Aus dem Zentralinstitut für Seelische Gesundheit

Institut für Neuropsychologie und Klinische Psychologie

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Contextual Modulation of Associative Learning and the Role of Resting State Brain Activity in Posttraumatic Stress Disorder

Inauguraldissertation

zur Erlangung des Doctor scientiarum humanarum (Dr. sc. hum.)

an der Medizinischen Fakultät Mannheim

der Ruprecht-Karls-Universität

zu

Heidelberg

Vorgelegt am 27.04.2016

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1. Introduction

1.1. The relevance of posttraumatic stress disorder

Recent conflicts resulted in approximately 60 million forcibly displaced people worldwide (UNHCR, 2016) – many of whom most likely survived potentially life-threatening events. Being exposed to such a stressor is the prerequisite for the development of posttraumatic stress disorder (PTSD), which is further characterized by symptoms of intrusion (re-experiencing of the eliciting traumatic event), persistent effortful avoidance of distressing trauma-related stimuli, negative alterations in cognition and mood, and chronic hyperarousal/ hyperreactivity (American Psychiatric Association, 2013). These symptoms have debilitating effects on almost every aspect of everyday life. In light of the current worldwide political situation, the incidence of PTSD might be even higher than previously reported in epidemiological studies (i.e., USA: 8.7% (Kessler et al., 2005), Germany 2.3-3.4% (Maercker, Forstmeier, Wagner, Glaesmer, & Brahler, 2008)). It is important to note that not every person who is exposed to a trauma (i.e., USA: 80%, Germany and Switzerland: 20-30% (Breslau, 2009)) will develop full-blown PTSD, indicating an important role for individual vulnerability or risk and resilience factors for the manifestation of the disorder. However, once PTSD has emerged, even future generations can be affected by the initial trauma through latent transmission and continue to show heightened vulnerability to stress (Kellermann, 2013). Research investigating the underlying psychobiological mechanisms is needed to advance our understanding of the disorder and to create innovative therapeutic interventions that are necessary to successfully deal with its presumably increasing incidence.

1.2. Psychological Theories

There is general acceptance that memory is severely altered by traumatic events and that traumatic memories are accompanied by aberrant learning processes and changes in neurobiologal mechanisms (Bremner & Vermetten, 2001; Hayes, VanElzakker, & Shin, 2012).

1.2.1. Learning processes

Pavlovian conditioning describes a universal learning mechanism where a conditioned response (CR) is acquired through multiple pairings of an initially neutral stimulus (conditioned stimulus; CS) with a biologically relevant stimulus (unconditioned stimulus; US). In the case of PTSD, the traumatic event (e.g. a shipwreck) serves as a US and trauma reminders (e.g. a life vest, open water, screaming sounds) serve as CSs resulting in a continued fear response, which can be considered a CR (Grillon, Southwick, & Charney, 1996; Pitman, 1988). Since its original description in 1927 (Pavlov, 1972), pavlovian conditioning has been used extensively to investigate PTSD and other anxiety disorders in rodents, healthy humans and patient groups (for review see VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). In laboratory situations, fear is often induced by mildly painful stimuli such as electric stimulation that are paired with one of two (or more) neutral stimuli, such as pictures or sounds (Sehlmeyer et al., 2009). In human studies, differential delay conditioning is often employed. Here, CS and US overlap in time, and the CS that predicts the occurrence of the US acts as a danger signal (CS+) whereas the second stimulus, which is never followed by the US, acts as a safety signal (CS-).

Repeated presentations of the CS without subsequent US presentations will extinguish the CR, but not erase the link between the CS and the US (Bouton & Bolles, 1979b; Rescorla & Heth, 1975). Instead, successful conditioning and extinction establish two distinct memory traces; the originally acquired CS-US association and a newly formed CS-noUS association (Quirk, 2002; Rescorla, 2001). In a given situation, the behavioral response (fear vs. no fear) depends on the memory trace that is retrieved, which in turn depends on the interpretation of the CS (danger vs. safety). Accordingly, rodent and human research shows that the original fear response can be elicited again after extinction (Bouton, 2002; VanElzakker et al., 2014) after the mere passage of time (spontaneous recovery; Pavlov, 1972; Rescorla, 2004), after confrontation with the US alone (reinstatement; Rescorla & Heth, 1975) and after a change of context (renewal, see 1.2.2.; Bouton & Ricker, 1994).

PTSD patients are characterized by increased conditionability (Orr et al., 2000), deficient extinction learning (Norrholm et al., 2011; Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000) and reduced discrimination learning (Grillon & Morgan, 1999; Peri et al., 2000). Increased conditionability refers to increased acquisition (magnitude or speed of

the response) of CS-US contingencies in PTSD patients compared to healthy controls that has been observed in fear conditioning studies (Orr et al., 2000; Peri et al., 2000). An implication of these observations is that some people might develop strong CRs to a traumatic event more readily than others. Indeed, the development of PTSD can be considered an extreme case of one-trial-learning, where alterations in the amygdala enhance memory consolidation for the traumatic event leading to re-experiencing rather than remembering (Grillon et al., 1996; Yehuda, 2002).

Furthermore, it is generally accepted that deficient extinction (memory) may be responsible for the development and maintenance of PTSD (Flor & Nees, 2014; VanElzakker et al., 2014). It has been repeatedly shown that PTSD patients preserve a fear response after a conditioning and extinction procedure, indicating insufficient extinction memory (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Milad et al., 2008; Milad et al., 2009; Steiger, Nees, Wicking, Lang, & Flor, 2015). Without successful extinction, trauma-related cues continue to evoke the conditioned fear response, which becomes manifest or re-occurs in PTSD symptoms like re-experiencing, avoidance or hyperarousal (e.g. after seeing life vests in the news daily, being confronted with a life vest on a plane results in a flashback). In this way, even originally neutral stimuli (e.g. a plane) can be associated with trauma reminders (e.g. a life vest) – that now serve as USs – through second-order conditioning (Wessa & Flor, 2007). As a result, the newly conditioned stimulus acts as a danger signal that induces fear in situations unrelated to the traumatic event (e.g. fear of flying). Hence, second-order conditioning and insufficient extinction may lead to the generalization of fear in PTSD (Lissek et al., 2005; Peri et al., 2000).

In line with this, PTSD patients seem unable to correctly interpret and use danger and safety signals to modulate fear (Bremner et al., 2005; Garfinkel et al., 2014; Jovanovic, Kazama, Bachevalier, & Davis, 2012; Weike, Schupp, & Hamm, 2008). In laboratory studies, PTSD patients have been shown to generalize the acquired fear response to the CS-, resulting in a sensitized reaction to the actual safety cue (Blechert et al., 2007; Grillon & Morgan, 1999; Peri et al., 2000) or safety context (Garfinkel et al., 2014). As a consequence, PTSD patients fail to inhibit acquired fear responses – even in the presence of safety signals (i.e. confrontation with a life vest may evoke a fear response despite being on “safe” solid floor).

PTSD treatment such as (cognitive) behavioral therapy is based on the above described learning mechanisms: Prolonged exposure (intensive extinction training) and discrimination training (differentiation of safety and danger cues) are among the most successful therapeutic strategies for PTSD (Committee on Treatment of Posttraumatic Stress Disorder, 2008).

1.2.2. The role of context

After successful acquisition and extinction, the CS is associated with danger (US) as well as with safety (no US) and this ambiguity needs to be resolved by the individual in order to react appropriately upon subsequent confrontations with the CS. Among other factors, the context is a reliable indicator for which memory trace is to be retrieved (Bouton, 2002, 2004): the presentation of the CS in the acquisition (or a novel) context will lead to retrieval of the CS-US association and result in a fear response, whereas the presentation of the CS in the extinction context will lead to retrieval of the CS-noUS association and result in suppression of the fear response (see Figure 1). Thus, retrieval of the extinction memory is related to the presentation of the CS in the context in which extinction training occurred; outside this context, renewal of fear is observed (Bouton & Bolles, 1979a; Bouton & Ricker, 1994; Mineka & Oehlberg, 2008; Mystkowski, Craske, Echiverri, & Labus, 2006; Rauhut, Thomas, & Ayres, 2001). As a consequence, the correct identification of the (extinction) context sets the stage for the selection of the appropriate reaction.

Interestingly, this process seems to be disrupted in PTSD, as patients show inadequate fear responses when they are confronted with trauma reminders in contexts in which these cues do not have a predictive meaning (e.g. a life vest presented in a psychotherapy practice during exposure therapy elicits a fear response, even though drowning is not predicted, not even possible, in this environment).

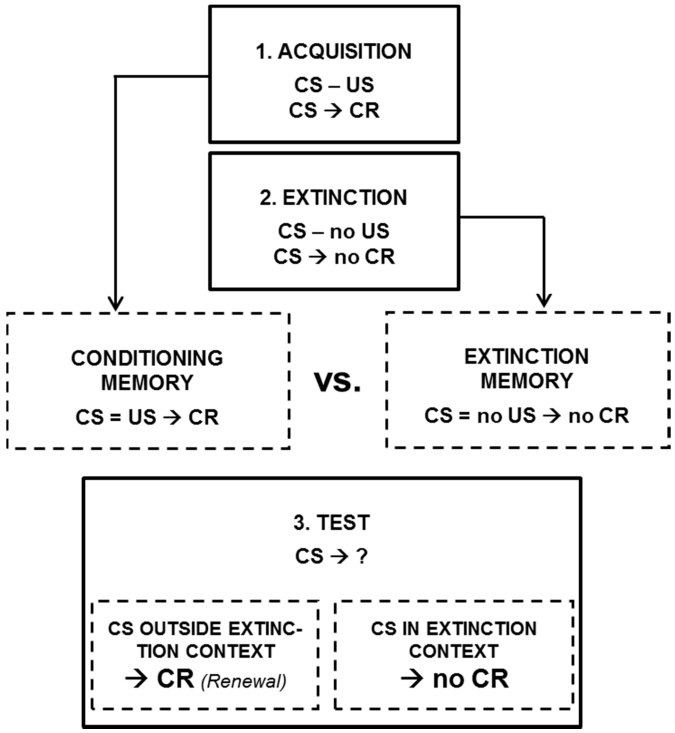

Figure 1. Schematic illustration of the retrieval failure model (Bouton, 2002, 2004). After successful acquisition and extinction of a fear response, two memory traces compete against each other: the conditioning memory trace where the conditioned stimulus (CS) predicts the occurrence of the unconditioned stimulus (US) and therefore results in a conditioned response (CR); and the extinction memory trace where the CS predicts the absence of the US and therefore does not result in a CR. During test, the context indicates which memory trace is to retrieve. If the CS is again presented outside the extinction context, the conditioning memory trace is activated and elicits a CR (renewal of fear). If the CS is presented in the extinction context, the extinction memory trace is activated and suppresses the CR.

Psychological explanations for this deficit are based on how a context is perceived. Rudy et al. (2004) have noted that "physical elements of a conditioning context are represented in the brain as either (a) a set of independent features or (b) features bound into a conjunctive representation by the hippocampus which supports pattern completion" (p. 675). During fear conditioning, one of two processes may occur: the single elements (CSs) are separately associated with the fearful event (US) or the single elements are bound into a new unitary representation that comprises the co-occurrence of all features including the fearful event. As a result, (a) one single feature (e.g. a life vest) may be sufficient to elicit a fear response or (b) the confrontation with a complete scene that matches the full representation (e.g. a shipwreck: life vests, open water, screaming noises) is needed to evoke this reaction. According to Acheson, Gresack, and

Risbrough (2012), PTSD patients have difficulties in forming a conjunctive representation of the context due to hippocampal abnormalities (see 1.2.3.) and are therefore more likely to utilize the elementary strategy when encoding the traumatic event and thus ignore the context. As a result, the "threshold" for a fear response is reduced and situations unrelated to the trauma have the potential to evoke the conditioned fear response. In accordance with this, they show deficient contextual conditioning, i.e. they are unable to acquire a differential conditioned response to the context (Steiger et al., 2015).

In studies by Milad et al. (2008; 2009), where contexts and cues were combined, PTSD patients have repeatedly shown reduced recall of fear extinction. In these experiments, digital photographs of two different rooms were used as contexts and the colors of a lighted lampshade (blue or red) that appeared within each room were used as CS+ and CS-. One day after acquisition and extinction, subjects were again confronted with the CSs in the extinction context. PTSD patients failed to retain extinction memory during these tests as indicated by elevated skin conductance responses. Taken together, these and other studies (Steiger et al., 2015) reflect a failure to learn – or retain learning – that a context in which a shock does not occur is safe. The above described extinction deficit in PTSD patients may therefore in part be a result of deficient contextual conditioning: PTSD patients seem unable to correctly associate a context with conditioned stimuli, which is a prerequisite for successful extinction learning (Acheson et al., 2012; Bouton & Moody, 2004; Flor & Wessa, 2010; Maren, Phan, & Liberzon, 2013).

Interestingly, these observations explain an effect known from behavioral therapy: exposure treatment is more effective when performed in "multiple contexts, especially those in which the previously feared stimulus is likely to be encountered once treatment is over" (Craske et al., 2008, p. 20).

1.2.3. Functional neuroanatomy

Brain circuits involved in PTSD involve the amygdala, ventromedial prefrontal cortex (vmPFC) and hippocampus (Rauch, Shin, & Phelps, 2006; Rauch, Shin, Whalen, & Pitman, 1998).

Amygdala. The amygdala is crucial for fear learning, i.e. the acquisition of a CS-US association (Helmstetter & Bellgowan, 1994; Muller, Corodimas, Fridel, & LeDoux, 1997). It is active during fear acquisition and early extinction (Knight, Cheng, Smith,

Stein, & Helmstetter, 2004; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998), but undergoes rapid habituation over acquisition trials (Büchel & Dolan, 2000). Activation of the amygdala has been linked to electrodermal activity during fear acquisition (LaBar et al., 1998; Phelps et al., 2001) and has been observed even when study participants were perceptually unaware of the conditioned stimuli (Öhman, Carlsson, Lundqvist, & Ingvar, 2007; Tabbert et al., 2011; Tabbert, Stark, Kirsch, & Vaitl, 2006). In rodents, neurochemical lesions as well as reversible inactivation of the amygdala disrupt the acquisition of fear (Maren, Yap, & Goosens, 2001; Muller et al., 1997; Sparta et al., 2014; Wilensky, Schafe, & LeDoux, 1999). Amygdala reactivity is exaggerated in individuals with PTSD (Liberzon et al., 1999; Rauch et al., 2000) and is positively correlated with symptom severity (Protopopescu et al., 2005). Increased amygdala activity has been reported during fear acquisition in PTSD (Milad et al., 2009). Bremner et al. (2005) examined women with childhood sexual-abuse-related PTSD in a simple fear conditioning and extinction procedure and simultaneous positron emission tomography measurement. Compared to women without abuse or PTSD, the patients exhibited increased amygdala activation during the acquisition of a fear response (whereas decreased PFC activation was related to sustained fear during extinction in PTSD).

vmPFC. The vmPFC exhibits inhibitory control over the amygdala during fear extinction, reducing the emergence of the conditioned fear response (Quirk & Beer, 2006; Quirk, Russo, Barron, & Lebron, 2000). In addition to its role during extinction (Gottfried & Dolan, 2004; Molchan, Sunderland, McIntosh, Herscovitch, & Schreurs, 1994), rodent and human studies suggest that the vmPFC is also a key area for the recall of fear extinction (Lebron, Milad, & Quirk, 2004; Milad, Rauch, Pitman, & Quirk, 2006; Phelps, Delgado, Nearing, & LeDoux, 2004; Quirk & Beer, 2006; Rauch et al., 2006; Sotres-Bayon, Cain, & LeDoux, 2006). In PTSD, vmPFC activity is negatively correlated with symptom severity and the inhibition of the amygdala is diminished during extinction (Liberzon & Sripada, 2008). More importantly, in PTSD, the vmPFC fails to maintain extinction of conditioned fear (Liberzon & Sripada, 2008). In a study by Milad et al. (2009), for example, PTSD patients showed impaired extinction retention compared to trauma-exposed healthy controls indicated by a lack of differential SCRs to an extinguished and an unextinguished CS+, which was positively correlated with reduced activity in the bilateral vmPFC (as well as reduced activity in the hippocampus and increased activity in the dorsal anterior cingulate cortex (dACC)).

Hippocampus. The hippocampus is involved in the encoding and recognition of episodic memories and environmental cues (i.e. recognizing safe and dangerous contexts) (Burgess, Maguire, & O'Keefe, 2002; Jovanovic et al., 2012). During fear conditioning, the hippocampus binds together different elements of a context into a conjunctive representation (Acheson et al., 2012; Maren, 2001; Rudy, 2009) which then can be associated with a US (or the absence of a US) in the amygdala (Maren, 2001). In line with this, the hippocampus plays a crucial role during extinction learning and retrieval by regulating the context-specific activation of the amygdala (Bremner et al., 2003; Corcoran & Maren, 2001, 2004; Ji & Maren, 2005, 2007; Milad et al., 2009; Shin & Liberzon, 2010). Indeed, hippocampal impairments found in PTSD patients and those vulnerable for PTSD seem to impede the contextual processing of stimuli and therefore mediate the failure to maintain extinction (Bremner et al., 2003; Gilbertson et al., 2002; Milad et al., 2009; Steiger et al., 2015). Figure 2 illustrates how hippocampal deficits are thought to cause disrupted contextual modulation of fear conditioning in PTSD (Acheson et al., 2012). Poor hippocampal functioning leads to the usage of a predominantly elemental strategy for encoding the traumatic event. As described above, the devastating result of this strategy is that each discrete cue encoded during trauma is later able to induce conditioned fear responses independent of the context. In this regard, even the presence of a safety cue would not enable PTSD patients to reduce fear (cf. chapter 1.2.1.) (Blechert et al., 2007; Grillon & Morgan, 1999; Peri et al., 2000). Despite this theoretical framework, the specific impact of the hippocampus on PTSD remains a subject of debate. Whereas structural findings have consistently shown bilateral hippocampal volume reduction in PTSD (Bremner et al., 2005; Karl et al., 2006; Kitayama, Vaccarino, Kutner, Weiss, & Bremner, 2005; Smith, 2005), functional neuroimaging studies have reported both hypo- and hyper-reactivity (Bremner et al., 2003; Shin & Liberzon, 2010). Even though this discrepancy might stem from methodological differences across studies, caution is needed when interpreting fMRI results in terms of task performance or stimulus processing. Increases in brain activation could reflect a compensatory mechanism (Bokde et al., 2010; Fanselow, 2010), resulting from dysfunctional brain circuits as described above.

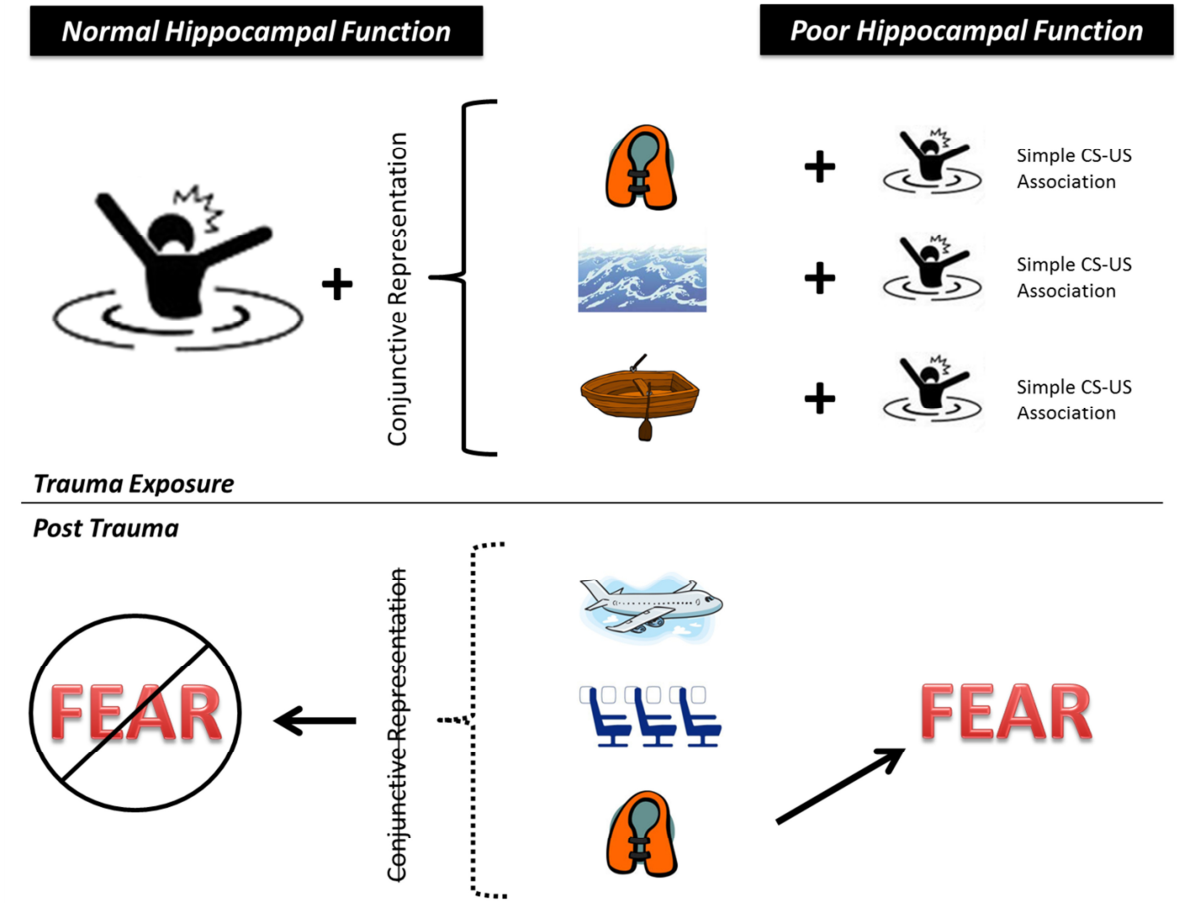

Figure 2. Schematic illustration of hippocampal impact on contextual fear learning. The left side of the figure represents normal hippocampal function that allows for a conjunctive representation of contextual cues, i.e. a combination of individual elements including the traumatic experience (in this case a shipwreck). Later exposure to a single element of the traumatic situation (in this case a life vest) will not trigger a fear response, because the entire representation does not match the original context. The right side of the figure illustrates deficient hippocampal function leading to single associations of each element of the context with the traumatic experience. As a result of this elemental strategy, later exposure to a single element of the traumatic situation is sufficient to trigger a fear response. The model suggests that there is a shift to a predominantly elemental strategy in PTSD. As a result, each discrete cue that was encoded during the traumatic experience may induce a conditioned fear response irrespective of the context. The figure was adapted and the example modified from Acheson, Gresack, & Risbrough (2012).

Other brain regions that are involved in a network modulating human (contextual) fear conditioning such as the dACC or the insula are also hyperreactive in PTSD (Pitman et al., 2012). These structures may modulate fear processing within the amygdala, which in the case of PTSD leads to an exaggerated fear response (Aupperle et al., 2012; Fonzo et

al., 2010; Milad et al., 2009; Rougemont-Bücking et al., 2011; Shin et al., 2009; Strigo et al., 2010).

Insula. Among other functions, the insula is involved in interoceptive awareness (Menon & Uddin, 2010) as well as fear acquisition (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Büchel, Dolan, Armony, & Friston, 1999; Büchel, Morris, Dolan, & Friston, 1998; Critchley, Mathias, & Dolan, 2002; Dunsmoor, Bandettini, & Knight, 2007; Gottfried & Dolan, 2004; Klucken, Tabbert, et al., 2009; Knight, Waters, & Bandettini, 2009; Marschner, Kalisch, Vervliet, Vansteenwegen, & Buchel, 2008; Morris & Dolan, 2004; Phelps, et al., 2004; Phelps, et al., 2001) and extinction (Gottfried & Dolan, 2004; LaBar, et al., 1998; Milad, Wright, et al., 2007; Phelps et al., 2004). During fear conditioning, the insula and the amygdala are activated in concert (Etkin & Wager, 2007; Pohlack, Nees, Ruttorf, Schad, & Flor, 2012). Interestingly, in PTSD, the associated increases of activation in both structures are greater compared to control groups (Bremner et al., 2005; Fonzo et al., 2010). Furthermore, insular activation has been linked with PTSD symptom severity, such as heightened detection of bodily arousal (Simmons et al., 2008).

dACC. The dACC mediates response selection, error detection, pain perception and fear learning and expression (Carter, Botvinick, & Cohen, 1999). Activation in the dACC is increased during fear learning (Alvarez et al., 2008; Büchel et al., 1999; Büchel et al., 1998; Dunsmoor et al., 2007; Klucken, Kagerer, et al., 2009; LaBar et al., 1998; Marschner, et al., 2008; Milad, Quirk, et al., 2007; Milad, Wright, et al., 2007; Morris & Dolan, 2004; Olsson, Nearing, & Phelps, 2007; Phelps et al., 2004). PTSD patients exhibit heightened dACC activity during fear conditioning (Rougemont-Bücking et al., 2011) and recall of extinction learning (Milad et al., 2009). Increased dACC activity has been suggested as a biomarker reflecting familial risk of developing PTSD after trauma (Shin et al., 2009).

Given the fact that cognitive behavior therapy relies on fear extinction mechanisms and that the above described brain structures underlie fear conditioning and extinction, research should aim to determine precisely how the involved neural circuitries are differentially altered by various behavioral interventions. As stated by Rauch, Shin and Phelps (2006), these results could be used to customize treatment by predicting the subsequent differential response to a given intervention.

1.2.4. Functional Resting State Connectivity

In functional neuroimaging, subjects are commonly confronted with an experimental task (studies including patients usually expose the participants to stimuli with direct diagnostic relevance to the relevant condition) and blood-oxygen-level-dependent (BOLD) signals in a distinct brain region of interest are measured. An alternative approach is to examine and identify intrinsic connectivity networks (ICNs) underling sensory, motor, and cognitive functions during resting state (Beckmann, DeLuca, Devlin, & Smith, 2005; Cordes et al., 2000; Lowe, Mock, & Sorenson, 1998) or during task performance (Allen et al., 2011; Calhoun, Liu, & Adali, 2009; Calhoun et al., 2011). Key methods for the identification of ICNs in (resting-state) fMRI BOLD data are independent component analysis (ICA) (Damoiseaux et al., 2006) and seed-based functional interdependence analysis (Fox et al., 2005; Greicius, Krasnow, Reiss, & Menon, 2003).

