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Neurobiological, attentional and memory changes in posttraumatic stress disorder

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1 INTRODUCTION

Experiencing a traumatic event is not a rare eventuality, estimates of lifetime traumatic event prevalence rate range from around 54% (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Kilpatrick et al., 2013) to 89% varying by country, historical, political and social factors (Atwoli, Stein, Koenen, & McLaughlin, 2015; Burri & Maercker, 2014). Moreover, more than 30% of individuals worldwide have a history of multiple traumatic experiences (Kessler et al., 2017). Virtually, at least one of two in the general population is a traumatized individual. Maladaptive reactions to these events can lead to the development of posttraumatic stress disorder (PTSD). PTSD patients suffer from the trauma, re-experiencing unwanted details, thoughts and even dreaming of it (see chapter 1 below for a full clinical description of the disorder). They live in a constant enhanced physiological state, continuously monitoring for possible threats in the environment. Moreover, they start avoiding situations or people that could remind them of the trauma. Finally, individuals who develop persistent PTSD, also experience several emotional responses (such as fear, sadness, guilt) (Ehlers & Clark, 2000). Fear and anxiety mechanisms are clearly at the core of this disorder, such that it was previously classified as an anxiety disorder. Together with these symptoms, there are also several parallel underlying cognitive changes taking place that affect the way the world is attended to and could be responsible for the development and maintenance of the disorder.

Worth noting, PTSD is one of the rare circumstances in which the etiological factor, which triggered the disorder, is known (Ehlers & Clark, 2000) and consequently perceptual and attentional processing of it can more easily be object of investigation, together with its sequelae.

An impairment in autobiographical memory, theorized in several “dual representation” models has been established to be at the core of the disorder, with an exacerbation in processing and representing traumatic cues accompanied by a poorer integrated analysis and representation of the related contexts, or at least of a scarce integrated representation of their association.

Nevertheless, little is still known regarding how processing of fear or traumatic information in the first place affects our memory of it and consequently future information processing and behavior.

The way we encode and represent every experience depends critically on our perceptual and attentional processes (Kanwisher & Wojciulik, 2000), and even at rest, we constantly monitor our internal and external environment in individually different intrinsic ways (Gusnard, Raichle, & Raichle, 2001) and probably process off-line past events for memory consolidation (Miall & Robertson, 2006).

It is worth noting that, among exposed individuals only 5.6% develops PTSD (this percentage increases depending on gender and sociodemographic characteristics) and only half of these develop persistent PTSD (Koenen et al., 2017). This suggests that individual differences exist in vulnerability and resilience to traumata and increased understanding of these differences with the associated mechanisms is clearly an important goal.

It is thus important to study individual differences in the way we attend the world regardless of a specific task and see how these differences are associated with basic mechanisms in making fear and anxiety associations. Differentiating predispositions at rest can be insightful in understanding proneness to develop these associations in a maladaptive way. Further, we tried to connect encoding mechanisms (perceptual and attentional processes) of high adaptive value events with memory and learning.

In the present work, we aimed at answering some of these open questions by investigating:

- cued and contextual conditioning mechanisms (subserving fear and anxiety learning) in association with neural patterns at rest, using functional magnetic resonance imaging (fMRI) in healthy controls;
- encoding mechanisms (subserving perceptual and attentional processes) and their association with learning and memory through the means of brain event-related potentials (ERPs) and eye tracking measures of cues and contexts in PTSD and traumatized individuals who did not develop the disorder (NPTSD).

1.1 Posttraumatic stress disorder

After a traumatic experience individuals that develop PTSD experience a number of distressing symptoms included in three main categories re-experiencing (intrusive memories, thoughts and/or flashbacks), heightened general arousal (hyperattention and continuous monitoring for potential threats, enhanced startle reactivity), emotional numbing and avoidance regarding reminders of the traumatic event (American Psychiatric Association, 2010). In the last update of the DSM (Diagnostic and Statistical Manual of Mental Disorders), DSM-5 (American Psychiatric Association, 2013), PTSD has been moved from the category of anxiety disorders into a new one of “trauma and stressor-related disorders”. This related to the fact that the presence of a known stressor triggers the development of the disorder. Previously described symptoms in DSM-IV were mostly kept and another cluster of symptoms regarding negative alterations in cognitions and mood has been added. According to the cognitive model of PTSD proposed from Ehlers and Clark (2000), there are two main processes at play: individual differences in the appraisal of the trauma (including sensory processing) and/or its sequelae and individual differences in the memory for the event and its connection to other memories (Ehlers & Clark, 2000). The authors propose that “the trauma memory is poorly elaborated and inadequately integrated into its context in time, place, subsequent and previous information and other autobiographical memories”. These differences are responsible for creating the perception of a current threat, through “situational fear” and avoidance through generalization, even though the traumatic event is in the past.

As a neurobiological correlate of PTSD impaired prefrontal cortex (PFC) top-down modulation of an hyper-responsive amygdala, together with alterations of hippocampal function and structure that leads to an impairment in contextual processing mechanisms have been proposed (Rauch, Shin, & Phelps, 2006).

1.2 Dual representation models of learning and memory

A “dual representation” theory of PTSD has been proposed (Brewin, Dalgleish, & Joseph, 1996) and recently updated (Brewin, Gregory, Lipton, & Burgess, 2010) according to which, an imbalance or even a dissociation between sensorial and contextual representation of the information is responsible for the mnemonic sequelae (intrusions, flashbacks etc.) of the disorder.

The first representation of the information present and processed during the traumatic event is made through binding sensorial details (S-rep) with emotional/affective states. The second representation is a contextual one (C-rep), a spatially less structured and abstract representation of where the event is happening. The C-rep, that would be mainly encoded in a viewpoint-independent (allocentric) and retrieved in a viewpoint-dependent (egocentric) perspective, is considered to be poorly encoded in individuals who develop PTSD or at least poorly associated with the related S-reps.

Other authors, in a similar fashion, presented a model describing the existence of unitary and conjunctive representations referring to the main salient events and backgrounds/contexts of the encoded scene (Rudy, Huff, & Matus-Amat, 2004; Rudy & O'Reilly, 2001). Flor and Wessa (2010) reinterpreted these previous models and in line with them theorized that individuals who develop PTSD might have impaired contextual processing and others (Acheson, Gresack, & Risbrough, 2012) suggested that processing in PTSD might depend mostly on an elemental representation strategy probably due to impaired hippocampal processing that weakens the conjunctive one. Thus, the properties of these models have also been integrated in a mechanistic and neurobiological manner with fear conditioning mechanisms and it has been assumed that the amygdala is mainly mediating elemental representations and contextual conditioning and the hippocampal formation is mainly mediating contextual representations (Acheson et al., 2012; Maren, 2001; Maren, Phan, & Liberzon, 2013; Maren & Quirk, 2004).This hippocampal impairment would also explain why these patients cannot correctly differentiate dangerous and safe contexts (Rudy, 2009). A major function of this circuit is in the disambiguation of cues that have different meanings in different contexts (Maren et al., 2013).

Interestingly, even though these models assume that the development and maintenance of PTSD is based on mnemonic and retrieval features of the traumatic

event (choosing the hippocampus as a possible main vulnerability factor), they mention perceptual and attentional aspects as been relevantly involved but not relevantly affected in the disorder. S-rep, unitary and elemental representations are individually encoded perceptions in the different sensorial modalities (such as tactile, visual, odor, spatial or temporal stimuli) selected as salient (emotionally charged), while conjunctive representation would refer to an integrated perception of the different elements associated together and with the environment in a more abstract unstructured form (Acheson et al., 2012; Rudy et al., 2004).

More recently it has also been proposed a dissociation between perceptual and episodic memory as accounting for flashbacks and intrusions. The contextualization processes would be led from selective attention and recoding of the sensory input, thus pointing to a more organized and integrated information representation (more easily consciously accessible and reducing involuntary intrusions) (Brewin, 2014).

Despite this, perceptual and attentional processes and the way they could differently interact with memory have only partially been taken into account in these models.

1.3 Neurocircuitry of stress and anxiety disorders (or trauma related disorders)

Preclinical studies of stress and conditioned fear by researchers such as Davis (1992) and LeDoux (2003) informed nowadays neuroimaging investigations in humans of what is called “fear network”, a basic model of normal fear responding focused on the critical role of the amygdala in fear acquisition and expression.

Cortical feedback to the amygdala is provided by specific brain regions, including the medial prefrontal cortex (mPFC), the anterior cingulate; the hippocampus provides information about the context of a potentially threatening stimulus or situation, and draws on information about the environment retrieved from explicit memory caches (Kent & Rauch, 2003).

Studies in humans quite unanimously confirmed the role of amygdala, hippocampus and frontal control regions in the pathophysiology of anxiety and stress/trauma related disorders (Sehlmeyer et al., 2009; Shin & Liberzon, 2010).

PTSD and other anxiety disorders (e.g. panic disorders, social anxiety disorder) have been linked to different pattern of activation but sharing the core of this neurocircuitry, amygdala, hippocampus and mPFC (Kent & Rauch, 2003).
PTSD especially has been associated with atypical connectivity between amygdala hippocampus and mPFC (Michopoulos, Norrholm, & Jovanovic, 2015).

1.4 Cued and contextual fear conditioning

Fear is an adaptive essential emotion in humans and fear learning mechanisms are representative of basic learning mechanisms in what concerns making associations between internal or external stimuli and/or situations.
It has been proposed that pathological anxiety could emerge from dysregulated patterns of fear learning (Shin & Liberzon, 2010) thus, cued and contextual classical conditioning paradigms have been extensively used as experimental models for anxiety disorders (Glotzbach-Schoon et al., 2013; Grillon, 2002b; Indovina, Robbins, Nunez-Elizalde, Dunn, & Bishop, 2011).
In classical conditioning, an initially neutral stimulus becomes conditioned (CS) after being paired with a biologically relevant stimulus called unconditioned (US) and becomes able to elicit a conditioned response (CR) that may be similar but can also be antagonistic to the original or unconditioned response (UR).
In discrimination paradigms, two CSs are used, not only the one paired with the US (CS+) but also another one that it is never paired with it (CS−) (Lissek et al., 2005). The difference between CRs to the CS+ and CS− will provide a differential index indicative of discriminative learning (Lissek et al., 2005). In order to avoid sensitization phenomena only a part of the CSs+ is constantly paired with the US. A low differential value between CS+ and CS- could then be indicative either of enhanced conditionability, to both dangerous and safe signals, or of enhanced generalization through an inability of inhibiting fear towards safety cues (Duits et al., 2015). Either conceptualization (i.e., stimulus generalization or lack to inhibit fear) supports impaired discrimination learning.
The output of the autonomic nervous system associated with fear conditioning can be measured through recording of the skin conductance response (SCR) (Marin et al., 2017; Orr et al., 2000). This peripheral outcome can be also used in differential conditioning studies, providing a differential SCR (Michopoulos et al., 2015).

Importantly, it has been proposed that different type of conditioning could better model different aspects of fear learning and consequently different mental disorders (Indovina et al., 2011). Cued conditioning, in which there is learning of an association between a discrete stimulus (cue) and a predictable danger (US), might better model aspects of phobic fear. In contrast, context conditioning involves the association between an internal or external context with an unpredictable danger (aversive event delivered with variable onsets) and thus could better model sustained anxiety (Grillon, 2002a). Individual differences in healthy individuals affecting these mechanisms have been proposed to be also associated with higher risk of developing an anxiety disorder (Mineka & Oehlberg, 2008).

An important further step in understanding the pathophysiology of these disorders can be made by understanding neural differences associated with these conditioning mechanisms and the activity/connectivity of the brain at rest.

1.5 Perceptual and attentional mechanisms in PTSD

Already back of several decades an information processing model of anxiety was proposed (Beck & Clark, 1997) in which the biased perception of a threatening stimulus was the core explanation for development and maintenance of anxiety disorders "… *pathological anxiety… is a biased or overestimated perception of danger which does not correspond to the exigencies of the internal or external environment…* " (p .51).

Clinical observations showed that some stimuli can trigger intrusions (Foa, Steketee, & Rothbaum, 1989) in PTSD and that these sensations are predominantly visual and seem to happen in the 'here and now' rather than being memories of past (Ehlers et al., 2002). Intrusive re-experiencing in PTSD has been linked to perceptual (priming) processing of trauma-related material (Ehlers and Clark (2000)) and recently associated with the hypothesized lack of conceptual processing (Lyttle, Dorahy, Hanna, & Huntjens, 2010). A study that assessed perceptual bias using a blurred picture identification task in a large cohort of trauma survivors concluded that a processing bias exists specifically for trauma-related stimuli compared to neutral or negative in PTSD and acute stress disorder patients compared to NPTSD (Kleim, Ehring, & Ehlers, 2012). The authors refer to it as a processing advantage leading to

an enhanced readiness for trauma-related information. A study using fMRI reported an atypical visual sensory processing in PTSD in the ventral visual stream, thought to be responsible for object property processing during a picture-viewing task (Mueller-Pfeiffer et al., 2013). The authors linked this deficit to dysfunctional attention processes.

Indeed, the description of PTSD symptoms frequently includes a constant search for threat in the everyday environment beyond those related to the original trauma, suggesting that PTSD patients might be generally oversensitive to threat (Zukerman, Itzchak, Fostick, & Armony-Sivan, 2017); this comes together with extreme avoidance of possible trauma reminder exposure. Hyperarousal (hyper-sensitivity) and avoidance symptoms, core features of PTSD and of anxiety disorders in general, are thought to work through associated atypical attentional (covert or overt) processes (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Thus, vigilance-avoidance models of anxiety have been proposed prompted from the work of Williams, Watts, MacLeod and Matthews (1988). These models have been tested and challenged especially in studies employing eye tracking methodologies because eye tracking can provide a more direct measure of attentional bias without requiring verbal or motor responses (Felmingham, Rennie, Manor, & Bryant, 2011). Eye tracking associated with free viewing paradigms can therefore delineate different mechanisms of attention bias and its effects (hypervigilance, maintenance/disengagement and attentional avoidance) with minimal interference.

As reported in a recent meta-analysis (Armstrong & Olatunji, 2012), the most frequently observed effect in eye tracking free viewing studies of anxiety disorders is hypervigilance, with sooner fixation towards threat in anxious versus non-anxious individuals and towards threatening stimuli, instead of positive. In contrast, this spatial orienting bias has not been consistently reported regarding maintenance or avoidance of aspects of attention.

Findings regarding PTSD point in the same direction. One study showed a significantly higher number of initial fixations to trauma-related words in the PTSD group compared to traumatized (NPTSD) controls. Another study found differences in in a sample of individuals with anxiety disorder in a dimensional fashion; individuals with high compared to low number of PTSD symptoms had larger pupillary dilatation and fixation time (Kimble, Fleming, Bandy, Kim, & Zambetti, 2010).

