when administered prior to fear learning (Eckstein et al., 2016; Striepens et al., 2012), but facilitation of extinction when administered prior to extinction learning (Acheson et al., 2013; Eckstein et al., 2014). Notably, medication-enhanced psychotherapy for PTSD with
oxytocin would involve administration of oxytocin prior to each exposure-based therapy session, i.e. prior to fear extinction learning. Whether (and under which circumstances) oxytocin administration results in improved extinction learning in PTSD patients, is an important and exciting avenue for future research.

Oxytocin could potentially also enhance treatment response for PTSD by increasing the therapeutic alliance and motivation for treatment (Olff et al., 2010). The strength of therapeutic alliance (i.e. the emotional bond between patient and therapist) is an important predictor of treatment success (Ormhaug et al., 2014). Oxytocin has been found to increase pro-social behavior, such as in-group trust and cooperation (De Dreu and Kret, 2016; Van IJzendoorn and Bakermans-Kranenburg, 2012), (neural) reward sensitivity towards social stimuli in healthy individuals (Groppe et al., 2013; Scheele et al., 2013) and empathic responses towards women in PTSD patients (Palgi et al., 2016). Moreover, in the same group of participants described in this PhD thesis, oxytocin enhanced neural processing of monetary reward and loss anticipation, as indicated by increased activity in key regions of the reward pathway (i.e. the striatum, anterior insula and dorsal anterior cingulate cortex) (Nawijn et al., 2016). Therefore, oxytocin could potentially enhance motivation for goal-directed approach behavior in PTSD (such as motivation for treatment), which may result in enhanced treatment response in PTSD. However, recent clinical (pilot) studies investigating oxytocin effects on psychotherapy for mood- and anxiety disorders showed mixed effects, such as increased subjective anxiety and symptom severity, nominally decreased therapeutic alliance and confidence in treatment, but also decreased social avoidance behavior (Acheson et al., 2015; Macdonald et al., 2013). Future clinical studies are therefore needed to investigate whether oxytocin can indeed increase motivation for treatment and the therapeutic alliance in PTSD patients.

Taken together, oxytocin holds therapeutic promise to enhance treatment response in PTSD: oxytocin has anxiolytic properties (this PhD thesis), may enhance extinction learning (Acheson et al., 2013; Eckstein et al., 2014) and could potentially improve motivation for treatment and the therapeutic alliance (Nawijn et al., 2016; Olff et al., 2010). However, careful attention should be paid to elucidating the effects of inter-individual and contextual factors of oxytocin effects on the abovementioned mechanisms. Clinical studies should investigate whether oxytocin administration indeed results in improved treatment response in PTSD, and under which circumstances and for which individuals.

**Limitations**

We included a specific, but homogeneous, sample of highly trauma-exposed police officers, thereby controlling for the potential confounding effect of trauma exposure on neurobiology. However, this may limit the generalizability of our findings regarding neurobiological alterations and oxytocin effects in PTSD to other PTSD patient groups (e.g. civilian trauma),
as different types of traumatic events may have different neurobiological correlates. On the same note, comparison of oxytocin findings in our trauma-exposed control sample to previous oxytocin findings in healthy participants was hindered by the specific characteristics of our samples, such as high police work-related trauma-exposure and high resilience. Additionally, although our overall sample size was reasonably large for an oxytocin administration study in psychiatric patients, it was relatively small to investigate oxytocin effects dependent on both sex and PTSD status. Taken together, our results should be interpreted with caution and future studies should replicate our (oxytocin) findings in other PTSD patient groups, preferably with other types of trauma exposure.

Finally, we included female participants taking oral contraceptives, which may have influenced oxytocin receptor affinity and basal oxytocin levels and therefore oxytocin administration effects (Caldwell et al., 1994). Fortunately, female trauma-exposed controls and PTSD patients did not differ regarding hormonal contraceptive use, which is therefore unlikely to account for the differential oxytocin findings between these groups. Additionally, we did not control for phase of menstrual cycle our female participants were tested in, as we tried to minimize the time interval and hence fluctuations in symptom severity between the two scanning sessions. This could potentially have confounded our findings, although similar oxytocin effects on amygdala reactivity were previously observed for healthy females tested in the luteal (Bertsch et al., 2013) and follicular (Domes et al., 2010) phase. Future studies investigating the (clinical) effects of oxytocin in PTSD should take phase of menstrual cycle and contraceptive use into account, especially since extinction learning was previously found to be influenced by phase of menstrual cycle, both in humans (Glover et al., 2015; Milad et al., 2006) and rodents (Gruene et al., 2015).