Whereas anatomical connectivity refers to structural connections between distinct brain regions through white matter fibers, functional connectivity is a statistical concept, capturing temporal correlations between distributed and often spatially remote neuronal units (Fox et al., 2005). Brain regions that belong to the same functional network show synchronized spontaneous low-frequency fluctuations (Rogers, Morgan, Newton, & Gore, 2007). However, functional connectivity does not reflect directionality, i.e. causal influences of one neural element over another (cf. effective connectivity).

The most important ICNs are the default mode network (DMN), the salience network (SN), and the central executive network (CEN). The DMN (Raichle et al., 2001), comprises the medial prefrontal cortex (mPFC), posterior cingulate cortex/precuneus, inferior parietal cortices, lateral temporal cortices and hippocampus. DMN activation is linked to functions such as self-referential processing, autobiographical memory, and emotion regulation (Daniels et al., 2010; Menon, 2011; Spreng, Mar, & Kim, 2009). It is temporally anti-correlated with the so-called task-positive networks (Dosenbach et al., 2007; Seeley et al., 2007; Sridharan, Levitin, & Menon, 2008). Of those, the SN involves the anterior insula and the ACC as well as extensive connectivity with subcortical structures (amygdala, substantia nigra or ventral tegmental area and thalamus). The SN is important for the detection of and directing attention to biologically salient stimuli in the environment and is involved in reward and motivation (Eckert et al., 2009; Menon & Uddin, 2010; Seeley et al., 2007). In contrast, the CEN involves the dorsolateral prefrontal cortex and the posterior parietal cortex as well as subcortical coupling that is

distinct from that of the salience network (dorsal caudate and anterior thalamus) (Seeley et al., 2007). The CEN is important for higher-order cognitive and attentional control.

According to Menon's (2011) triple network model, changes in the connectivity patterns within and between the three main ICNs are positively correlated with a number of affective and neurocognitive symptoms seen in mental disorders, such as PTSD. Observed PTSD-related changes within the DMN include increased and decreased connectivity between key anterior (e.g., mPFC and ACC) and posterior nodes (e.g., posterior cingulate cortex and precuneus) as well as other connectivity alterations, including subcortical areas such as the hippocampus and the thalamus (Daniels et al., 2010; Peterson, Thome, Frewen, & Lanius, 2014; Sripada, King, Welsh, et al., 2012). In addition, within-network connectivity of the SN and the CEN has been found to be altered in PTSD samples across both resting state and task conditions, but the direction of these effects has not always been consistent (Birn, Patriat, Phillips, Germain, & Herringa, 2014; Cisler, Scott Steele, Smitherman, Lenow, & Kilts, 2013; Fonzo et al., 2010; Fox & Raichle, 2007; Greicius et al., 2003; Kim et al., 2007; Lindauer et al., 2004; Rabellino et al., 2015; Schuff et al., 2011; Seeley et al., 2007; Shin, Rauch, & Pitman, 2006; Sripada, King, Welsh, et al., 2012; Yin, Li, et al., 2011). Importantly, PTSD patients show increased coupling of the DMN and SN (Rabellino et al., 2015), resulting in impaired task-induced suppression of DMN activity and a failure to switch to task-positive networks (Daniels et al., 2010). According to a meta-analysis by Koch et al. (2016) "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)" (p. 9). Furthermore, the SN mediates switching between the DMN and the CEN (Zhu et al., 2015; Zhu et al., 2014), which seems to be insufficient in PTSD (Menon, 2011). Taken together, these findings are in line with the triple network model, i.e. the assumption that aberrant connectivity patterns within and between the DMN, the SN and the CEN represent a key neurofunctional deficit in PTSD.

Abnormal resting state functional connectivity has also been observed through seed-based analyses in relevant brain areas that have been identified as key circuits for PTSD such as amygdala, hippocampus and PFC regions (Koch et al., 2016). Findings on amygdala connectivity revealed increased connectivity with the insula (Rabinak et al.,

2011; Sripada, King, Garfinkel, et al., 2012; Sripada, King, Welsh, et al., 2012), decreased connectivity with the medial PFC (Jin et al., 2014) and decreased anticorrelation with the rACC (Sripada, King, Garfinkel, et al., 2012). In addition, atypical hyperconnectivity of the hippocampus as well as temporal and frontal regions have been observed in soldiers with PTSD compared to healthy combat controls and were linked to symptom severity (Dunkley et al., 2014). Together, these seed-based results are consistent with the idea of a disrupted network in PTSD indicating, for example, reduced prefrontal inhibitory control over the amygdala.

The fact that alterations in functional connectivity have repeatedly been linked to the severity of PTSD symptoms emphasizes the relevance of such findings. In particular, scores in the Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995) have been associated with resting state and task-related functional connectivity (Koch et al., 2016; Tursich et al., 2015). For example, it has been suggested that the strength of DMN connectivity is associated with symptom severity (Lanius et al., 2010; Qin et al., 2012; Yin et al., 2011; Zhou et al., 2012). Negative correlations have been found between CAPS scores and connectivity of DMN seed regions with the left hippocampus (Sripada, King, Welsh, et al., 2012) and right amygdala (Calhoun et al., 2009). For the SN, connectivity seems to be associated with symptom severity as well, but with inconsistent directions of the effect (Greicius et al., 2003; Seeley et al., 2007; Sripada, King, Garfinkel, et al., 2012; Yan et al., 2013). Notably, associations of functional connectivity and symptom severity seem to have predictive value. Two studies (Lanius et al., 2010; Qin et al., 2012) have shown that enhanced connectivity between DMN and amygdala measured relatively shortly after the trauma (2 days, 5-6 weeks) predicts the development of PTSD symptoms and symptom severity at a later stage (1-6 months, 12 weeks post trauma).

If increased DMN–SN connectivity shortly post trauma represents a vulnerability factor for the development of PTSD, as the aforementioned studies suggest, it could be possible to identify individuals with a high risk of developing PTSD and offer special care to these individuals in order to prevent the manifestation of the disorder. But, in order to establish reliable predictions, further research is needed.

1.3. Hypotheses

As outlined above, functional neuroimaging and connectivity analyses are used to investigate psychopathology, targeting different mechanisms. In PTSD, such research includes neural correlates of (contextual) fear conditioning or symptom severity (Norrholm et al., 2011; Steiger et al., 2015).

A large body of research on return of fear has been accumulated in rodents and in healthy humans, expanding our insight in processes like renewal (Alvarez, Johnson, & Grillon, 2007; Baeyens et al., 2005; Claassen, Mazilescu, Thieme, Bracha, & Timmann, 2016; Effting & Kindt, 2007; Nakajima, Tanaka, Urushihara, & Imada, 2000; Neumann & Longbottom, 2008; Vansteenwegen et al., 2006), reinstatement, (Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2004, 2007; Dirikx, Vansteenwegen, Eelen, & Hermans, 2009; Lonsdorf, Haaker, & Kalisch, 2014; Westbrook, Iordanova, McNally, Richardson, & Harris, 2002) and generalization (Lissek et al., 2008; Torrents-Rodas et al., 2012; Vervliet, Vansteenwegen, & Eelen, 2004, 2006). Even though the results can be applied to PTSD, research on patient groups is needed to understand the relevance of return of fear for the disorder. For example, deficient extinction retention in PTSD has been linked to reduced activity in the hippocampus and vmPFC and increased activity in the dACC (Milad et al., 2009) and the amygdala (Garfinkel et al., 2014). Furthermore, one study showed reduced renewal in PTSD that was associated with lower activity in the amygdala and the vmPFC (Garfinkel et al., 2014).

Most studies that investigated context-dependent fear conditioning, extinction and return of fear presented CSs within stationary contexts (Baeuchl, Meyer, Hoppstadter, Diener, & Flor, 2015; Garfinkel, et al., 2014; Haaker, Lonsdorf, Thanellou, & Kalisch, 2013; Meir Drexler et al., 2014; Milad et al., 2008; Milad et al., 2009; Steiger et al., 2015). A promising tool, however, are computer generated environments (Glotzbach-Schoon et al., 2013; Grillon, Baas, Cornwell, & Johnson, 2006; Indovina, Robbins, Nunez-Elizalde, Dunn, & Bishop, 2011). Such virtual realities (VRs) are advantageous, because the person can navigate through an environment without actually moving, so that laboratory and fMRI measurements are feasible even when different spatial contexts need to be presented (Pine et al., 2001; Pine et al., 2002). Indeed, when investigating extinction memory, the context is an important factor (see 1.2.2.).

In the first study of this dissertation, the focus was on context-dependent learning. An ABC conditioning procedure with high ecological validity was employed by mimicking

the real life course of learning, i.e. acquisition of fear at the location of the trauma (= A, e.g. shipwreck on the ocean), extinction of fear through exposure therapy (= B, e.g. a clinic and/or a psychotherapy practice) and return of fear (PTSD symptoms) at a third unrelated situation (= C, e.g. the patients home or work place). Unlike previous studies that compared PTSD patients either with trauma-survivors without PTSD or with healthy, trauma-naïve controls (e.g. Blechert et al., 2007; Garfinkel et al., 2014; Grillon & Morgan, 1999; Milad et al., 2009; Milad, Wright, et al., 2007; Rougemont-Bücking et al., 2011), our sample included all three of these groups, thus, protective mechanisms could also be investigated (Nalloor, Bunting, & Vazdarjanova, 2011; Yehuda, Flory, Southwick, & Charney, 2006; Yehuda & LeDoux, 2007). It is still unclear whether the mere experience of trauma leads to neurobiological changes and altered learning mechanisms or if these are characteristics of the disorder (and probably have existed before the trauma) (Kremen, Koenen, Afari, & Lyons, 2012).

The main hypothesis of the first study was:

1) PTSD patients compared to traumatized persons without PTSD and healthy trauma-naïve controls show heightened return of fear as indicated by (a) higher differential SCRs, (b) higher ratings of arousal, valence and CS-US contingency and (c) higher differential amygdala activation at the beginning of the test phase.

Additionally, we hypothesized:

2) Trauma-exposed but unaffected controls show intermediate results, presumably as a consequence of the stress experience at the time of trauma.
3) The psychobiological changes in PTSD are associated with symptom severity.

As elaborated in chapter 1.2.4., the investigation of functional networks adds important insights on the underlying neurobiology of PTSD. Based on these previous results, the second study included an ICA and a seed-based analysis of resting brain activity in these samples and furthermore a correlation analysis of the resulting findings with CAPS-scores.

The main hypotheses of the second study were:

1) PTSD patients compared to traumatized persons without PTSD and healthy trauma- naïve controls show aberrant resting state network connectivity (i.e., decreased DMN connectivity and increased SN connectivity).
2) PTSD patients compared to traumatized persons without PTSD and healthy trauma- naïve controls show increased functional connectivity between the amygdala (as seed region) and other brain regions such as the anterior insula.
3) Changes in amygdala-connectivity within the PTSD group are positively correlated with symptom severity.

2. Empirical studies

2.1. Study1

Deficient fear extinction memory in posttraumatic stress disorder

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Keywords: Posttraumatic stress disorder (PTSD), fear conditioning, renewal, safety learning, amygdala, hippocampus

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Abstract

Background: Posttraumatic stress disorder (PTSD) might be maintained by deficient extinction memory. We used a cued fear conditioning design with extinction and a post-extinction phase to provoke the return of fear and examined the role of the interplay of amygdala, hippocampus and prefrontal regions**.**

Methods: We compared 18 PTSD patients with two healthy control groups: 18 trauma-exposed subjects without PTSD (nonPTSD) and 18 healthy controls (HC) without trauma experience. They underwent a three-day ABC-conditioning procedure in a functional magnetic resonance imaging scanner. Two geometric shapes that served as conditioned stimuli (CS) were presented in the context of virtual reality scenes. Electric painful stimuli were delivered after one of the two shapes (CS+) during acquisition (in context A), while the other (CS-) was never paired with pain. Extinction was performed in context B and extinction memory was tested in a novel context C.

Results: The PTSD patients showed significantly higher differential skin conductance responses than the non-PTSD and HC and higher differential amygdala and hippocampus activity than the HC in context C. In addition, elevated arousal to the CS+ during extinction and to the CS- throughout the experiment was present in the PTSD patients but self-reported differential valence or contingency were not different. During extinction recall, differential amygdala activity correlated positively with the intensity of numbing and ventromedial prefrontal cortex activity correlated positively with behavioral avoidance.

Conclusions: PTSD patients show heightened return of fear in neural and peripheral measures. In addition, self-reported arousal was high to both danger (CS+) and safety (CS-) cues. These results suggest that a deficient maintenance of extinction and a failure to identify safety signals might contribute to PTSD symptoms, whereas non-PTSD subjects seem show normal responses.

1. Introduction

Posttraumatic stress disorder (PTSD) is characterized by re-experiencing of the eliciting traumatic event, chronic hyperarousal, avoidance behaviors and negative alterations in cognition and mood (American Psychiatric Association, 2013). Prevalent theories about the disorder suggest that enhanced acquisition and delayed extinction of conditioned fear (Orr, Metzger, Lasko, Macklin, Peri, and Pitman, 2000), disturbed trauma and extinction memories (Ehlers and Steil, 1995; Milad, Orr, Lasko, Chang, Rauch, and Pitman, 2008; Milad, Pitman, Ellis, Gold, Shin, Lasko, Zeidan, Handwerger, Orr, and Rauch, 2009) and deficient processing of contextual information and safety cues play a role in the disorder (Acheson, Gresack, and Risbrough, 2012; Flor and Wessa, 2010a; Jovanovic, Norrholm, Blanding, Davis, Duncan, Bradley, and Ressler, 2010; Rougemont-Bücking, Linnman, Zeffiro, Zeidan, Lebron-Milad, Rodriguez-Romaguera, Rauch, Pitman, and Milad, 2011; Wessa, Jatzko, and Flor, 2006). In pavlovian fear conditioning, an initially neutral or conditioned stimulus (CS) is repeatedly paired with an aversive unconditioned stimulus (US) until its presentation alone elicits a conditioned response (CR), which is often but not always similar to the unconditioned response (UR). In differential delay fear conditioning one CS acts as danger signal and predicts the occurrence of the US (CS+) whereas a second stimulus is never followed by a US and acts as safety signal (CS-) and the CS and the US overlap in time. The CR can be extinguished by presenting the CS repeatedly without subsequent delivery of the US. However, this procedure does not erase the originally acquired CS-US association, which can be reactivated and elicit the CR under certain circumstances (Bouton and Bolles, 1979; Lovibond, Davis, and O'Flaherty, 2000; Norrholm, Anderson, Olin, Jovanovic, Kwon, Warren, McCarthy, Bosshardt, Sabree, Duncan, Rothbaum, and Bradley, 2011a; Rescorla and Heth, 1975).

Several mechanisms can disturb extinction memory and can trigger the originally acquired CR after extinction. These include spontaneous recovery (the mere passage of time) (Pavlov, 1972; Rescorla, 2004), reinstatement (post-extinction confrontation with the US alone) (Rescorla and Heth, 1975) and renewal (context change after extinction) (Bouton and Ricker, 1994). In the case of renewal, it is important to note that the extinction of a fear response is context-specific (Bouton, 2002; 2004; Corcoran and Maren, 2001; Huff, Hernandez, Blanding, and LaBar, 2009) and a mere change of context can revoke the extinguished CR, whereas the acquisition of a fear response generalizes

to different contexts. One explanation for this phenomenon is that after successful conditioning and extinction, two memory traces exist that compete against each other (Bouton, 1993): 1) the originally acquired CS-US association and 2) a newly formed CS-noUS association. Therefore the CS becomes ambiguous and external factors such as the context are thought to regulate the behavioral response (Bouton, 2002).

Brain imaging studies (Bremner, 2003; Liberzon and Sripada, 2008; Pitman, Rasmusson, Koenen, Shin, Orr, Gilbertson, Milad, and Liberzon, 2012; Shin, Rauch, and Pitman, 2006; Yehuda and LeDoux, 2007) have shown that (a) heightened amygdala activity results in exaggerated acquisition of fear and hyper-responsivity to threat-related stimuli (Bremner, Vermetten, Schmahl, Vaccarino, Vythilingam, Afzal, Grillon, and Charney, 2005; Protopopescu, Pan, Tuescher, Cloitre, Goldstein, Engelien, Epstein, Yang, Gorman, LeDoux, Silbersweig, and Stern, 2005; Rauch, Whalen, Shin, McInerney, Macklin, Lasko, Orr, and Pitman, 2000; Shin, Orr, Carson, Rauch, Macklin, Lasko, Peters, Metzger, Dougherty, Cannistraro, Alpert, Fischman, and Pitman, 2004; Yang, Mozhui, Karlsson, Cameron, Williams, and Holmes, 2008), (b) deficient frontal cortical function mediates a failure to maintain extinction and to suppress amygdala-driven responses to trauma-related stimuli (Bremner et al., 2005; Shin, Wright, Cannistraro, Wedig, McMullin, Martis, Macklin, Lasko, Cavanagh, Krangel, Orr, Pitman, Whalen, and Rauch, 2005) and, (c) deficient hippocampal function leads to deficits in explicit learning/memory (Vermetten, Vythilingam, Southwick, Charney, and Bremner, 2003) and a failure to modulate cue-related memories by the context, i.e. to appreciate safe contexts (Acheson et al., 2012; Flor and Wessa, 2010b; Steiger, Nees, Wicking, Lang, and Flor, 2015) in PTSD.

In line with this it was proposed (Acheson et al., 2012; Flor and Wessa, 2010a) that in PTSD deficient hippocampal function at the time of trauma may lead to exaggerated associations between cues and the trauma and a deficient association of the context and the trauma. As a result, later exposure to a single element of the context can trigger a fear response and this is experienced out of the corrective context. Indeed, Milad et al. (2008; 2009) showed that recall of fear extinction is reduced in PTSD patients compared to trauma-exposed but unaffected controls in a context-dependent conditioning paradigm. During acquisition and extinction, the CSs (different colors of a lighted lampshade) where presented within two different contexts (stationary pictures of two different rooms). When subjects were again confronted with the CSs one day later in the extinction context (ABB), the PTSD patients showed elevated skin conductance

responses. In accordance with the theoretical framework, this failure to retain extinction memory was accompanied by reduced activation in the hippocampus, as well as reduced activation in the ventromedial prefrontal cortex and enhanced activation in the dorsal anterior cingulate cortex (Milad et al., 2009).

Jovanovic et al. (2012) proposed that PTSD patients fail to inhibit fear responses to an acquired danger stimulus in the presence of a safety signal as a result of impaired safety learning and an inability to modulate fear responses with safety cues, which presumably requires a cognitive, cortical component (Bremner et al., 2005; Weike, Schupp, and Hamm, 2008). Indeed, several studies (Blechert, Michael, Vriends, Margraf, and Wilhelm, 2007; Grillon and Morgan, 1999; Peri, Ben-Shakhar, Orr, and Shalev, 2000) found evidence for a generalized (or sensitized) reaction to the unreinforced stimulus in PTSD.

Thus, in PTSD, the failure to maintain extinction could be enhanced since the contextual processing of stimuli may be compromised due to the hippocampal impairments found in PTSD patients and those vulnerable for PTSD (Acheson et al., 2012; Bremner, Vythilingam, Vermetten, Southwick, McGlashan, Nazeer, Khan, Vaccarino, Soufer, Garg, Ng, Staib, Duncan, and Charney, 2003a; Gilbertson, Shenton, Ciszewski, Kasai, Lasko, Orr, and Pitman, 2002; Steiger et al., 2015).

In our study, we presented two CSs in the context of virtual reality (VR) scenes and used an ABC conditioning procedure, where acquisition, extinction, and extinction memory were tested in three different contexts to increase the ecological validity of the experimental design. We further used a traumatized control group without PTSD and a never traumatized control group to differentiate the impact of and coping with trauma per se from the influence of PTSD symptomatology. We hypothesized that PTSD patients compared to traumatized persons without PTSD and healthy trauma-naïve controls show heightened return of fear in self-report, peripheral and central indicators of fear such as verbal ratings, differential SCR and amygdala activation.

2. Methods and materials

2.1. Participants

Eighteen persons with PTSD, 18 trauma-exposed subjects without PTSD (nonPTSD) and 18 healthy controls without trauma experience (HC) participated in the study. PTSD and nonPTSD were included if they had experienced a traumatic event meeting the A-criterion for PTSD in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; American Psychiatric Association; American Psychiatric Association, 2013). The PTSD group additionally fulfilled criteria B through F verified by the Clinician-Administered PTSD Scale (CAPS; Blake, Weathers, Nagy, Kaloupek, Gusman, Charney, and Keane, 1995). All traumatic events were experienced in adulthood, i.e. after 18 years of age. The HC group was matched to the trauma-experienced samples but had never been confronted with a trauma. All participants underwent the Structured Clinical Interview for DSM-IV I and II (Fydrich, Renneberg, Schmitz, and Wittchen, 1997; Wittchen, Wunderlich, Gruschwitz, and Zaudig, 1997). Exclusion criteria were comorbid current or lifetime psychotic symptoms, borderline personality disorder and current alcohol/drug dependence or abuse, cardiovascular or neurological disorders, brain injury, acute pain, continuous pain or medication for attention deficit hyperactivity disorder, pregnancy and metal implants. All subjects completed a cognitive test battery in order to test memory and general cognitive function, including the Culture Fair Intelligence Test (CFT; Weiß, 1998), the Multiple Choice Word Fluency Test (MWT-B; Lehrl, 2005), and the "Kurztest für allgemeine Basisgrößen der Informationsverarbeitung" [Short Test for General Factors of Information Processing] (KAI; Lehrl, Gallwitz, Blaha, and Fischer, 1991). There were no differences in cognitive and memory function as well as general intelligence (IQ) between the three groups. Subjects additionally answered questionnaires between sessions: PTSD patients scored higher in the Center for Epidemiological Studies Depression Scale (ADS; Hautzinger and Bailer, 1993), the Childhood Trauma Questionnaire (Bernstein and Fink, 1998) and the trait version of the State-Trait-Anxiety Inventory (Spielberger, 2010). The ethics committee of the Medical Faculty Mannheim, Heidelberg University approved the study. All participants gave written informed consent. The patients were offered treatment, controls were reimbursed (€80) for travel and other expenses. Table 1 presents the demographic and clinical characteristics of the participants (for patient flow see

supplemental Figure 1). The study conformed to the Code of Ethics of the World Medical Association (World Medical Association, Declaration of Helsinki, seventh revision, 2013).

Table 1. Demographic and psychometric data for the posttraumatic stress disorder-patients (PTSD), the trauma-exposed subjects without PTSD (nonPTSD) and the healhy controls (HC).

	PTSD (n=18)	**nonPTSD** (n=18)	**HC** (n=18)	**group statistic**
Demographics				
sex (m/f)	9/9	9/9	11/7	χ^2 (2) = .59, p = .746
age, mean (SD)	39.39 (12.36)	40.61 (14.21)	36.61 (12.21)	F(2, 51) = .45, p = .640
Education, N° general education/ secondary modern school/ grammar school	1/7/8	1/5/10	1/4/11	χ^2 (2) = 1.93, p = .381
Handedness, mean (SD)	82.87 (22.36)	76.52 (50.86)	76.24 (41.67)	F(2, 51) = .16, p = .855
Intelligence quotient, mean (SD) MWT-B	108.18 (16.25)	109.11 (14.44)	112.94 (14.80)	F(2, 49) = .35, p = .709
Intelligence quotient, mean (SD) KAI	108.11 (15.92)	111.56 (14.50)	116.44 (15.04)	F(2, 51) = 1.37, p = .263
Intelligence quotient, mean (SD) CFT	114.28 (9.98)	120.72 (14.83)	115.72 (10.63)	F(2, 51) = 1.43, p = .249
Trauma severity				
Months since trauma, mean (SD)	125.44 (130.32)	103.61 (141.66)		F(1, 34) = .23, p = .633
Trauma type (I/II)	15/3	18/0		χ^2 (1) = 3.18, p = .074
- N° accident (car, plane, fire, other)	4 (2, 1, 1, 0)	12 (5, 2, 1, 4)		
- N° rape	2	0		
- N° (gun) violence	6	6		
- N° war (attack, helicopter crash)	3 (2, 1)	0		
- N° imprisonment	2	0		
- N° aggressive stalking	1	0		
Loss of control, mean (SD)	91.47 (24.22)	74.12 (39.38)		F(1, 32) = 2.40, p = .132
Helplessness, mean (SD)	92.12 (24.32)	86.76 (20.69)		F(1, 32) = .48, p = .494
Fear, mean (SD)	77.35 (36.32)	57.06 (44.55)		F(1, 32) = 2.12, p = .155
The feeling to die, mean (SD)	61.18 (46.89)	47.35 (44.20)		F(1, 32) = .78, p = .383
N° injuries during trauma	11	8		
Clinician Administered PTSD Scale				
Re-experiencing, items 1-5, mean (SD)	1.92 (0.89)	0.64 (0.14)		F(1, 33) = 72.16, p < .001
Avoidance, items 6-12, mean (SD)	1.56 (0.70)	0.41 (0.13)		F(1, 33) = 78.74, p < .001

Hyperarousal, items 13-17, mean (SD)	2.01 (0.84)	0.14 (0.28)		F(1, 33) = 80.17, p < .001
Emotional numbing, items 9-12, mean (SD)	1.55 (1.04)	0.00 (0.00)		F(1, 33) = 37.76, p < .001
Posttraumatic Diagnostic Scale, mean (SD)	32.76 (9.44)	4.44 (4.10)		F(1, 33) = 135.2, p < .001
Comorbidities/ Medication				
N°comorbid major depression	5	0	0	
N° remitted major depression	5	1	1	
N° other acute or remitted comorbid disorder	9	0	0	
Depression Scale (ADS), sum (SD)	25.53 (9.59)	8.61 (5.28)	6.83 (5.14)	F(2, 50) = 38.52, p < .001
Childhood Trauma Questionnaire, mean (SD)	33.94 (14.01)	27.82 (5.49)	27.11 (6.28)	F(2, 50) = 2.81, p = .070
State-Trait Anxiety Inventory (Trait), sum (SD)	50.19 (9.09)	32.25 (7.42)	33.22 (10.29)	F(2, 47) = 20.14, p < .001
N° antidepressant medication	7	1	3	
N° other medication	6	5	9	

Note. CFT = Culture Fair Intelligence Test, *MWTB* = the Multiple Choice Word Fluency Test, *KAI* = "Kurztest für allgemeine Basisgrößen der Informationsverarbeitung" [Short Test for General Factors of Information Processing], *ADS* = Center for Epidemiological Studies Depression Scale

2.2. Stimuli and experimental procedure

We used a differential delay cued fear conditioning paradigm that was administered in three different VR environments (ABC renewal) built in NeuroVR 1.5 (Riva, Gaggioli, Villani, Preziosa, Morganti, Corsi, Faletti, and Vezzadini, 2007). The three virtual contexts depicted an apartment, an office and a classroom, consisting of several rooms each (Figure 1). Two transparent geometric shapes (square and rhombus) were presented in front of the contexts and served as CS+ and CS-. The CSs were built in MS Office Power Point (2007, Redmond, WA, USA) and inserted into the virtual rooms byVirtualDub32 bit (www.virtualdub.org), covering about 15% of the screen. The shape filling was white with a black frame and the whole figure was 20% transparent, so that the CSs did not cover any item of the background context. The subjects could navigate through the contexts in an initial habituation phase (see supplement). During fMRI, pre-recorded (Fraps® 2.9.9; www.fraps.com) paths including the CSs were presented using Presentation 14.4 software (Neurobehavioral Systems, Albany, CA, USA). The US was a slightly painful electric stimulus applied at the right thumb (Pohlack, Nees, Liebscher, Cacciaglia, Diener, Ridder, Woermann, and Flor, 2012).