Importantly, it has been explicitly stated that the usage of threatening cues embedded in naturalistic scenes would be an advantage in attentional eye tracking studies (Williams et al., 1988) because it would provide alternative of locations to be fixated apart from threat in a more ecological way.

1.6 Neurobiological and neurophysiological correlates of relevant mechanisms for PTSD

1.6.1 Resting-state fMRI

Resting state (rs) fMRI measures spontaneous and synchronous low frequency fluctuations (<0.1 Hz) in the blood oxygen level dependent (BOLD) signal to investigate the functional connectivity of the brain. “Resting state” refers to the initially surprising finding that the brain is very active even while not involved in any specific task (Raichle & Mintun, 2006) and “connectivity” refers to the fact that regions of the brain spatially distinct can be temporally correlated.

Since Biswal, Yetkin, Haughton, and Hyde (1995) discovery of a resting state signal in the brain and Raichle et al. (2001) publication of a “default mode brain function” while spontaneously and constantly monitoring external and internal stimuli, much work has been published to document the relevance of resting-state functional connectivity (rs-FC) in basic and clinical neuroscience (Lee, Smyser, & Shimony, 2013).

It has been proposed that connectivity in rs networks is associated with activity and performance during tasks through specific cognitive mechanisms (Madhyastha, Askren, Boord, & Grabowski, 2015; Mennes et al., 2010; Schultz, Balderston, & Helmstetter, 2012).

This concept has also been extended to anxiety disorders and it has been proposed that investigating changes in specific networks at rest relates to general cognitive functioning and can highlight modulation of fear responses (Schultz et al., 2012; Sylvester et al., 2012). In this direction, some studies showed that rs-FC of the amygdala (with mPFC and ACC) was altered following a cue conditioning paradigm (Schultz et al., 2012) and after fear reminder exposure (P. Feng, Zheng, & Feng, 2016). Enhanced rs-FC connectivity between the amygdala and a region in the right anterolateral temporal cortex in patients with vmPFC lesions was found and

interpreted as relevant for psychopathology, connecting a loss or reduction of functionality of the first with more activity in the second (Motzkin et al., 2015). Especially, the default mode network (DMN) is thought to support a state of readiness in responding to environmental demands (Kluetsch et al., 2012) and was specifically shown to be relevant for affective and cue safety learning (Fullana et al., 2016). A study reported that the DMN was anti-correlated with a fear-processing network and was described as active when feeling safe and thus necessary for conceptualizing safe memories (Marstaller, Williams, Rich, Savage, & Burianova, 2015). None of these studies, however, differentiated the contribution of contextual and cued learning mechanisms with respect to relevant resting state networks and their implications for anxiety disorders.

1.6.2 Electroencephalographic (EEG) and event-related perceptual studies in anxiety and/or trauma related disorders

EEG is, due its accurate temporal resolution in the order of milliseconds (ms) and low number of exclusion criteria, an elective method for studying information processing abnormalities in clinical populations.

Several electroencephalographic (EEG) studies of event-related potentials (ERPs) have investigated differences in information processing between individuals diagnosed with PTSD, traumatized individuals that did not develop the disorder and healthy controls and showed relevant abnormalities in the amplitudes and latencies of several components (from 50 (mainly auditory) to 300 ms) (for a review, Javanbakht, Liberzon, Amirsadri, Gjini, & Boutros, 2011).

Despite this, trauma-related changes in PTSD have been mostly investigated in amplitudes and latencies of the attention related P300 component (with onset around 250-300 ms after stimulus presentation) (Johnson, Allana, Medlin, Harris, & Karl, 2013).

In this regard, it is worth mentioning the recent interest in assessing early perceptual top-down modulation from higher order cognitive areas to primary visual areas through changes in amplitudes, latencies and polarity of the component C1 in visual tasks. The C1 is the earliest described visual deflection in the ERP and mainly

generated from activity in the primary visual cortex (V1) (Clark, Fan, & Hillyard, 1994). It shows a characteristic change in polarity in response to stimuli presented in the upper or lower visual field (due to the retinotopic organization of V1) (Bayer et al., 2017; Clark et al., 1994; Jeffreys & Axford, 1972).

Several studies showed different processes affecting this component, such as spatial attention (Kelly, Gomez-Ramirez, & Foxe, 2008; Proverbio & Adorni, 2009), aversive learning (Pourtois, Grandjean, Sander, & Vuilleumier, 2004; Stolarova, Keil, & Moratti, 2006), anxiety (Rossi & Pourtois, 2017), mood state and emotional processing (Brosch, Sander, Pourtois, & Scherer, 2008; Vanlessen, Rossi, De Raedt, & Pourtois, 2014) and emotionally complex and competing stimuli in the same visual field (West, Anderson, Ferber, & Pratt, 2011).

Other ERP, EEG and magnetoencephalographic (MEG) studies that focused on the emotional modulation of C1 in high and lower level of anxiety, or of fearful ecological stimuli reported that the onset of this component is in such cases shifted even earlier, possibly due to the adaptive significance of these stimuli (West et al., 2011; Weymar, Keil, & Hamm, 2014).

These findings suggest that plasticity of the visual cortex and its neural connectivity act to optimize early perception of specific features indicative of emotional relevance (Stolarova et al., 2006) and make it an interesting target in PTSD patients considering their hypersensitivity to threat.

1.7 Hypotheses

This dissertation aimed at investigating the role of cued and contextual fear- and anxiety-related mechanisms (underlying maladaptive learning in the development and maintenance of anxiety disorders) in association with resting state connectivity in healthy individuals and encoding mechanisms at play in PTSD versus NPTSD groups.

Specifically, in the first study we tested the association between DMN connectivity and learning physiological indicators of cue and context conditioning paradigms (recording differential skin conductance responses (SCR)). We also investigated the role of trait anxiety though mean of linear regressions. Individual differences in neural networks associated with these mechanisms already at rest can elucidate

vulnerability in the same mechanisms involved in the development of an anxiety disorder.

We expected healthy individuals to show a different predisposition in their brain activity at rest depending on their conditionability and trait anxiety scores. Because of the reported neurocircuitry in anxiety disorders and conditioning and the role of the DMN, we expected:

- individuals with high differential SCR during cue conditioning to show a reduced connectivity within the DMN involving the amygdala and mPFC;
- individuals with high differential SCR during context conditioning to show a reduced connectivity within the DMN involving the hippocampus.

In the second study we examined the information processing patterns of cue and contextual features in both traumatized individuals that developed PTSD compared to those who did not develop the disorder (NPTSD). We used high-density EEG recordings with simultaneous eye tracking during free viewing of trauma-related cues embedded in naturalistic contexts. On the following day we tested retrieval and memory manipulating cue and context associations.

We expected PTSD patients to show an early encoding bias with respect to NPSD:

- at the perceptual level in the polarity/amplitude of the earliest visual component (C1), processing of the traumatic parts of the pictures (lower visual field) in PTSD would lead to a less negative C1 (ideally a polarity inversion) as indicator of which part of the visual field is processed;
- in the behavioral/attentional profile through the indices of eye tracking, we expected faster attending to traumatic cues than contexts;
- finally, this atypical encoding should account for variance in the memory impairment, with shorter attending and processing time of the contexts predicting a worse conjunctive representation of the tested material.

2 EMPIRICAL STUDIES

2.1 Study 1: Default mode network connectivity of fear- and anxiety-related cue and context conditioning.[1]

[1] **Publication:** Zidda, F., Andoh, J., Pohlack, S., Winkelmann, T., Dinu-Biringer, R., Cavalli, J., Ruttorf, M., Nees, F., Flor, H. (2016). Default mode network connectivity of fear- and anxiety-related cue and context conditioning. *NeuroImage*. 165, 190-199.
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Abstract

Classical fear conditioning is an important mechanism to adequately respond and adapt to environmental threats and has been related to the development of fear and anxiety. Both cue and context conditioning have been studied but little is known about their relation to relevant resting state networks. The default mode network (DMN) has been reported to be involved in affective learning and described as facilitating a state of readiness in responding to environmental changes.

We examined resting state brain connectivity patterns of the default mode network (DMN) in 119 healthy volunteers. Specifically, we carried out correlation analyses between the DMN and skin conductance responses (SCRs) as well as arousal, valence and contingency ratings during learning. In addition, we examined the role of trait anxiety. Two different DMN patterns were identified in which stronger connectivity was linked to lower differential SCRs during fear and anxiety learning. One was related to cue conditioning and involved the amygdala and the medial prefrontal cortex, and one was associated with context conditioning and included the hippocampal formation and sensorimotor areas. These results were replicated in an independent sample. Functional connectivity of the DMN with these key regions at rest was also predictive of trait anxiety but this association could not be replicated in the second sample.

We showed that DMN connectivity is differently associated with cued versus contextual learning mechanisms. Uncovering individual differences in baseline network connectivity of the DMN with these key regions might lead to a better understanding of fear and anxiety. Such findings could indeed help to identify vulnerability factors linked to network alterations at rest with dysregulation of learning processes involved in the pathophysiology of stress and anxiety disorders.

Keywords:

Highlights

- Default mode network (DMN) connectivity linked to fear and anxiety learning
- DMN functional connectivity (FC) with amygdala relates to cue conditioning
- FC between DMN and hippocampus associates with context conditioning
- FC in amygdala and hippocampus with DMN is predictive of trait anxiety
- Important implications for mechanisms involved in stress and anxiety disorders

Introduction

Cued and contextual conditioning paradigms have been used as experimental models for anxiety disorders (Glotzbach-Schoon et al.,2013; Grillon, 2002b; Indovina et al., 2011). Aversive classical conditioning is a well-established laboratory procedure in which emotionally neutral stimuli that occur in connection with harmful or otherwise aversive events acquire the capacity to elicit defensive responses (Fanselow and LeDoux, 1999). In particular, cued conditioning might model aspects of phobic fear because it involves the learning of an association between a discrete stimulus (cue) and a highly predictable danger (the aversive event or unconditioned stimulus (US)). In contrast, context conditioning involves the association between a diffuse and not easily discriminable surrounding, an internal or external context, with an unpredictable danger (aversive event delivered with variable onsets) and has thus been related to sustained anxiety (Grillon, 2002a). The neural correlates of fear learning have been well established with a pivotal role of the amygdala and the insula in the acquisition and expression of conditioned fear as well as the modulatory role of the medial prefrontal cortex (mPFC) and the dorsal anterior cingulate cortex (dACC) on these limbic regions (Kumar et al., 2013; Sehlmeyer et al., 2009; Shankman et al., 2014; Shin and Liberzon, 2010). In addition, the hippocampal formation has been established as a core region in contextual conditioning (Acheson et al., 2012; Rudy, 2009; Rudy et al., 2002). Dissociable roles for hippocampus and amygdala were also described in structural magnetic resonance imaging (MRI) studies. Increased amygdalar volume in particular was associated with higher skin conductance responses (SCRs) during cued fear acquisition (Cacciaglia et al., 2015; Winkelmann et al., 2016) and increased hippocampal volume was linked to a greater ability in discriminating contexts and context conditioning (Pohlack et al., 2012a).

However, it still remains unclear how and if individual differences of regional brain activation of these key regions at rest and in a network perspective are associated with learning performance and if they may act through these aversive learning mechanisms to confer vulnerability to anxiety- and stress-related disorders. A previous study showed that resting state functional connectivity (rs-FC) of the amygdala (with mPFC and ACC) was altered in a cue conditioning paradigm (Schultz et al., 2012). The authors found an increased connectivity between the superior frontal gyrus and the amygdala following conditioning, possibly as a consequence of

the memory strength of newly acquired material. Recently, resting state connectivity between the amygdala and ventromedial prefrontal cortex (vmPFC), after fear reminder exposure, was suggested to be a predictor of the subsequent extinction effect (Feng et al., 2016). Motzkin et al. showed enhanced rs-FC connectivity between the amygdala and a region in the right anterolateral temporal cortex in patients with vmPFC lesions. The authors interpreted this finding as directly relevant for psychopathology, delineating a detailed relationship between mPFC and amygdala, loss or reduction of functionality of the first would result in more activity in the second (Motzkin et al., 2015). Recently, the default mode network (DMN) was shown to be relevant for affective and cue safety learning (Fullana et al., 2016). The DMN participates in internal modes of cognition (Buckner et al., 2008), is thought to facilitate a state of readiness in responding to environmental changes (Kluetsch et al., 2012) and has also been linked to certain aspects of social cognition (Mars et al., 2012). Marstaller et al. (2017) described the DMN as necessary for conceptualizing safe memories and interpreted it as a mind-wandering state possible when individuals consider themselves to be safe (Marstaller et al., 2017). The authors reported that the DMN was anti-correlated with a fear-processing network. These studies, however, all used discrete cues and/or predictive stimuli in their experimental paradigms but so far contextual and cued learning mechanisms have not been differentiated with respect to relevant resting state networks. It is still unclear if changes in the functional connectivity of the DMN are differently related to fear and anxiety learning and how this relates to trait anxiety measures.

In the present study we tested the link between rs-FC in the DMN and cue as well as context conditioning as important mechanisms of fear- and anxiety-related processes. We combined rs-fMRI assessments with two subsequent conditioning sessions, using differential skin conductance scores of cue and context conditioning as indices of different types of associative learning.

Our hypotheses state that increased functional connectivity of the DMN (1) with the amygdala and frontal control regions would be associated with a decrease in the magnitude of cue aversive learning, and (2) that another DMN connectivity pattern including the hippocampal formation, would negatively correlate with the strength of contextual conditioning indices.

Materials and methods

Participants

One-hundred-twenty-two healthy adults (35 females; mean age 21.77, s.d. 2.95, range 18–39 years) participated in the study. They were recruited in schools for rescue workers in the context of a longitudinal study on predictors of posttraumatic stress disorder (PTSD). The sample overlaps with that of previous studies (Pohlack et al., 2012b, 2015; Winkelmann et al., 2016). Exclusion criteria included mental disorders such as major depressive disorder, current or chronic substance abuse, schizophrenia or any personality disorder, as assessed with the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders-IV (Wittchen et al., 1997). The trait scale of the German version of the State-Trait-Anxiety Inventory (STAI) (Laux et al., 1981) was also administered (STAI scores, mean 34.77 s.d. 8,22, range 23–56). Three persons had to be excluded from data analysis due to technical issues during the acquisition of rs-fMRI data, leading to 119 participants.
The Ethical Review Board of the Medical Faculty Mannheim, Heidelberg University, approved the study and written informed consent was obtained from all participants.