**Future perspectives**

It remains to be investigated whether the neurobiological alterations observed in PTSD have developed as consequence of the disorder or represent pre-existing neurobiological vulnerability factors for PTSD development. For example, lower uncinate fasciculus white matter integrity was previously associated with a genetic polymorphism, related to increased risk for stress-related psychopathology such as PTSD (i.e. 5HTTLPR, indicating low expression of the serotonin transporter) (Pacheco et al., 2009). On the other hand, soldiers with increased development of PTSD symptoms over the course of military deployment showed decreased uncinate fasciculus white matter integrity compared to pre-deployment baseline (Admon et al., 2013). Due to the cross-sectional nature of our study, however, we were not able to address this issue. Therefore, prospective studies investigating neurobiological vulnerability factors and neurobiological alterations as consequence of trauma exposure and/or PTSD development are important in elucidating these open questions. Additionally, our findings strongly suggest that the neurobiology of PTSD may be different for male and female PTSD patients.
Chapter 8

Given the anxiolytic oxytocin effects in PTSD patients described in this PhD thesis, investigating the therapeutic potential of oxytocin in PTSD is a promising avenue for future clinical research. However, our findings also emphasized that the effects of oxytocin may depend on contextual and inter-individual differences, such as sex and (severity of) PTSD psychopathology (Chapter 3-5). Clinical studies should therefore be aimed at investigating the effects of oxytocin on psychotherapy outcomes for PTSD, as well as elucidating the moderating factors influencing oxytocin effects on clinical outcomes. Of note, oxytocin administration may also prevent or decrease development of PTSD symptoms after recent trauma exposure, via the same anxiolytic and pro-social mechanisms described above (Frijling et al, 2014; Olff et al, 2010). Research into prevention of PTSD development seems especially important since only few preventive interventions are available to date. Interestingly, preliminary results on the effects of oxytocin for PTSD prevention seem promising: an 8-day treatment regime, initiated within 12 days post-trauma, resulted in decreased clinician-rated PTSD symptoms at follow-up in those participants with high initial distress (van Zuiden et al, in preparation).

Finally, more fundamental (human and animal) research should be conducted to investigate the pharmacodynamic response function, as well as the suggested dose-response curve of oxytocin administration (Cardoso et al, 2013). To date, it remains unknown whether intranasal oxytocin passes the blood-brain-barrier (Chini et al, 2014; Quintana et al, 2016), although elevated oxytocin levels in CSF have been observed after intranasal oxytocin administration (Striepens et al, 2013). Additionally, oxytocin effects may be time-dependent (Toth et al, 2012), and seem to depend on baseline fear regulation and oxytocin system functioning (Labuschagne et al, 2010; Rilling et al, 2014). More fundamental knowledge into the pharmacodynamic response of oxytocin, timing and dosage effects of oxytocin is therefore of paramount importance before routine clinical application of oxytocin to enhance response to psychotherapy can be initiated.

CONCLUSION

This PhD thesis described the effects of a single intranasal oxytocin administration on fear neurocircuitry in male and female PTSD patients, as a first step to investigate the clinical potential of oxytocin in PTSD. Our findings indicate anxiolytic effects of oxytocin administration in PTSD: oxytocin resulted in dampened subjective anxiety and nervousness, decreased amygdala activity towards emotional stimuli, and normalized aberrant amygdala subregion functional connectivity with prefrontal areas. Additionally, oxytocin altered neural processing of negative stimuli during distraction as cognitive emotion regulation strategy in PTSD patients in a sex-dependent manner. These anxiolytic effects of oxytocin indicate therapeutic promise for medication-enhanced psychotherapy in PTSD. Notably, the effects of oxytocin
were dependent on baseline neural fear regulation abilities, which were related to sex and (severity of) PTSD status. Therefore, investigating the effects of oxytocin on psychotherapy in PTSD, while elucidating potential moderating effects of contextual and inter-individual factors, is a promising avenue for future clinical research.
Nederlandse samenvatting
(Dutch summary)