The fMRI assessments were spread across three days (Figure 1). The first session began by determining the intensity of the painful stimulus (see supplement). In the habituation (HAB) phase each CS was presented five times for eight seconds with an inter-trial-interval (ITI) of 18 (+/-2) seconds in front of a grey background. The electric stimulus was presented six times for 500 milliseconds in the ITI and with no connection to either of the geometric shapes. The acquisition (ACQ) phase followed immediately in virtual context A. The geometric shapes were presented in front of the scene for eight seconds with an ITI of 10 (+/-2) seconds (i.e., 8, 9.5, 10.5, 12). One geometric shape (CS+) was always paired with the pain-US whereas the other shape was never paired with the painful stimulus (CS-). The US was presented for 500 milliseconds 7.5 seconds after the onset of the CS+, so that both stimuli co-terminated (delay conditioning). Acquisition consisted of 30 CS+US and 30 CS- presentations (ACQ1: first 15 CS+US/CS-, ACQ2: last 15 CS+US/CS-). The participants were uninformed about the CS-US contingency and were told to passively watch the pre-recorded path through the virtual environment. They returned to the scanner on the following day for extinction (EXT). The procedure of day 1 was repeated in virtual context B without presentation of the pain-US (EXT1: first 15 CS+noUS/CS-, EXT2: last 15 CS+noUS/CS-). Differences in the speed of acquisition and extinction between the patients and controls were accounted for by employin a large number of trials in each condition. This permitted a comparable baseline for the extinction memory test, which followed one week later. Extinction memory ("renewal"; REN) was tested in virtual context C, where 10 CS+ and 10 CS- were presented (REN1: first 2 CS+noUS/CS-, REN2: last 8 CS+noUS/CS-) without the pain-US. The order of the initial CS+/CS- appearances was counterbalanced so that the participants started with one of two sequences (CS+ CS- CS- CS+ or CS- CS+ CS+ CS-) (92) in a randomized fashion. Contexts and cues were randomized across subjects.

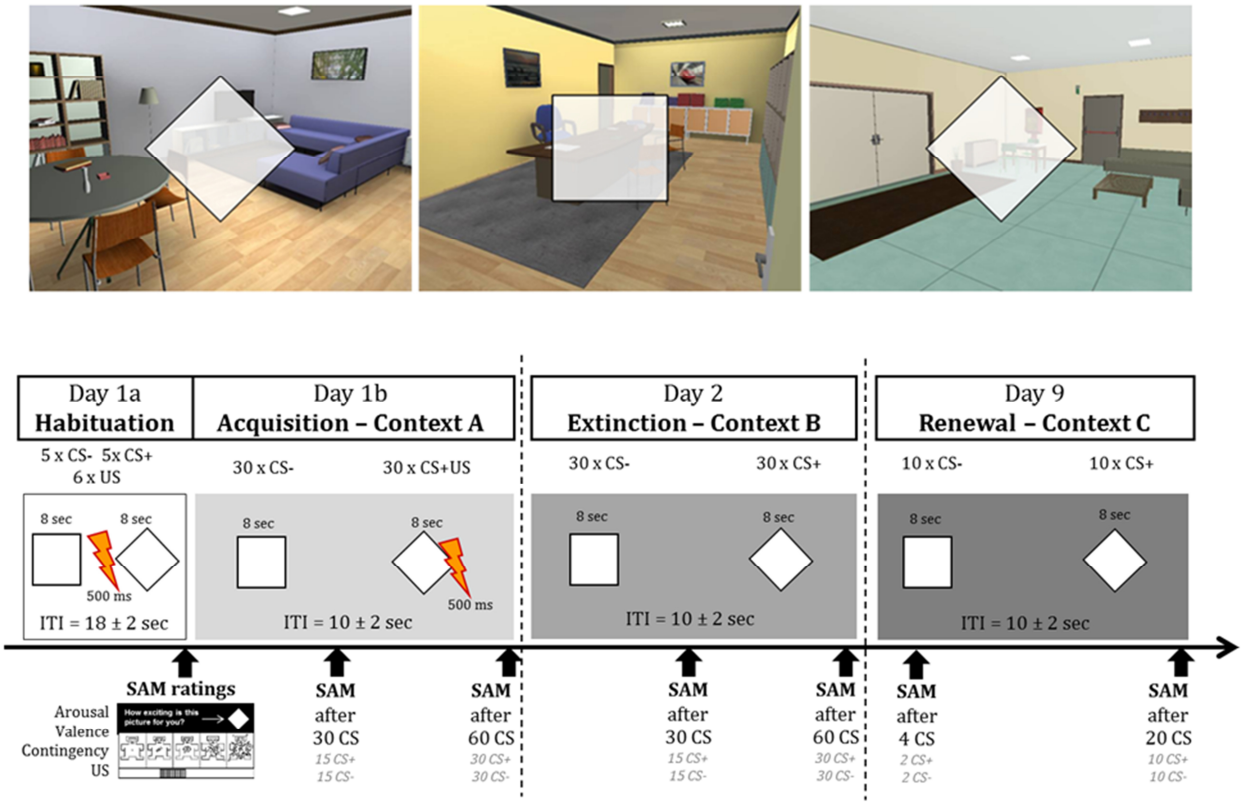

Figure 1. Top: Examples of the three virtual contexts (apartment, office, classroom), including the two transparent conditioned stimuli (square and rhombus). Bottom: Schematic illustration of the experimental procedure. US = unconditioned stimulus, CS+ = conditioned stimulus paired with the US, CS- = conditioned stimulus NOT paired with the US, ITI = inter trial interval, SAM = Self-Assessment Manikin.

2.3. Skin conductance responses (SCR)

SCRs were continuously recorded using a Brain Products galvanic skin response magnetic resonance module and Brain Vision Recorder 1.05 (Brainproducts, Munich, Germany). Two silver/silver chloride electrodes were placed on the thenar and hypothenar eminence of the participants' right hand. We used a sampling rate of 5000 Hz, filters were DC and 250 Hz. The data were downsampled to 10Hz using BrainVision Analyzer 1.05 and analyzed using the Matlab R2010b (The MathWorks Inc., Natick, MA, USA) based software Ledalab V3.4.3 (*www.ledalab.de*). After manual artefact correction we used the batch mode including smoothing with a Gaussian window width of 40 samples and 6-fold optimization to perform a continuous decomposition analysis (Benedek and Kaernbach, 2010). The SCRs were quantified as the sum of amplitudes above a minimum amplitude threshold criterion of 0.01 µS within a predefined response window of 1–7.5s (Pineles, Vogt, and Orr, 2009) after stimulus onset and normalized using a logarithmic [y=log(x+1)] transformation. For the SCR data, REN1

was defined as the first CS+/CS- presentation to account for the short duration of the expected return of fear. Accordingly, EXT2 comprises the last CS+/CS- presentation to describe the endpoint of the extinction procedure. A total of 8 PTSD patients, 6 nonPTSD subjects and 6 healthy controls did not show sufficient SCRs to the experimental stimuli and were excluded from the SCR analysis (Phelps, Delgado, Nearing, and LeDoux, 2004).

2.4. Self -reports

The participants rated arousal and emotional valence of the CSs using the self-assessment manikin (SAM; Bradley and Lang, 1994). CS/US contingency was assessed by asking "how likely is this picture [picture of the CS+ or CS- within the current VR background] followed by a painful stimulus". Ratings were given on a visual analogue scale (VAS) ranging from "not likely" to "extremely likely". The unpleasantness and intensity of the US were rated from "just painful/ unpleasant" to "extremely painful/ unpleasant". The ratings were administered at HAB, ACQ1, ACQ2, EXT1, EXT2, REN1 and REN2and were later transformed to a 9-point scale.

2.5. Magnetic resonance imaging (MRI)

Whole-brain MRI images were acquired using a 3T Magnetom TRIO whole body MR-scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a standard 12-channel head coil. A gradient-echo echo planar imaging (EPI) sequence (protocol parameters: TR = 2700 ms; TE = 27 ms; matrix size = 96 x 96; field of view = 220 x 220 mm^2; flip angle = 90°, GRAPPA PAT 2) was used to record 1180 functional volumes: 100 for habituation, 450 for acquisition and extinction each and 180 for the extinction memory test. Each volume consisted of 40 axial slices (slice thickness = 2.3 mm; gap = 0.7 mm, voxel size = 2.3mm^3) measured in descending slice order and positioned along a tilted line to the anterior-posterior commissure (AC-PC orientation). An automated high-order shimming technique was used to maximize magnetic field homogeneity.

Functional volumes were analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB R2010B. After discarding the first four volumes to account for T_1-saturation effects, images were realigned to the fifth volume by minimizing the mean square error (rigid body transformation). Participants with motion estimates exceeding 2.3 mm and 2° were excluded from analyses. The images were slice time corrected to

reference slice 20 and normalized to the standard space of the Montreal Neurological Institute (MNI) using EPI template provided by SPM8. To reduce spatial noise (and allow for corrected statistical inference), the volumes were smoothed with a 7.0 x 7.0 x 9.0 mm^3 Gaussian kernel. For statistical analyses the fMRI time series were high-pass filtered (temporal cut off: 128 s) and corrected for serial autocorrelations using first-order autoregressive functions AR(1). On the first level, we set up a general linear model (GLM) including the following experimental conditions: CS+ first and second half, CS- first and second half and baseline in AQC, EXT and REN and CS+, CS-, US in HAB. These inputs were convolved with a canonical hemodynamic response function (first order expansion) to create the design matrix. The six parameters describing the rigid body transformation were implemented as confound variables in the statistical analyses to covary out signal that is correlated with head motion. For the CSs, we analyzed the blood-oxygen-level-dependent (BOLD) signal within a response window of 1-7 seconds after stimulus onset, so that the response to the US would not interfere with the response to the CSs.

The data analysis was limited to the first part of each phase to reduce motion artefacts, except for habituation, which was short. Second-level whole brain analyses were performed for each group separately in order to detect conditioning-specific activation. Functional region of interest (ROI) analyses were used for group comparisons. In line with our a priori hypothesis, the ROIs were amygdala and hippocampus as defined by masks taken from the Wake Forest University Pick Atlas 3.0.4 (Maldjian, Laurienti, Kraft, and Burdette, 2003). The mask for the vmPFC targeted Brodman areas (BA) 10, 14, 25 and 32 (as well as parts of BA 11, 12 and 13) as defined by Nielsen-Hansen masks (Nielsen and Hansen, 2002). For each subject, mean β-weights were extracted from these predefined ROIs using the REX toolbox for SPM (Duff, Cunnington, and Egan, 2007).

2.6. Statistical data analysis

The analysis focused on the beginning of the test phase (REN1) (Bouton and Ricker, 1994; Neumann and Longbottom, 2008). The primary outcome variable for all measures was a difference score (D-Score) that describes the net difference between the CS+ and the CS- [f(D-score) = CS+ – CS- for self-reports and SCR or f(D-score) = CS+ > CS- for functional MRI (fMRI)] and hence conditioning-specific ("differential") effects.

For self-reports and SCR, we conducted repeated-measures ANOVAS with CS type (CS+, CS-) as within subjects factor and group (HC, nonPTSD, PTSD) as between subjects factor. When applicable, we used one-sided paired-samples t-tests for post-hoc analyses. Within group analyses were performed using one-sided one sample t-tests. The statistical fMRI analysis included whole brain analyses and functional ROI analyses of the test phase (REN1) for the three groups and an additional comparison of the mean β-weights of the pre-defined ROIs between groups using independent samples t-tests.
The CS+ and CS- alone during REN1, as well as the phases preceding the context change, specifically the endpoints of conditioning (ACQ2) and extinction (EXT2), were treated as control variables. The summarized results are presented within this paper. Details on the statistics exceed the scope of this paper and can be found in the supplement.
To assess interrelations between symptom severity and return of fear, we calculated one-tailed bivariate correlations between CAPS scores (PTSD only) and brain activity (mean beta-weights) in the amygdala, the hippocampus and the vmPFC during REN1.
The general significance level was set to alpha = .05. Greenhouse–Geisser corrections were implemented when appropriate and corrections of the α-level were performed where necessary. For the fMRI analysis, we used family-wise-error (FWE) corrected α-levels at the cluster (whole-brain) or the peak level (ROI). All further statistical analyses were performed with SPSS 20 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

2.7. Laboratory study

An additional experiment was conducted in the laboratory, where SCR, but no fMRI was recorded. The general procedure remained the same as in the main study, but on day 3, the subjects (N = 7 PTSD patients) re-entered context B (the extinction context) instead of the novel context C (ABB). Ten CS+noUS and 10 CS- were presented with SAM ratings after the first two CS+noUS/CS- (SPON1) and at the end of the phase (SPON2). The US was not delivered in this phase. The SCR was recorded using Biobench (http://www.ece.umd.edu/biobench/), subsequent data analysis was performed according to the main study. Since there was only one experimental group, statistical data analysis included repeated-measures ANOVAs with CS-type (CS+, CS-) and phase (EXT2, SPON1) as within-subject factors. Post-hoc analyses were conducted using Bonferroni-corrected paired-samples t-tests. Since the return of fear habituated quickly,

further tests in other contexts were not feasible in the scanner and necessitated a separate test for spontaneous recovery.

3. Results

3.1. Skin conductance responses

We found a significant CS type x group interaction at the beginning of the extinction memory test ($F(2,31) = 3.403$, $p = .046$). Post-hoc group comparisons revealed significantly higher differential SCRs (D-scores) in the PTSD patients than in either healthy group (PTSD vs. HC = : $t(20) = 1.86$, $p = .039$; PTSD vs. nonPTSD : $t(20) = 2.07$, $p = .026$). Within group analyses confirmed a significant differentiation between CS+ and CS- only in the PTSD patients ($t(9)= 1.91$, $p=.04$), but not the nonPTSD and HC (Figure 2).

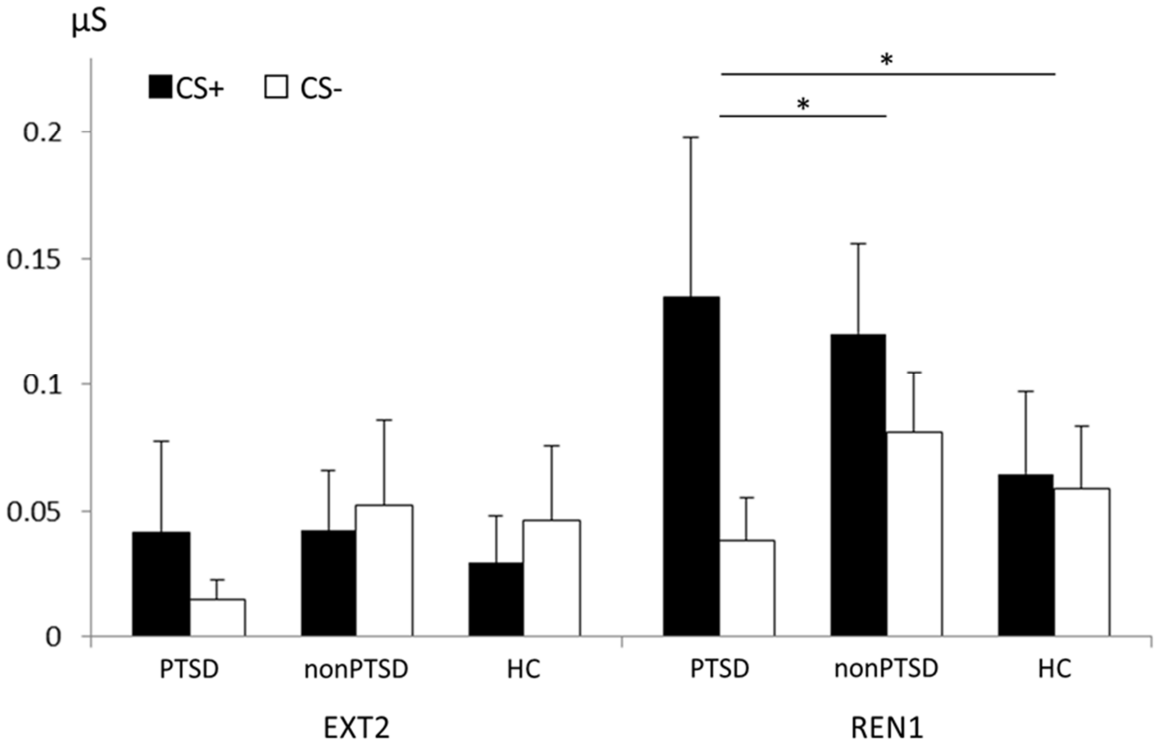

Figure 2. Skin conductance responses (SCRs) of the CS+ (reinforced conditioned stimulus) and the CS- (unreinforced conditioned stimulus) at the end of the extinction training (EXT2) and at the beginning of the extinction memory test (REN1) for posttraumatic stress disorder (PTSD) patients, trauma-exposed non-PTSD subjects (nonPTSD) and non-trauma healthy controls (HC). * = $p < .05$, error bars represent standard errors. Note: For exact values of the SCR data, please refer to supplemental table S1.

3.2. Self-report

There were no significant CS type x group interactions in arousal, valence and contingency ratings at REN1. Within-group analyses showed significant differential responses in the PTSD group for arousal ($t(17) = 2.83$, $p = .012$) (Figure 3) and contingency ($t(17) = 2.35$, $p = .031$) and in the nonPTSD group for contingency ($t(17) = 2.53$, $p = .01$) (Figure 3).

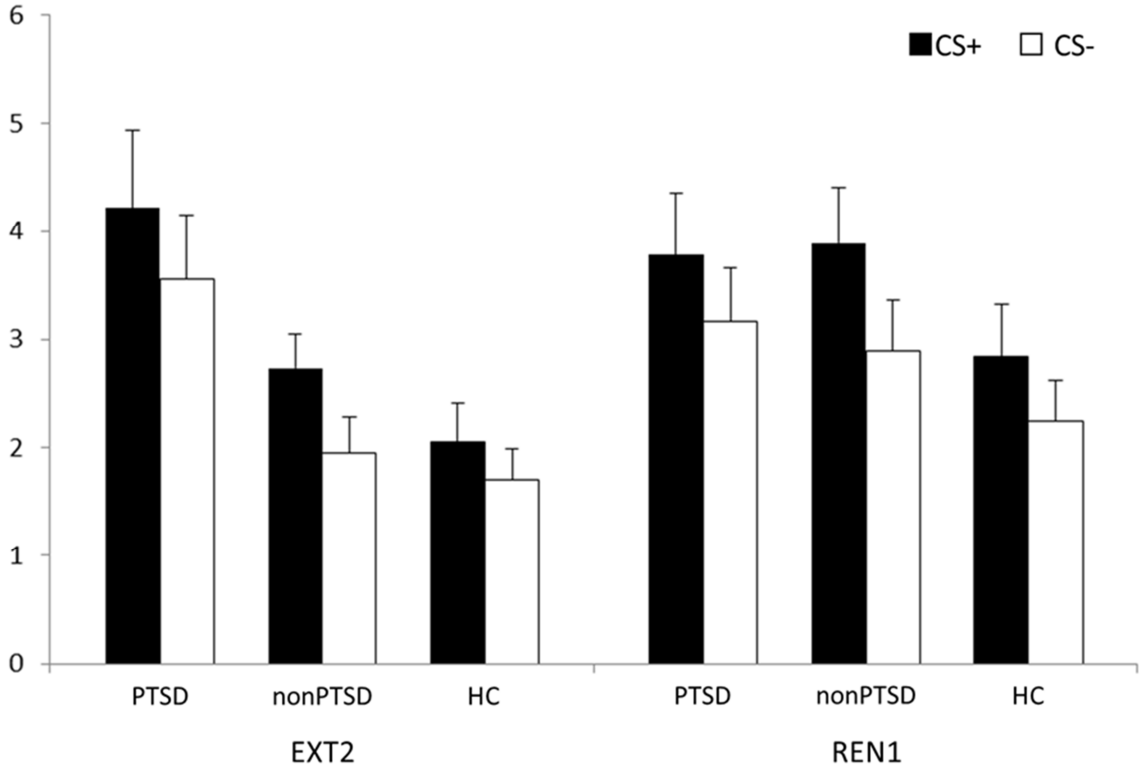

Figure 3. Arousal ratings of the CS+ (reinforced conditioned stimulus) and the CS- (unreinforced conditioned stimulus) at the end of the extinction training (EXT2) and at the beginning of the extinction memory test (REN1) for posttraumatic stress disorder (PTSD) patients, trauma-exposed non-PTSD subjects (nonPTSD) and non-trauma healthy controls (HC). * = $p < .05$, error bars represent standard deviations. Note: For exact values of arousal, valence, contingency ratings as well as ratings of the unconditioned stimulus, please refer to supplemental table S1.

3.3. Functional magnetic resonance imaging (fMRI)

Whole brain activation during REN1 revealed significant activations in the thalamus, hippocampus as well as frontal and temporal areas. Table 2 lists all brain regions that showed significant activation in REN1 separately for each group.

Table 2. Significant whole brain activations during return of fear

Group	Area of Activation	MNI coordinates x,y,z	*p*
PTSD	Temporal pole: superior temporal gyrus-R	46, 5, -17	4.68^{-6}
	Fusiform gyrus-L	-37, -45, -23	5.53^{-6}
	Superior frontal gyrus, orbital part-R	16, 28, -14	1.61^{-4}
	Thalamus-R	5, -13, -2	1.01^{-4}
	Middle temporal gyrus-R	44, -66, 19	2.33^{-4}
	Middle temporal gyrus-R	-27, -2, -29	2.46^{-4}
	Middle temporal gyrus-R	46, -59, 1	2.96^{-4}
	Parahippocampalgyrus-R	23, 5, -26	3.05^{-4}
	Middle frontal gyrus-R	35, 35, 43	3.88^{-4}
	Inferior frontal gyrus, triangular part-L	-34, 28, -2	6.12^{-4}
	Lobule 3-R	9, -43, -14	6.45^{-4}
	Middle frontal gyrus-R	51, -4, 52	7.46^{-4}
	Middle frontal gyrus-R	30, 19, 43	9.26^{-4}
	Hippocampus-L	-14, -9, -17	9.36^{-4}
	Superior temporal gyrus-R	55, -20, -2	9.93^{-4}
nonPTSD	Thalamus-L	-9, -27, 10	2.61^{-4}
	Superior frontal gyrus, dorsolateral-R	16, 21, 58	3.31^{-4}
	Supplementary motor area-R	12, 19, 55	3.96^{-4}
HC	Inferior frontal gyrus, opercular part-R	32, 8, 31	2.00^{-5}
	Hippocampus-R	37, -15, -17	2.97^{-4}
	Inferior frontal gyrus, triangular part-R	44, 31, 4	3.45^{-4}
	Inferior parietal, but supramarginal and angular gyri-L	-53, -36, 43	5.07^{-4}
	Median cingulate and paracingulategyri-L	-7, -11, 31	6.07^{-4}
	Inferior parietal, but supramarginal and angular gyri-L	-57, -34, 46	6.35^{-4}
	Inferior frontal gyrus, opercular part-R	44, 10, 7	9.24^{-4}
	Inferior parietal, but supramarginal and angular gyri-L	-32, -59, 52	9.55^{-4}
	Inferior frontal gyrus, opercular part-L	-50, 8, 22	9.65^{-4}

Note: Contrast: CS+ >CS-; threshold for peak voxel p < .001, two-tailed, uncorrected; cerebellum not included; PTSD = posttraumatic stress disorder patients, nonPTSD = trauma-exposed non-PTSD subjects, HC = non-trauma healthy controls

ROI analyses at REN1 revealed significantly higher activation in the right hippocampus (p = .044) in PTSD compared to HC.

The results of the extracted mean β-weights from the a priori defined ROIs can be found in Figure 4. There was a significant difference between PTSD and HC (t(34) = 2.24, p =

.032) in amygdala activity at the beginning of the test phase. No significant group differences were found for the hippocampus and the vmPFC.

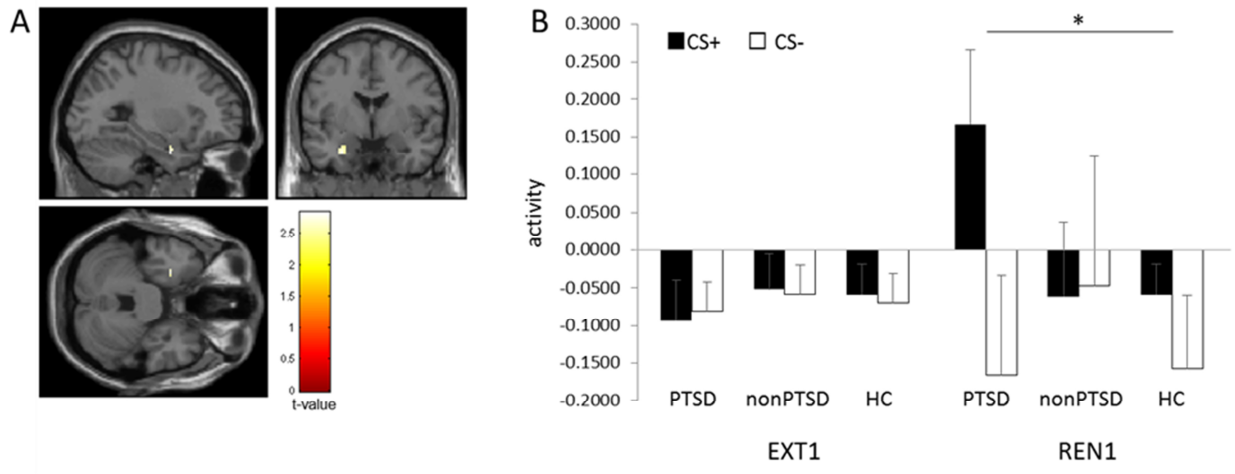

Figure 4. **A**. Higher left amygdala activity in posttraumatic stress disorder (PTSD) patients compared to healthy controls (HC) in a region of interest (ROI) analysis. **B**. Activity in the left amygdala (mean β-weights) in posttraumatic stress disorder (PTSD) patients, trauma-exposed non-PTSD subjects and non-trauma healthy controls (HC) over the course of the experimental procedure. ACQ1 = first 15 reinforced conditioned stimulus (CS+)/unreinforced conditioned stimulus (CS-) trials of acquisition, EXT1 = first 15 CS+/CS- trials of extinction, REN1 = first 2 CS+/CS- trials of renewal. * = $p < .05$, error bars represent standard errors.