Experimental design

The participants were instructed to rest quietly without sleeping during a resting state fMRI measurement for 5.32 min (for details on the acquisition parameters see section below). Subsequently, SCR measurements were carried out during cue and context differential aversive conditioning paradigms. The order of presentation for cue and context conditioning was counterbalanced across subjects. We used a well-established fear conditioning procedures consisting of four phases - habituation, early and late acquisition (i.e. ACQ1-2), and extinction - for both cue and context conditioning (Cacciaglia et al., 2014; Lang et al., 2009; Pohlack et al., 2015) (see Fig. 1A and B). A painful electric stimulus on the right thumb of each participant using a cupric electrode connected to an electric device (Digitimer, DS7A, Welwyn GardenCity, UK) served as unconditioned stimulus (US). Intensities of stimulation were determined for each participant using an individually pre-determined threshold as follows: increasingly intense stimuli were administered (50-ms bursts, 12 Hz) starting with a mild stimulus until each participant indicated it as “painful” (pain threshold) and then further until the pain became unbearable (pain tolerance). This

procedure was repeated three times, and the values of the last two trials were averaged. The chosen stimulus intensity was defined at 80% between pain threshold and pain tolerance such that the sensation was painful but tolerable. The participants were then asked to rate the intensity and unpleasantness of the US on two Likert scales ranging from 0 (not painful or unpleasant) to 10 (extremely painful or unpleasant). The stimuli had to be rated at least as seven on both scales to be administered during the experimental session and if not the intensity was increased until this level was reached.

For cue conditioning, two geometric figures (a blue square and a yellow rhombus) served as conditioned stimulus (CS). During habituation, 10 CS followed by the US (CS+) and 10 CS never followed by the US (CS–) were presented for 6 s; 4 US were presented for 2.9 s in random order. The acquisition and extinction phases comprised 18 presentations of each CS-type (CS+ (CS paired with the US) and CS- (never paired with the US)). During acquisition, the CS+ was coupled with the US in 50% of the cases (called CS + paired) (starting 3.1 s after cue onset and terminating with the CS) in the other 50% the CS+ was presented without the US (called CS + unpaired) (Fig. 1A).

In the context condition, we used two colors, which were filling the entire visual field. To give the participants a stronger feeling of context, both CSs were blended into each other and were reaching the full color spectrum only after a fading phase (see Fig. 1B). This temporal component is considered an essential part of conceptualizing a context because it allows the viewer, even while using a simple color, to experience a complete meaningful percept (Maren et al., 2013). In the habituation phase, the US (2.9 s) was delivered 10 times during the interstimulus interval (4–12 s); in early and late acquisition, the US was paired to 50% of the CS+. To enhance unpredictability the onset of the US was randomly assigned over the time course of the CS+ (3–8 s after CS+ onset) (Grillon et al., 2006; Pohlack et al., 2015). The CS- was never accompanied by the US (safe condition), neither in cue nor in context conditioning. During extinction, no US was presented.

Skin conductance response (SCR)

During each conditioning phase, we recorded SCRs using a BrainAmp ExG MR device in combination with a GSR MR module (Brain Products, Gilching, Germany) at

a sampling rate of 16 Hz. A constant current of 0.5 V passed through 13 mm Ag/AgCl electrodes placed on the thenar and hypothenar eminence of the participants' left hand. An electrolyte gel consisting of 0.5% saline in a neutral base (Brain Products, Gilching, Germany) was placed in each electrode cup prior to electrode attachment. The recording procedure followed previously published guidelines (Boucsein et al., 2012).

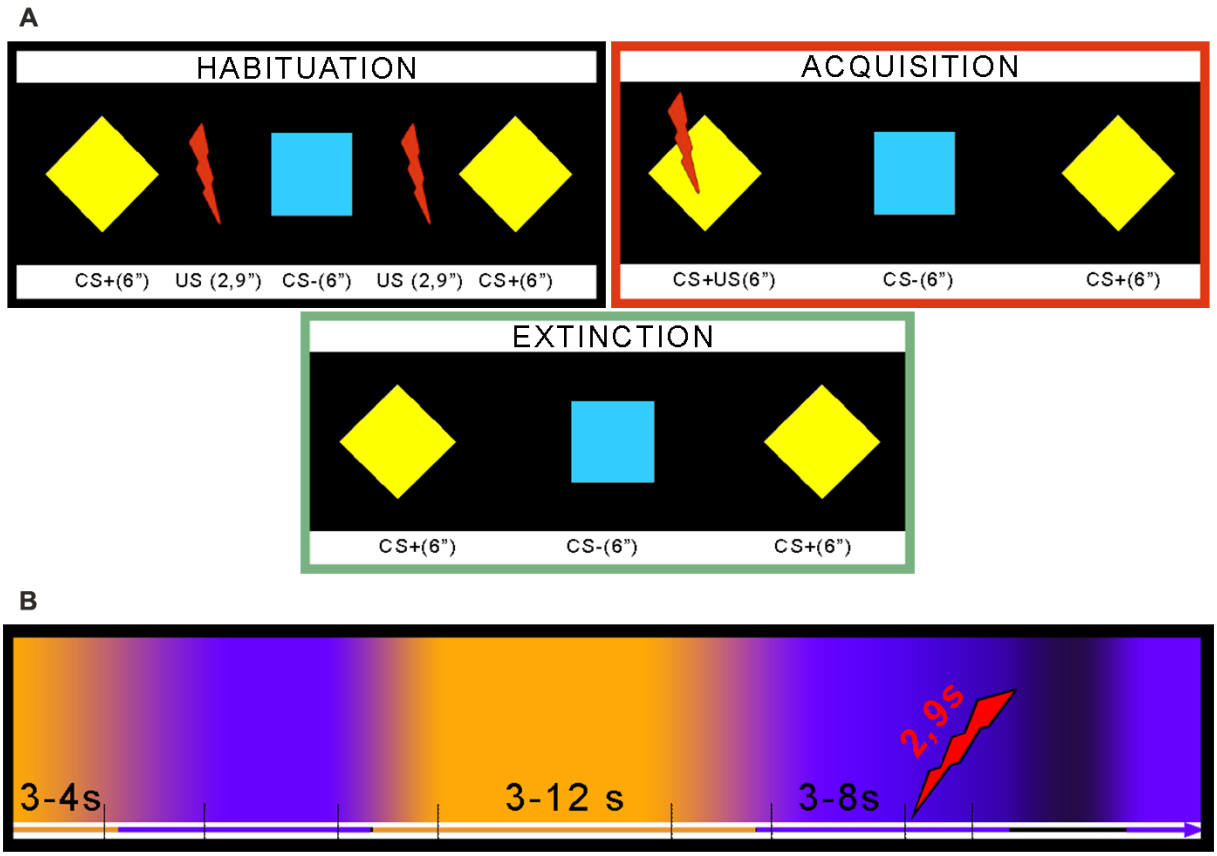

Fig. 1. Aversive conditioning paradigms: (**A**) Cued conditioning paradigm consisting of habituation, early (ACQ1) and late acquisition (ACQ2), and extinction phases. CS±: conditioned stimulus; US: unconditioned stimulus. Two different colors (yellow and blue) served as CS±. (**B**) Context conditioning paradigm. Two different colors (orange and lilac) served as CS±. Contexts were blended into each other before they reach their maximum spectrum. CS ± presentation varied between 3 and 12 s. US onset was unpredictable, i.e. varied between 3 and 8 s after full spectrum was reached and lasted 2.9 s. During habituation and extinction phases, the two colors were always separated by a black screen.

Skin conductance responses (SCRs) were analyzed using the Ledalab software (Benedek and Kaernbach, 2010) and were defined as the maximum response amplitude between 1 and 9 s after CS onset with a criterion of the smallest recordable SCR set at 0.01 μS. For each individual, the amplitudes of the SCRs were averaged across trials. SCR values were normalized using a logarithmic transformation [ln (1 + SCR)]. For each subject, differential SCRs were obtained by

subtracting the SCRs elicited by the CS- from those triggered by the CS þ unpaired (CS + unpaired/CS-). Participants whose data were not usable due to artefacts were excluded (N = 41 in cue and N = 36 in context). Additionally, non-responders (14 in cue and 34 in context) in the SCR differential measures were also excluded in the correlation analyses still leading to a substantial subsample of 64 participants in cue and 49 in context.

Ratings of arousal, valence and contingency

After each of the four phases, self-reports (valence and arousal) based on the Self-Assessment Manikin (Bradley and Lang, 1994) were collected for both CSs and converted to a nine-point scale. US expectancy was rated on a visual analogue scale of 100 mm length converted to a range from 1 (very unlikely) to 9 (very likely).

Acquisition of MRI data

A high-resolution T1-weighted 3D image was acquired for each participant on a 3T MAGNETOM Trio whole body scanner (Siemens Medical Solutions Healthineers, Erlangen, Germany) with a 12-channel head coil using a magnetization prepared rapid gradient echo sequence (1 mm isotropic voxel size, TR/TE = 2300/2.98 ms, 160 slices, matrix = 256 X 256). Resting-state fMRI data were acquired using a T2*-weighted gradient-echo echo planar imaging sequence with the following parameters: 2.3 mm isotropic voxel size, 40 slices; FOV 220 mm; TR/TE = 2700/ 27 ms, lasting 5.32 min resulting in 120 acquired volumes.

Data analysis

SCRs analysis

To assess differences in the reaction to CS + unpaired/CS,

Bonferroni-corrected paired t-tests were employed for each conditioning phase. For all statistical analyses we used the Predictive Analytic Software (PASW, SPSS Inc., Chicago, IL) for windows, version 20.0.0 and Psych: R package version 3.2.0.

Analysis of functional MRI data

Whole brain analyses. Resting-state fMRI data were analyzed using Multivariate Exploratory Linear Optimized Decomposition into Independent Components

(MELODIC) independent components analysis (ICA) from the FSL software package (Beckmann et al., 2005, 2009; Beckmann and Smith, 2004). The preprocessing of fMRI data included motion correction, high-pass temporal filtering (with a cut-off of 100 s) and removal of non-brain structures from the echo planar imaging volumes using Brain Extraction Tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET). The images were subsequently smoothed with a Gaussian kernel of full-width at half-maximum of 5 mm. fMRI volumes were registered to the individual's structural scan and to MNI-152 standard space images using FMRIB's Linear Image Registration Tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT). MELODIC ICA was applied using all fMRI scans together (n= 119) to obtain robust group-ICA spatial maps. Correlation analyses were carried out between the independent component maps relevant to our hypotheses (i.e. DMN) and the average differential SCRs (CS+ unpaired/CS-), acquired during the conditioning experiment, using dual regression and 1000 permutations in randomize (FMRIB Software Library randomise v2.9) with threshold-free cluster enhancement (TFCE) and family-wise error correction (FWE) ($p < 0.05$) (Nichols and Holmes, 2002). Uncorrected exploratory thresholds ($p_{uncorrected} < 0.005$) were employed when appropriate based on a priori hypotheses and reported.

Region of interest analyses (ROIs). In order to test specifically the differential role of amygdala and hippocampus within the DMN, we additionally extracted the mean signal values representing functional connectivity coefficients from selected ROIs: 1) left and right hippocampus, dentate gyrus/cornu ammonis and subiculum; 2) left and right amygdala, centromedial and basolateral group, through the means of the Jülich histological atlas masks (Amunts et al., 2005; Eickhoff et al., 2005). The FC coefficients of the selected ROIs were then used to conduct linear regression analyses with trait anxiety. Before running the linear regression test, we assessed the linearity assumption plotting scatterplots of the mean extracted signal values in these ROIs against the sum of the STAI scores (normalized, using a natural logarithmic transformation) with a superimposed regression line. Visual inspection of these two plots indicated a linear relationship between the variables. There was homoscedasticity and normality of the residuals.

Direct comparison of correlation coefficients of amygdalar and hippocampal ROIs with conditioning scores. In order to directly test how within subject differences in the

conditioning indicators were associated specifically with hippocampus and amygdala in the same participant, we conducted additional analyses computing a direct comparison of these correlation coefficients within the same person. For this purpose we employed Pearson correlations to examine whether the degree of functional connectivity of the amygdala and the hippocampus with the DMN correlated differently with differential SCRs in cue and context conditioning, using a subset of 32 subjects, consisting of participants who successfully underwent both conditioning experiments and showed significant conditioned responses in both designs. This was done, taking also into account the dependence due to repeated measures on the same sample (t-test on correlated correlation coefficients), in order to draw direct statistical comparisons between the degree of rs-FC in certain nodes of the networks associated with cue and context conditions.

Replication of results in an independent sample

To avoid any bias due to sample selection (fire workers are suggested to be more resilient than the general population (Wagner et al., 1998)), we repeated our ROI analyses in a representative healthy population sample, which was matched for age, gender and geographical location. Forty-two healthy adults (14 females; mean age 22.93, s.d. 2.93, range 19–29 years) who met the same in- and exclusion criteria were recruited and participated in the same procedures described for the main sample. From this sample, we excluded SCR non-responders or participants whose data were not usable due to SCR artefacts (N = 12 in cue and N = 10 in context) from the correlation analyses that involved SCRs. This still resulted in a sample of N= 30 participants for cue and N= 32 for context conditioning. To extract coefficients within the DMN, and run correlations between connectivity coefficients in these regions and differential SCRs in cue and context conditioning, we used the same functional ROIs mentioned above (bilateral amygdala and hippocampus). Additionally, we computed a linear regression analysis with trait anxiety (STAI scores, mean 34.87, s.d. 7.29, range 24–55) as already described above.

Results

SCR and self-report data

Successful fear conditioning was shown by both SCRs and self-reports as shown here below.

Cue conditioning

In the habituation phase, no significant SCR differences were found between CS+ unpaired and CS-. Arousal was not significantly different between the two CSs, while the valence of the CS- was significantly higher than that of the CS+ (resp. $t116 = -3.05$; $p = 0.003$) and contingency ($t116 = -3.2$; $p = 0.001$). During both early and late acquisition differences between CS + unpaired and CS- were found to be statistically significant (ACQ1: $t63 = 8.36$; $p < 0.001$; ACQ2: $t63 = 4.99$; $p < 0.001$) for SCR. The same applied to the self-report data, such that compared with CS-, the CS+ was rated significantly more arousing (ACQ1: $t117 = 13.02$; $p < 0.001$; ACQ2: $t114 = 16.18$; $p < 0.001$), more charged on emotional valence (ACQ1: $t117 = 15.58$; $p < 0.0001$; ACQ2: $t114 = -16.94$; $p < 0.0001$) and more likely associated (contingency) to the US (ACQ1: $t117 = 23.34$; $p < 0.0001$; ACQ2 = $t114 = 34.95$; $p < 0.0001$).
During the extinction phase, no significant differences were found.