‘BOOSTING OXYTOCIN AFTER TRAUMA - Effects of oxytocin on fear neurocircuitry in patients with post-traumatic stress disorder’
Ongeveer tachtig procent van de Nederlandse bevolking maakt gedurende zijn of haar leven één of meerdere traumatische gebeurtenissen mee (de Vries and Olff, 2009). Een traumatische gebeurtenis bestaat uit een directe of indirecte blootstelling aan een feitelijke of dreigende dood, ernstige verwonding of seksueel geweld (American Psychiatric Association, 2013). Tijdens en vlak na het meemaken van een traumatische gebeurtenis, zijn gevoelens van angst en stress een normale reactie op de dreigende situatie, die geleidelijk weer afnemen. Echter, bij ongeveer tien procent van de mensen resulteert blootstelling aan een traumatische gebeurtenis in een posttraumatische stress stoornis (PTSS), waarbij symptomen van verhoogde angst aanhouden (de Vries and Olff, 2009). PTSS bestaat uit vier symptoom clusters: (1) intrusieve herinneringen aan de traumatische gebeurtenis; (2) vermijding van stimuli die doen herinneren aan de traumatische gebeurtenis; (3) negatieve veranderingen in cognities en stemming en (4) veranderingen in prikkelbaarheid en reactief vermogen. Voor een officiële diagnose van PTSS moeten deze symptomen minimaal gedurende één maand aanhouden en zorgen voor een significante beperking in het functioneren (American Psychiatric Association, 2013).

ning bij gezonde mannen (Sripada et al, 2013). Daarom werd er verondersteld dat oxytocine via een duaal mechanisme behandelingseffecten bij PTSS kan versterken: (1) oxytocine kan de verhoogde angstrespons in PTSS patiënten verminderen, bijvoorbeeld door amygdala activiteit te dempen en (2) oxytocine kan motivatie voor therapie en de band tussen de therapeut en patiënt verbeteren (therapeutische alliantie). Dit proefschrift focust op het eerste mechanisme.

Als eerste stap om de (therapeutische) effecten van oxytocine toediening bij PTSS patiënten te onderzoeken, hebben we de neurobiologische effecten van oxytocine op angst en beloningsgevoeligheid bij PTSS patiënten onderzocht: de BOOSTER studie. Dit was een gerandomiseerde, placebo-gecontroleerde fMRI studie bij 77 mannelijke en vrouwelijke politieagenten met en zonder een PTSS. Als eerste hulpverleners op de noodhulp worden politieagenten met grote regelmaat blootgesteld aan traumatische gebeurtenissen, zoals schietincidenten, overlijdensgevallen, auto-ongelukken en ernstig verwonde slachtoffers. Politieagenten hebben hierdoor een verhoogd risico op PTSS. De BOOSTER studie bestond uit drie meetmomenten: tijdens het eerste meetmoment (T0) werd mondelinge en schriftelijke toestemming voor deelname verkregen en werden de in- en exclusiecriteria voor deelname gecheckt. In de twee daaropvolgende fMRI afspraken (T1 en T2) kregen deelnemers tijdens de ene sessie oxytocine en tijdens de andere sessie een nepmiddel (placebo) toegediend via een neusspray. Welke deelnemers oxytocine kregen tijdens de eerste sessie en welke deelnemers tijdens de tweede sessie was door toeval bepaald en onbekend voor de onderzoekers. Ongeveer 40 minuten na neusspray toediening werd de MRI sessie gestart met een anatomische scan. Hierna werd er begonnen met de verschillende taken om het neurale angstcircuit in kaart te brengen (Zie Figuur 1.1 voor de opzet van de BOOSTER studie en uitleg van de taken).

Het doel van dit proefschrift is om de effecten van oxytocine op het neurale angstcircuit bij mannelijke en vrouwelijke politieagenten met PTSS te beschrijven (Deel 1: Hoofdstuk 2-5). In het tweede deel van dit proefschrift is de neurobiologie van PTSS onderzocht, met specifieke focus op hersenactiviteit en functionele connectiviteit tijdens rust ('resting-state') en anatomische integriteit van witte stof banen (Deel 2: Hoofdstuk 6-7).