The analysis of the control variables (CS+ and CS- alone during the test phase, phases preceding the context change) revealed that the differences at REN1 were mainly driven by high CS+ ratings and that there were no significant group differences directly prior to the context change, as all groups showed successful extinction. Specifically, in the SCR data, all groups displayed significant or trend-level reactions to the CS+ and CS- alone during the context change, but there were no group differences in the individual CSs. No significant CS-type x group interaction was found at the end of acquisition or extinction. Analyses of the CS+ and CS- alone during ACQ2 and EXT2 did not reveal any significant group differences. See supplemental Table S1 for the course of the individual SCRs throughout the experiment. In the self-reports, all three groups showed significant CS+ ratings for REN1 in arousal, valence and contingency. CS+ arousal ratings were higher in PTSD compared to HC. All three groups showed significant CS- ratings at REN1 in arousal, valence and contingency. CS- arousal ratings were higher in PTSD compared to HC. There were no significant CS-type x group interactions in arousal, valence and

contingency ratings at ACQ2 and EXT2 (see supplemental table S1). Analyses of the CS+ and the CS- alone showed that CS+ ratings in arousal, valence and contingency were significantly higher during acquisition as compared to all other time points. CS- ratings were significantly higher during habituation as compared to the following phases for valence and contingency, but not arousal, where PTSD patients rated the CS- as highly arousing. A subsequent analysis of the CS+ at the end points of conditioning (ACQ2) and extinction (EXT2) revealed a significant phase x group interaction. Post-hoc analyses showed a significant main effect of phase for the two healthy groups, but not for the PTSD patients, where the ACQ2 did not differ significantly from EXT1 (Figure S3). All subjects rated the US as equally unpleasant. In the fMRI, we found significant group differences for the CS+ alone during context change in the left (PTSD > HC) and right amygdala (PTSD > nonPTSD) and the left and right hippocampus (PTSD > HC), but not for the CS-. There were no significant group differences during acquisition and extinction. For details on the statistical analysis and results of this section, please refer to the supplement.

3.4. Laboratory study

The SCR data suggested successful conditioning and extinction and showed a rise of both CSs during the extinction test. However, since the reactions to the CS+ and the CS- were both elevated to approximately the same level (see Figure 5), this effect was probably a mere orienting response and not (differential) spontaneous recovery. The statistical analysis confirmed that there was no significant CS type (CS+, CS-) x phase (EXT2, SPON1) effect ($F(1,6) = .21$, p = n.s.) and that the CS+ and the CS- did not significantly differ during EXT2 ($t(6) = .033$, p = n.s.) and SPON1 ($t(6) = -.446$, p = n.s.).
In the SAM ratings (arousal, valence, contingency), the repeated measures ANOVA revealed a significant main effect for CS type only for arousal ($F(1,6) = 6.08$, $p = .049$) but not for contingency and valence (see supplemental Figure S2). Indeed, a slight elevation of the CS+ and hence the difference between CS+ and CS- can be seen at SPON1, but seems irrelevant when taking the high standard deviation into account. Accordingly, post-hoc comparisons did not reveal significant differences between CS+ and CS- at EXT2 ($t(6) = 2.12$, p = n.s.) and SPON1 ($t(6) = 1.87$, p = n.s.).

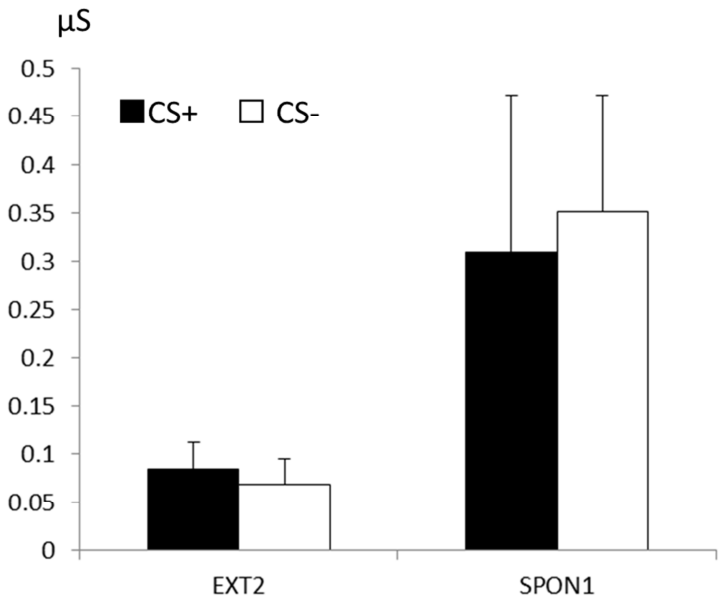

Figure 5. Results from the laboratory study: Skin conductance responses (SCRs) of the CS+ (reinforced conditioned stimulus) and the CS- (unreinforced conditioned stimulus) at the end of the extinction training (EXT2) and at the beginning of the extinction recall test within the same context (SPON1). PTSD = posttraumatic stress disorder patients, nonPTSD = trauma-exposed non-PTSD subjects, HC = non-trauma healthy controls, error bars represent standard errors.

3.5. Correlational analyses

In the PTSD group there were significant positive correlations between the intensity of numbing and differential left amygdala activity at REN1 (r = .424, p =.040) and between the frequency and intensity of avoidance (avoidance total) and differential vmPFC activity at REN1 (frequency: r = .608, p =.014, intensity: r = .509, p =.022, total: r = .406, p =.046).

4. Discussion

In this imaging study we investigated the return of fear after conditioning and extinction in PTSD patients using VR environments. PTSD patients showed elevated return of fear as indicated by a larger differential SCR compared to non-PTSD and HC and greater differential left amygdala activity during the early test phase than HC. On the verbal level, however, PTSD patients did not exhibit higher differential responses during the context change.

In the present study, PTSD patients demonstrated to be more vulnerable to the return of fear when being confronted with the CS+ after extinction as they exhibited higher differential SCRs and higher amygdala activity in context C than non PTSD and HC, respectively. One likely explanation for the reactivation of the CR is the context change after extinction (renewal). However, other mechanisms, such as spontaneous recovery might also be responsible for the result (Milad, Orr, Pitman, and Rauch, 2005). To test the specificity of the effect, we conducted an extinction recall phase where we presented the CS again in the extinction context. The PTSD patients in this experiment did not show differential spontaneous recovery (albeit showing elevated SCRs to both CSs suggesting a strong orienting response in this phase). Hence, we suggest that the context change after extinction most likely elicited the CR in the original study. However, since the laboratory study was only conducted in a small number of subjects (none of whom was part of the original study) and did not include control groups, we cannot completely rule out other mechanisms for the explanation of the increased return of fear in our PTSD sample.

A study in veterans with PTSD and healthy combat controls, that included an extinction recall phase in a fear conditioning paradigm (ABBA) found reduced renewal of fear on the original learning context (Garfinkel, Abelson, King, Sripada, Wang, Gaines, and Liberzon, 2014). The authors suggested that the PTSD patients are unable to use contextual cues to modulate fear responses and thus fail to appropriately use both safety and danger context information, resulting in impaired extinction recall in the safety context and a lack of fear renewal in the danger context. However, there may have been an interaction of the responses to the A and B context that were both presented and the presentation of the extinction context (B) might have reinforced extinction, thus reducing renewal. Therefore further studies are needed to determine under which conditions (i.e., number of trials/phases, type of trauma, type of context)

and prior experiences (i.e., successful conditioning and extinction) fear renewal occurs in PTSD patients.

In the current study, PTSD patients displayed higher differential amygdala activity in the new context, suggesting more fear recall in this group. This result leads to the assumption that relapse after extinction in PTSD may be associated with hyperresponsivity of the amygdala and that amygdala activity prevails in PTSD patients when extinction learning needs to be maintained. These deficits may be associated with more return of fear and might be a reason for relapse in PTSD (Hayes, Hayes, and Mikedis, 2012). Interestingly, amygdala activity at REN1 correlated significantly positively with emotional numbing in the PTSD patients, emphasizing its role for PTSD symptoms.

Structural findings have consistently shown bilateral hippocampal volume reduction in PTSD compared to trauma-naïve as well as trauma-exposed controls (Karl, Schaefer, Malta, Dorfel, Rohleder, and Werner, 2006; Kitayama, Vaccarino, Kutner, Weiss, and Bremner, 2005; Smith, 2005). In healthy humans, hippocampal volume was found to be positively associated with contextual conditioning (Pohlack et al., 2012). Functional neuroimaging studies on hippocampal function in PTSD, however, have produced mixed results. Both, reduced (Shin and Liberzon, 2010) and (potentially compensatory) increased activation (Bremner et al., 2003a) compared to controls have been reported. In the present study, we found higher hippocampal activity during renewal in PTSD. Rauch, Shin and Phelps (2006) reason that "diminished hippocampal function could be paradoxically protective by interfering with fear conditioning and promoting the generalization of extinction" (p. 379). Indeed, it has been shown in rodents that inactivation of the hippocampus facilitates the generalization of extinction across contexts and interferes with renewal and contextual reinstatement of conditioned fear (Corcoran and Maren, 2001; 2004; LaBar and Phelps, 2005). Our result might indicate that in turn elevated hippocampal function interrupts the generalization of extinction so that a novel context more easily promotes the return of fear. However, one needs to be cautious when interpreting higher brain activation, as more hippocampal engagement in a task could reflect a compensatory mechanism in response to a lack of efficiency which – in the case of PTSD – could be a result of volume reduction. Indeed, compensatory mechanisms as a consequence of dysfunctional brain circuits have

previously been described (Bokde, Lopez-Bayo, Born, Ewers, Meindl, Teipel, Faltraco, Reiser, Moller, and Hampel, 2010; Fanselow, 2010).

In the PTSD sample we observed a positive relationship between differential vmPFC activity during renewal and behavioral avoidance. However, we did not find significant group differences in the differentiation of CS+ and CS- during test. Others (Milad et al., 2009; Rougemont-Bücking et al., 2011) have reported vmPFC deactivation in PTSD but have not directly compared a reinforced with an unreinforced stimulus. Our result therefore is in line with previous research that highlighted the role of the vmPFC for PTSD.

Even though elevated return of fear was present in physiological measures, the PTSD patients did not show this effect in fear ratings. This result might be related to group differences prior to the test phase. The arousal ratings of the PTSD patients differed significantly from that of the two healthy groups. The PTSD patients continued to rate the CS+ as highly arousing during extinction and rated the CS- as highly arousing throughout the experiment (even exceeding the level of arousal to the CS+ in the healthy groups). We can therefore assume deficient explicit differential learning in PTSD that could account for the lack of change in the new context in this measure. The result further confirms previous research suggesting that PTSD patients are impaired in identifying safety signals, such as specific cues (CS-) or general contexts (extinction context) (Grillon and Morgan, 1999; Jovanovic et al., 2012; Norrholm, Jovanovic, Olin, Sands, Karapanou, Bradley, and Ressler, 2011b; Peri et al., 2000). As a consequence, safe situations might seem ambiguous to them and therefore constitute a potential threat. The fact that chronic hyperarousal is one of the core symptoms of PTSD is in line with these findings.

In the SCR data, trauma-exposed non-PTSD controls did not significantly differ from healthy controls but showed significantly smaller SCRs than the PTSD patients. In the amygdala and hippocampus ROI analyses, however, the non-PTSD group did not differ from PTSD, even though the healthy controls did. This observation is in accordance with previous research where trauma-exposed non-PTSD subjects have produced mixed results either differing from PTSD (Diener, Wessa, Ridder, Lang, Diers, Steil, and Flor, 2012; Milad et al., 2009; Norrholm et al., 2011b; Wessa and Flor, 2007) or showing intermediate responses (Diener, Nees, Wessa, Wirtz, Frommberger, Penga, Ruttorf, Ruf, Schmahl, and Flor, 2014; Steiger et al., 2015) or differing only in one measure but not

the other (Rougemont-Bücking et al., 2011). This inconsistent picture might indicate that the mere experience of a traumatic event can lead to changes in fear conditioning and extinction, but that the impact is not as uniform as in full-blown PTSD, presumably depending on individual factors of the trauma itself and the vulnerability of the individual. Our study indicates that the experience of trauma alone leads to peripheral but not central physiological change in conditionability and extintionability. Furthermore, non-PTSD subjects seem to possess mechanisms protecting them from harmful consequences of these physiological changes. One candidate might be explicit mechanisms that compensate for possible effects on the behavioral level. In our study, non-PTSD and HC subjects showed identical patterns in verbal arousal ratings, clearly differing from PTSD patients. Hence, one can assume that potentially frontal-cortical mechanisms might be capable of compensating for deteriorating sub-cortical effects. However, the present study cannot answer this question, but the result offers questions for further research aiming at characterizing the difference between the experience of trauma and PTSD symptomatology.

Research conducted in healthy participant groups has repeatedly produced renewal effects in the absence of an anxiety disorder (Alvarez, Johnson, and Grillon, 2007; Effting and Kindt, 2007; Grillon, Alvarez, Johnson, and Chavis, 2008; Hermann, Stark, Milad, and Merz, 2016; Milad et al., 2005; Vansteenwegen, Hermans, Vervliet, Francken, Beckers, Baeyens, and Eelen, 2005). In the present study, we did not observe such a return of fear in the healthy controls. A possible explanation for this negative result is the extensive extinction training. It has previously been shown that healthy subjects fail to show significant renewal effects after a high number of extinction trials (Claassen, Mazilescu, Thieme, Bracha, and Timmann, 2016).

The study has several limitations. We had a small sample size and had to further reduce the sample for the SCR analysis due to non-responders (25.9%) (Milad et al., 2005; Raio, Brignoni-Perez, Goldman, and Phelps, 2014). Even though this clearly reduces the quality of the data, it is a common problem in combined fMRI-SCR studies (Dziobek, Preissler, Grozdanovic, Heuser, Heekeren, and Roepke, 2011; Haaker, Lonsdorf, Thanellou, and Kalisch, 2013; Hartley, Fischl, and Phelps, 2011; Kalisch, Korenfeld, Stephan, Weiskopf, Seymour, and Dolan, 2006; LaBar and Phelps, 2005; Lonsdorf, Haaker, and Kalisch, 2014; Phelps et al., 2004; Winkelmann, Grimm, Pohlack, Nees, Cacciaglia, Dinu-Biringer, Steiger, Wicking, Ruttorf, Schad, and Flor, 2015) as high

filtering is necessary to reduce scanner-derived artifacts. Also, we included medicated (n = 11) patients in our sample (Bremner et al., 2003a; Bremner, Vythilingam, Vermetten, Southwick, McGlashan, Staib, Soufer, and Charney, 2003b; Grillon and Morgan, 1999; Milad et al., 2009) which might have had a debilitating influence on the extinction learning of our human sample through altered brain activity (Burghardt, Sigurdsson, Gorman, McEwen, and LeDoux, 2013). However, there are some strengths of our sample. The traumatic experiences of our subjects were diverse compared to previous studies using only male war survivors (Eckart, Stoppel, Kaufmann, Tempelmann, Hinrichs, Elbert, Heinze, and Kolassa, 2011; Geuze, Westenberg, Jochims, de Kloet, Bohus, Vermetten, and Schmahl, 2007; Gilbertson, Williston, Paulus, Lasko, Gurvits, Shenton, Pitman, and Orr, 2007; Grillon and Morgan, 1999; Milad et al., 2008) or women with childhood sexual abuse (Bremner et al., 2003a; Bremner et al., 2003b; Gurvits, Lasko, Repak, Metzger, Orr, and Pitman, 2002), thus allowing for more generalizability of the findings.

To conclude, we provide evidence for possibly context related return of fear (renewal) in PTSD patients that is correlated with hyperactivity of the amygdala and elevated arousal as measured by SCR and subjective ratings. This finding supports the hypothesis that PTSD is characterized by deficient extinction maintenance which is a probable cause for relapse. Nevertheless, the lack of fear renewal in our healthy comparison groups shows that fear renewal is not a simple and universal phenomenon like animal studies suggest (Vervliet, Baeyens, Van den Bergh, and Hermans, 2012), and that further research is needed to understand its neural underpinnings.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft: grant SFB636/C1 to H.F. and grant SFB636/Z3 to L.R.S.

Aspects of this work were presented at the Fifth European Meeting on Human Fear Conditioning, May 22nd – 24th 2013, Leuven, Belgium (Centre for the Psychology of Learning and Experimental Psychopathology, Katholieke Universiteit Leuven) and the 39thTagungPsychologie&Gehirn, May 30th – June 1st 2013,Würzburg, Germany.

Financial Disclosure

All authors reported no financial or potential conflicts of interest.

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Supplemental Information

Supplemental methods

Experimental procedure (VR habituation task)

On day one, before the functional magnetic resonance (fMRI) measurement, subjects were habituated to the contexts and tested for memory of the contexts outside the scanner in an initial active exploration phase. They were equipped with a head-mounted display and a game pad and asked to walk through all rooms of one context for five minutes using the NeuroVR player 1.5. (Riva, Gaggioli, Villani, Preziosa, Morganti, Corsi, Faletti, and Vezzadini, 2007). To ensure that subjects paid attention to the virtual rooms, we included a memory task. Before the walkthrough started, subjects were instructed to remember the location of all furniture. After each context, they had to identify the actual furniture on a two-dimensional map of the three virtual environments with a time limit of again five minutes. Each map included eleven target and ten distractor items. Subjects were instructed to name the targets (i.e, when they remembered a piece of furniture at a certain location) and cross out the distractors (i.e. when they did not remember any object at a certain location). Objects and distractor items were depicted by squares of different sizes, so that identification based solely on the shape of the objects was not possible.

Determination and statistical analysis of painful stimulation

The slightly painful electric stimulus was delivered by an electrical stimulus generator (Digitimer, DS7A, Welwyn Garden City, UK) through a cupric (copper) electrode attached to the participants' right hand. Pain threshold and pain tolerance were obtained through the administration of increasingly painful stimuli (50 ms bursts, 12 Hz) and the participants indicated how painful the stimulus was until it was unbearable. The procedure was repeated three times. The values of the last two runs were entered into the formula

*(mean pain tolerance-mean pain threshold)*0.8+pain threshold*

in order to obtain a value of 80% of the pain tolerance. Subjects were then asked to rate the pain intensity and unpleasantness on a Likert scale ranging from 0 (just painful or unpleasant) to 10 (extremely painful or unpleasant). We only used pain ratings that were equal to or above 7 on both scales. When subjects did not rate the pain as aversive after habituation, the intensity was increased by 0.4 mA.

Group differences in US ratings were analyzed via oneway ANOVAs.

Statistical analysis of the control variables

The control analysis included acquisition (primarily ACQ2 as the endpoint of conditioning) and extinction (primarily EXT 2 as the endpoint of extinction) as well as individual analyses of the CS+ and CS- during all experimental phases. According to the analysis of SCR and self-reports during the test phase, we conducted repeated-measures ANOVAS with CS type (CS+, CS-) as within subjects factor and group (HC, nonPTSD, PTSD) as between subjects factor. When applicable, we used one-sided paired-samples t-tests for post hoc analyses. The statistical fMRI analysis was also conducted according to the test phase. It included whole brain analyses and functional ROI analyses of the test phase (REN1) for the three groups and an additional comparison of the mean β-weights of the pre-defined ROIs between groups using independent samples t-tests.

CS+ and CS- were analyzed separately by one-sample t-tests for within group effects and independent-samples t-tests for between-group effects in all measures. Because we observed an interesting pattern in the SAM ratings of the PTSD patients, we performed a data driven in-depth analysis of this measure: We conducted repeated-measures ANOVAs of the D-score with phase (HAB, ACQ1, ACQ2, EXT1, EXT2, REN1, REN2) as within-subject factor and group (PTSD, nonPTSD, HC) as between-subject factor. Furthermore, we conducted repeated-measures ANOVAs of the CS+ alone including ACQ2 and EXT2 to capture the endpoints of learning. When applicable, post-hoc t-tests were conducted.

As in the main study, the general significance level was set to alpha = .05. Greenhouse–Geisser corrections were implemented when appropriate and corrections of the α-level were performed where necessary. For the spm-based fMRI, we used family-wise-error (FWE) corrected α-levels at the cluster (whole-brain) or the peak level (ROI), respectively. All further statistical analyses were performed with SPSS 20 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

Supplemental results

VR habituation task

Correct recognition was operationalized as 1) identification of a target and 2) crossing out a distractor item. There were no significant group differences in both measures in any of the three contexts (one-way ANOVA: all $F > 1.12$, all $p < .131$). There was, however, a trend towards a group difference in distractor recognition in the "office" context (nonPTSD = 6.65, PTSD = 5.0, HC = 4.76; $F(2, 51) = 2.91$, $p = 0.71$).

Painful stimulation

All subjects rated the US as unpleasant (means [standard deviations]: HAB=5.22 [1.57], ACQ1=5.57 [1.88], ACQ2=5.80 [1.98]) and painful (means [standard deviations]: HAB=4.6 [1.69], ACQ1=4.98 [1.80], ACQ2=5.52 [1.90]) and there were no significant group differences (all $F<1.279$, all $p>.287$).

Skin conductance responses

CS+ alone: During context change, nonPTSD showed significant activation to the CS+ ($t(10)= 3.696$, $p=.004$). PTSD and HC showed a trend towards significant activation to the CS+ (PTSD: $t(7)= 2.293$, $p=.056$; HC: $t(8)= 2.073$, $p=.072$). There were no significant group differences.

CS- alone: During context change, nonPTSD and HC showed significant activation to the CS- (nonPTSD: $t(10)= 3.181$, $p=.01$; HC: $t(8)= 2.417$, $p=.042$). PTSD patients showed a trend towards significant activation to the CS- ($t(7)= 2.133$, $p=.07$). There were no significant group differences.

PTSD patients displayed higher differential skin conductance than HC during habituation. ($t(15) = -2.769$, $p = .014$). There were no differences between PTSD and nonPTSD and no group differences in SCR to the US. Furthermore, there were no group differences during ACQ2 and EXT2 in the difference score, the CS+ alone and the CS- alone.

Self-reports

CS+ alone: All three groups showed significant CS+ ratings for REN1 in arousal (all ts>5.718, all ps<.001), valence (all ts> 8.481, all ps<.001) and contingency (all ts>5.500, all ps< .001) (Table 2). CS+ arousal ratings were higher in PTSD compared to HC ($t(34) = 1.77$, $p = .043$).

CS- alone: All three groups showed significant CS- ratings at REN1 in arousal (all ts> 6.022, all ps<.001), valence (all ts> 7.903, all ps<.001) and contingency (all ts> 4.366, all ps< .001) (Table 2). CS- arousal ratings were higher in PTSD compared to HC ($t(29.04) = 1,89$, $p = .034$).

The in-depth analysis of the arousal, valence and contingency ratings indicated successful conditioning for the entire sample in all three dimensions as indicated by significant main effects of phase (all $F > 13.89$, all $p < .0001$) for the D-score. Post-hoc Bonferroni corrected pairwise comparisons indicated significantly higher differentiation of the CS+ and the CS- during acquisition (ACQ1 and ACQ2) as compared to habituation (HAB) and extinction (EXT1 and EXT2) (all $p < .01$). Similar analyses of the CS+ and the CS- alone showed that CS+ ratings were significantly higher during acquisition (ACQ1 and ACQ2) as compared to all other time points (all $p < .001$) and CS- ratings were significantly higher during habituation as compared to the following phases (all $p <.048$) in valence (ACQ1, ACQ2, EXT1, REN2) and contingency (ACQ1, ACQ2, EXT1, EXT2, REN1, REN2). The CS- effect was not observed in arousal ratings. Individual analyses of the three groups replicated the overall findings for the most part, the two healthy groups showed the expected conditioning pattern (i.e. higher D-scores in ACQ compared to the other phases reaching significance or trend level) in arousal, valence and contingency (all $p < .097$). The PTSD patients only showed such successful conditioning and extinction in valence and contingency (all $p < .071$), but not in arousal.

Subsequent analyses of group effects in arousal ratings were performed, since PTSD patients did not show successful learning in this measure. We chose to analyze the CS+ alone, because CS- ratings have already revealed a PTSD specific pattern in the overall analysis that distorts the D-score. The analysis of the endpoints of conditioning (ACQ2) and extinction (EXT2) revealed a significant phase*group interaction ($F(2, 51) = 4.415$, $p = .017$, $\eta^2 = .1485$). Individual group analyses showed a significant main effects of phase only for the two healthy groups (HC: $F(1, 17) = 19.013$, $p < .001$, $\eta^2 = .528$; nonPTSD: $F(1, 17) = 16.498$, $p < .001$, $\eta^2 = .493$), but not for the PTSD patients

fMRI

Results for the individual CS during context change show the following: In the ROI analysis PTSD patients displayed significant activation to the CS+ alone in the right amygdala (p = .04) and the HC displayed a trend towards significant activation to the CS- alone in the right amygdala (p = .089). After extraction of the mean β-weights, we found significant group differences for te CS+ alone during context change for the left (PTSD > HC: t(22.32) = -2.11, p = .046) and right amygdala (PTSD > nonPTSD: : t(34) = -2,23, p = .032) and the left (PTSD > HC: : t(22.38) = -2.26, p = .035) and right (PTSD > HC: : t(20.18) = -2.39, p = .027) hippocampus, but no group differences for the CS-.