Context conditioning

In the habituation phase, no significant differences between CS+/CS in the SCRs were found neither in arousal nor in valence measures but a significant difference was found in contingency ($t114 = 2.87$; $p < 0.005$) with the mean of the CS+ higher than the CS-. During both early and late acquisition, CS+ unpaired was significantly higher than CS- (resp. $t48 = 5.90$; $p < 0.0001$; $t47 = 2.74$; $p = 0.009$). In line with these results, arousal (ACQ1: $t115 = 10.25$; $p < 0.0001$; ACQ2 = $t115 = 11.09$; $p < 0.0001$), valence (ACQ1 = $t115 = 4.68$; $p < 0.0001$; ACQ2 = $t115 = 5.08$; $p < 0.001$) and contingency (ACQ1: $t115 = 23.05$; $p < 0.0001$; ACQ2: $t115 = 31.79$; $p < 0.0001$) were significantly higher for the CS + than for the CS-. During extinction, differences between CS+ and CS- in the SCRs were not statistically different, but reached significance in all three self-report measures (arousal: $t115 = 4.91$; $p < 0.0001$; valence: $t115 = 2.15$; $p = 0.03$; contingency: $t115 = 4.77$; $p < 0.0001$).

MRI data

Identification of the DMN

Melodic estimated 25 independent components using a Laplacian approximation (http://fsl.fmrib.ox.ac.uk/fsl). Three IC maps identified as artefacts were excluded as voxels with high values within these ICs were mainly located in the cerebral spinal fluid, white matter or large vessels, leaving a total of 22 components. We identified 10 IC maps which were covering most of the explained variance and which largely conformed to expected networks identified in other studies: visual network, auditory network, DMN (Fig. 2), extrastriate/visual cortex, executive control network, right and left lateralized fronto-parietal networks, somatosensory network (Beckmann et al., 2005; Cole et al., 2010; Damoiseaux et al., 2006; Smith et al., 2013).

Whole brain correlations with SCRs and ratings

Cue conditioning. For cue conditioning, we found a negative correlation between the DMN (e.g. amygdala, mPFC, occipital cortex) and differential SCR values in ACQ1 ($p_{uncorrected} < 0.005$) (Fig. 3A; Table 1). Correlation analyses carried out between rs-IC maps and differential contingency ratings (obtained during ACQ1) revealed a significant negative correlation with the DMN, including PCC and precuneus ($p < 0.05$ corrected) (see Supplement, Fig. 1). Such correlations were not found for habituation, ACQ2 or the extinction phase.

Context conditioning. For context conditioning, we found a significant negative correlation between the DMN (bilateral hippocampi, occipital and somatosensory and motor cortices) and differential SCR values ($p < 0.05$ corrected) (Fig. 3B; Table 2), in ACQ1. In addition, we observed a significant negative correlation between the DMN (including the thalamus) and differential arousal values in extinction ($p < 0.05$ corrected).

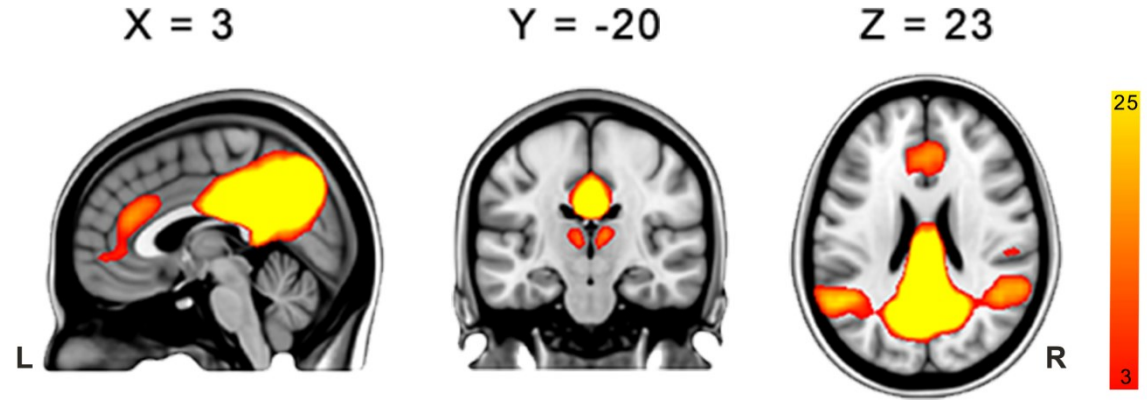

Fig. 2. Independent component (IC) maps representing the default mode network (DMN) detected by group-independent component analysis (ICA) in Melodic (FSL) (119 subjects). Statistical images are z values overlaid on a MNI152 brain template.

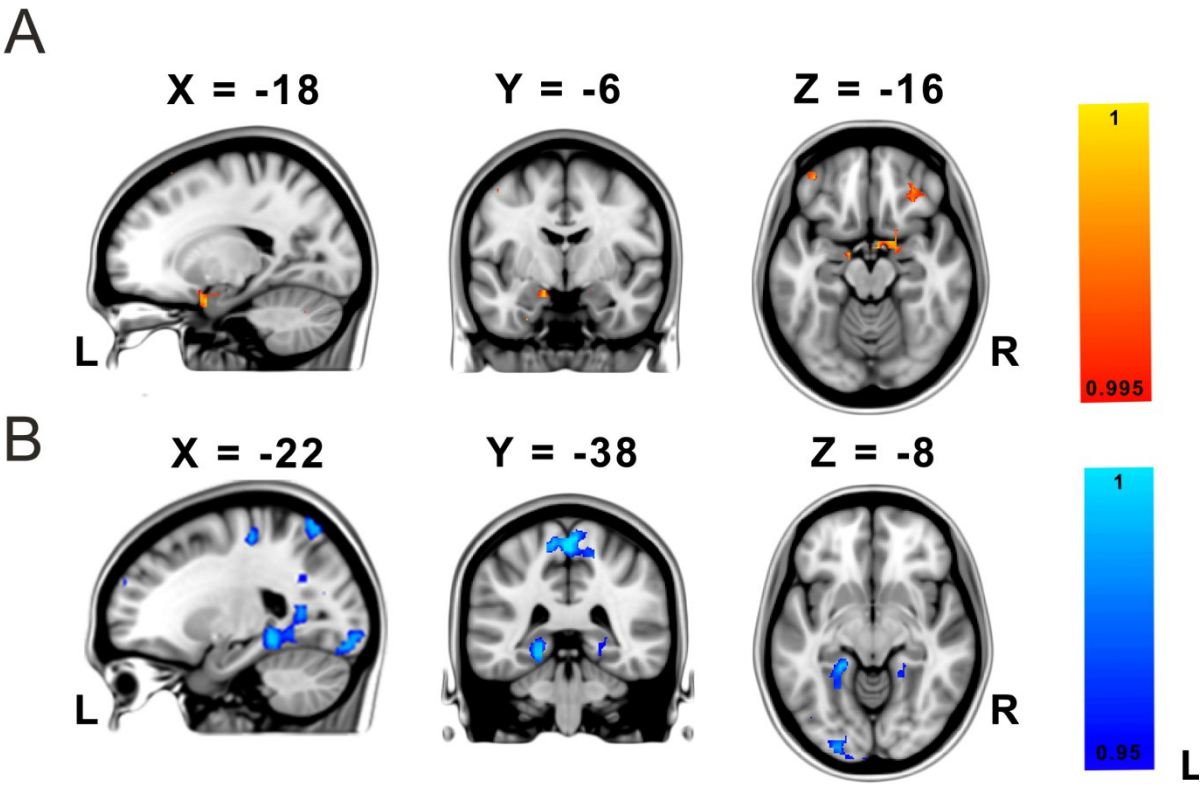

Fig. 3. Results from dual regression analysis (whole brain correlation with differential SCRs): (**A**) Negative correlations between the amygdala and the frontal cortex (within the DMN) with differential SCRs in cue conditioning: p-values uncorrected< 0.005, slices are shown at [x = -18, y = -6, z = -16; MNI152 coordinates]). (**B**) Negative correlations between the hippocampus, precuneus, visual and somatosensory cortices (within the DMN) and differential skin conductance responses (SCRs) in context conditioning (p-values corrected< 0.05; [x = -22, y = -38, z = -8]). Color bars represent signal intensity (one–P-value).

Table 1. Peak voxels (MNI coordinates), *t*-values and cluster size of brain areas (part of the DMN), which significantly correlated with differential skin conductance responses (SCRs) during the cue conditioning phase.

Brain areas (CUE)	**X (mm)**	**Y (mm)**	**Z (mm)**	***t*-values**	**Cluster size (voxels)**
Left Amygdala	-18	-6	-16	1.98	2192
Right Amygdala	18	-2	-16	3.36	
Right Frontal pole, inc. superior frontal gyrus	30	38	-16	3.40	1640
	6	22	52	3.73	
Left Frontal pole, inc. superior frontal gyrus	-38	46	-16	4.82	
	-10	30	60	3.50	
Right Visual cortex	22	-74	8	3.13	1394
Left Visual cortex	-10	-74	8	3.51	

Table 2. Peak voxels (MNI coordinates), *t*-values and cluster size of brain areas (part of the DMN), which significantly correlated with differential skin conductance responses (SCRs) during context conditioning phase.

Brain areas (CONTEXT)	**X (mm)**	**Y (mm)**	**Z (mm)**	***t*-values**	**Cluster size (voxels)**
Right hemisphere					
Primary somatosensory cortex inc.	58	-14	40	2.73	4876
	2	-38	60	4.36	
superior parietal lobule	2	-34	68	4.64	
Primary motor cortex	-42	-22	52	3.36	
Left hemisphere	-2	-38	60	3.96	
Primary somatosensory cortex inc.	-2	-34	68	4.64	
superior parietal lobule					
Primary motor cortex					
Visual cortex (Left hemisphere)	-14	-86	-16	4.51	2765
Right Hippocampus	22	-34	-8	3.30	2395
Left Hippocampus	-18	-38	-8	4.69	

Direct comparison of the correlation coefficients of amygdala ROI and hippocampus ROI with cue and context SCRs. A comparison of correlation coefficients in the amygdala in cue vs. context conditioning did not reach significance ($t(31) = 0.53$). However, the correlation between the functional connectivity coefficient in the hippocampus and differential SCRs was significantly stronger in context vs. cue ($t(31) = -2.45$, $p < 0.02$) conditioning.

Linear regression between functional regions of interest (ROIs) and trait anxiety

A linear regression established that the FC coefficients in the right amygdala (centromedial and basolateral group) were significantly associated with trait anxiety, (β= - 0.322; F (1, 100) = 11.55, p < 0.001; 95% confidence interval = from - 0.42 to - 0.11; Fig. 4A), accounting for 10.4% of the explained variability in trait anxiety scores (adjusted R^2 = 9.5%; d = - 0.392, medium to large size effect according to Cohen (1992)). For the right hippocampus (dentate gyrus/cornus ammonis), there was a statistically significant association with trait anxiety, (β= - 0.215; F (1, 100) = 4.83, p < 0.05; 95% confidence interval = from - 0.34 to - 0.018; Fig. 4B), accounting for 4.6% of the variation in anxiety sensitivity scores (adjusted R^2 = 3.7%; d = - 0.273, small to medium size effect).

Replication of results in an independent sample

The DMN map (Fig. 5), was identified as one of the first 10 independent components (out of 25) which were covering most of the explained variance and which largely conformed to expected networks identified in other studies (Beckmann et al., 2005; Smith et al., 2013). We found a significant negative correlation, within the DMN, for the left and right amygdala and differential SCRs during cue conditioning in ACQ1 (resp: r - 0.42, p = 0.02; 95% confidence interval = from - 0.68 to - 0.07; r= - 0.49, p = 0.006; 95% confidence interval = from - 0.72 to - 0.15) (Fig. 6A; Table 3). We also found significant negative correlation, within the DMN, with the right hippocampus and differential SCRs during context conditioning in ACQ1 (r = - 0.37, p = 0.03; 95% confidence interval = from - 0.64 to -0.01) (Fig. 6B; Table 4). A linear regression established that the FC coefficients in the left hippocampus (dentate gyrus/cornus ammonis), were significantly associated with trait anxiety, (β = 0.42; F (1, 32) = 8.46, p = 0.006; 95% confidence interval = from 0.25 to 1.38), accounting for 17.5% of the explained variability in trait anxiety scores (adjusted R^2 = 14.5%; d = 0.482, medium to large size effect according to Cohen (1992)). No statistically significant association with trait anxiety was found for the amygdala.

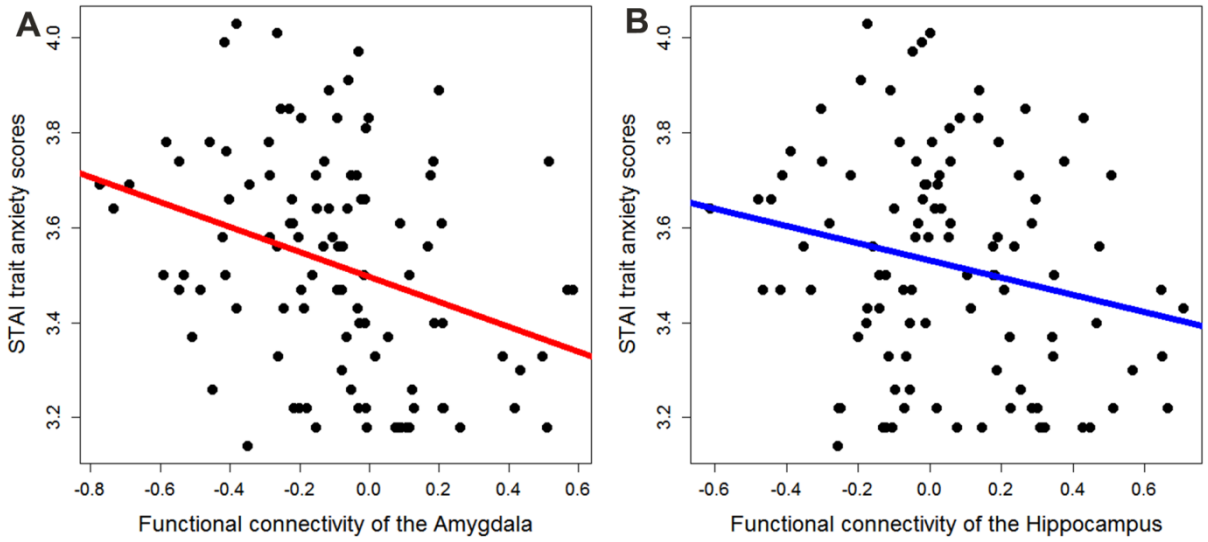

Fig. 4. Functional connectivity as a predictor of trait anxiety: (A) Linear regression between resting-state functional connectivity (rs-FC) strength of the right amygdala, basolateral group (β = - 0.322; F (1, 100) = 11.55, p < 0.001; r^2 = 0.104) and STAI trait anxiety scores normalized. High trait anxious individuals show reduced connectivity with the Amygdala. (B) Linear regression between rs-FC strength of the right hippocampus (β = - 0.215; F (1, 100) = 4.83, p < 0.05; r^2 = 0.046) and STAI trait anxiety scores normalized. High trait anxious individuals show reduced connectivity with the Hippocampus.