**SAMENVATTING VAN BEVINDINGEN**

**Hoofdstuk 2** is een literatuurstudie naar het neurale angstcircuit bij gezonde mensen, en hoe dit veranderd is bij PTSS patiënten. Daarnaast onderzochten we hoe oxytocine op het neurale angstcircuit van PTSS patiënten zou kunnen ingrijpen om behandeling te verbeteren. Neurobiologisch gezien wordt PTSS geassocieerd met hyperactiviteit in hersengebieden die zorgen
voor alertheid en waakzaamheid (‘salience processing’), zoals de amygdala, dorsale anterior cingulate cortex (dACC) en anterior insula (Hayes et al., 2012; Patel et al., 2012). Daarnaast is er verminderde prefrontale controle over de angstrespons, welke met name gekarakteriseerd wordt door hypoactiviteit in de ventromediale prefrontale cortex (vmPFC) en verminderde functionele connectiviteit tussen de vmPFC, amygdala en hippocampus (Hayes et al., 2012; Sripada et al., 2012b). Behandeling van PTSS zou kunnen worden verbeterd door in te grijpen op deze neurale mechanismen: het verminderen van de angstrespons van de amygdala en het versterken van prefrontale controle over de amygdala. Intranasale oxytocine toediening bij gezonde mannen leidt tot verminderde amygdala activiteit (Domes et al., 2007; Kirsch et al., 2005) en versterkte connectiviteit tussen de amygdala en vmPFC (Sripada et al., 2013). Daarnaast kan oxytocine de therapeutische alliantie (d.w.z. de band tussen therapeut en cliënt) verbeteren, wat ook tot een verbeterde behandelingssrespons zou kunnen leiden. Op basis van het literatuuroverzicht in dit hoofdstuk concludeerden we dat oxytocine mogelijk de effectiviteit van bestaande behandeling voor PTSS zou kunnen verbeteren, maar dat voorafgaand aan klinische toepassing hier eerst nog uitgebreid neurobiologisch en klinisch onderzoek naar gedaan moet worden.

In Hoofdstuk 3 (Koch et al., 2016a) beschrijven we het effect van oxytocine toediening op amygdala activiteit bij het zien van emotionele gezichten bij mannelijke en vrouwelijke politieagenten met en zonder PTSS. In tegenstelling tot onze verwachtingen, vonden we geen verhoogde activiteit (hyperactiviteit) van de amygdala onder placebo bij PTSS patiënten. Bij getraumatiserde controle deelnemers vonden we meer amygdala activiteit bij het zien van boze en angstige gezichten, dan bij het zien van blij en neutrale gezichten. Echter, bij PTSS patiënten observeerden we evenveel amygdala activiteit bij het zien van boze en angstige gezichten als bij blij en neutrale gezichten, wat betekent dat PTSS patiënten geen onderscheid maakten tussen negatieve en positieve emotionele gezichten. Oxytocine toediening resulteerde in verminderde linker amygdala activiteit ten opzichte van alle emotionele gezichten bij PTSS patiënten, maar in toegenomen amygdala activiteit bij getraumatiserde controle deelnemers. Deze effecten waren onafhankelijk van geslacht van de deelnemers en emotie van de gezichten. De vermindering van amygdala activiteit na oxytocine toediening bij PTSS patiënten was groter bij patiënten die meer subjectieve angst rapporteerden tijdens de placebo conditie. Samenvattend vonden we potentieel anxiolytische (angstdempende) effecten van oxytocine toediening bij PTSS patiënten, waarbij oxytocine toediening resulteerde in demping van linker amygdala activiteit bij het zien van emotionele gezichten.

In Hoofdstuk 4 (Koch et al., 2016c) hebben we gekeken naar het effect van oxytocine toediening bij politieagenten met en zonder PTSS op subjectieve angst en communicatie (functionele connectiviteit) van de basolaterale (BLA) en centromediale (CeM) amygdala subnuclei met prefrontale hersengebieden. De amygdala wordt vaak geconceptualiseerd als één