During acquisition, whole brain analyses revealed conditioning-specific activation in the anterior cingulated cortex (ACC), amygdala, insula, striatum, thalamus and frontal/ motor/ sensory cortices (Sehlmeyer, Schoning, Zwitserlood, Pfleiderer, Kircher, Arolt, and Konrad, 2009) in all three groups, which largely disappeared during extinction. Group comparisons of the ROIs in ACQ revealed a trend towards higher activation of the bilateral amygdala (left: p = .078, right: p = .098) in the PTSD patients compared to HC. Group comparisons of the ROIs in EXT revealed a trend towards higher activation of the left hippocampus (p = .094) in nonPTSD compared to PTSD. CS+ alone: In ACQ, PTSD patients compared to HC displayed significantly higher activation in the bilateral amygdala (left: p = .017, right: p = .026) and nonPTSD compared to PTSD displayed a trend towards higher activation in the right hippocampus (p = .076). There were no group differences in these ROIs in EXT. CS- alone: In ACQ, PTSD displayed a trend towards significantly higher activation in the left amygdala (p = .071) than HC and significantly higher activation in the vmPFC (p = .008) than nonPTSD. The latter effect was also visible in the whole brain comparison (p = .064 at [-18;56;22]). In EXT, there was higher activation of the left hippocampus (p = .042) in the PTSD patients, compared to HC.

Supplemental References

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Supplemental table

Table S1. Means (and standard deviations) for self-reports (arousal, valence, contingency) and skin conductance responses.

	HAB			ACQ1			ACQ2			EXT1		EXT2		REN1		REN2	
	CS+	CS-	US	CS+	CS-	US	CS+	CS-	US	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
arousal																	
PTSD	3.61 (2.00)	4.06 (1.63)	4.56# (1.79)	4.89 (2.00)	3.06 (1.92)	4.61# (1.85)	4.83 (2.28)	3.83 (2.50)	5.06# (2.04)	3.83 (2.43)	3.78 (2.24)	4.22 (3.06)	3.56 (2.53)	3.78 (2.44)	3.17 (2.09)	3.94 (2.78)	3.83 (2.38)
nonPTSD	3.61 (2.25)	2.89 (1.97)	4.94# (1.66)	5.39 (2.30)	2.56 (1.92)	5.50# (1.76)	5.50 (2.64)	2.22 (1.96)	6.06# (1.80)	3.22 (1.90)	2.83 (2.15)	2.72 (1.36)	2.00 (1.50)	3.72 (2.19)	2.67 (1.88)	2.78 (1.87)	2.39 (2.00)
HC	3.61 (2.17)	3.33 (1.97)	4.33# (1.65)	5.22 (2.18)	2.39 (1.85)	4.83# (1.76)	5.06 (2.82)	2.39 (2.17)	5.44# (1.82)	2.50 (1.92)	1.78 (1.35)	1.94 (1.51)	1.56 (1.20)	2.50 (1.86)	2.06 (1.35)	2.00 (1.53)	1.67 (1.28)
valence																	
PTSD	5.33 (1.94)	4.89 (1.53)	5.22° (1.93)	5.89 (2.35)	3.11 (1.88)	5.11° (1.91)	6.61 (1.91)	4.44 (2.83)	5.39° (1.91)	4.56 (2.43)	4.56 (2.46)	5.17 (2.94)	4.44 (2.79)	4.78 (2.39)	4.33 (2.33)	4.78 (2.77)	4.06 (2.51)
nonPTSD	4.33 (1.57)	4.00 (1.53)	5.17° (1.38)	6.39 (2.30)	2.67 (1.85)	6.06° (1.73)	6.50 (2.01)	2.33 (1.65)	6.33° (1.72)	4.06 (1.59)	3.06 (1.59)	4.00 (1.28)	3.39 (1.54)	4.28 (1.36)	3.67 (1.72)	4.17 (1.69)	3.06 (1.63)
HC	5.06 (2.31)	4.22 (2.18)	5.06° (1.43)	6.33 (1.94)	3.17 (2.56)	5.56° (1.98)	6.83 (2.15)	2.78 (1.93)	5.67° (2.25)	3.67 (1.94)	2.89 (1.68)	3.11 (1.97)	3.00 (1.85)	3.78 (1.87)	3.72 (1.64)	3.44 (1.79)	3.33 (1.72)
contingency																	
PTSD	4.72 (2.16)	5.11 (2.03)	---	7.06 (2.99)	1.67 (1.65)	---	7.89 (2.30)	2.00 (2.57)	---	3.50 (2.77)	2.56 (2.12)	3.11 (2.74)	2.00 (1.75)	3.56 (2.57)	2.33 (1.88)	2.56 (2.20)	2.22 (1.96)
nonPTSD	4.44 (1.95)	5.28 (2.47)	---	8.11 (1.53)	2.39 (2.55)	---	8.83 (.51)	1.78 (2.05)	---	2.22 (1.87)	1.61 (1.04)	2.11 (1.49)	1.56 (1.10)	2.61 (1.91)	1.72 (1.67)	1.94 (1.43)	1.11 (.47)
HC	5.83 (2.23)	4.67 (2.03)	---	8.00 (1.50)	2.56 (2.75)	---	7.83 (2.26)	1.67 (1.33)	---	2.61 (2.43)	1.94 (1.59)	1.44 (1.20)	1.39 (.98)	2.44 (1.89)	1.89 (1.53)	2.00 (1.97)	1.22 (.55)
SCR (µS)																	
PTSD	.109 (.14)	.140 (.18)	.375 (.47)	.077 (.11)	.050 (.07)	.156 (.07)	.053 (.09)	.025 (.03)	.092 (.09)	.054 (.10)	.304 (.05)	.050 (.09)	.024 (.039)	.083 (.10)	.034 (.04)	.015 (.02)	.012 (.02)
nonPTSD	.093 (.09)	.090 (.08)	.208 (.13)	.090 (.11)	.062 (.07)	.235 (.05)	.072 (.08)	.043 (.04)	.166 (.04)	.057 (.05)	.042 (.04)	.046 (.06)	.037 (.05)	.107 (.09)	.091 (.095)	.041 (.05)	.025 (.03)
HC	.067 (.04)	.049 (.04)	.213 (.10)	.047 (.03)	.031 (.03)	.169 (.09)	.038 (.05)	.024 (.03)	.126 (.08)	.059 (.06)	.037 (.03)	.032 (.048)	.030 (.03)	.041 (.06)	.036 (.04)	.027 (.04)	.020 (.03)

Note: CS+ = reinforced stimulus, CS- = unreinforced stimulus, US = unconditioned stimulus; HAB = habituation, ACQ = Acquisition: 1 = first 15 CS+/CS- trials, 2 = last 15 15 CS+/CS- trials, EXT = extinction trials: 1 = first 15 CS+/CS- trials, 2 = last 15 15 CS+/CS- trials, REN = renewal: 1 = first 2 CS+/CS- trials, 2 = last 8 15 CS+/CS- trials; PTSD: patients with posttraumatic stress disorder, nonPTSD: healthy subjects with trauma-experience, but no PTSD symptoms, HC: healthy controls without trauma experience. # painfulness rating, °unpleasantness rating.

Supplemental Figure Legends

Figure S1: Participant flow chart. The initial contact was made through public announcements (flyer, newspaper articles, talks), the in- and outpatient clinics of the Central Institute of Mental Health (Mannheim) and cooperating clinics (e.g. SRH Klink Karlsbad-Langensteinbach).The clinical interview and cognitive testing were generally administered on the first day of the experiment. In individual cases, i.e. when the diagnosis was unclear, the clinical interview was done on a fourth session prior to the experiment. Also, due to organizational issues, cognitive testing had to be moved to another day of assessment in single cases. Emphasis was laid on the stable timing of the fMRI assessment (i.e., one day between acquisition and extinction, seven days between extinction and renewal). However, in a total of twelve cases, the third day of measuring had to be pre- or postponed because of individual factors (sickness, work commitments of the participants, measurement slots in the scanner etc.) with a minimum of five and a maximum of ten days (mean = 6.94 days) between extinction and renewal. PTSD = posttraumatic stress disorder patients, nonPTSD = trauma-exposed non-PTSD subjects, HC = non-trauma healthy controls.

Figure S2: Course of arousal, valence and contingency ratings throughout the laboratory study for the PTSD sample. HAB = habituation, ACQ = Acquisition (1 = first 15 CS+/CS- trials, 2 = last 15 15 CS+/CS- trials), EXT = extinction trials (1 = first 15 CS+/CS- trials, 2 = last 15 15 CS+/CS- trials), SPON = spontaneous recovery (1 = first 2 CS+/CS- trials, 2 = last 8 15 CS+/CS- trials), REN = renewal (1 = first 2 CS+/CS- trials, 2 = last 8 15 CS+/CS- trials). CS = conditioned stimulus: solid lines = CS+ (paired with the unconditioned stimulus (US)), dashed lines = CS- (NOT paired with the US).

Figure S1

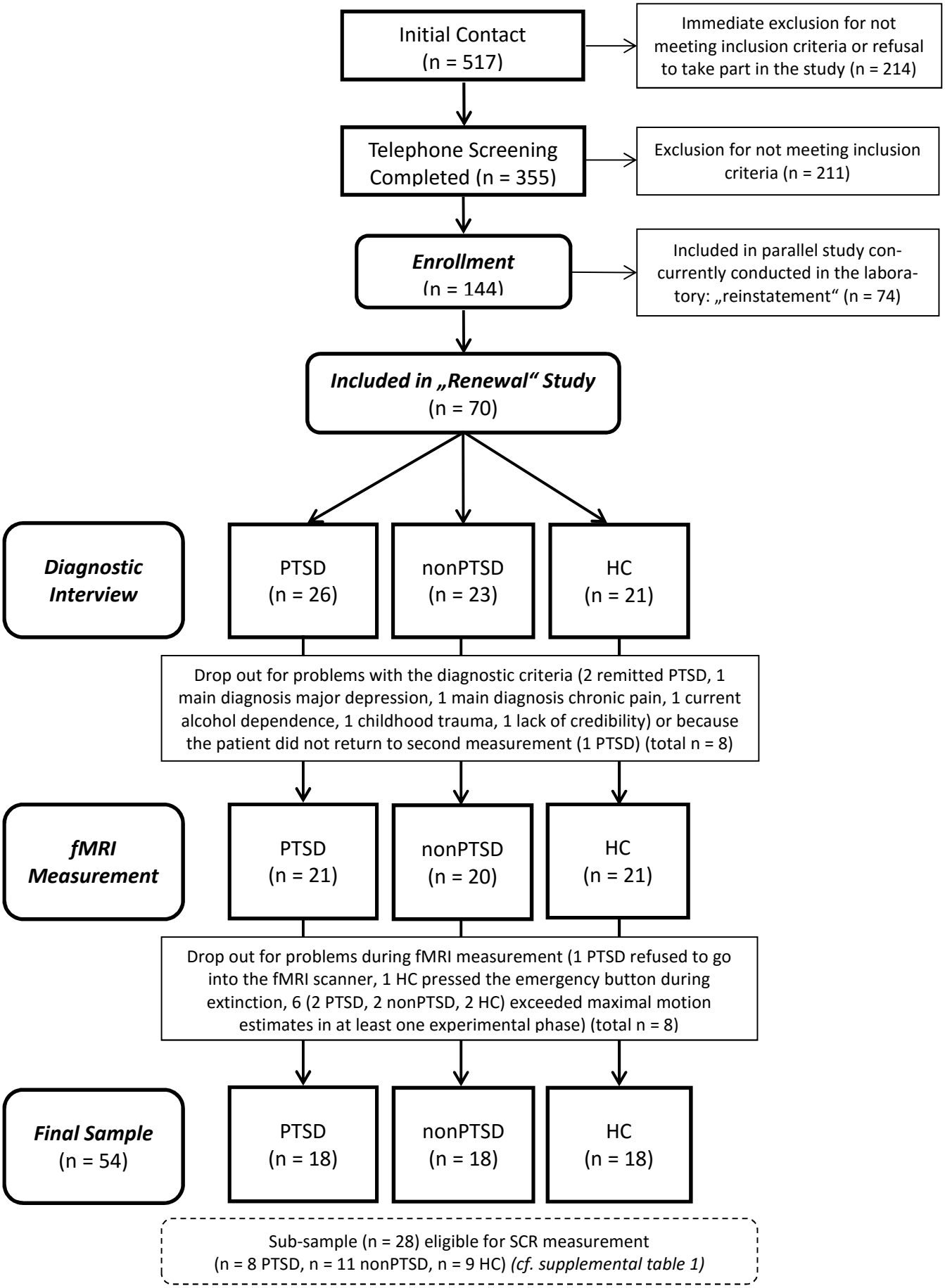
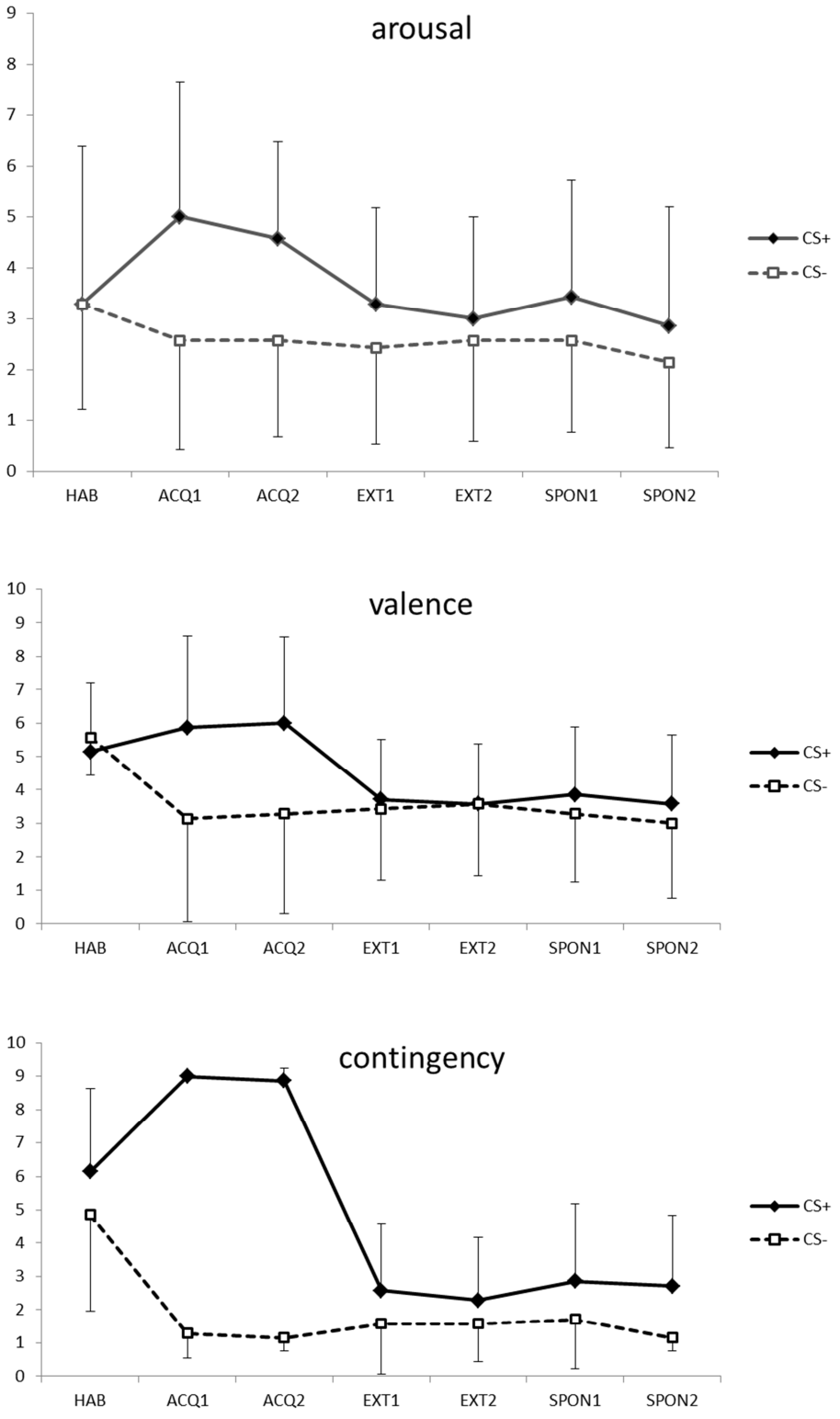

Initial Contact
(n = 517)
Immediate exclusion for not meeting inclusion criteria or refusal to take part in the study (n = 214)
Telephone Screening Completed (n = 355)
Exclusion for not meeting inclusion criteria (n = 211)
Enrollment
(n = 144)
Included in parallel study concurrently conducted in the laboratory: „reinstatement“ (n = 74)
Included in „Renewal“ Study
(n = 70)
Diagnostic Interview
PTSD
(n = 26)
nonPTSD
(n = 23)
HC
(n = 21)
Drop out for problems with the diagnostic criteria (2 remitted PTSD, 1 main diagnosis major depression, 1 main diagnosis chronic pain, 1 current alcohol dependence, 1 childhood trauma, 1 lack of credibility) or because the patient did not return to second measurement (1 PTSD) (total n = 8)
fMRI Measurement
PTSD
(n = 21)
nonPTSD
(n = 20)
HC
(n = 21)
Drop out for problems during fMRI measurement (1 PTSD refused to go into the fMRI scanner, 1 HC pressed the emergency button during extinction, 6 (2 PTSD, 2 nonPTSD, 2 HC) exceeded maximal motion estimates in at least one experimental phase) (total n = 8)
Final Sample
(n = 54)
PTSD
(n = 18)
nonPTSD
(n = 18)
HC
(n = 18)
Sub-sample (n = 28) eligible for SCR measurement
(n = 8 PTSD, n = 11 nonPTSD, n = 9 HC) (cf. supplemental table 1)

Figure S2


arousal
9
8
7
6
5
4
3
2
1
0
HAB
ACQ1
ACQ2
EXT1
EXT2
SPON1
SPON2
CS+
CS-
valence
10
9
8
7
6
5
4
3
2
1
0
HAB
ACQ1
ACQ2
EXT1
EXT2
SPON1
SPON2
CS+
CS-
contingency
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HAB
ACQ1
ACQ2
EXT1
EXT2
SPON1
SPON2
CS+
CS-

2.2. Study 2

Increased amygdala-insula functional connectivity correlates with symptom severity in posttraumatic stress disorder

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Keywords: Posttraumatic stress disorder (PTSD), fear conditioning, renewal, safety learning, amygdala, hippocampus

Formatted for "Journal of Psychiatry & Neuroscience"

Abstract

Background: Post-traumatic stress disorder (PTSD) is characterized by altered brain responses to emotional stimuli. Recent studies implied changes in resting state connectivity, including large-scale networks (e.g. the default mode network (DMN) and the salience network (SN)) and amygdala seeds, that were associated with PTSD symptoms. However, the direction of those effects remained equivocal. The goal of this study was to investigate resting-state functional connectivity and its relationship to symptom severity in DMN, SN and the amygdala in PTSD patients compared to trauma trauma-exposed subjects without PTSD and healthy controls without traumatic experience (HC).

Methods: All subjects (18 PTSD, 18 nonPTSD and 18 HC) participated in a 5.5 minute resting state scan. To investigate DMN and SN connectivity, we performed independent component analysis (ICA). A seed-based analysis of amygdala connectivity was performed by extracting spatially averaged time series for the amygdala seed and obtaining voxelwise correlations of the seed time courses with all other brain regions.

Results: On the network level, we did not find relevant alterations in connectivity (the DMN analysis did not yield significant group differences and the SN component was not detected in our sample). The seed-based analysis revealed heightened connectivity of the left amygdala with the left insula in PTSD versus nonPTSD. This connectivity correlated significantly positively with the intensity of re-experiencing. HC showed significantly higher positive correlations of the left amygdala with the right putamen and the right insula than both trauma-experienced groups.

Limitations: The sample size was small and we included PTSD patients with comorbid disorders. The methods allow for a description of correlational, but not causal relationships. We did not monitor cardiac and respiratory cycles online.

Conclusion: Our results indicate that altered amygdala-insula coupling and decreased amygdala-putamen coupling, but not DMN connectivity, relate to the pathophysiology of PTSD. Increased connectivity between the left amygdala and the left insula might influence re-experiencing intensity presumably through a stronger functional link between somatic sensations and emotional states.

Introduction

A better understanding of the neuronal correlates of mental disorders advances etiological psychobiological models and can generate new options for innovative therapeutic interventions (1). In recent years, the investigation of functional networks in the resting brain has become increasingly important. It is generally assumed that, when "doing nothing", spontaneous low-frequency (< 0.1 Hz) fluctuations are synchronized in time between distant brain regions that belong to the same functional network (2). Analogous structural connections have been demonstrated (3-5) and it has been shown that the signal changes arise from fluctuations in metabolic demand, unrelated to cardiac and respiratory effects (6). This baseline activity (7) provides a valuable tool for the understanding of human neural functional architecture and hence an opportunity to identify brain processes underlying mental disorders that are presumably interfering with active task performance (8). The Default Mode Network (DMN) (9) is one of the resting state networks that has received a lot of attention. The DMN includes the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC)/precuneus (PrC), inferior parietal cortices, lateral temporal cortices and hippocampus – areas that are generally deactivated when a person engages in a cognitively demanding task (10, 11). The DMN is thought to represent a self-referential and introspective state (12). Activity in the DMN is temporally anti-correlated with so-called task-positive networks that are activated during task performance and reflect extraspective attentional orienting, (13-15). The salience network (SN) ensures that the individual stays alert to changing environmental demands by detecting of and directing attention to biologically salient stimuli in the environment (14, 16, 17).

In PTSD patients, resting state findings on the network level yielded decreased as well as increased connectivity within different regions of the DMN (18) (18-22). Importantly, correlations between the strength of DMN connectivity and the severity of PTSD symptoms were reported in most studies (18-20, 22-24) and one study demonstrated that the type of trauma as well as the time from trauma modulate connectivity patterns (25). Lower anti-correlations between the DMN and the SN have also been reported (21, 26) and may indicate a relative dominance of threat-sensitive circuitry even in task-free conditions which might underlie the characteristic hypervigilance towards potential threat (18, 21, 27).

Additionally, research has started to investigate alterations in connectivity patterns directly addressing the PTSD-relevant brain areas (28). Of special interest in this regard is the amygdala, which is crucial for perceiving threat and generating fear responses (29) and has a pivotal role for the disorder (30). Rabinak et al. (31) found significantly higher positive connectivity between an anatomically derived right amygdala seed and the ipsilateral insula in veterans with PTSD compared to veterans without PTSD, but did not include a trauma-naïve control group. Another study investigating veterans with and without PTSD but no healthy controls (32) found stronger functional coupling between the bilateral amygdala and the right insula, as well as reduced functional coupling between the amygdala and the hippocampus, and decreased anti-correlation between the amygdala and ACC subregions. Decreased connectivity between the amygdala and the insula has also been reported (33-35), but in these studies connectivity was analyzed during emotional paradigms. Taken together, these and other (23, 36) results seem to confirm the idea of a disrupted neural circuit in PTSD including the amygdala, the hippocampus, the ventromedial prefrontal cortex (vmPFC) and the anterior insula (37-39) not only in task-related conditions but also under rest.

The relevance of amygdala connectivity for PTSD is furthermore evident in its correlation with symptom severity (19, 22, 40). Lanius et al. (19) found that resting state connectivity of the PCC – an important node of the DMN – with the right amygdala and the perigenual ACC positively correlated with acute PTSD symptoms in a group of patients who had experienced the traumatic event 6-12 weeks before the scan. Moreover, PCC – right amygdala connectivity 6 weeks post-trauma predicted PTSD symptoms as assessed by the Clinician Administered PTSD Scale (CAPS) (41) 12 weeks post-trauma. Zhou et al. (22) showed that the same connectivity pattern (PCC – right amygdala) was negatively correlated with CAPS scores. However, their participants were scanned 2 days post-trauma and underwent CAPS interviews at 1 and 6 months. Both results suggest that connectivity of the amygdala with other brain structures is related to PTSD symptomatology, but the direction of the relationship might depend on the time of scanning, and therefore the development of the (full-blown) disorder. Thus, altered resting state activity might be a consequence and develop differentially over time and not a pre-existing characteristic of the disorder. Undoubtedly, methodological differences (e.g. hardware, software, instructions, frequency range, pre-processing steps) might have contributed to these different findings.

In the present study, we examined resting state data of PTSD patients, trauma-exposed subjects without PTSD (nonPTSD) and healthy controls without prior trauma experience (HC), thus providing important comparisons for the understanding of traumatic experience and the development of PTSD symptoms or resilience. In search of unique biomarkers related to PTSD, resting state data have provided promising results (REF Tursich). By investigating both, resting-state functional connectivity of large-scale brain networks (ICA) and seed-based connectivity of the amygdala including its relation to symptom severity, we aim to contribute further insight into the alterations of connectivity patterns related to PTSD, thereby tackling the discrepancies observed in previous research (e.g. the direction of connectivity within the DMN, the direction of amygdala and SN connectivity with symptom severity) (28). Concerning large scale resting state networks, we hypothesized decreased DMN and increased SN connectivity in the PTSD group. In the seed-based analysis of amygdala connectivity, we hypothesized increased coupling with other brain regions such as the anterior insula in PTSD. Furthermore, we hypothesized that changes in amygdala-connectivity would be positively correlated with symptom severity.

Methods and materials

Participants

The present resting state study included 18 patients with acute PTSD, 18 trauma-exposed subjects without PTSD (nonPTSD) and 18 healthy controls without previous trauma experience (HC) (Table 1). All PTSD patients met criteria for current PTSB based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) (42). NonPTSD subjects fulfilled the A-Criterion for PTSD, but not Criteria B through F. Diagnoses were based on CAPS outcomes. All traumatic events occurred after 18 years of age and ranged from single events to episodes of chronic stress. Exclusion criteria were cardiovascular or neurological disorders, brain injury, acute pain, continuous pain, attention deficit hyperactivity disorder, medication, pregnancy and metal implants. All subjects completed a cognitive test battery in order to test memory and general cognitive function, including the Culture Fair Intelligence Test (CFT; 43), the Multiple Choice Word Fluency Test (MWT-B; 44), and the "Kurztest für allgemeine Basisgrößen der Informationsverarbeitung" [Short Test for General Factors of Information Processing] (KAI; 45). There were no differences in cognitive and memory function as well as general intelligence (IQ) between the three groups. The study was approved by the Ethics Committee of the Medical Faculty Mannheim, Heidelberg University. All participants gave written informed consent. Patients were offered psychotherapy in our outpatient clinic; control participants received €80 for travel and other expenses. The study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki, seventh revision, 2013) (46).