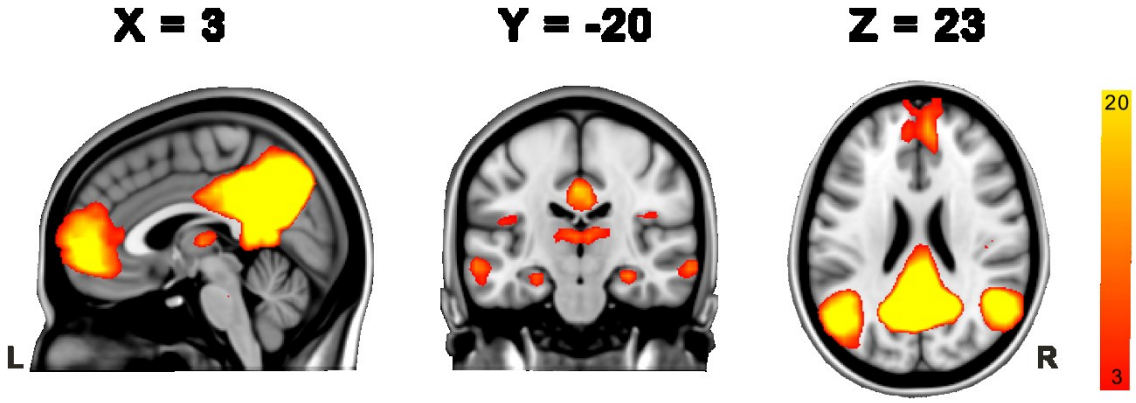

Fig. 5. Independent component (IC) maps representing the default mode network (DMN) detected by group-independent component analysis (ICA) in Melodic (FSL) (42 subjects) in the replication sample. Statistical images are z values overlaid on a MNI152 brain template.

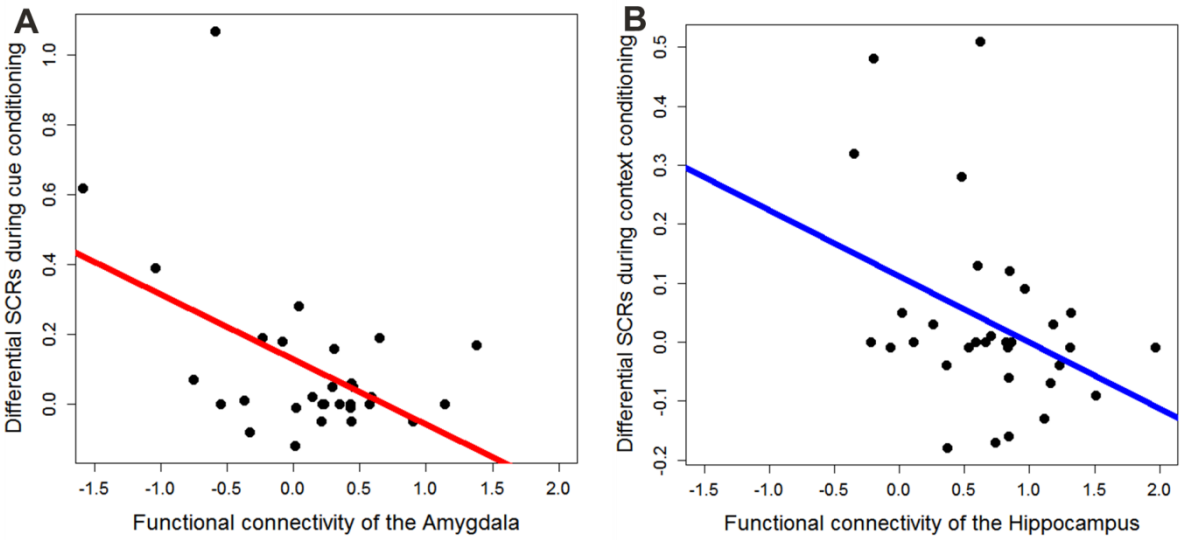

Fig. 6. Analyses in the replication sample (A) Negative correlation between resting-state functional connectivity (rsFC) strength of the right amygdala, (r = - 0.49, p = 0.006) and differential skin conductance responses (SCRs) in microsiemens (μS) during cue conditioning. (B) Negative correlations between rsFC strength of the right hippocampus (r = - 0.37, p = 0.03) and differential SCRs in μS during context conditioning.

Table 3. Peak voxels (MNI coordinates), *t*-values and cluster size of brain areas (part of the DMN), which significantly correlated with differential skin conductance responses (SCRs) during cue conditioning.

Brain areas (CUE)	**X (mm)**	**Y (mm)**	**Z (mm)**	***t*-values**	**Cluster size (voxels)**
Right Amygdala	26	-2	-8	2.89	10
Left Amygdala	-18	-2	-16	4.69	

Table 4. Peak voxels (MNI coordinates), *t*-values and cluster size of hippocampal region of interest (part of the DMN), which significantly correlated with differential skin conductance responses (SCRs) during context conditioning.

Brain areas (CONTEXT)	**X (mm)**	**Y (mm)**	**Z (mm)**	***t*-values**	**Cluster size (voxels)**
Right Hippocampus	22	-30	0	3.10	35

Discussion

We investigated the association between the strength of functional connectivity in the DMN and two central associative learning mechanisms – cue and context conditioning. Different brain patterns at rest, one including the amygdala and the other one including the hippocampal formation, were found to be differentially associated with cue and context conditioning. In particular, we showed that increases in connectivity within the DMN, with prefrontal and limbic regions (amygdala) were negatively associated with cue conditioning scores while connectivity between sensory-motor regions and the hippocampus were negatively correlating with context conditioning scores. These two experimental models of associative aversive learning can be seen as the two sides, phasic/discrete and sustained/continuous, of a continuum of phenomena involved in processing environmental threats (Grillon, 2002a). The DMN is active during internal processing and mind wandering but at the same time it supports a state of readiness to situational demands. We showed that strength in connectivity of the main reported DMN regions with key regions relevant for affective learning is associated with less capacity in distinguishing dangerous and safe stimuli, possibly easing generalization phenomena. This also suggests that reduced connectivity between the DMN and regions relevant for aversive learning at rest is a good indicator of better discriminability, as shown before, while assessing dangerous and safe cues separately (Marstaller et al., 2017). Accordingly, connectivity strength of amygdala and hippocampus with the DMN was also a good predictor of trait anxiety. While at rest and not engaged in any specific task or thought, processes strengthening fear and anxiety learning might still be at play. Importantly, our findings can help in differentiating the role that amygdala and hippocampus, key regions essential for affective processing, might play also at rest in association with respect to different aversive associative learning mechanisms in healthy controls. This distinction may be of further interest to explore in elucidating intrinsic individual network differences that may at rest represent vulnerability factors for developing a certain anxiety disorder, along the wide and heterogeneous spectrum they belong to, from specific phobias to generalized anxiety disorders.

In cue conditioning, during early acquisition, we found, according to our hypotheses, a network including the amygdala, the mPFC and occipital areas, which was negatively correlated with differential SCRs between CS+unpaired/CS-, the latter

directly representing an autonomic measure of strength of the learnt aversive association. The stronger the connectivity among these regions at rest, the less was the differentiation between safe and threat signals in the subjects while they were engaged in a fear conditioning task. People who showed this pattern at rest might be less able to discriminate a specific potential danger when encountered.

Other neuroimaging studies pointed out the relevance of functional connections between the PFC, either in its dorsolateral (Kim and Hamann, 2007; Eippert et al., 2007), or medial subdivisions (for a review see (Ochsner and Gross, 2005)), while delineating the neural circuitry underlying the regulation of conditioned fear and supporting a general role for this region in mediating inhibition of the amygdala response.

In context conditioning during early acquisition, as expected, the hippocampus was involved in the association within the DMN and conditionability SCR indices. The stronger the connectivity of the hippocampus and sensory-motor areas with the DMN, the less was the capability of the subjects in distinguishing between safe and dangerous contexts. This is in accordance with other resting state studies in PTSD, where altered hippocampal connections with frontal, temporal and parietal brain areas have been associated with symptom severity (Dunkley et al., 2014; Spielberg et al., 2015). Interestingly, increased resting state connectivity of the DMN with the amygdala and the hippocampus may also negatively and separately predict trait anxiety, strengthening the notion that they represent different aspects of fear and anxiety learning. Individuals high in trait anxiety showed reduced connectivity within the DMN with amygdala and hippocampus, connectivity with these regions is known to be relevant not only for aversive learning but also for anxiety psychopathology (for a review see (Lissek, 2012)). This is also in line with previous fMRI literature on conditioning and anxiety in healthy individuals (Indovina et al., 2011), using task-based analyses.

The negative association between trait anxiety and functional connectivity of the amygdala within the DMN was found in previous studies (Kim et al., 2011). Our association between amygdala functional connectivity and trait anxiety could not be replicated in our independent sample. This might be related to the smaller sample size or the different recruitment strategy. However, other studies also found not entirely consistent associations. For example, Kim et al. (2011), reported a significant

amygdala-mPFC association with state anxiety measures and a more controversial one with trait anxiety. They also found a dissociation between connectivity of the amygdala with the dorsomedial or ventromedial PFC with one showing a positive and the other a negative correlation with anxiety. Similarly, another study investigating insular-amygdalar connectivity reported state and trait anxiety as being differentially linked to dynamic functional and more static structural neural aspects (Baur et al., 2013). Future studies need to differentiate the specific pathways of amygdala connectivity that might be necessary to fully highlight the important role this region plays as part of different fear- and anxiety-related networks and associated anxiety measures.

Whereas we observed a negative association between trait anxiety and the functional connectivity of the right hippocampus in the discovery sample, we found a positive association between trait anxiety and the connectivity of the left hippocampus in the replication sample. This finding, while supporting the association between the hippocampal formation and trait anxiety in healthy individuals, leads to a more cautious interpretation of our results and raises interesting questions regarding the functional lateralization of the hippocampal formation. Recent studies already started to investigate this issue also at the level of task independent functional connectivity showing that the left hippocampus is part of a fronto-limbic network and that the right hippocampus is instead involved in a larger integrated network of areas that includes the right insula, the right caudate, the thalamus and bilateral lentiform nuclei (Robinson et al., 2016). Moreover, other studies supported the hypothesis of a hemispheric specialization of the hippocampal formation associating verbal memory with the left and spatial memory with the right hippocampus (Ezzati et al., 2016; Ushakov et al., 2016). The dissociation found in our study might reflects these different aspects, as individuals high in trait anxiety might be more prone to sequential processing due to higher connectivity with the left hippocampus while at the same time reduced connectivity with right hippocampus might be related to worse spatial processing. Both might lead to differences in handling uncertainty and unpredictability. The differential correlations in our samples might also be related to the different sample sizes and recruitment strategies. Further investigation is needed to clarify these aspects of network lateralization with respect to fear and anxiety learning.

Taken together our results suggest that a high non task-related engagement may hinder task performance, maybe due to the intrinsic processing already present at rest (de Voogd et al., 2017; von Rhein et al., 2017). This strengthens the proposition that the development of phasic fear and tonic anxiety may be modeled by experimental paradigms involving cued and contextual fear conditioning (Duval et al., 2015; Grillon, 2002b, 2008; Indovina et al., 2011). This is also in line with previous structural studies in which a dissociable role of amygdala and hippocampus for cue and context conditioning was described (Cacciaglia et al., 2014). We suggest that people who have a higher connectivity between the hippocampus and sensory-motor areas at rest are less able to deal with sustained anxiety and unpredictability (anticipatory) when exposed to threat even if they are able to acquire discrete fear memories. This was shown from the inverse directionality of our correlation between hippocampus and cue and context learning measures. In line with previous studies (for review (Duval et al., 2015)), our results add to the differentiation of these two qualitatively distinct types of learning, produced by different learning conditions and support the notion that these mechanisms might differently relate to anxiety disorders (Mineka and Oehlberg, 2008; Nees et al., 2015).

The predictive value of indices of the strength of conditionability has been successfully related to the likelihood of developing mood and anxiety disorders (Indovina et al., 2011), and was often directly correlated with the amount of symptoms in clinical samples (Steiger et al., 2015). Here we clarified that different patterns of activity of the DMN are associated with different emotional learning and modulatory processes in humans. Resting state networks are associated with many known brain functions including sensory, cognitive or reward processes (Beckmann et al., 2005; Schmidt et al., 2015; Smith et al., 2009). An increase or decrease in connectivity in brain networks at rest or during disengagement from tasks was already shown to predict reactivity during specific tasks, as described in many studies (Feng et al., 2013; Schultz et al., 2012), and in line with our interpretation.

For the ratings, we found a significant negative correlation between PCC and precuneus and differential US expectancy values, such that higher connectivity in these regions related to lower awareness in discriminating CSþ and CS-. Interestingly, the PCC and precuneus have been shown to be part of the so-called DMN (Beckmann et al., 2005), and have been associated with the processing of and creation of a representation of the environment (Gusnard et al., 2001). In addition, we

found a negative correlation between the DMN (including the thalamus) and differential arousal values in extinction.

Taken together these results show the essential role of the hippocampal formation in integrating sensory inputs. Because of the unpredictability of the US, contextual fear conditioning has been described as inducing a state of chronic anticipatory anxiety (Grillon, 2008) which might be reduced when a better encoding and integration of contextual spatio-temporal inputs is achieved.

Moreover, a better understanding of the association between brain networks at rest and responses to threat exposure, cue- or context-related, may be of great importance in identifying vulnerability factors involved in the etiology and maintenance of stress and anxiety disorders such as PTSD (Flor and Nees, 2014).

Specific phobias or panic disorder could be related to phasic fear while posttraumatic stress disorder and generalized anxiety disorder to more diffuse and sustained anxiety. Studies using longitudinal designs to collect pre- and post-morbid conditioning rates among anxiety-disordered individuals (of which this study belongs) are needed in order to test the predictive value of these assumptions.