In Hoofdstuk 5 bestudeerden we de effecten van oxytocine toediening bij PTSS patiënten tijdens het uitvoeren van een distractie taak als cognitieve emotieregulatie strategie. Eerder onderzoek had namelijk aangetoond dat het verbeteren van emotieregulatie capaciteiten bij PTSS patiënten voorafgaand aan psychotherapie resulteerde in betere behandelingsresultaten (Bryant *et al.*, 2013). Deelnemers kregen zowel neutrale als negatieve plaatjes te zien. Tijdens de presentatie van een deel van de negatieve plaatjes moesten ze een werkgeheugentaak uitvoeren: de distractie conditie. Tijdens deze conditie moesten de deelnemers een rij van zes letters in gedachten houden, terwijl ze een negatief plaatje te zien kregen. Hierna verscheen er één letter in beeld en moesten de deelnemers aangeven of die letter deel uitmaakte van de vooraf getoonde rij met letters. Distractie leidde tot gereduceerde amygdala activiteit bij alle deelnemers, behalve bij mannelijke PTSS patiënten. Deze patiënten groep liet geen demping van amygdala en posterior insula activiteit zien tijdens het uitvoeren van de distractie taak. Na oxytocine toediening lieten mannelijke PTSS patiënten wel een demping van posterior insula (maar niet amygdala) activiteit zien tijdens de distractie conditie. Daarnaast beoordeelden ze alle (neutrale en negatieve) plaatjes positiever na oxytocine. Vrouwelijke PTSS patiënten lieten onder placebo juist meer rechter amygdala demping zien tijdens de distractie conditie dan vrouwelijke controles. De mate van rechter amygdala demping tijdens distractie in vrouwelijke patiënten was positief gecorreleerd met de ernst van vermijdings-symptomen. Na oxytocine toediening was er geen groepsverschil tussen vrouwelijke patiënten en vrouwelijke getraumatiseerde controles meer. Daarnaast vonden we toegenomen activiteit in de linkera amygdala na oxytocine bij alle vrouwelijke deelnemers. Samenvattend
laten deze resultaten zien dat oxytocine toediening bij mannelijke patiënten zorgde voor een verbeterde stemming, en voor betere demping van neurale activiteit bij het zien van negatieve stimuli tijdens de distractie conditie. Bij vrouwelijke PTSS patiënten resulteerde oxytocine juist in verhoogde amygdala activiteit tijdens distractie.

**Hoofdstuk 6** (Koch *et al*, 2016b) bestaat uit een meta-analyse en systematische literatuur review naar hersenactiviteit bij PTSS patiënten tijdens rust (‘resting-state’). We voerden een meta-analyse uit op 14 resting-state studies bij PTSS patiënten, waarbij activiteit in het hele brein tijdens rust was gemeten (whole-brain resting-state studies). Hierdoor konden we in kaart brengen welke hersengebieden minder (hypoactiviteit) of meer (hyperactiviteit) actief zijn bij PTSS patiënten, vergeleken met gezonde mensen. Uit de meta-analyse kwam naar voren dat PTSS patiënten consistente hyperactiviteit lieten zien in de ventrale anterior cingulate cortex (vACC) en parahippocampus/amygdala, en tevens consistente hypoactiviteit in de posterior insula, de pyramidis van het cerebellum en de middle frontal gyrus. Daarnaast hebben we een systematische review uitgevoerd naar negen ‘seed-based’ functionele connectiviteits-studies bij PTSS patiënten. In deze studies werd onderzocht met welke hersengebieden een hersengebied van interesse (‘seed’) communiceert. Uit deze systematische review kwam naar voren dat PTSS geassocieerd is met toegenomen communicatie tussen gebieden van het salience netwerk (SN), dat betrokken is bij waakzaamheid en alertheid, maar met verminderde communicatie tussen gebieden van het default mode netwerk (DMN), dat geactiveerd wordt tijdens rust en intern gerichte dachten. Op basis van de bevindingen van de meta-analyse en systematische review concludeerden we dat PTSS patiënten tijdens rust verhoogde activiteit in en communicatie tussen hersengebieden lieten zien die betrokken zijn bij waakzaamheid en alertheid, maar juist minder activiteit in en communicatie tussen hersengebieden die betrokken zijn bij emotieregulatie en intern gerichte gedachten tijdens rust.

Tot slot onderzochten we in **Hoofdstuk 7** de witte stof integriteit bij PTSS patiënten met diffusie tensor imaging (DTI). Witte stof banen maken communicatie tussen verschillende hersengebieden mogelijk. Er wordt verondersteld dat verminderde witte stof integriteit resulteert in minder effectieve communicatie tussen hersengebieden. Op basis van eerdere DTI bevindingen bij PTSS patiënten en het neurobiologische model van PTSS, hebben we gekeken naar de volgende anatomische witte stof banen: de bilateral uncinate fasciculus, cingulum en superior longitudinale fasciculus (zowel temporale als pariëtale projecties). Vergeleken met getraumatiseerde controle deelnemers, vonden we significant verhoogde diffusiviteit (mean diffusivity: MD) van de rechter uncinate fasciculus (d.w.z. de witte stof baan die de laterale amygdala met de ventromediale prefrontale cortex (vmPFC) en orbitofrontale cortex verbindt) bij mannelijke en vrouwelijke PTSS patiënten. Bij PTSS patiënten was MD van de rechter uncinate fasciculus positief gerelateerd aan mate van angst, en amygdala en vmPFC activiteit bij het zien van vrolijke en neutrale gezichten. Deze bevindingen komen
overeen met het huidige neurobiologische model van PTSS, waarin verondersteld wordt dat ineffectieve communicatie tussen de amygdala en vmPFC resulteert in verminderde prefrontale controle over de angstrespons en dus verhoogde angst, met name in dreigende situaties.