Table 1. Demographic and psychometric data for the posttraumatic stress disorder-patients (PTSD), the trauma-exposed subjects without PTSD (nonPTSD) and the healhy controls (HC).

	PTSD (n=18)	**nonPTSD** (n=18)	**HC** (n=18)	**group statistic**
Demographics				
sex (m/f)	9/9	9/9	11/7	χ^2 (2) = .59, p = .746
age, mean (SD)	39.39 (12.36)	40.61 (14.21)	36.61 (12.21)	F(2, 51) = .45, p = .640
Education, N° general education/ secondary modern school/ grammar school	1/7/8	1/5/10	1/4/11	χ^2 (2) = 1.93, p = .381
Handedness, mean (SD)	82.87 (22.36)	76.52 (50.86)	76.24 (41.67)	F(2, 51) = .16, p = .855

Intelligence quotient, mean (SD) MWT-B	108.18 (16.25)	109.11 (14.44)	112.94 (14.80)	F(2, 49) = .35, p = .709
Intelligence quotient, mean (SD) KAI	108.11 (15.92)	111.56 (14.50)	116.44 (15.04)	F(2, 51) = 1.37, p = .263
Intelligence quotient, mean (SD) CFT	114.28 (9.98)	120.72 (14.83)	115.72 (10.63)	F(2, 51) = 1.43, p = .249
PTSD Symptomatology				
Posttraumatic Diagnostic Scale, mean (SD)	32.76 (9.44)	4.44 (4.10)		F(1, 33) = 135.20, p < .001
PDS re-experiencing, item1-5 , mean (SD)	1.87 (0.65)	0.31 (0.40)		F(1, 33) = 73.87, p < .001
PDS avoidance, item 6-12, mean (SD)	1.90 (0.62)	0.15 (0.25)		F(1, 33) = 121.90, p < .001
PDS hyperarousal, item 13-17, mean (SD)	2.14 (0.84)	0.37 (0.37)		F(1, 33) = 66.80, p < .001
PDS emotional numbin, item 9-11, mean (SD)	1.63 (0.91)	0.11 (0.05)		F(1, 33) = 47.73, p < .001
CAPS re-experiencing, items 1-5, mean (SD)	1.92 (0.89)	0.64 (0.14)		F(1, 33) = 72.16, p < .001
CAPS avoidance, items 6-12, mean (SD)	1.56 (0.70)	0.41 (0.13)		F(1, 33) = 78.74, p < .001
CAPS hyperarousal, items 13-17, mean (SD)	2.01 (0.84)	0.14 (0.28)		F(1, 33) = 80.17, p < .001
CAPS emotional numbing, items 9-12, mean (SD)	1.55 (1.04)	0.00 (0.00)		F(1, 33) = 37.76, p < .001
Trauma severity				
Trauma type (I/II)	15/3	18/0		χ^2 (1) = 3.18, p = .074
Months since trauma, mean (SD)	125.44 (130.32)	103.61 (141.66)		F(1, 34) = .23, p = .633
Loss of control, mean (SD)	91.47 (24.22)	74.12 (39.38)		F(1, 32) = 2.40, p = .132
Helplessness, mean (SD)	92.12 (24.32)	86.76 (20.69)		F(1, 32) = .48, p = .494
Fear, mean (SD)	77.35 (36.32)	57.06 (44.55)		F(1, 32) = 2.12, p = .155
The feeling to die, mean (SD)	61.18 (46.89)	47.35 (44.20)		F(1, 32) = .78, p = .383
N° injuries during trauma	11	8		
Comorbidities/ Medication				
N°comorbid major depression	5	0	0	
N° remitted major depression	5	1	1	
N° other acute or remitted comorbid disorder	9	0	0	

Note. CAPS = Clinician Administered PTSD Scale, PDS = Posttraumatic Diagnostic Scale, CFT = Culture Fair Intelligence Test, MWT-B = Multiple Choice Word Fluency Test, KAI = "Kurztest für allgemeine Basisgrößen der Informationsverarbeitung" [Short Test for General Factors of Information Processing].

Procedure

Whole brain imaging data were acquired on a 3T Magnetom TRIO whole body MR-scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a standard 12-channel head coil. One-hundred-and-twenty functional volumes were recorded using a gradient-echo echo planar imaging (EPI) sequence (protocol parameters: TR = 2700ms; TE = 27ms; matrix size = 96 x 96; field of view = 220 x 220 mm^2; flip angle = 90°, GRAPPA PAT 2). There were 40 axial slices per volume and a slice thickness of 2.3 mm

(gap = 0.7 mm). Measurement was performed in descending slice order and positioned along a tilted line to the anterior-posterior commissure (AC-PC orientation).

All subjects received a high-resolution anatomical scan, obtained prior to the resting-state functional scan. When subjects confirmed their well-being, 5.5 minutes of resting state scan followed where they were instructed to relax, stay awake, remain still and keep their eyes closed. Further fMRI measurements were performed after the resting state procedure and are not reported here.

Preprocessing

Image preprocessing and statistical data analysis were performed using MATLAB R2010B (The MathWorks Inc., Natick, MA, USA) and Statistical Parametric Mapping (SPM8, Wellcome Department of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). The first four images were discarded to account for T_1-saturation effects. Preprocessing included realignment to the fifth volume by minimizing the mean square error (rigid body transformation). No participant exceeded motion estimates of 2.3 mm and 2°. The images were normalized to the standard space of the Montreal Neurological Institute (MNI) using the EPI template provided by SPM8, the voxel size was kept according to the one measured. Smoothing was performed with a 7.0 x 7.0 x 9.0 mm^3 Gaussian kernel to reduce spatial noise (and allow for corrected statistical inference). Resting state functional connectivity measures low-frequency spontaneous BOLD oscillations, which is why a band of .01 – .10 Hz was examined.

Independent Component Analysis

We used the infomax algorithm (47) within the Group ICA/IVA of fMRI toolbox (GIFT) version 2.0a software (http://icatb.sourceforge.net/) to perform group spatial ICA for all 54 subjects (48, 49). To determine the optimal number of components, dimension estimation was performed using minimum description length criteria, modified to account for spatial correlation (50). This procedure resulted in 26 components. The ICASSO algorithm implemented in the GIFT software was run four times to increase robustness. Each subject's component image and time course were computed (back reconstructed) and converted to z-scores for further analysis

The components of interest (DMN, SN) were identified through visual inspection and spatial sorting. First, two independent raters selected the component that best

represented the DMN and the SN. Second, all component images were spatially correlated (two-tailed) with the respective component masks. For the DMN we used a binary mask derived from Bluhm et al., also utilized by Kluetsch et al. (51), containing the PCC/PrC, mPFC, bilateral lateral parietal cortices, and bilateral temporal gyri. For the SN we used a binary mask including the anterior insula and the dorsal anterior cingulate cortex (dACC) (44; available at http://findlab.stanford.edu/functional_ROIs.html).

Group differences in network connectivity were tested in spm8 (http://www.fil.ion.ucl.ac.uk/spm/). First, one-sample t-tests were performed to create overall spatial maps of the components of interest for all subjects (45, 52). Then, the individual subject maps of the respective component were entered into second level analyses to investigate group differences (PTSD vs. HC, PTSD vs. nonPTSD, HC vs. nonPTSD). Two-sample t-tests were conducted. The overall component spatial maps were used to mask the respective contrasts (51). Region of interest (ROI) analyses were performed using the above mentioned binary DMN and SN masks.

Seed-based Connectivity Analysis

In line with our a priori hypothesis, we used an amygdala seed. The region of interest (ROI) was defined by masks taken from the Wake Forest University Pick Atlas 3.0.4 (53) On the individual level, we extracted spatially averaged time series for each amygdala seed as well as from white matter (WM) and cerebrospinal fluid (CSF) masks. The latter ones were added to the model as nuisance covariates in order to control for physiological noise caused by breathing and heart-beat changes. To estimate the correlation of the seed region time series with other brain regions an individual GLM was set up for each subject and each seed region. The according seed region time course was entered as regressor in the fMRI model together with the WM and CSF time courses as well as the realignment parameters. No further conditions were added in the model. A contrast image was set up for the first regressor of each model, representing the voxelwise correlation of the seed time courses with all other brain regions.

Within-group analyses where performed on the whole brain level. For group comparisons (PTSD vs. HC, PTSD vs. nonPTSD, HC vs. nonPTSD) of functional amygdala connectivity, we additionally conducted small volume analyses in theory-driven ROIs (hippocampus, insula, ACC, vmPFC) as well as a data-driven ROI (putamen) that

marginally failed to reach significance in whole-brain second-level contrasts. Masks were again obtained from the WFU pickatlas 3.0.4 (54).

Correlation of Amygdala Connectivity and Symptom Severity

Mean beta weights of significant left amygdala correlations were extracted with the REX toolbox for SPM (55) and entered into SPSS 20 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) to calculate hypothesis-based one-tailed bivariate correlations with CAPS scores.

Statistical Analysis

The general significance level was set to $p<.05$ (values smaller than .1 were defined as trend level). For fMRI, we used family-wise-error (FWE) corrected α-levels at the cluster (whole-brain) or the peak level (ROI).

Results

Independent Component Analysis

Two independent raters selected component 8 to best represent the DMN. Additionally, component 8 showed the highest correlation with our DMN mask ($r = 0.6$, $p < .001$). Hence, this component was selected for further DMN analyses. Component 8 included mainly positive correlations in the bilateral PrC, PCC, angular gyrus, ACC and dorsomedial prefrontal cortex as well as smaller clusters in the bilateral hippocampus, parahippocampus, superior frontal gyrus, rolandic operculum and postcentral gyrus. The main negative correlations included in component 8 were the bilateral inferior parietal cortex, supplemental motor area and anterior insula, smaller clusters were in the bilateral middle frontal gyrus and superior occipital gyrus (Figure 2, Supplemental Table 2). Those main areas have previously been implicated in the default network. These overall brain areas were also present in the individual groups (see Supplemental Table 2 and Figure 1). Direct group comparisons did not yield significant differences between PTSD and HC, PTSD and nonPTSD, HC and nonPTSD in the connectivity of component 8.

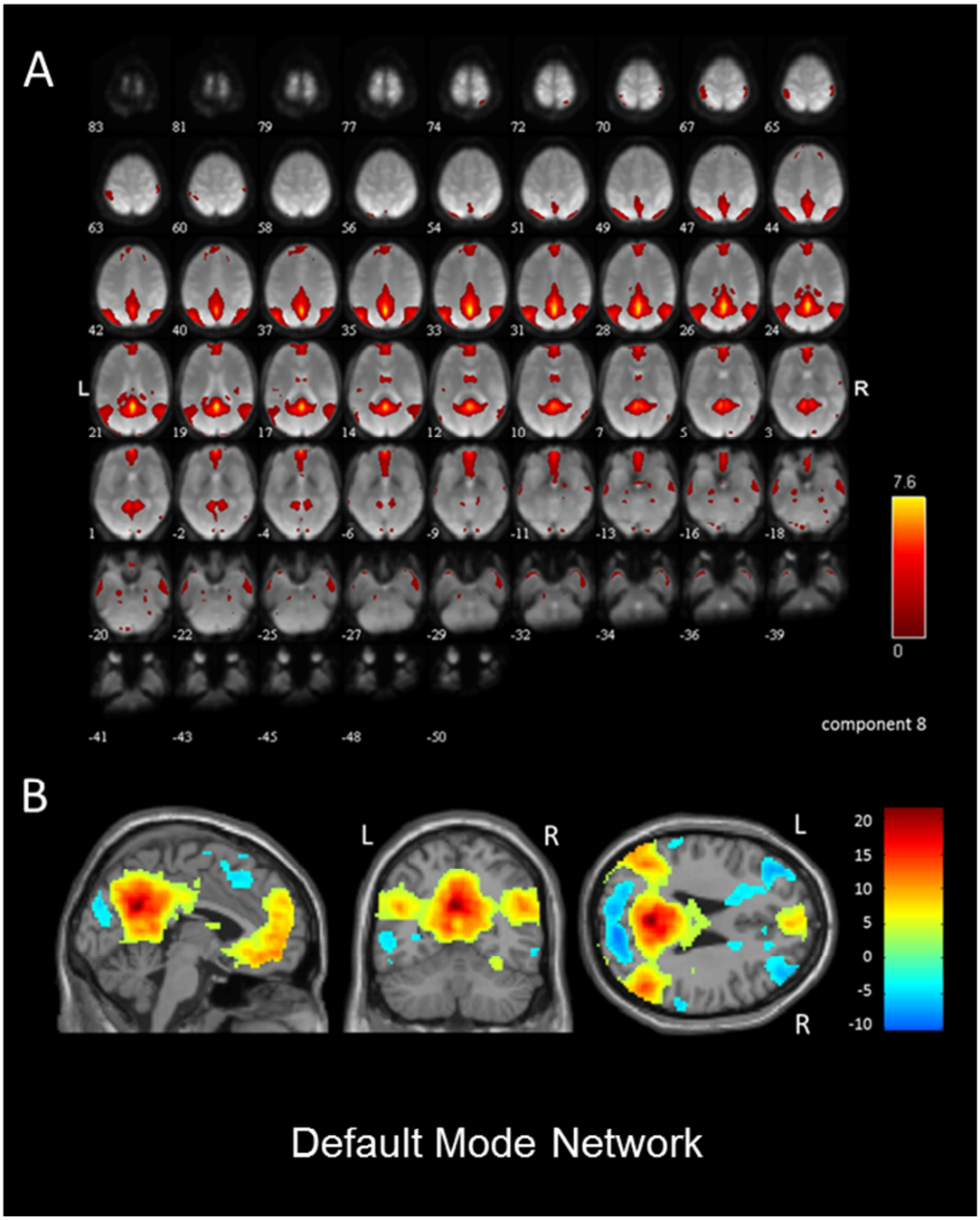

Figure 2. The component identified as the default mode network (component 8). Analyses included the entire sample (N =54), i.e. posttraumatic stress disorder (PTSD) patients, trauma-exposed non-PTSD subjects and healthy control participants. [A] Spatial map of the mean component estimates of the default component identified by group independent component analysis (ICA). [B] Statistical parametric map of the default component resulting from one-sample t-test. T-values are displayed, L = left, R = right

The SN was not identified by the raters and the highest correlation of the ICA-derived components with our SN mask did not reach significance (component 16: r = 0.2, p = n.s.).

Amygdala Seed Regions

We found positive functional correlations with the bilateral amygdala seeds in widespread areas of the grey matter in each of the three experimental groups. For details on left amygdala connectivity, see Table 1. (For details on right amygdala connectivity see Supplemental Table 1.)

Table 2. Areas positively correlated with the left amygdala seed region

Group	Area ofActivation	MNI coordinates x,y,z	*Cluster* $p_{FWE\text{-}corr.}$	k_E
PTSD	Sub cortical gray nuclei / Amygdala-L	-20,1,-20	< .001	855
(N = 18)	Limbic lobe / Hippocampus-L	-16, -9, -20		
	Limbic lobe / Parahippocampalgyrus-L	-18, -22, -18		
	Sub cortical gray nuclei / Amygdala-R	23, -2, -22		
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	65, -13, -16	< .001	22
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-R	3, -68, -2	< .001	23
	Parietal lobe / Medial surface / Precuneus-L	-4, -48, 49	< .001	58
	Parietal lobe / Medial surface / Precuneus-L	-4, -55, 53		
	Parietal lobe / Medial surface / Precuneus-R	3, -64, 49		
	Frontal lobe / Orbital surface / Inferior frontal gyrus, orbital part-R	25, 24, -16	< .001	17
	Occipital lobe / Medial and inferior surfaces / Fusiform gyrus-R	23, -73, -16	< .001	56
	Occipital lobe / Medial and inferior surfaces / Fusiform gyrus-R	35, -66,-18		
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	55, -4, -22	< .001	20
	Occipital lobe / Lateral surface / Superior occipital gyrus-R	23, -87, 26	< .001	11
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	58, -61, 3	< .001	10
nonPTSD	Limbic lobe / Hippocampus-R	30, -9, -18	< .001	821
(N = 18)	Sub cortical gray nuclei / Amygdala-R	21, -4, -18		
	Limbic lobe / Hippocampus-R	30, -18, -20		
	Limbic lobe / Hippocampus-L	-23, -9, -18	< .001	876
	Limbic lobe / Hippocampus-L	-32, -11, -22		
	Limbic lobe / Hippocampus-L	-18, -15, -18		
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-11, -103, -2	< .001	110
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-2, -96, -2		
	Occipital lobe / Lateral surface / Middle occipital gyrus-L	-32, -98, -2		
	Sub cortical gray nuclei / Lenticular nucleus, pallidum-L	-16, 5, 1		
	Central region / Postcentralgyrus-L	-55, -6, 26	< .001	37
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	62, -20, -9	< .001	68
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	58, -32, -6		
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	53, -22, -9		
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-L	-9, -84, -6	< .001	51
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-L	-9, -75, -11		
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-L	-14, -66, -6		
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	0, -64, 10	< .001	15
	Temporal lobe / Lateral surface / Inferior temporal gyrus-R	62, -50, -11	< .001	10
	Occipital lobe / Medial and inferior surfaces / Fusiform gyrus-R	30, -41, -16	< .001	14
	Limbic lobe / Parahippocampalgyrus-R	21, -43, -11		
	Limbic lobe / Temporal pole: middle temporal gyrus-R	51, 14, -27	< .001	12
	Parietal lobe / Medial surface / Precuneus-R	16, -71, 42	< .001	10
	Occipital lobe / Lateral surface / Superior occipital gyrus-R	25, -78, 37	< .001	21
	Occipital lobe / Lateral surface / Inferior occipital gyrus-R	25, -101, -4	< .001	14
HC	Limbic lobe / Hippocampus-L	-20, -6, -22	< .001	4473
(N = 18)	Limbic lobe / Parahippocampalgyrus-L	-25, -22, -20		
	Frontal lobe / Orbital surface / Olfactory cortex-L	-23, 3, -16		
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	69 ,-38 , 1	< .001	283
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	55, -34 ,1		
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	58, -27, -4		

Limbic lobe / Anterior cingulate and paracingulategyri-R	5, 44 ,28	< .001	110
Frontal lobe / Medial surface / Superior frontal gyrus, medial-R	5, 47, 40		
Frontal lobe / Medial surface / Superior frontal gyrus, medial-L	-9, 47, 37		
Central region / Postcentralgyrus-L	-53, -11, 30	< .001	186
Central region / Postcentralgyrus-L	-50, -11, 21		
Central region / Postcentralgyrus-L	-55, -6, 44		
Frontal lobe / Lateral surface / Superior frontal gyrus, dorsolateral-L	-16, 54, 7	< .001	18
Frontal lobe / Medial surface / Superior frontal gyrus, medial-L	-7, 54, 5		
Occipital lobe / Medial and inferior surfaces / Cuneus-R	14, -78, 42	< .001	82
Occipital lobe / Lateral surface / Superior occipital gyrus-R	23, -75, 44		
Central region / Precentralgyrus-R	48, -6, 49	< .001	138
Central region / Precentralgyrus-R	58, -2, 37		
Central region / Precentralgyrus-R	44, -11, 35		
Sub cortical gray nuclei / Thalamus-R	9, -11, 1	< .001	53
Frontal lobe / Lateral surface / Middle frontal gyrus-R	37, 31, 35	< .001	23
Limbic lobe / Median cingulate and paracingulategyri-L	-7, 10, 33	< .001	27
Central region / Postcentralgyrus-R	53, -25, 56	< .001	11
Frontal lobe / Lateral surface / Middle frontal gyrus-L	-39, 33, 28	< .001	30
Frontal lobe / Lateral surface / Inferior frontal gyrus, triangular part-L	-46, 26, 30		
Central region / Postcentralgyrus-L	-55, -25, 28	< .001	30
Central region / Postcentralgyrus-L	-64, -20, 28		
Frontal lobe / Orbital surface / Inferior frontal gyrus, orbital part-L	-30, 28, -16	< .001	15
Frontal lobe / Lateral surface / Inferior frontal gyrus, triangular part-R	51, 28, 28	< .001	28
Cerebellum / Hemisphere / Lobule 4-5-L	-7, -43, -9	< .001	10
Parietal lobe / Lateral surface / Supramarginalgyrus-R	51, -18, 28	< .001	15
Parietal lobe / Lateral surface / Angular gyrus-R	42, -66, 44	< .001	19
Occipital lobe / Lateral surface / Superior occipital gyrus-L	-16, -78, 42	< .001	57
Occipital lobe / Lateral surface / Middle occipital gyrus-L	-25, -82, 40		
Occipital lobe / Medial and inferior surfaces / Fusiform gyrus-R	23, -73, -13	< .001	88
Cerebellum / Hemisphere / Lobule 6-R	16, -68, -16		
Occipital lobe / Medial and inferior surfaces / Lingual gyrus-R	7, -71, -6		
Limbic lobe / Anterior cingulate and paracingulategyri-R	3, 21, -9	< .001	10
Frontal lobe / Lateral surface / Superior frontal gyrus, dorsolateral-L	-18, 44, 40	< .001	13
Occipital lobe / Medial and inferior surfaces / Cuneus-L	3, -91, 14	< .001	18
Frontal lobe / Lateral surface / Middle frontal gyrus-R	28, 33, 44	< .001	15
Limbic lobe / Median cingulate and paracingulategyri-L	0, -45, 33	< .001	14
Limbic lobe / Median cingulate and paracingulategyri-L	-4, -45, 42	< .001	10
Frontal lobe / Medial surface / Paracentral lobule-R	14, -38, 56	< .001	17
Occipital lobe / Medial and inferior surfaces / Lingual gyrus-L	-23, -59, -6	< .001	21
Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-20, -55, 3		
Frontal lobe / Lateral surface / Inferior frontal gyrus, opercular part-R	42, 12, 30	< .001	11
Central region / Postcentralgyrus-L	-57, -4, 14	< .001	12
Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-7, -94, -2	< .001	18
Frontal lobe / Orbital surface / Gyrus rectus-L	-2, 28, -16	< .001	13
Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-R	3, -61, 14	< .001	14
Occipital lobe / Medial and inferior surfaces / Lingual gyrus-R	7, -64, 7		
Central region / Precentralgyrus-R	35, -27, 67	< .001	10

Threshold cluster p < .001, two-tailed, FWE-corrected; k_E = cluster size; MNI = Montreal Neurological institute; PTSD = posttraumatic stress disorder patients, nonPTSD = trauma-exposed non-PTSD subjects, HC = non-trauma healthy controls, R = right, L = left.

Group comparisons revealed significantly greater left amygdala connectivity with the right putamen and the right insula in HC than in PTSD (putamen [35,-15,3]: t(34) = 5.22, p = .002; insula [35,-15,5]: t(34) = 4.30, p = .038) and in nonPTSD (putamen [32,-9,1]: t(34) = 5.00, p = .004; insula [46,19,-11]: t(34) = 4.24, p = .045). PTSD exhibited greater

left amygdala – left insula connectivity than nonPTSD (insula [-25,24,5]: t(34) = 4.56, p = .022), see Figure 1. No significant group differences were found for right amygdala functional connectivity.

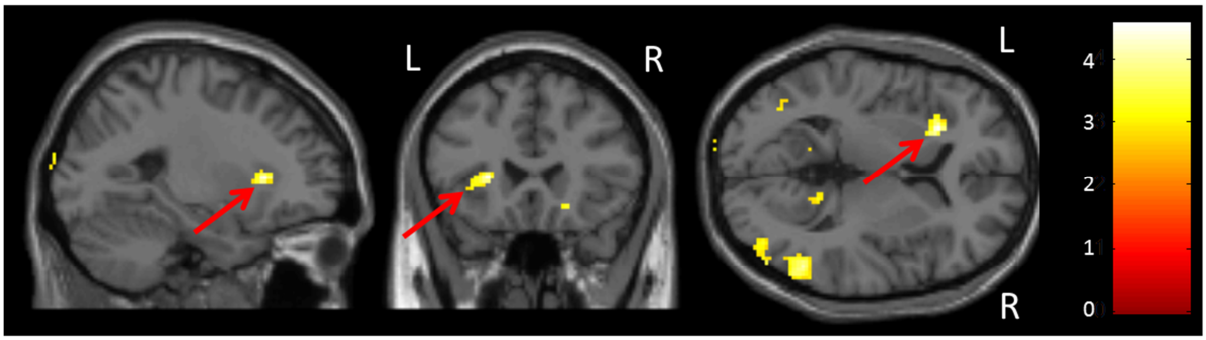

Figure 1. Higher connectivity of the left amygdala with the left insula (arrow) in posttraumatic stress disorder (PTSD) patients compared to trauma-exposed subjects without PTSD in a small volume analysis. Amygdala and insula masks were defined using the Montreal Neurological Institute (MNI) template Automated Anatomical Labeling (AAL) map. $p_{FWE} < .05$ (peak voxel), t-values are displayed, L = left, R = right.

Correlation of Amygdala Connectivity With Symptom Severity

Left amygdala – left insula connectivity differed between PTSD and nonPTSD and showed a marginally significant positive correlation with the intensity of re-experiencing in the PTSD group (r = .396, p = .05). No significant correlations were found with avoidance, hyperarousal and emotional numbing.

Discussion

In the present study, we investigated resting state connectivity of large-scale networks and an amygdala seed in trauma-experienced subjects with and without PTSD as well as trauma-naïve control participants. On the network level (DMN and SN connectivity), no significant differences between the experimental groups were found. But PTSD patients showed heightened connectivity of the left amygdala with the left insula compared to nonPTSD subjects. Interestingly, this enhanced connectivity correlated positively with symptom severity, specifically the intensity of re-experiencing. In comparison to HC, we found reduced left amygdala connectivity with the right putamen and the right insula in both trauma-experienced groups. These results indicate that aberrant resting state amygdala-connectivity might be related to the pathophysiology of PTSD.