Some limitations exist in this work. In cue conditioning, during early acquisition, the association between the DMN and differential SCRs was observed at an uncorrected threshold. This outcome is comparable with a previous study investigating resting state metabolism in association with autonomic fear responses during fear acquisition (Linnman et al., 2012). Our results are also in line with previous literature on task-based fMRI stating that the amygdala, mPFC and orbitofrontal cortex have a central influence and are closely related to the control and expression of SCRs (Cheng et al., 2007; Linnman et al., 2012).

The participants in this study were predominantly male. An unpaired t-test analysis on the connectivity maps showed no significant differences for the regions of interest. We attempted to further control for these effects by repeating the analyses and removing variances that could be explained by gender using general linear models. These yielded no significant differences in our regions of interest. Nevertheless, we believe that future gender balanced studies will better resolve potential gender related differences.

Finally, although our sample was part of a longitudinal study on predictors of developing PTSD, our participants were healthy at the date of the measurement and we cannot derive direct conclusions with respect to clinical populations. Further

research and longitudinal assessments are needed to understand to what extent the DMN characteristics we observed are vulnerability factors or consequences of the conditioning and anxiety measures. Such information could help to understand the development of anxiety disorders.

Conclusion

We showed that, while DMN connectivity with the amygdala and the mPFC appears to be associated with the strength of learning of discrete cues, mediating phasic fear learning and an immediate response system, DMN connectivity at rest with the hippocampal formation together with sensory-motor areas may be more involved in contextual learning mechanisms, related to sustained states of anxiety. Moreover, DMN functional connectivity with both amygdala and hippocampus was separately predictive of trait anxiety scores. Thus, a better understanding of these dysregulation mechanisms and how they interact in the development and maintenance of fear and anxiety might provide important insights not only on the pathophysiology of these disorders (Indovina et al., 2011; Schiller et al., 2010) but also into designing more successful treatment strategies. Resting state networks could be considered useful biomarkers of the association between brain activity patterns and psychophysiological reactivity and in a longitudinal perspective might be predictors of developing stress and anxiety disorders.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2017.10.024.

Zidda et al.

Default mode network connectivity of fear- and anxiety-related cue and context conditioning.

Supplementary material

1. Results

1. 1 Whole brain correlations with SCRs and ratings

Cue conditioning:

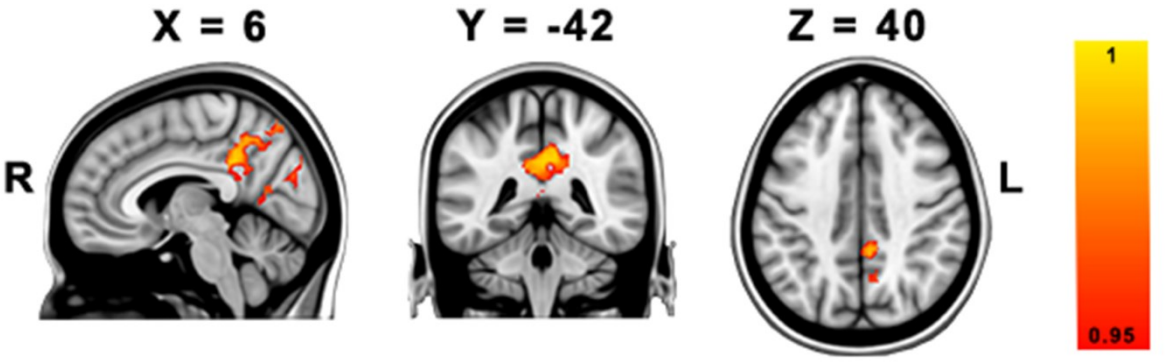

Figure 1. Results from dual regression analysis (whole brain correlation with differential contingency ratings): negative correlations between the posterior parietal cortex and the precuneous (within the DMN) and differential contingency ratings in cue conditioning: *p*-values corrected< 0.05, slices are shown at [x= 6, y= -42, z= 40; MNI152 coordinates]) Color bars represent signal intensity (one–P-value).

Context conditioning:

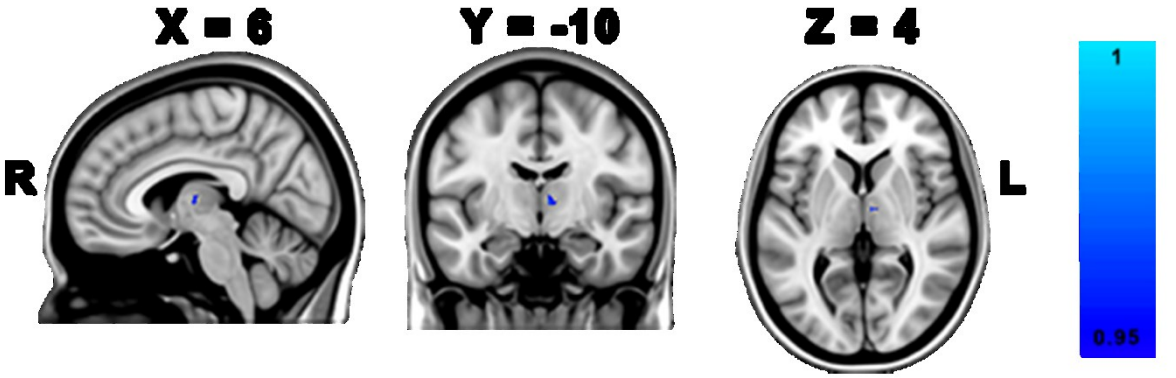

Figure 2. Results from dual regression analysis (whole brain correlation with differential arousal ratings): negative correlations between the thalamus (within the DMN) and differential arousal ratings in context conditioning (*p*-values corrected< 0.05; [x= 6, y= -10, z= 4]). Color bars represent signal intensity (one–P-value).

1. 2 Region of interest analysis in the replication sample

Linear regression analysis between the left hippocampus and trait anxiety:

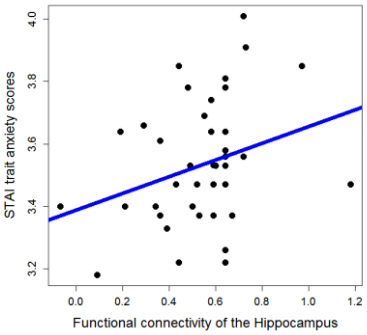

Figure 3. Linear regression between rs-FC strength of the left hippocampus (β = .42; F(1, 32) = 8.46, p = .006) and normalized STAI trait anxiety-scores.

2.2 Study 2: Early atypical encoding of traumatic material in post-traumatic stress disorder and its relation to memory impairments: an ERP-eye-tracker (preliminary title).[2]

[2] **Publication:** Zidda, F., Steiger, F., Winkelmann, T., Ruttorf, M., Andoh, J., Nees, F. and Flor, H.: Early atypical encoding of traumatic material in post-traumatic stress disorder and its relation to memory impairments: an ERP-eye-tracker study. Manuscript in preparation for submission.

Abstract

Objective: Posttraumatic stress disorder (PTSD) is characterized by an exaggerated response to trauma-relevant cues and an impaired processing of contexts. However, it is not clear if this dysfunction is related to memory processes or if the encoding of cues and contexts is already impaired.

Method: We examined encoding and retrieval of trauma-related cues and neutral contexts in patients with PTSD and traumatized controls without PTSD (NPTSD) using simultaneous high-density electroencephalography and eye-tracking. After encoding on day 1, retrieval for known and unknown cue-context associations was assessed. In order to control the trauma-specificity of these effects we used trauma-unrelated cues and neutral contexts as a control condition.

Results: Our analyses revealed an early difference in the morphology of the scalp event-related potentials (ERPs) of the earliest visual deflection (C1) to trauma cues in PTSD compared to NPTSD. Moreover, PTSD but not NPTSD looked significantly faster at the trauma cues than at the contexts as indicated by the time to first fixation of the eye-tracker data. The PTSD group also performed significantly worse than the NPTSD in retrieving cue/context associations. Memory performance was significantly predicted by the ERPs and eye-tracking data related to the processing of contexts. This effect was not found for neutral cues.

Conclusions: We showed that the encoding of cues and contexts contributes significantly to the impairment in cue- and context-related memories in PTSD patients. Thus, treatments aiming at improving contextual associations need to take into account both the encoding and the formation of associations about contextual information.

Key Words

Posttraumatic stress disorder, attention, eye-tracker, perception, memory, affective processing, ERP.

INTRODUCTION

Posttraumatic stress disorder (PTSD) develops as a response to a traumatic event and is characterized by 'hyper'-arousal, avoidance, intrusive thoughts, nightmares and memories related to the traumatic experience and a cluster of symptoms regarding negative alterations in cognitions and mood.

Importantly, it has been proposed that an autobiographical memory disturbance exists towards specific trauma aspects occurring at the expense of the contexts, not allowing persons to realize that a such trauma reminders in a safe context are no longer a threat, causing PTSD patients to never feel safe (Ehlers & Clark, 2000). More specifically, a "dual representation" model of PTSD has been proposed (Brewin, Dalgleish, & Joseph, 1996) and recently updated (Brewin, Gregory, Lipton, & Burgess, 2010) according to which, an imbalance or even a dissociation between sensorial emotionally charged (S-rep) and contextual representation (C-rep) of the information is responsible for the mnemonic sequelae (intrusions, flashbacks etc.) of the disorder.

The C-rep, that would be mainly encoded in a view-point independent (allocentric) and retrieved in a viewpoint-dependent (egocentric) perspective, is considered to be poorly encoded in individuals that develop PTSD or at least poorly associated with the related S-reps.

Other authors, in the same direction, described the existence of elemental and conjunctive representations referring to the main salient events and backgrounds/contexts of the encoded scene (Rudy, Huff, & Matus-Amat, 2004; Rudy & O'Reilly, 2001). Wessa and Flor (2002) theorized that individuals who develop PTSD might have impaired contextual processing and (Acheson, Gresack, & Risbrough, 2012) suggested that processing in PTSD might depend mostly on an elemental representation strategy probably due to impaired hippocampal processing that weakens the conjunctive one. Support came also from neurobiological studies showing that the first item-emotion binding process (elemental representation) works through upregulation of the amygdala whist the second item-context binding process (associative representation) is supported by hippocampal downregulation (Bisby, Horner, Horlyck, & Burgess, 2016). This hippocampal impairment would also explain why these patients cannot correctly differentiate dangerous and safe contexts (Rudy, 2009).

Consequently, while these aspects have been extensively investigated in memory tasks, also with EEG studies (Tsivilis, Otten, & Rugg, 2001), early perceptual and attentional processes that could account for this mnemonic bias have not been thoroughly investigated, in PTSD and in light of these influential elemental/conjunctive theories.

S-rep, unitary and elemental representations are individually encoded perceptions in the different sensorial modalities (such as tactile, visual, odor, spatial or temporal stimuli) selected as salient (emotionally charged) (Brewin, 2014), while conjunctive representation would refer to an integrated perception of the different elements associated together and with the environment in a more abstract unstructured form (Acheson et al., 2012; Rudy et al., 2004).

Perceptual and attentional top-down modulation has been shown at very early stages of visual processing involving the very first hundreds milliseconds (ms) of the processing stream (Kelly, Gomez-Ramirez, & Foxe, 2008; Rauss, Pourtois, Vuilleumier, & Schwartz, 2009). Indeed, effects of affective material have been shown already in the amplitudes and polarity of the C1, the first identified visual deflection, and in its sources (Keil et al., 2007; Stolarova, Keil, & Moratti, 2006). A distinct characteristic of C1 is its polarity reversal when stimuli are presented in different parts of the visual field (e.g. upper versus lower or different hemi-fields) (Clark, Fan, & Hillyard, 1994; Jeffreys & Axford, 1972). Therefore, C1 changes would suggest not only learning-induced neural plasticity in one or more primary visual areas of V1–V3 (Zhang, Li, Song, & Yu, 2015) due to a bias towards traumatic information but would also represent an indicator of the processed part of the visual field, such as cue and context. Therefore, if changes in early perceptual modulations occur in PTSD, these might be indicated by polarity inversions of the C1.

Eye tracker studies were used to validate attentional (hypervigilance-avoidance) models in anxiety disorders (Armstrong & Olatunji, 2012; Williams, Watts, MacLeod, & Mathews, 1988) and are also suited to analyze early processing related to cues and contexts in PTSD.

We aimed at examining perceptual and attentional processes and the way they could differently interact with memory by combining eye-tracker and EEG recordings during free viewing of traumatic cues embedded in neutral contexts (suggested as a more ecological way than separating them (Williams et al., 1988). This encoding session

was followed by a memory test on the same material controlled manipulating cue/context associations.

We expected an excessive focus on trauma-related cues at the expense of the context to cause early perceptual biases in PTSD compared to NPTSD as visible in the modulation of polarity/amplitudes of the visual C1 and in eye tracking early fixation measures. Referring to the memory performance we expected the PTSD group to better retrieve pictures requiring a more elemental/unitary strategy (aka where the association between cues and contexts was kept constant) and consequently in being especially worse than NPTSD in retrieving cue-context modified associations. We finally expected a link between different perceptual and attentional strategies in PTSD possibly accounting for the memory performance. Specifically, we expected EEG amplitudes and eye-tracker contextual data to predict memory for cue-context associations while ERP and eye-tracker of cue could predict memory processes for the more elemental based pictures.

METHOD

Participants

We compared 20 PTSD patients (12 female; mean age 41.85, s.d. 8.72, range 30–55 years) and 20 trauma-exposed healthy subjects without PTSD (NPTSD: trauma control group) (13 female; mean age 44.40, s.d. 12.58, range 19–62 years). The PTSD patients were recruited via the outpatient clinic of the Central Institute of Mental Health, Mannheim and self-referred based on press coverage and information on the web site of the institution. None of the participants was medicated and all were clinically screened by trained psychologists. The traumatized persons had experienced various types of single episode traumatic experiences (see Supplement for detailed sample information).

Procedure

The participants underwent to a two-day experimental paradigm. On day 1, imultaneous high density 128-Channel EEG and eye-tracker recordings were performed during free viewing of visual stimuli (see below and the Supplemental Methods section for details). On day 2, they participated in a memory test of the pictures seen on the previous day. After the memory test, we collected self-report data of valence, arousal, self-relevance and self-reported item/background

prevalence to assess to which they paid more attention; see below and the Supplemental Methods section where details on the ratings and further neuropsychological and psychometric assessments are given).

Simultaneous EEG and eye-tracking paradigm (day 1): stimuli

In order to create a well-tailored trauma-related picture set 30 traumatic cues per trauma type were embedded in neutral contexts (details in the Supplement). The presentation of every image lasted 6 seconds. The inter-trial (ITI) interval was varying from 2 to 4 seconds.