**DISCUSSIE EN CONCLUSIE**

Onze bevindingen met betrekking tot de neurobiologie van PTSS leveren een belangrijke bijdrage aan het neurobiologische model van PTSS. Ten eerste observeerden we zowel verminderde structurele als functionele connectiviteit tussen de amygdala en prefrontale cortex bij PTSS patiënten (*Hoofdstuk 3, 4, 6 & 7*), wat kan resulteren in verlaagde prefrontale controle over de angstrespons van de amygdala, en dus verhoogde angst. In overeenstemming met deze hypothese hing de verlaagde structurele connectiviteit tussen de prefrontale cortex en amygdala bij PTSS samen met verhoogde angst en amygdala activiteit bij het zien van emotionele gezichten (*Hoofdstuk 7*). Daarnaast vonden we verlaagde communicatie tussen de centromediale nucleus (CeM) van de amygdala en vmPFC bij mannelijke PTSS patiënten, wat wederom zou kunnen duiden op verminderde prefrontale controle over de amygdala (*Hoofdstuk 4*). Bij vrouwelijke PTSS patiënten vonden we verhoogde communicatie tussen de basolaterale nucleus van de amygdala (BLA) en dACC, welke beiden onderdeel uitmaken van het salience netwerk. Ook in de systematische review vonden we verhoogde communicatie tussen belangrijke gebieden van het salience netwerk, wat het onderliggende neurale mechanisme van verhoogde waakzaamheid bij PTSS zou kunnen representeren. Echter, onze resultaten spreken de (in het model veronderstelde) verhoogde amygdala activiteit als belangrijk neurobiologisch kenmerk van PTSS (Rauch *et al.*, 2006) tegen. Amygdala activiteit lijkt eerder op een subtielere manier veranderd te zijn bij PTSS: PTSS patiënten hebben met name moeite met het dempen van amygdala activiteit bij het zien van emotionele stimuli in veilige contexten (*Hoofdstuk 3, 5 & 6*), wat symptomen van verhoogde waakzaamheid en alertheid zou kunnen verklaren. Tot slot vonden we aanwijzingen dat de neurobiologie van PTSS verschillend is voor mannelijke en vrouwelijke PTSS patiënten. Onze resultaten benadrukken daarom het belang om rekening te houden met geslachtsverschillen als de neurobiologie van PTSS wordt onderzocht.

We observeerden angstremmende effecten van oxytocine toediening bij PTSS patiënten: oxytocine resulteerde in verminderde subjectieve angst en zenuwachtigheid (*Hoofdstuk 4*) en in verlaagde amygdala activiteit bij het zien van emotionele gezichten (*Hoofdstuk 3*). Resultaten van de functionele connectiviteitsstudie (*Hoofdstuk 4*) suggereerden dat oxytocine resulteerde in verminderde amygdala activiteit via twee verschillende mechanismen bij mannelijke en vrouwelijke PTSS patiënten: oxytocine zorgde mogelijk voor toegenomen controle
van de vmPFC over de CeM (mannen), maar in afgenomen verhoogde waakzaamheid van de dACC en BLA (vrouwen). In overeenstemming met recente oxytocine literatuur, lijken de effecten van oxytocine in onze studie afhankelijk te zijn van angstregulatie capaciteiten, wat gerelateerd kan zijn aan individuele eigenschappen zoals psychopathologie, geslacht en persoonlijkheidsschalenmerken (Bartz et al., 2011b; Labuschagne et al., 2010; Olff et al., 2013; Rilling et al., 2014). Zo werd eerder gevonden dat oxytocine een angstremmend effect kan hebben bij mensen met suboptimale angstregulatie, maar geen of zelfs een anxiogene effect bij mensen met optimale angstregulatie (Bertsch et al., 2013; Labuschagne et al., 2010). Wij vonden bijvoorbeeld grotere demping van amygdala activiteit na oxytocine toediening bij PTSS patiënten, en in het bijzonder bij patiënten met hogere angst en amygdala activiteit onder placebo. Ook de verschillende oxytocine effecten op distractie als emotieregulatie strategie kunnen vanuit dit gezichtspunt verklaard worden (Hoofdstuk 5): oxytocine herstelde de demping van posterior insulaactiviteit tijdens distractie in mannelijke PTSS patiënten, terwijl oxytocine amygdala demping tijdens distractie verminderde bij vrouwelijke participanten, die onder placebo al amygdala demping tijdens distractie lieten zien. Tot slot resulteerde oxytocine in toegenomen amygdala activiteit ten opzichte van emotionele gezichten bij gezonde, getraumatiserde controles, die vermoedelijk een veerkrachtige groep vormden (Hoofdstuk 3).