The negative result concerning large scale networks was surprising considering previous reports about alterations in DMN and SN connectivity in PTSD (27). In fact, we did not detect the SN component in our analysis, probably due to the small sample size and/ or the heterogeneity within our sample (see Table 1). The lack of significant group differences in DMN connectivity suggests that there are no influences of trauma or PTSD on the brain's overall resting activity in our sample. However, the fact that DMN differences between PTSD patients and trauma-exposed or unexposed controls have previously been reported (18, 21, 56), raises the question why we did not detect such differences. One explanation for the lack of significant group differences in network connectivity might be the small sub-group sizes in our study and genuinely different patient and control groups between studies (early-life trauma, veterans, and mixed groups; trauma-exposed and trauma-naïve controls). Another reason might be essential methodical differences between studies. Whereas we used group level ICA, others have conducted psychophysiological interactions (PPI) -like analyses to identify aberrant coupling of the key network nodes, such as the PCC/PrC and vmPFC for the DMN and the anterior insula for the SN (18, 21). Both approaches analyze functional connectivity, i.e. the temporal correlation between spatially remote neurophysiological events. The PPI-like approach, however, is seed-based and therefore limited in its conclusions on network activity. ICA on the other hand, has been shown to reliably extract a variety of networks with very high consistency, including the DMN and the SN (57). Hence, one can assume that our negative result may reflect a real lack of DMN differentiation between

the investigated groups. This might indicate that the increased amygdala-insula coupling in our PTSD patients is specific for the disorder and not a mere result of general changes in the brain's resting connectivity. To make that assumption, however, one would have to rule out the possibility that the amygdala-insula coupling is part of a different component (that could differ between groups).

Increased amygdala – insula connectivity in PTSD compared to nonPTSD has previously been reported by Rabinak et al. (31) and Sripada et al. (32). Our result provides further evidence for an influence of a functional relationship between the two regions for PTSD.

Both, the amygdala and the insula have repeatedly been found to be involved in the disorder (for an overview see 58). The insula plays an important role in higher sensory functions (59, 60), emotion processing (61) and anticipation of emotionally aversive stimuli (62). PTSD patients exhibit increased insula activity during script-driven imagery (63, 64), retrieval of emotional or neutral stimuli (65-67), aversive smells and painful stimuli (68), anticipation of negative images (69) and negative emotional faces (34). It has been observed that increases in insula and amygdala activity during fear conditioning (70, 71) and confrontation with trauma reminders (68) are not only associated with one another, but the co-occurring increases are greater in PTSD than in controls (34, 72). Interestingly, the amygdala is hyperresponsive to threat, irrespective of whether trauma-related or -unrelated materials are presented and insula activity is increased during anticipation of negative events (35). Our own result underlines the importance of an interplay of the two structures that exhibit strong reciprocal physiological connections including projections from the anterior insula to the main output nuclei of the amygdala (73-76) for the development of the disorder. Importantly, we found enhanced connectivity when we compared the PTSD patients to trauma-exposed unaffected control subjects. Therefore, we can assume that an increase in amygdala-insula coupling is related to PTSD and not the mere experience of trauma. From a clinical point of view, it is important to understand why some individuals are resilient. The fact that we found less functional connectivity of the amygdala and the insula in the nonPTSD group might represent a possible resilience factor and indicate that in turn hyperconnectivity may promote the development and maintenance of PTSD symptoms.

Given the evidence for a significant relationship between PTSD symptoms and amygdala activity (77) as well as insular responsivity (35, 78-80) in active tasks, it is plausible that

amygdala-insula coupling also relates to PTSD symptomatology (19, 22). Indeed, we found a positive relationship between amygdala-insula connectivity and symptom severity in our sample. The result indicates that hyper-connectivity between the amygdala and the insula is related to an increase in the intensity of re-experiencing symptoms. Even though the result was only marginally significant, it is in line with the literature indicating a link between insula activation and re-experiencing severity (79). Increased connectivity between the amygdala and the insula could underlie this observation by providing a stronger functional link between somatic sensations and emotional states. Activity in the anterior insula reflects explicit awareness of bodily processes including subjective emotional experience (55), but it is noteworthy that other brain regions, such as the amygdala, ACC and ventral striatum, are co-activated in almost all imaging studies of emotion (54). It has been suggested that the amygdala and the insula form a functional network mediating the anxious anticipation of aversive events, which is hyperactive in highly anxious individuals (81). In their experiment with healthy subjects, Carlson et al. (81) found that activity in the insula was predictive of neutral and aversive acoustic stimulation, whereas the amygdala was only involved in aversive trials. Hence, combined amygdala-insula activity might mediate the negative (fearful) valence of an anticipated event (such as re-experiencing) and enable the individual to engage in action planning to avoid the aversive stimulus (82). Resting state connectivity might constitute an underlying prerequisite for this process, but research converging task-dependent and -independent methods is needed to further elucidate the link between brain structure and function. Notably, we did not find significant correlations of amygdala-insula coupling and other PTSD symptoms such as hyperarousal or avoidance. One possible explanation for this lack is that the relationship with re-experiencing is very specific, but this assumption needs to be studied in more detail. Interestingly, previous studies linking amygdala connectivity to symptomatology have produced mixed results; i.e. positive (19) and negative (22) correlations with a variety of symptoms. All these studies have investigated amygdala-PCC connectivity. The correlation with re-experiencing intensity might be specific for amygdala-insula coupling. However, this assumptions needs replication and experiments designed to directly investigate the relationship between amygdala-insula connectivity and re-experiencing are needed.

We did not find significant correlations of the amygdala seed with any other than the above mentioned a priori ROIs (hippocampus, ACC, vmPFC). The same negative result has been reported by Rabinak et al. (31) and might indicate that functional activation differences between healthy (trauma-experienced or -inexperienced) subjects and PTSD are not equivalently found in resting state analyses and probably rely on task-related functions or specific cognitive processes.

A positive feature of our study is that we not only investigated trauma-experienced subjects, but added a trauma-naïve control group. In addition to identifying the factors that lead to the development of full-blown PTSD, the comparison of trauma-naïve and trauma-experienced subjects helps to analyze the impact of a traumatic event per se. In our HC group, we found higher positive correlations of the amygdala and the putamen than in both trauma-experienced groups. The putamen plays an important role in reward prediction (83-88) and the prediction of outcome in general (89). In aversive conditioning, the ventral striatum (nucleus accumbens, ventral caudate and ventral putamen) is activated during anticipation of a negative event (90) and this activation is positively correlated with the magnitude of the prediction error (91). The prediction error is essential for learning (92) and it has been shown that synaptic mechanisms in the lateral amygdala are sensitive to contingencies, i.e. predictive relationships (93). The reduced amygdala-putamen coupling at rest might indicate that the correct prediction of an outcome, appetitive or aversive (94), relies on baseline connectivity between the two regions. Indeed, PTSD patients overestimate CS-US contingencies (95) and generalize potential threat (96-98), probably as a result of insufficient striatal modulation. However, even though nonPTSD subjects also exhibited less amygdala-putamen coupling than HC in the resting condition, their behavioral pattern is often (but not always) similar to HC in fear conditioning studies (95, 99-104). The role of functional coupling between the amygdala and the putamen in fear conditioning and PTSD remains unclear and needs to be assessed in more detail.

Surprisingly, the trauma-naïve healthy controls showed more coupling of the left amygdala seed and the right insula than both trauma-experienced groups. The peaks within in the insula are located in the anterior part, preventing an argumentation along the posterior-to-mid-to-anterior model of integration of interoceptive information (105) to explain why we found higher amygdala-insula coupling in HC when compared to PTSD and nonPTSD, as well as higher amygdala-insula coupling in the patients when

comparing PTSD and nonPTSD. However, there was a laterality difference: Whereas PTSD patients exhibited increased functional connectivity of the left amygdala and the left insula than non PTSD, HC showed more left amygdala coupling with the right insula than both trauma-experienced groups. Interestingly, PTSD patients have shown deficits in inhibition during a Go/No-Go task and activated the left lateral frontal cortex while healthy controls activated a right-lateralized cortical inhibitory network (106). Our results indicate that this effect may have a neuronal basis in the resting brain. However, this dissociation requires replication in order to understand the precise role of laterality for resting state functional connectivity of the amygdala and the insula in PTSD.

Our study has several limitations. First, we investigated a relatively small sample, which is a general problem of clinical neuroimaging studies (70). However, we included three relevant groups of participants for comparison so that patient specific statements can be considered reliable. Unlike other studies that comprised only veterans (21, 31, 32, 107), we included a diversity of trauma types so that our results apply to a broad range of PTSD patients. Second, we included PTSD patients with comorbid disorders, which undoubtedly is a common characteristic of this patient group, especially comorbid depression (108). Third, functional connectivity describes correlational relationships and does not allow for causal conclusions, so that assumptions about the directionality of the interplay of our amygdala seed with other pre-defined brain regions rely on literature-based knowledge and not the data itself. Fourth, we tested literature-derived assumptions, i.e. predefined ROIs, and cannot make statements about other potentially correlated brain regions. Fifth, we did not monitor cardiac and respiratory cycles, which lead to structured noise during fMRI scanning. However, we did include white matter and cerebrospinal fluid masks in the seed-based analysis and excluded the respective components from the ICA in order to control for non-specific scanner-derived noise (21).

Despite these limitations, our results demonstrate that abnormalities in connectivity patterns with the amygdala underlie the pathophysiology of PTSD. Especially hyperconnectivity with the insula seems to differentiate patients from resilient subjects and influence symptom severity presumably by mediating stronger anticipation of negative events. Further research is needed to investigate whether connectivity analyses can predict the development of PTSD symptoms in traumatized individuals.

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Supplemental Material

Altered amygdala-insula functional connectivity correlates with symptom severity in posttraumatic stress disorder

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Supplemental Table 1. Areas positively correlated with the right amygdala seed region

Group	Area of Activation	MNI coordinates x,y,z	*Cluster* $p_{FWE\text{-}corr.}$	k_E
PTSD	Sub cortical gray nuclei / Amygdala-R	32, -2, -22	< .001	2101
(N = 18)	Sub cortical gray nuclei / Amygdala-R	19, 3, -16		
	Limbic lobe / Hippocampus-R	25, -6, -18		
	Limbic lobe / Temporal pole: superior temporal gyrus-L	-43, 12, -22	< .001	58
	Limbic lobe / Temporal pole: superior temporal gyrus-L	-48, 3, -16		
	Sub cortical gray nuclei / Lenticular nucleus, pallidum-R	16, 3, 3	< .001	56
	Sub cortical gray nuclei / Lenticular nucleus, pallidum-R	12, 3, -4		
	Sub cortical gray nuclei / Caudate nucleus-R	9, 10, 1		
	Sub cortical gray nuclei / Lenticular nucleus, putamen-L	-27, 14, 3	< .001	46
	Sub cortical gray nuclei / Lenticular nucleus, pallidum-L	-16, 3, 1	< .001	69
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-7, -98, -9	< .001	9
nonPTSD	Sub cortical gray nuclei / Amygdala-R	28, -4, -20	< .001	1962
(N = 18)	Sub cortical gray nuclei / Lenticular nucleus, putamen-R	28, 3, -11		
	Limbic lobe / Hippocampus-L	-18, -13, -18	< .001	780
	Limbic lobe / Parahippocampal gyrus-L	-27, -25, -20		
	Limbic lobe / Parahippocampal gyrus-L	-25, -41, -11		
	Parietal lobe / Lateral surface / Superior parietal gyrus-L	-14, -73, 44	< .001	72
	Parietal lobe / Medial surface / Precuneus-R	-4, -71, 47		
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-9, -103, -2	< .001	89
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-4, -96, -2		
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	58, -2, -22	< .001	41
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	53, -9, -16		
	Limbic lobe / Temporal pole: middle temporal gyrus-R	55, 8, -22		
	Frontal lobe / Lateral surface / Inferior frontal gyrus, triangular part-L	-41, 26, 14	< .001	95
	Frontal lobe / Lateral surface / Inferior frontal gyrus, triangular part-L	-46, 35, 17		
	Frontal lobe / Lateral surface / Middle frontal gyrus-L	-34, 58, 10		
	Occipital lobe / Lateral surface / Superior occipital gyrus-R	28, -75, 37	< .001	105
	Occipital lobe / Lateral surface / Superior occipital gyrus-R	23, -68, 42		
	Sub cortical gray nuclei / Lenticular nucleus, putamen-L	-14, 10, 1	< .001	44
	Frontal lobe / Lateral surface / Inferior frontal gyrus, triangular part-L	-50, 19, 26	< .001	37
	Parietal lobe / Lateral surface / Inferior parietal, but supramarginal and angular gyri-L	-30, -57, 51	< .001	24
	Occipital lobe / Medial and inferior surfaces / Fusiform gyrus-L	-37, -9, 2-9	< .001	12
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-R	21, -64, -4	< .001	15
	Occipital lobe / Medial and inferior surfaces / Fusiform gyrus-R	21, -64, -13		
	Temporal lobe / Lateral surface / Superior temporal gyrus-L	-64, -27, 7	< .001	13
	Frontal lobe / Lateral surface / Inferior frontal gyrus, triangular part-R	48, 31, 19	< .001	27
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-L	-7, -84, -6	< .001	22
	Frontal lobe / Medial surface / Superior frontal gyrus, medial-L	-7, 40, 26	< .001	12
	Sub cortical gray nuclei / Lenticular nucleus, putamen-L	-25, 5, -9	< .001	16
	Parietal lobe / Lateral surface / Angular gyrus-L	-46, -61, 40	< .001	11
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-R	21, -96, -4	< .001	38
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-R	16, -103, 5		
HC	Sub cortical gray nuclei / Amygdala-R	25, 1, -18	< .001	4488
(N = 18)	Frontal lobe / Medial surface / Superior frontal gyrus, medial-R	5, 47, 28	< .001	56
	Cerebellum / Hemisphere / Lobule Crus1-R	39, -66, -27	< .001	344
	Cerebellum / Hemisphere / Lobule 6-R	28, -75, -20		
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-R	21, -68, -13		
	Central region / Postcentral gyrus-L	-55, -11, 24	< .001	204
	Central region / Postcentral gyrus-L	-60, -9, 33		
	Central region / Postcentral gyrus-L	-48, -11, 30		

Central region / Postcentral gyrus-R	51, -25, 58	< .001	13
Frontal lobe / Lateral surface / Middle frontal gyrus-R	39, 33, 35	< .001	25
Frontal lobe / Lateral surface / Middle frontal gyrus-R	32, 31, 30		
Temporal lobe / Lateral surface / Superior temporal gyrus-R	67, -27, 1	< .001	58
Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-4, -96, 3	< .001	124
Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-R	5, -91, 5		
Frontal lobe / Lateral surface / Middle frontal gyrus-L	-39, 31, 30	< .001	18
Temporal lobe / Lateral surface / Superior temporal gyrus-R	51, -29, -2	< .001	22
Parietal lobe / Medial surface / Precuneus-R	7, -59, 47	< .001	97
Parietal lobe / Medial surface / Precuneus-R	9, -64, 35		
Occipital lobe / Medial and inferior surfaces / Lingual gyrus-L	-7, -84, -6	< .001	49
Cerebellum / Hemisphere / Lobule 6-L	-11, -82, -16		
Central region / Precentral gyrus-R	60, 8, 28	< .001	124
Central region / Postcentral gyrus-R	51, -9, 37		
Central region / Precentral gyrus-R	42, -11, 37		
Frontal lobe / Lateral surface / Inferior frontal gyrus, opercular part-R	39, 12, 30	< .001	28
Limbic lobe / Posterior cingulate gyrus-L	-7, -48, 30	< .001	18
Parietal lobe / Lateral surface / Angular gyrus-R	44, -68, 42	< .001	123
Occipital lobe / Lateral surface / Superior occipital gyrus-R	21, -73, 42		
Parietal lobe / Lateral surface / Inferior parietal, but supramarginal and angular gyri-R	37, -55, 40		
Occipital lobe / Lateral surface / Middle occipital gyrus-L	-43, -84, 1	< .001	19
Cerebellum / Hemisphere / Lobule Crus1-L	-32, -82, -20	< .001	93
Cerebellum / Hemisphere / Lobule Crus1-L	-41, -73, -22		
Cerebellum / Hemisphere / Lobule 6-L	-20, -73, -18		
Limbic lobe / Median cingulate and paracingulate gyri-L	-11, 8, 35	< .001	17
Limbic lobe / Anterior cingulate and paracingulate gyri-L	-4, 10, 28		
Frontal lobe / Medial surface / Superior frontal gyrus, medial-R	3, 56, 5	< .001	12
Temporal lobe / Lateral surface / Middle temporal gyrus-R	69, -41, 5	< .001	22
Parietal lobe / Medial surface / Precuneus-L	-9, -64, 30	< .001	17
Central region / Precentral gyrus-R	39, -13, 65	< .001	13
Parietal lobe / Medial surface / Precuneus-L	-7, -64, 47	< .001	29
Occipital lobe / Lateral surface / Middle occipital gyrus-L	-37, -78, 35	< .001	14

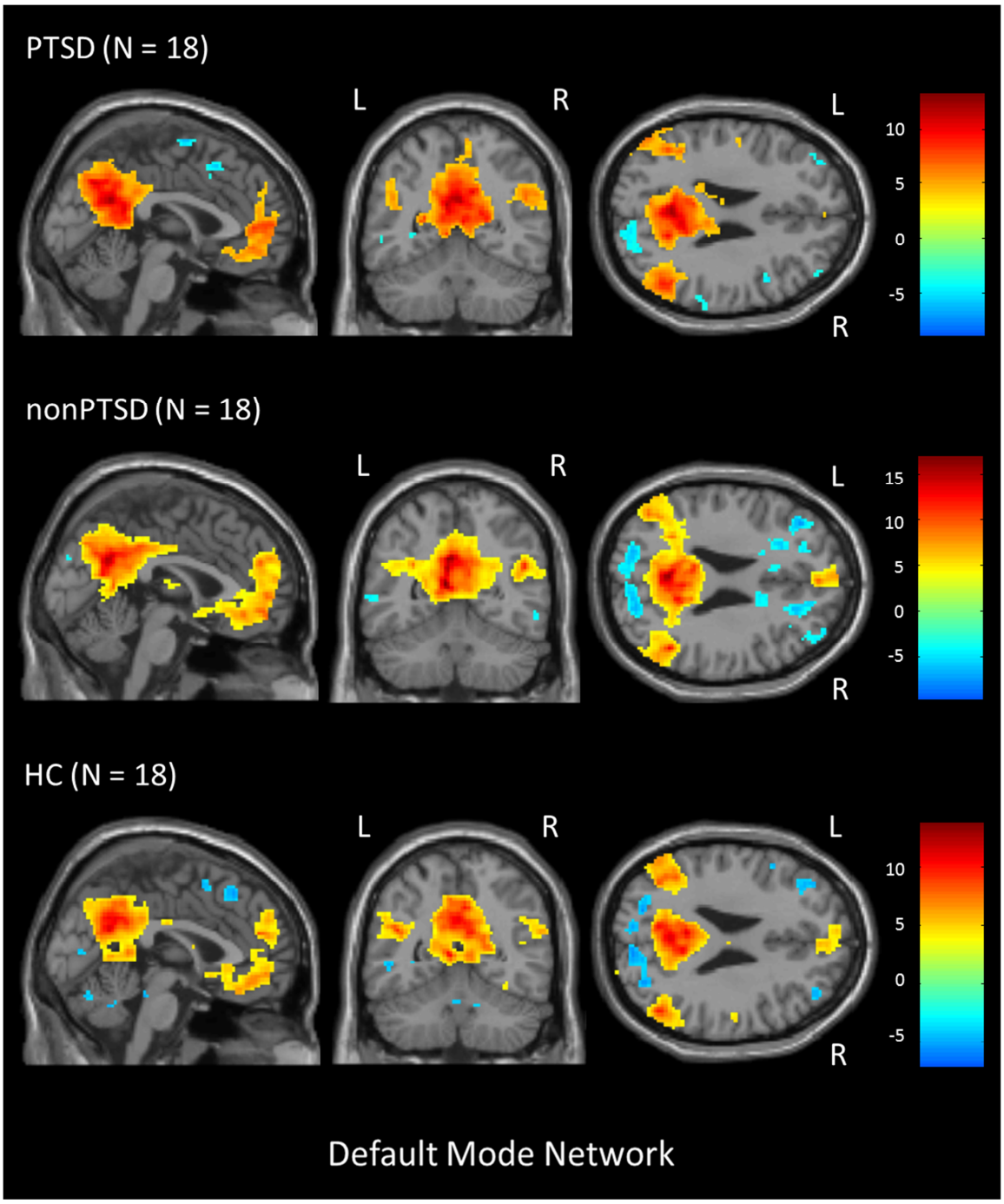
Threshold cluster p < .001, two-tailed, FWE-corrected; k_E = cluster size; MNI = Montreal Neurological institute; PTSD = posttraumatic stress disorder patients, nonPTSD = trauma-exposed non-PTSD subjects, HC = non-trauma healthy controls, R = right, L = left.

Supplemental Table 2. Brain regions identified in the default mode network component

Group	Area of Activation	MNI coordinates x,y,z	*Cluster* $p_{FWE\text{-}corr.}$	k_E
All	Parietal lobe / Medial surface / Precuneus-L	-2, -57, 26	< .001	4144
(N = 54)	Parietal lobe / Medial surface / Precuneus-L	-7, -48, 7		
	Parietal lobe / Medial surface / Precuneus-R	9, -55, 21		
	Parietal lobe / Lateral surface / Angular gyrus-R	51, -64, 28	< .001	970
	Parietal lobe / Lateral surface / Angular gyrus-R	44, -66, 42		
	Parietal lobe / Lateral surface / Angular gyrus-R	44, -78, 37		
	Parietal lobe / Lateral surface / Angular gyrus-L	-50, -68, 37	< .001	1072
	Parietal lobe / Lateral surface / Angular gyrus-L	-43, -61, 26		
	Parietal lobe / Lateral surface / Angular gyrus-L	-43, -73, 44		
	Frontal lobe / Orbital surface / Superior frontal gyrus, medial orbital-L	0, 47, -9	< .001	1514
	Frontal lobe / Orbital surface / Superior frontal gyrus, medial orbital-L	-2, 37, -13		
	Frontal lobe / Medial surface / Superior frontal gyrus, medial-L	0, 54, 1		
	Temporal lobe / Lateral surface / Middle temporal gyrus-L	-62, -9, -20	< .001	107
	Temporal lobe / Lateral surface / Middle temporal gyrus-L	-64, -15, -16		
	Temporal lobe / Lateral surface / Middle temporal gyrus-R	62, -6, -20		
PTSD	Parietal lobe / Medial surface / Precuneus-R	3, -64, 37	< .001	827
(N = 18)	Parietal lobe / Medial surface / Precuneus-R	5, -50, 7		
	Parietal lobe / Medial surface / Precuneus-L	-2, -57, 26		
	Parietal lobe / Lateral surface / Angular gyrus-L	-50, -68, 37	< .001	83
	Occipital lobe / Lateral surface / Middle occipital gyrus-L	-46, -75, 37		
	Parietal lobe / Lateral surface / Angular gyrus-R	51, -64, 28	< .001	48
	Limbic lobe / Anterior cingulate and paracingulate gyri-L	-2, 44, 5	< .001	15
	Frontal lobe / Medial surface / Superior frontal gyrus, medial-R	3, 54, 7		
nonPTSD	Parietal lobe / Medial surface / Precuneus-L	-2, -57, 28	< .001	1338
(N = 18)	Parietal lobe / Medial surface / Precuneus-L	-7, -55, 12		
	Parietal lobe / Medial surface / Precuneus-L	-2, -61, 17		
	Parietal lobe / Lateral surface / Angular gyrus-R	48, -57, 24	< .001	51
	Parietal lobe / Lateral surface / Angular gyrus-R	51, -64, 33		
	Frontal lobe / Orbital surface / Superior frontal gyrus, medial orbital-L	-4, 49, -9	< .001	102
	Frontal lobe / Orbital surface / Superior frontal gyrus, medial orbital-L	-9, 42, -11		
	Frontal lobe / Orbital surface / Superior frontal gyrus, medial orbital-R	3, 54, -11		
	Parietal lobe / Lateral surface / Angular gyrus-L	-46, -71, 28	< .001	17
	Frontal lobe / Medial surface / Superior frontal gyrus, medial-L	0, 54, 1	< .001	13
HC	Limbic lobe / Posterior cingulate gyrus-R	7, -50, 30	< .001	510
(N = 18)	Parietal lobe / Medial surface / Precuneus-L	-2, -59, 26		
	Limbic lobe / Posterior cingulate gyrus-L	-4, -48, 26		
	Occipital lobe / Medial and inferior surfaces / Calcarine fissure and surrounding cortex-L	-4, -55, 5	< .001	39
	Parietal lobe / Lateral surface / Angular gyrus-R	51, -66, 26	< .001	16
	Occipital lobe / Medial and inferior surfaces / Lingual gyrus-R	7, -43, 3	< .001	20
	Parietal lobe / Lateral surface / Angular gyrus-L	-43, -68, 33	< .001	12
	Occipital lobe / Lateral surface / Middle occipital gyrus-L	-39, -66, 26		

Threshold cluster p < .001, two-tailed, FWE-corrected; k_E = cluster size; MNI = Montreal Neurological institute; PTSD = posttraumatic stress disorder patients, nonPTSD = trauma-exposed non-PTSD subjects, HC = non-trauma healthy controls, R = right, L = left.

Supplemental Figure 1



3. General Discussion

3.1. Main findings

The present studies underline the value of neuropsychological research for our understanding of mental disorders such as PTSD. We could link the diverse symptoms that characterize the disorder to brain activity and connectivity, suggesting that different approaches are necessary to capture the neurobiology of PTSD. In addition, psychological theories offered a comprehensive background for our research. Context-dependent and cued fear conditioning, for example, represent important processes for PTSD – not only for the understanding of the disorder as such, but also for the understanding of the underlying (dysfunctional) mechanisms on various levels. Especially in the case of fMRI, sophisticated designs are needed to investigate specific disorder-related processes. Fear conditioning in combination with modern technologies such as VR offer valuable tools for this objective.