Memory paradigm (day 2): stimuli

On the second day, the subjects performed a memory test including five picture categories composed of 24 slides each (see below) (identical – new/new – new/old – old/new – old/old). Stimulus size was 1024 X 768 pixels.

The pictures were created manipulating the presence of old/new cues and contexts and their association in order to assess the different impact of the cue/context manipulation on memory retention. The identical category simply included pictures which were exactly the same as the day before; the new-new category referred to pictures not seen on the previous day; new-old and old-new categories included an old cue inserted in a new background and a new cue inserted in a previously seen background; in the old-old category, both, cue and context were already seen on the previous day but rearranged in a different pair (i.e., the cue was in a different context and the context was paired with a different cue).

Every image was shown for 6 seconds, followed by a forced choice with the following question “Please choose which one of the following sentences better describes this picture”. The answers included: identical (the same pair), completely new (a new pair), context old (only the background is old), cue old (only the object is old) or both old, cue and context (the rearranged pair).

Structural magnetic resonance imaging (MRI)

Based on previous findings on hippocampal volume in PTSD (Logue et al., 2018) and work on the role of hippocampal and amygdala volume in cue and context learning (Cacciaglia, Pohlack, Flor, & Nees, 2015; Maren et al., 2013; Pohlack et al., 2012),

amygdala and hippocampal volumes were assessed in a subgroup of 17 participants (9 PTSD, 8 NPTSD) who were willing to participate in a separate structural scan session and related to the picture processing and retrieval (see Supplement for details).

Data analysis

EEG

The EEG data were analyzed off-line with EEGLAB (Delorme & Makeig, 2004) running under Matlab 8.2 (The Mathworks) (see Supplement). Independent component source locations were estimated by creating an equivalent current dipole model for each component with the DIPFIT 2.2 (EEGLAB plug-in using Fieldtrip toolbox functions, (Oostenvelt, 2003)) that estimates dipole location by applying inverse source modeling methods to a standard boundary element head model using the Montreal Neurological Institute (MNI) template. Only components with scalp maps with <15% residual variance from the best fitting forward model scalp projection were considered for further analysis.

The scalp channel data were computed using conventional trial averaging procedures within the STUDY structure in EEGLAB. Stimulus-locked ERPs were computed for each subject and channel followed by grand-average channel ERPs for each group. In order to specifically assess early visual activity, peak-to-baseline mean values were extracted from parieto-occipital and occipital posterior channels and grouped in two hemispheric clusters (E63,E64,E65,E66,E68,E69,E70,E73,E74; for the left and E82,O2,E84,E89,E90,E94,E95, E99 for the right hemisphere), which are all surrounding and approximating Oz and averaged according to the latency of interest (□ 20 - 60ms) (Braeutigam, Bailey, & Swithenby, 2001; Foxe & Simpson, 2002; Weymar, Keil, & Hamm, 2014). A mixed ANOVA was used with hemisphere (left, right) as within-subject factor and group (PTSD, NPTSD) as between factor in order to test for differences in the C1 mean amplitudes.

EYE-TRACKING

A mixed ANOVA was applied to the eye-tracking total time to first fixation data (TTtFF; see Supplement for more details) with cue/context values as within-subject factor and group as between-subject factor.

MEMORY

Singles values for each of the five memory categories (identical, new/new, new/old, old/new and old/old) were summed up and then averaged, in order to have a single value per category and subject. Moreover, following our theoretical assumptions regarding memory for items and memory for context/item associations, we computed a confirmatory factor analysis on the five mentioned subcategories which showed that two main factors were separately accounting for the variance present in the data. Based on these results, we combined the 5 categories into 2 more global categories: the first one (called "Item" = identical + newnew) represented the memory for pictures in which the association between cue and context was kept constant and thus, could rely on the processing of the main element without explicit association with the context; the second one (called "Association" = new/old + old/new + old/old) represented categories where the correct association between the cue and context was needed to retrieve the correct response. This allowed to more clearly differentiate between the weight of cue/context in a more elemental versus a more conjunctive associative representation.

MRI

We extracted the volume of subcortical brain structures using the Freesurfer 6.0 image analysis suite (Dale, Fischl, & Sereno, 1999) (see Supplement for details). Independent sample t-tests two-tailed with $p < 0.05$ were used. One-tailed Pearson correlations with the memory performance (*item* and *association*) were also tested.

Multiple hierarchical regressions

In order to assess the effect of the encoding, as recorded via EEG and eye-tracking, on the memory performance on the second day, we employed two multiple hierarchical regressions. In one regression model, we used the memory scores for the category association as dependent variable and in the second model the memory scores for the item category. Independent variables included the eye-tracker TTtFF values for cue and context and the C1 ERP amplitudes. These variables were initially entered alone and then in blocks in order to highlight the respective proportion of change in the explained variance of the dependent variable.

Outliers which had studentized residual values above 2.8 SD (N = 3; one in the memory category association, two in the TTtFF of cue and context), were replaced by

the mean. Logarithmic transformations and range corrections were used when necessary to achieve normality. All statistical analyses were performed in IBM SPSS Statistics for Windows version 20.0 the statistical significance level was set to $p < .05$.

RESULTS

Encoding of combined cues and contexts ERPs

In the ERPs, we identified a very early C1 deflection in the trauma category with an onset peaking at ~40 ms, maximal over posterior parieto-occipital and occipital sites. The main effect of group showed that there was a statistically significant difference in the mean of the C1 amplitude between the PTSD and NPTSD groups F(1, 30) = 4,939, p < .05, partial η2 = .141. The early C1 was statistically significantly different (more positive) in the PTSD compared to the NPTSD group (negative) for both the left and right clusters (Fig. 1ABCD). There was no statistically significant interaction between laterality (left, right) and group (PTSD, NPTSD), nor a laterality main effect (resp: F(1, 30) = 0,119, p = .732, partial η2 = .004; F(1, 30) = 0,254, p = .732, partial η2 = .042).

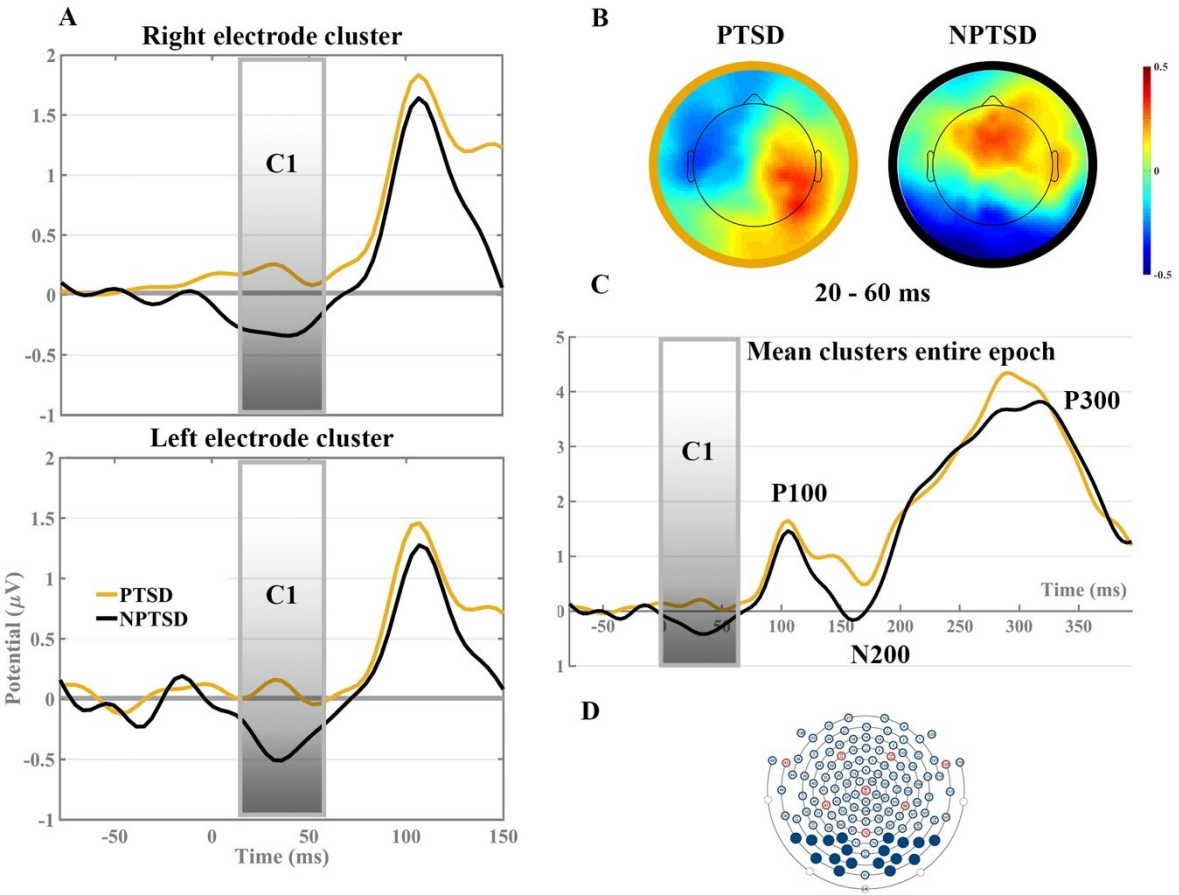

Fig. 1: (A) Grand average event-related potentials (ERPs) for left and right parieto-occipital clusters, highlighted in grey is the time window of the C1 that was significantly different between the post traumatic stress disorder (PTSD) and the

traumatized control group (NPTSD). (B) Scalp maps at specified latencies in the PTSD (orange) and NPTSD (black) groups. (C) Mean waveform of the two clusters showing the entire epoch and the identified visual components. (D) Layout of the electrode array, in blue the electrodes used for statistical analyses with midline electrodes separating right and left clusters.

The independent components were grouped into several clusters on the basis of dipole location, power, average ERP in the 20-60 ms window of interest, mean Event Related Spectral Perturbation (ERSP) and Inter-Trial phase coherence as shown in other studies (Milne, Scope, Pascalis, Buckley, & Makeig, 2009).
Six clusters of components explained most of the variance in our ERP epoch following stimulus onset (see Fig.2). Two clusters had dipoles that were primarily located in the visual cortex. The other clusters were located laterally and medially subcortically in line with limbic anterior (ACC), posterior cingulate (PCC), and subcortical (Thalamus and Hippocampus) regions, (see Table 1). Mean dipole locations and dipole clusters are shown in Fig.4. The approximated estimated Talairach coordinates, as defined in the Yale BioImage Suite software website (http://www.bioimagesuite.org), and the nearest grey matter of the mean equivalent current dipole of each cluster are presented in Table 1 comparable to those from previous studies (Milne et al., 2009; Rissling et al., 2014).

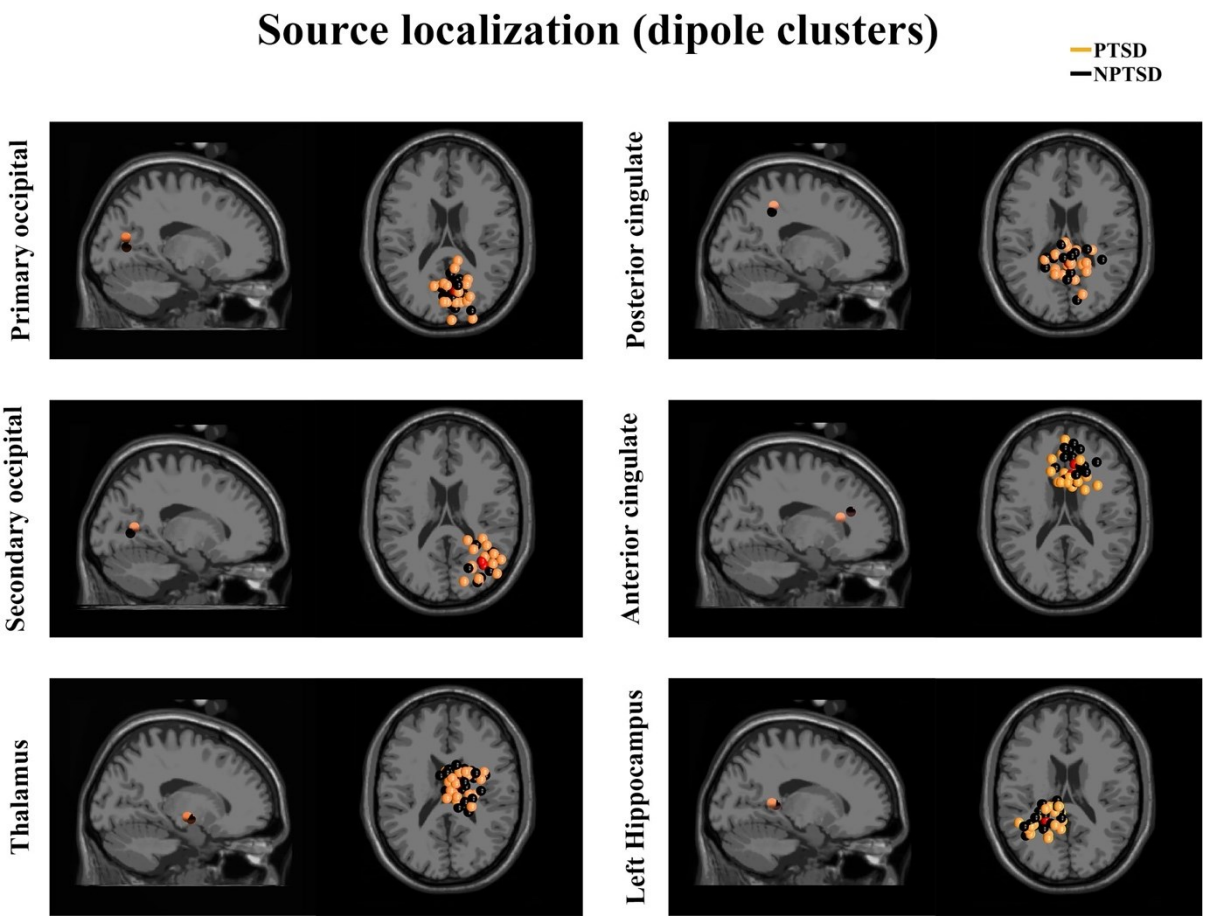

Fig. 2: Equivalent dipole localization plots showing the centroid of (left) and the source cluster (right) of each independent component (IC) in the Montreal Neurological Institute (MNI) template brain.

Table 1: Talaiarach coordinates and nearest grey matter to the average dipole location of each of the four clusters of independent components.