De effecten van oxytocine toediening bij PTSS patiënten lijken over het algemeen veelbelovend om respons op bestaande therapieën voor PTSS te verbeteren. Oxytocine zou behandeling van PTSS kunnen verbeteren via anxiolytische eigenschappen (dit proefschrift) en motivatie voor behandeling en de therapeutische band tussen patiënt en therapeut kunnen versterken (Nawijn et al., 2016; Olff et al., 2010). Echter, onze resultaten laten ook het belang van inter-individuele en contextuele factoren op de effecten van oxytocine zien. Toekomstige klinische studies zijn nodig om te onderzoeken of oxytocine daadwerkelijk de effectiviteit van behandeling van PTSS kan verbeteren, en of dit voor alle PTSS patiënten en alle behandelingen geldt.
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Appendix

B


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Appendix

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PUBLICATION LIST

Publications in this PhD thesis


Koch SBJ, Zuiden M van, Nawijn L, Frijling JL, Veltman DJ, Olff M. Effects of intranasal oxytocin on distraction as emotion regulation strategy in male and female patients with post-traumatic stress disorder. Submitted for publication. (Chapter 5)


Koch SBJ, Zuiden M van, Nawijn L, Frijling JL, Veltman DJ, Olff M. Decreased uncinate fasciculus tract integrity in male and female PTSD patients: A diffusion tensor imaging study. Submitted for publication. (Chapter 7)

Author contributions

1. Study concept and design. Acquisition, analysis or interpretation of data. Critical revision of the manuscript and important intellectual content: All authors
2. Drafting of the manuscript: Koch, van Zuiden
3. Statistical analysis: Koch
4. Obtained funding. Administrative, technical or material support: Olff
5. Study supervision: Olff, Veltman, van Zuiden
Appendix

Other publications


CURRICULUM VITAE

Saskia Koch was born on October 9th 1986 in Geldrop, the Netherlands. In 2005, she finished secondary school (VWO) at the Strabrecht College in Geldrop and started studying psychology at Tilburg University. After obtaining her Bachelor’s degree cum laude in 2008, she started the research master cognitive neuroscience at the Radboud University in Nijmegen. During this research master, Saskia did an internship at the Donders Center for Cognitive Neuroimaging where she investigated the involvement of the anterior prefrontal cortex in cognitive control over emotional actions with transcranial magnetic stimulation (TMS). She graduated cum laude in the summer of 2010. Hereafter, Saskia worked as research assistant at the Florey Institute of Neuroscience and Mental Health at the University of Melbourne, Australia.

In July 2011, Saskia started her PhD project at the psychiatry department of the Academic Medical Center (AMC) in Amsterdam, under supervision of prof. dr. Miranda Olff, prof. dr. Dick J. Veltman and dr. Mirjam van Zuiden. She was involved in two clinical studies on the (neural) effects of oxytocin administration in patients with post-traumatic stress disorder (PTSD) (BOOSTER study) and in acutely traumatized emergency department patients (BONDS study). For her PhD thesis, she investigated the effects of a single oxytocin administration on fear neurocircuitry in male and female police officers with and without PTSD. The results are described in this PhD-thesis and published in scientific international journals.

Currently, Saskia is working as postdoctoral researcher at the Affective Neuroscience group of prof. dr. Karin Roelofs at the Donders Institute of Cognitive Neuroimaging, where she investigates the role of automatic freeze-flight-flight reactions in the development of anxiety and aggression symptoms in police recruits after their first emergency work experience.