In the first study, we found elevated return of fear after acquisition and extinction in PTSD patients indicated by increased SCR and left amygdala activity in this group. Additionally, we found an inability to identify safety signals and an association between brain activity during return of fear and symptom severity. In light of the discrepant findings on the direction of hippocampal activation changes in PTSD (see 1.2.3.), our study confirms an elevation of hippocampal activation. During return of fear, the PTSD patients of our sample showed increased hippocampal activity. Even though this result might represent a compensatory upregulation, it might as well represent an underlying mechanism for the deficient generalization of extinction observed in PTSD (Schonfeld, Ehlers, Bollinghaus, & Rief, 2007). The reverse effect has been seen in rodent studies where an inactivation of the hippocampus led to facilitated generalization of extinction across contexts and an interference with renewal and contextual reinstatement of conditioned fear (Corcoran & Maren, 2001, 2004; LaBar & Phelps, 2005). This interpretation seems to contradict the model suggested by Acheson, Gresack and Risbrough (2012; see Figure 1) at first glance. However, the model primarily explains the dysfunctional context-related acquisition of trauma-related fear in PTSD, which is thought to reflect poor hippocampal function, whereas our study investigated extinction memory (renewal) for trauma-unrelated fear stimuli. Based on those differences, one

can assume that a general hippocampal dysfunction could have diverse consequences depending on the current task requirements such as the experimental phase (Zelikowsky, Pham, & Fanselow, 2012) or the stimulus material (Acheson et al., 2012). Indeed, previous findings suggest that hippocampal activation in PTSD is elevated when novel aversive stimuli are presented (Brohawn, Offringa, Pfaff, Hughes, & Shin, 2010) and lowered when trauma-related stimuli are presented (Hayes et al., 2011). In addition, (re)activation of the hippocampus seems to be related to fear renewal as opposed to fear acquisition (Hermann, Stark, Milad, & Merz, 2016). Hence, the increase in hippocampal activity to trauma-unrelated stimuli during return of fear in our study is in line with these supposed dissociations and therefore does not contradict the model of Acheson, Gresack and Risbrough (2012).

The fact that we observed increased renewal in our PTSD patients compared to both healthy groups is of particular interest, since the only other published study (Garfinkel et al., 2014) that investigated fear renewal in PTSD found impaired renewal compared to trauma-exposed but unaffected controls. The most striking difference between the two studies is that Garfinkel et al. (2014) conducted an extinction test prior to the context change and returned to the conditioning context for renewal (ABBA), whereas we did not directly assess extinction memory and introduced a novel context for renewal (ABC). The theoretical framework (see chapter 1.2) and the clinical observation that PTSD patients feel endangered in various situations and contexts, suggest increased renewal in PTSD rather than a reduction. Therefore our result seems to be in line with this assumption. However, since we did not include an extinction recall phase in our experiment, one could argue that we observed spontaneous recovery instead of renewal. Nevertheless, the absence of an increased return of fear in PTSD in the extinction test as observed in our laboratory study, suggests that the elevated fear response in the fMRI study was provoked by the novel context C and therefore indicated genuine renewal. On the other hand, there is a possibility that the extinction recall phase in the Garfinkel et al. (2014) study might have had an unpredicted impact on extinction learning and led to the unexpected result. Indeed, unlike our laboratory study, the PTSD patients did show elevated fear levels at this point compared to the non-PTSD group. Since the comparison to a trauma-naïve control group is missing in that study, interpretation and comparison of results is limited. This is particularly relevant since trauma-exposed control groups have previously been shown to display responses that neither completely reflect healthy

trauma-naïve controls nor PTSD patients (Diener et al., 2014; Diener et al., 2012; Garfinkel, et al., 2014; Milad et al., 2008; Milad et al., 2009; Norrholm et al., 2011; Rougemont-Bücking et al., 2011; Steiger et al., 2015; Wessa & Flor, 2007). As suggested in our study, the mere experience of a traumatic event might already lead to behavioral and neuronal changes (including the activation of protective mechanisms). Thus, the comparison with a trauma-naïve control group would have helped to detect the reasons for the contradictory results. Nonetheless, further research is needed to determine if and under which circumstances PTSD patients exhibit increased or decreased renewal, since there were many methodological differences between the two studies (i.e., ABC vs. ABBA, VRs vs. stationary pictures, number of trials).

Taken together, the first study showed that PTSD is associated with a deficient maintenance of extinction and a failure to identify safety signals, and that brain activity during renewal is linked to PTSD symptomatology. Besides confirming the theoretical framework for PTSD, we were able to link the neural correlates of deficient extinction maintenance to symptom severity and provide a possible explanation for an effect known from behavioral therapy, i.e. that exposure treatment is more successful, if it is conducted in multiple contexts (Craske et al., 2008; van Minnen, Zoellner, Harned, & Mills, 2015). Deficient extinction maintenance is a probable cause for relapse (return of fear), and exposure to the traumatic event in different contexts might reduce fear renewal by enforcing the generalization of extinction.

The central role of the amygdala for PTSD is indisputable and – in addition to activation studies – functional connectivity of the amygdala provides important insight on the pathophysiology of PTSD. We therefore conducted a second study on the above described sample, investigating the functional connectivity of the left amygdala (seed-based analysis) and important ICNs, such as the DMN and the SN (ICA).

We assumed that if elevated activity in the left amygdala was characteristic for our PTSD group in the first study, increased connectivity of the same region might constitute an additional marker for PTSD in our sample. Indeed, we found increased connectivity of the left amygdala with the left anterior insula in the PTSD patients compared to nonPTSD subjects. As elaborated in chapters 1.2.3. and 1.2.4., increased common activation and connectivity of both areas have previously been linked to PTSD (Bremner et al., 2005; Etkin & Wager, 2007; Fonzo et al., 2010; Pohlack et al., 2012; Rabinak et al., 2011; Sripada, King, Garfinkel, et al., 2012; Sripada, King, Welsh, et al., 2012). Our result,

including the positive correlation with the intensity of re-experiencing, is in line with those observations. Since both, the insula and the amygdala, are associated with the SN, increased connectivity within this network underpins the assumption that salience processing at rest is enhanced in PTSD (Tursich et al., 2015). From a clinical point of view, this suggests increased anxiety during the fMRI procedure, which is consistent with the general hypervigilance seen in PTSD (Koch et al., 2016). In our study the enhanced connectivity was found when comparing PTSD patients to trauma-exposed non-PTSD controls, indicating that increased left amygdala-left insula coupling is related to the disorder and not to the mere experience of trauma. Interestingly, we found decreased left amygdala-right anterior insula coupling in the PTSD patients when they were contrasted with the healthy trauma-naïve controls. This result shows that the role of functional amygdala-insula coupling might be more complex than previously assumed. Indeed, other studies have found increased connectivity of the right amygdala with the right (Rabinak et al., 2011) or bilateral insula (Sripada, King, Garfinkel, et al., 2012) and of the left amygdala with the bilateral insula (Sripada, King, Garfinkel, et al., 2012) or a lack of such connectivity (Rabinak et al., 2011) in PTSD compared to non-PTSD controls. The unpredicted decreased right-sided coupling compared to HC observed in our study further complicates the picture and shows that the comparison group might have an impact on the results and that laterality should be taken into consideration. Nonetheless, the studies show that aberrant functional amygdala – insula connectivity seems to be involved in the pathophysiology of PTSD and that further research is needed to understand the specific impact.

Another interesting result of our study is that amygdala-putamen connectivity seems to be decreased after experiencing a traumatic event. Both trauma-exposed groups showed a reduction of functional connectivity between the left amygdala and the right putamen compared to healthy controls. The role of the putamen for outcome prediction (Horvitz, 2002) together with the sensitivity of the amygdala for contingency (Bauer, LeDoux, & Nader, 2001) might lead to an overestimation of potential threat after trauma experience. Our study suggests that this could be a reaction to trauma per se and does not represent a vulnerability factor for PTSD, but the effect and its presumed implication need to be confirmed in further research.

The fact that we did not find significant group differences on the network level might indicate that there are no general changes in resting connectivity of the brain and that

the observed seed-based changes are specific for PTSD or trauma experience and are not a by-product of an underlying network alteration. However, a recent meta-analysis (Koch et al., 2016) states that "PTSD is associated with enhanced SN processing (…), decreased DMN connectivity (…) and altered connectivity between the nodes of the SN and DMN". The lack of such differences in our sample might be a result of the small sample size or methodological differences.

Our results are of clinical relevance as they indicate that PTSD symptoms can be associated with neuronal changes in the absence of a (trauma-related) task. This matches the clinical observation that PTSD patients suffer from re-experiencing and hypervigilance not only during potentially trauma-related situations but also during periods of rest.

Taken together, both studies underline the importance of the amygdala for the development and maintenance of PTSD. In our patient sample that comprised military and civilian trauma, we found alterations of amygdala activity and connectivity. Increases in amygdala activity were associated with a return of fear upon context change after successful extinction, and increased amygdala-insula connectivity was interpreted in respect to potentially enhanced SN processing, i.e. stronger salience processing at rest. Notably, these amygdala alterations were linked to PTSD symptoms in both studies. In the fMRI experiment, increased amygdala activity during return of fear was positively correlated with numbing intensity and in the resting state functional connectivity analysis, increased amygdala-insula coupling was positively correlated with re-experiencing. Directly linking functional changes to discrete symptoms in patient samples is of special relevance, because this connection cannot be drawn from preclinical research examining rodents or healthy humans. Even though this kind of research is highly valuable for developing models for PTSD, studies in actual patient groups are necessary to verify the theoretical framework and to detect (unexpected) disorder related mechanisms, such as the neuronal correlates of symptom severity.

In the fear conditioning experiment, increased amygdala activation was related to elevated return of fear and to the intensity of emotional numbing. Enhanced emotional numbing in PTSD has previously been linked to reduced ventral striatal activity during confrontation with happy faces (Felmingham et al., 2014). Interestingly, amygdala activity has also been reduced in this task, but was not directly related to emotional numbing. The fact that the subjects in our study were confronted with aversive instead

of appetitive stimuli might have led to the observed positive correlation of amygdala activity with numbing intensity. It is possible that intensified numbing constitutes a compensatory reaction to unbearable fear mediated by increases in amygdala activity in response to perceived threat, such as the presentation of the CS+ during extinction recall in study 1. This interpretation is highly speculative at this point and studies employing a direct measure of emotional numbing rather than CAPS scores are needed to verify this assumption.

In the resting state connectivity analysis, amygdala-insula connectivity was positively correlated with re-experiencing intensity in our PTSD sample. Given the role of the amygdala and the insula within the SN, one could assume that hyperarousal would be associated with hyperconnectivity between the two regions, but the correlation did not reach significance in our study. However, when taking general functions of the insula into account, it is not surprising that increased connectivity with the amygdala is correlated with re-experiencing. The anterior insula integrates and evaluates internal (bodily) and external (environmental) information (Uddin, 2015). In addition with (enhanced) emotional input from the amygdala, this could lead to stronger fear responses to internal or external trauma reminders and result in re-experiencing symptoms including flashbacks (Hopper, Frewen, van der Kolk, & Lanius, 2007). Our finding of increased amygdala-insula functional connectivity at rest might provide a neurobiological basis for this process, i.e. maladaptive coupling of visceral sensations/threat perception and emotion in PTSD symptoms. Again, further research is needed to verify this assumption.

Furthermore, we found differences between PTSD patients and trauma-exposed but unaffected controls in both studies. In the fear conditioning experiment, the non-PTSD group showed significantly less SCR during return of fear and – even though this did not reach significance – patterns of brain activation and subjective ratings that were very similar to those of the healthy controls and differed markedly from those of the PTSD patients. In the resting state study, the non-PTSD subjects showed reduced left amygdala – left insula coupling compared to PTSD. Interestingly, non-PTSD (and PTSD) further showed reduced positive left amygdala-right putamen and reduced left amygdala-right insula connectivity than HC. Taken together, these results suggest that the experience of trauma leads to alterations in the neuronal system, but that individuals who do not develop PTSD in the aftermath of trauma benefit from protective mechanisms. We

investigated both, connectivity and activation within the same sample and the combined data imply that a traumatic experience changes the brain's (the amygdala's) resting connectivity rather than its task-related activity. However, replication of the resting state functional connectivity result is needed, especially for the comparison of trauma-exposed but unaffected subjects with trauma-naïve healthy controls. And, contrary to our result, some activation studies have reported functional differences between the two trauma-exposed groups in fear conditioning paradigms (Garfinkel et al., 2014; Milad et al., 2008). One explanation for this difference might be the heterogeneity within our non-PTSD group. Whereas the aforementioned studies used veterans and combat controls, the range of traumatic experiences (including the time since trauma) within our control sample was quite diverse. In the past, research on non-PTSD controls with various trauma backgrounds has indeed produced mixed results (Diener et al., 2014; Diener et al., 2012; Milad et al., 2009; Norrholm et al., 2011; Rougemont-Bücking et al., 2011; Steiger et al., 2015; Wessa & Flor, 2007). In addition to the diverse circumstances of the traumatic event, several factors, such as experimental design (i.e., extinction test vs. renewal, number of CS-US pairings), stimulus material (stationary pictures vs. VR) and time frame (acquisition, extinction and extinction recall on the same day vs. different days) might have led to the observed difference. Nonetheless, our activation data suggests that possible resilience factors in non-PTSD may be associated with frontal regions explicit strategies for compensating potential trauma-related impairment, that are presumably mediated by frontal regions.

3.2. Limitations

Despite some strengths of the described studies, there are limitations that need to be taken into consideration when interpreting the results. The sample that was used for both analyses was rather small and included medicated and comorbid PTSD patients. Even though small sample sizes are not unusual in clinical fMRI research and medication as well as comorbidity are common in PTSD (Bremner et al., 2003; Bremner et al., 2003; Etkin & Wager, 2007; Grillon & Morgan, 1999; Milad et al., 2009), this undoubtedly influences the quality of the data. On the other hand, our data represent a characteristic PTSD population, which can be considered beneficial for the external validity of the results. In addition, selection effects in our samples have to be considered, since some

people are more prone to undergo potentially traumatic events than others due to *a priori* differences in personality or life circumstances.

In the fMRI conditioning study, the small sample size was even more pronounced for the SCR data, where 25.9% non-responders had to be excluded from the analysis. Again, even though this clearly limits the conclusion of the SCR results, this is a prevalent phenomenon in SCR measurements, especially when combined with fMRI (Dziobek et al., 2011; Haaker et al., 2013; Hartley, Fischl, & Phelps, 2011; Kalisch et al., 2006; LaBar & Phelps, 2005; Lonsdorf et al., 2014; Milad, Orr, Pitman, & Rauch, 2005; Phelps et al., 2004; Raio, Brignoni-Perez, Goldman, & Phelps, 2014; Winkelmann et al., 2015).

In the second study we investigated functional connectivity of predefined ROIs and were therefore not able to draw causal conclusions on the directionality of the amygdala coupling or make statements about other potentially correlated brain regions. We controlled for cardiac and respiratory cycles in the seed-based approach and the ICA, but did not directly monitor these potential confounders.

3.3. Relationship to other findings in the field

The results of this thesis are in line with previous research for the most part, but show some discrepancies that offer interesting research questions.

In the fear conditioning study, we confirmed the previously observed deficient identification of safety signals in PTSD patients (Bremner et al., 2005; Garfinkel et al., 2014; Jovanovic et al., 2012; Weike et al., 2008). The direction of hippocampal activity alterations in PTSD has been a matter of debate (Brohawn et al., 2010; Hayes et al., 2011; Hermann et al., 2016). Our data indicates an upregulation of the hippocampus that is associated with disrupted extinction maintenance of trauma-unrelated CSs (Milad et al., 2009). Concerning return of fear, our study contradicts a previous report. Whereas we observed enhanced renewal in PTSD compared to HC and nonPTSD, Garfinkel et al. (2014) found reduced renewal in PTSD compared to nonPTSD. The methodological differences between the two studies (e.g. ABC vs. ABBA renewal) imply that it is important to identify the mechanisms leading to increased or decreased return of fear in PTSD, including contextual and procedural factors.

The resting state connectivity study confirms the previously reported hyperconnectivity between the (left) amygdala and the (left) anterior insula under rest in PTSD compared to nonPTSD (Rabinak et al., 2011; Sripada, King, Garfinkel, et al., 2012). In addition, our

data revealed a downregulation of (left) amygdala-(right) insula coupling in PTSD compared to HC, suggesting a laterality difference. Since others (Rabinak et al., 2011; Sripada, King, Garfinkel, et al., 2012) have found an upregulation of this connection including the right amygdala in PTSD compared to nonPTSD, the results might indicate that laterality plays a role for PTSD-related changes in amygdala-insula coupling, but confirmation is needed. The fact that we found a positive correlation of increased amygdala-insula connectivity with the strength of re-experiencing in PTSD extends previous research showing a relationship between increased resting state connectivity of the amygdala (e.g. with the posterior cingulate/ precuneus) and PTSD symptoms (Lanius et al., 2010; Yin et al., 2011; Zhou et al., 2012), including associations of increased SN coupling with hyperarousal (Koch et al., 2016). Surprisingly, we did not find group differences in large scale networks (DMN and SN), even though alterations in both directions have previously been reported in PTSD. The small sample size and methodological differences might underlie this negative result.

3.4. Outlook

Despite the conclusions that can be drawn from the studies of this thesis, the results also raise questions for future research. Return of fear and specifically renewal are important obstacles for the long-term efficacy of exposure-based PTSD treatment (Vervliet, Craske, & Hermans, 2013). Yet, even though extensive preclinical studies have helped us understand many of the underlying mechanisms, the translational approach does not seem to have fully reached the clinical level. Patient studies on renewal are rare, in fact there is so far only one other study apart from ours and that study has produced opposing results. Thus, our experiment indicates that return of fear is not a simple and universal phenomenon like animal studies suggest (Vervliet, Baeyens, Van den Bergh, & Hermans, 2013) and future research in patient groups is needed to explain its role in PTSD. Comparing PTSD patients to both, trauma-exposed but unaffected and trauma-naïve controls, is desirable in this regard to disentangle trauma-experience from PTSD symptomatology. In light of the debate on the direction of hippocampal alterations in PTSD, the role of the conditioning context is crucial and should be elaborated further. VRs offer a promising tool for this objective, because they can generate environments that prevent cue-like associations of a context with a fear stimulus. For example, (stationary pictures of) different rooms might lead to simple verbal associations like

“apartment – shock” or “office – no shock”. One could argue that such labels do not represent contextual fear and therefore might not depend on the hippocampus. Hence, research on context-related return of fear should employ contexts that require normal hippocampal function, such as environments that contain an identical set of items and can only be separated by correctly identifying the individual arrangement of those items that is specific for a certain environment. Given intact hippocampal function, CSs presented within such contexts should only elicit fear in the acquisition context, but not in the extinction context (Acheson et al., 2012). As a consequence, deficit extinction or relapse of extinction could be linked more directly to the hippocampus. Furthermore, the conditioning protocol needs to be understood in more detail. On the one hand, an extinction memory test is needed to rule out spontaneous recovery, but on the other hand additional extinction training might modify the reaction to subsequent presentations of the CS. Directly investigating the impact of an extinction recall phase prior to the context change on renewal processes within one sample seems to be necessary in order to understand context-dependent return of fear in PTSD.

Altered resting state functional connectivity of the amygdala with the insula in PTSD needs to be replicated compared to trauma-exposed and trauma-naïve controls in order to verify the up- and down-regulation observed in our sample. Moreover, it would be beneficial to integrate altered amygdala coupling with further regions such as the putamen, hippocampus or ACC (Lanius et al., 2010; Sripada, King, Garfinkel, et al., 2012) into a comprehensive model or network for PTSD. As current research indicates, such connectivity patterns might be linked directly to symptomatology and could perhaps be used as a biomarker for PTSD in the future. However, caution is needed when interpreting resting state measures in PTSD. It is possible that PTSD patients experience trauma-related PTSD symptoms, including unwanted thoughts, hypervigilance, depersonalization and flashbacks during resting state scans. Even though this in itself is an interesting subject for research, it has not yet been directly investigated. Hence, the possibility that PTSD patients suffer in the scanner whereas control subjects are relaxed could confound the results and should be taken into consideration.

The presented studies further indicate that combined activation and connectivity analyses are beneficial and – since clinical samples are by nature highly valuable groups – future research should try to combine different measures in order to better explain the underlying neurobiology of PTSD. Results of such studies have the potential to improve

therapeutic interventions by providing means for more effective PTSD treatment (e.g. exposure therapy in various contexts, including virtual reality environments) and/or customized therapy programs (e.g. shorter therapy sessions to account for concentration deficits resulting from increased DMN-SN coupling). It might even be possible to prevent the development of PTSD by identifying high-risk individuals for example in military populations (e.g. through the detection of increased DMN-amygdala coupling prior to deployment). Increased efficacy of PTSD therapy is desirably not only in light of the presumably growing importance of the disorder, but also considering the misery patients have to endure.

4. Summary

In the present dissertation we addressed neuronal changes in PTSD using an activation-based and a resting state-based approach with a special focus on brain areas involved in abnormal activation in PTSD such as amygdala, hippocampus, ventromedial prefrontal cortex (vmPFC), dorsal anterior cingulate cortex (dACC) and insula. Our attention was directed to the mechanisms mediating increased return of fear and the association of PTSD symptoms with aberrant brain activity as well as aberrant resting state connectivity. In both studies we compared PTSD patients with trauma-exposed but unaffected controls (non-PTSD) and trauma-naïve healthy controls (HC).

In the first study, subjects underwent an ABC fear conditioning and extinction procedure, where two CSs were presented in front of virtual reality scenes. One of them (CS+) was paired with a slightly painful electrical stimulation (US) during acquisition, whereas the other one was never paired with the US (CS-). During extinction, there were no CS-US pairings. After acquisition (context A) and extinction (context B), the participants were brought to a novel context C and again confronted with the CSs. Self-reports, skin conductance responses (SCR) and functional magnetic resonance imaging (fMRI) were measured simultaneously. We found elevated return of fear in the PTSD patients indicated by larger differential SCR compared to non-PTSD and HC and larger differential amygdala and hippocampus activity compared to HC. Increased amygdala activation was positively correlated with numbing and vmPFC activity was positively correlated with behavioral avoidance even though there were no functional group differences in this region of interest. Additionally, PTSD patients failed to appropriately reduce subjective arousal to the CS- over the course of the experiment and to the CS+ during extinction. Taken together, the results of study 1 support the hypothesis that PTSD is characterized by aberrant activity within regions of the neurocircuitry model, which leads to deficient extinction maintenance. Furthermore, our data confirm a general inability of PTSD patients to correctly identify safety signals and modulate fear responses based on this information. Such dysfunctional mechanisms seem to contribute to PTSD symptoms and represent a probable cause for relapse, whereas resilient subjects appear to benefit from protective mechanisms.

In the second study, subjects underwent a resting state scan and functional connectivity was analyzed using an amygdala seed and independent component analysis (ICA) as well as correlations with symptom severity. The seed-based approach revealed

increased left amygdala – the left insula coupling in PTSD versus nonPTSD, which positively correlated with re-experiencing intensity. Compared to HC, both trauma-experienced groups showed higher positive correlations of the left amygdala and the right putamen as well as the right insula. The ICA did not reveal any group differences, i.e. in DMN connectivity. In summary, study 2 indicates that altered amygdala-insula coupling and decreased amygdala-putamen coupling, but not DMN connectivity, contribute to the pathophysiology of PTSD. Hyperconnectivity between the left amygdala and the left insula differentiated patients from resilient subjects and was linked to re-experiencing intensity. This result suggests that a stronger functional link between somatic sensations and emotional appraisal might lead to increased anticipation of negative events in PTSD, which potentially explains characteristic symptoms such as hyperarousal and negative alterations in mood and cognition.

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Bochum, den 24.04.2016

7. Danksagung

Meiner Doktormutter, Prof. Dr. Herta Flor, gilt der Dank für die einzigartige Möglichkeit, dass ich Forschung auf einem solch hohen Niveau betreiben durfte. Durch ihre kritischen Anregungen habe ich gelernt, meine Arbeit immer wieder zu hinterfragen und sowohl die Durchführung der Studien selbst als auch die Auswertung und Interpretation der Ergebnisse stetig zu verbessern.

Außerdem danke ich allen ehemaligen Mitgliedern von C1: Dr. Claudia Liebscher, Frauke Steiger, Dr. Slawomira Diener, Dr. Raffaele Cacciaglia, Dr. Stephanie Ridder, Dr. Tobias Winkelmann, Dr. Sebastian Pohlack, Francesca Zidda, Juliana Cavalli und Mario Rosero. Ohne die Zusammenarbeit im Team hätte die vorliegende Arbeit nicht entstehen können.
Besonderer Dank gilt an dieser Stelle Dr. Frauke Nees für ihre unermüdliche Unterstützung in allen fachlichen, aber auch praktischen und emotionalen Belangen.

Michaela Ruttorf, Dr. Traute Demirakca und Matthias Ruf danke ich für die verlässliche technische Hilfe bei der Programmierung, Implementierung und Auswertung der Studie.

Außerdem danke ich Claudia Stief, Birgül Sarun und Bettina Kirr für die vielfältige Unterstützung bei den Messungen, sowie Angelika Bauder, Artemis Tsoupas und Astrid Wolf für die stete Hilfe bei den notwendigen Formalitäten.

Meinen Mentoren im Graduiertenprogramm, Prof. Dr. Christine Kühner und Prof. Dr. Rainer Spanagel danke ich für die wertvollen Diskussionen und das Begleiten meines Fortschritts.

Ebenso wichtig für die Fertigstellung meiner Arbeit waren meine Kolleginnen und Kollegen, mit denen der Alltag im ZI viel Spaß gemacht hat und deren Rat ich immer gerne gesucht habe. Vielen Dank also an die „Kellerkinder“ Claudia, Martin, Robin, Angela und Maike und an meine lieben ZPP-Mitstreiterinnen Christiane und Leila.

Außerdem hätte ich die Arbeit sicher nicht fertigstellen können, hätte ich nicht immer auf die Unterstützung meiner Freunde zählen können. Sie ermöglichten mir den nötigen Ausgleich, damit ich neue Kraft und frische Ideen entwickeln konnte. Ich danke besonders den Basketball-Mädels der SG Mannheim (alle aufzuzählen würde an dieser Stelle den Rahmen sprengen…) und meinen „alten“ Bochumer Freunden Sören & Barbara, sowie Aga, Katha und Sahra.

Besonderer Dank gilt außerdem meiner Familie, die in allen Höhen und Tiefen für mich da war. Gerade in der finalen Phase der Dissertation waren meine Eltern Angela und Ulrich eine unschätzbare Hilfe – dafür bin ich ihnen sehr dankbar. Außerdem danke ich meinen Schwestern Maren, Miriam und Mona dafür, dass sie sich immer Zeit für mich genommen haben.

Zu guter Letzt gilt mein innigster Dank meiner eigenen kleinen Familie: Meinem Partner Alain, der bei allen Widrigkeiten ein Fels in der Brandung war, und unserem in der Schlussphase der Dissertation geborenen Sohn Jonathan Mbasha, der mich daran erinnert hat, was wirklich zählt.