Cluster number	Tailarach Coordinates X	Y	Z	Lobe	Nearest Grey Matter
5	7	-67	14	R-mid Occipital	Primary visual cortex
10	34	-61	6	R Occipital	Associative cortex
8	-22	-41	8	L Temporal	Hippocampus
6	13	-9	-5	Subcortical	Thalamus
3	7	26	15	R Limbic	Anterior cingulate
17	3	-40	43	R-mid Limbic	Posterior cingulate

Eye-tracking results

There was a statistically significant interaction between the TTtFF to traumatic cue/neutral context and group, $F(1,35) = 5.121$, $p < .05$, partial $\eta 2 = .128$ (Fig. 3A). The PTSD group was looking slower at the context compared to the NPTSD group.

Memory results

We found a significant memory per group interaction, $F(1, 36) = 4.956$, $p < .05$, partial $\eta 2 = .121$ (Fig. 3B). Compared to the NPTSD group, the PTSD group was worse at retrieving pictures of the *association* category than the ones of the *item* category.

Structural subcortical MRI results in a selected sample

The PTSD group showed significantly smaller hippocampal volumes ($t(15)= -2.330$; $p< 0.05$) but not smaller amygdalar volume ($t(15)= -1.153$; ns.) compared to NPTSD group. We found a significant negative correlation between hippocampal volume and memory for the *item* category ($r= -.44$; $p< .05$).

Hierarchical regression results

Memory for the category *association:*

The full model (model 3) with encoding measures, C1 mean ERP peaks (left and right), cued and contextual eye-tracking TTtFF, statistically significantly predicted the

mean memory *association* scores (model 3). R^2 = .551, F(4, 27) = 7.054, p < .005; adjusted R^2 = .473. The addition time to first contextual but not cue fixation (model 2) to the ERP C1 data led to statistically significant increase in the prediction of memory *association*, resulting in a ΔR^2 of .196, F(3, 27) = 9.811, p < .0005 (see Table 2).

Memory for the category *complex item:*

No model was able to significantly predict the performance in this memory category.

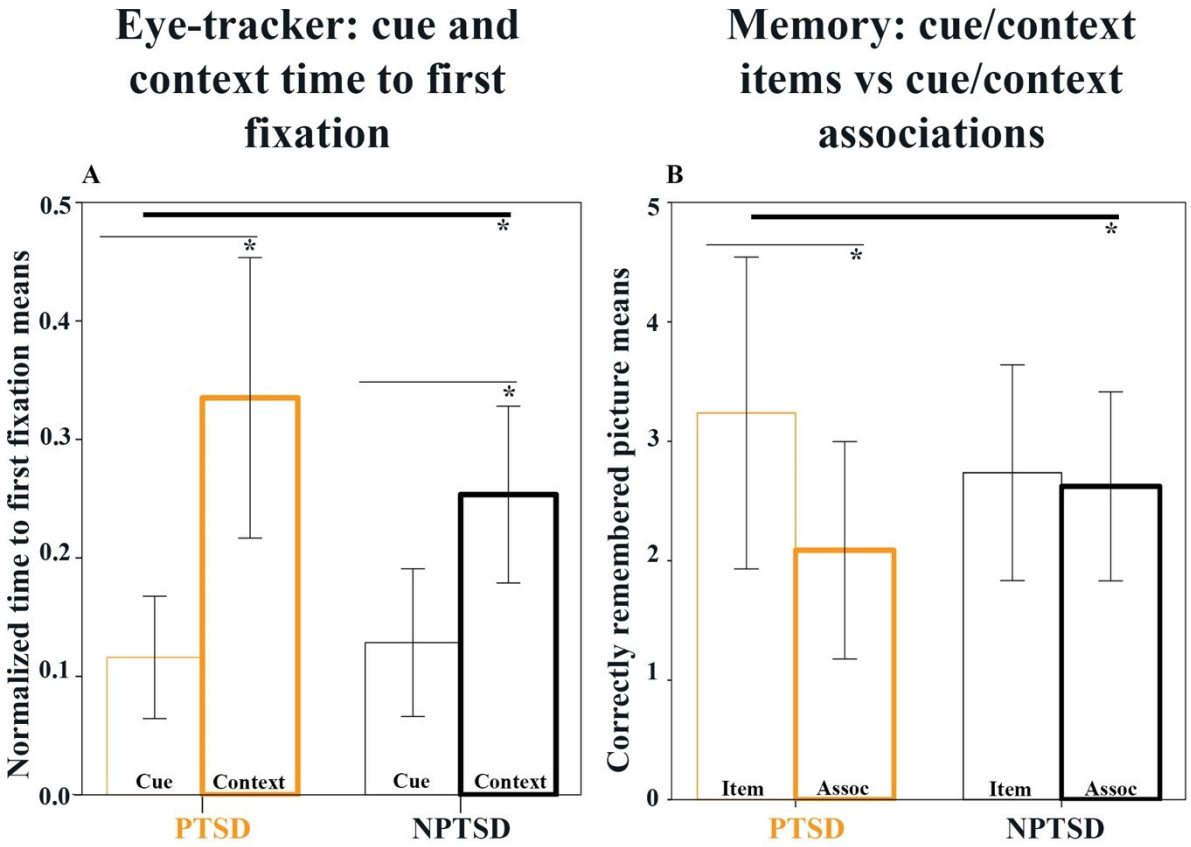

Fig. 3: (A) Mean eye-tracking time to first fixation. PTSD patients look earlier at the cue and significantly later at the context compared with the NPTSD group (B) PTSD patients perform significantly worse in correctly retrieving cue/context modified associations compared to cue/context unmodified items.

Table 2: Hierarchical regression between perceptual and attentional measures and memory performance of cue/context associations (* = p< .05; **= p<.005; ***= p<.0005).

Criterion Variable	**Memory Association category**				
Model	**N° predictors**	**R^2**	**p<**	**Change in R^2**	**ΔR^2 p<**
1 C1 (right/left)	**2**	**0.355**	**0.005****		**-**
2 C1 (right/left) Eye-tracker context	**3**	**0.551**	**0.0005*****	**0.196**	**0.005****
3 C1 (right/left) Eye-tracker context Eye-tracker cue	**4**	**0.551**	**0.005****	**0.003**	**0.954**

Control condition results

In the ERP data, it was not possible to reliably identify the early C1 deflection in the neutral non-traumatic category. There was no statistically significant interaction for neutral cues/neutral context and group ($F(1,35) = 1.135$, n.s., partial $\eta 2 = .031$) either in the eye-tracker data nor there for neutral cues/neutral context and group ($F(1,36) = .256$, n.s., partial $\eta 2 = .007$) in the memory performance.

DISCUSSION

We investigated encoding and retrieval of competing realistic information (trauma cues embedded in neutral contexts) in PTSD compared to traumatized NPTSD controls.
Our data on the earliest visual ERP (C1), the time to first fixation and the retrieval of cue/context manipulations indicate that contextual processing is impaired in PTSD compared to NPTSD. The poorer performance for memory of cue/context associations was also predicted by poorer contextual encoding

During encoding of the pictures, PTSD patients showed a very early perceptual difference in the polarity of the first identified visual ERP deflection (C1) in comparison to traumatized controls, who did not develop PTSD. The C1 has been described with latencies ranging between 40 and 70 ms (see (Woodman, 2010) for a review) for complex stimuli. Changes in the polarity of the C1 deflection were related to upper/lower visual field sensory processing due to its retinotopic properties also in recent studies (Bayer et al., 2017; Di Russo et al., 2012). In the case of complex stimuli such as pictures, the changes in polarity could be related to the processing of different but concurrent information in the visual field competing for neural representation (West, Anderson, Ferber, & Pratt, 2011). Our results indicate that PTSD processing starts from the emotional cues in the lower part at the expense of the context in the upper and surrounding parts of the visual field, while NPTSD show the opposite effect. The amplitude of the C1 deflection has also been associated before with affective top-down modulation in aversive learning (Stolarova et al., 2006), anxiety states and emotional processing (Rossi & Pourtois, 2012), perceptual learning (Zhang et al., 2015) and reward/motivation (Bayer et al., 2017). One study in particular showed C1 emotional modulation (in the 30–60 ms window) for fearful faces while they were competing with multiple complex stimuli (showed simultaneously) with a bias for fearful faces (West et al., 2011). Another study that focused on early perceptual processing of emotionally salient material compared anxious and non-anxious individuals and also found early differences in the C1 amplitude with latencies starting at 20 ms (Weymar et al., 2014). Yoneda et al. (1995), using EEG and magnetoencephalography (MEG), observed an early, non-specific visual response at ~ 40 ms after the stimulus, likely generated in the striate

cortex (Yoneda, Sekimoto, Yumoto, & Sugishita, 1995). Another MEG study also showed that a type and task-dependent (comparison of pairs of faces instead of objects or individual faces) early latency (30-60 ms) responses was present (Braeutigam et al., 2001). Interestingly and in line with our results, only the task that required an association and use of faces versus not neutral objects led to faster and higher neural responses.

Our deflection starts earlier and peaks at around 40 ms. We believe that this might show an important characteristic of how emotionally relevant and more ecologically valid material is processed. Braeutigam et al. (2001) described the finding of an earlier onset for the C1 as consistent with suggestions of anatomical pathways between thalamic nuclei and subcortical as well as cortical locations that may be activated simultaneously with or even before striate cortex. In our source location results, we found that cortical sensory and limbic regions (occipital and cingulate regions) as well as subcortical structures (thalamus and hippocampus) explained variance in the ERP signal. The anterior and posterior cingulate cortices (ACC, PCC) together with prefrontal regions form a fronto-parietal attention network involved in amplifying relevant and suppressing irrelevant input, therefore increasing related sensory representations (Bayer et al., 2017).

The behavioral eye-tracker results follow the neural ERP results. PTSD patients looked faster at cues and significantly slower at the neutral background while the NPTSD group did not show a significant temporal difference. This suggests that even if both groups look first at cue, the difference is in the contextual processing. These results are partially in line with previous studies using eye-tracking that assessed differences between stimuli with different valence. One study that compared PTSD patients with traumatized and non-traumatized controls showed that there was a significant difference in number of first fixations towards traumatic words in the PTSD group (Felmingham, Rennie, Manor, & Bryant, 2011). Another study (Thomas, Goegan, Newman, Arndt, & Sears, 2013) showed a significant difference in the percentage of initial fixations only for trauma-related images between PTSD and HC groups, with the traumatized NPTSD group in between not significantly different to either of the two other groups. No significant differences in other valenced or neutral categories were found. Many studies have shown that PTSD is associated with a heightened vigilance and increased attention to threat-related information, collectively

referred to as a threat-related attentional bias (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Cisler & Koster, 2010; Felmingham et al., 2011; Thomas et al., 2013). However, not many studies have investigated the relative ratio between traumatic cue and context-related eye-tracking parameters. Our results add to this line of research reporting a cue/context imbalance as a sensitive feature in the well reported attentional bias in PTSD.

The results of the memory tests follow theoretical “dual representation” accounts (Acheson et al., 2012; Brewin et al., 2010) of intrusive memories. PTSD patients performed significantly worse in retrieving item/background correct associations (impaired conjunctive representation) than pictures in which this association was not explicitly retrieved compared to NPTSD. This strengthens the idea of a more general association bias not directed towards certain parts of the scene only and due to an incomplete contextual processing, as previously proposed (Acheson et al., 2012). A recent study that, with fMRI, investigated the neural mechanisms which contribute to complex encoding and pattern of memory interaction for emotional events showing that memory for the associations between items and between items and their context relies on mechanisms that go beyond those supporting memory for a single item but in healthy volunteers (Bisby et al., 2016).

Our results from the control condition using neutral cues show that these effects are specific for trauma-related cues.

Finally, our hierarchical regression results show which of these encoding variables are associated with the memory impairment. Only encoding measures associated with contextual attentional processing significantly predicted variation in memory performance of the association category.

In conclusion, PTSD patients showed a fast orientation toward the trauma reminder and a significantly slower orientation toward the neutral background surrounding it, while individuals who did not develop the disorder look fast at the cue but also at the contexts. This type of indicator of perceptual processes might be very useful when assessing avoidance and generalization phenomena which are important in this disorder.

Researchers have already found that PTSD affects how people attend to the world around them, both at the perceptual and attentional levels and our findings add to

these insights that the encoding bias in cue versus context can also predict the memory impairment typical of PTSD.
In a treatment perspective, we can suggest that if encoding and retrieving certain parts of the environment are associated processes, which influence each other, they can therefore be targeted and modulated together for faster therapeutic outcomes.

Limitations

This work has some limitations. C1 studies usually require specific paradigms with extensive usage of simple stimulus repetition and in different parts of the visual field, which is not easy to implement with a clinical sample and using complex stimuli but also that goes often at the cost of ecological significance. In the attempt to overcome this limitation, we combined shorter EEG recordings with simultaneous eye-tracking and usage of ad hoc constructed pictures inclusive of traumatic cues in neutral contexts. Further studies with a pure perceptual focus on traumatic cues/contexts are needed to further investigate amplitude and morphology of the earliest visual ERP in clinical populations. Also, we believe further analyses comparing traumatic cues of different emotional valence (negative, positive) again in neutral contexts can better elucidate if these mechanisms are specifically trauma-related or not.
Lastly, we believe understanding how spatial processing and especially how subcortical limbic structures contribute to explained variance in the ERP signal is worth further consideration.

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Appendix B. Supplementary data

Zidda el al. Supplemental data

METHODS

Participants

Patients with post-traumatic stress disorder (PTSD) and the trauma control group (NPTSD) did not significantly differ in age, handedness or education. PTSD patients scored significantly higher in depressive symptoms as well as anxiety levels than NPTSD, (see Table 1). All analyses were then rerun using these variables as covariates, not changing the significance level of the results. Seven PTSD patients met criteria for current major depressive episode (MDE) and seven PTSD patients met criteria for panic disorder (PD), social phobia (SP) or specific phobia. Three traumatized controls also met criteria for PD, one for SP and one for current MDE.

The German version of the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) (Wittchen, 1997) was used to assess mental disorders including PTSD. Additionally, the German version of the Clinician-Administered PTSD Scale (CAPS; (Schnyder & Moergeli, 2002)) was employed to examine the current diagnosis of PTSD. Axis II diagnoses were determined using the SCID-II (SKID-II; (Wittchen, 1997)).The German version of the Center for Epidemiological Studies Depression Scale (Hautzinger & Bailer, 1993) was used to assess comorbid depressive symptoms. The German version of the State-Trait Anxiety Inventory trait version was used in order to assess the level of anxiety (Laux, Glanzmann, Schaffner, & Spielberger, 1981). Participants of the trauma control group were only included when they had a history of a criterion A trauma for at least 3 months before participation in the study.

Depending on the trauma experienced, the participants belonged to seven trauma clusters in total: car accident (PTSD = 4; NPTSD = 6), fire (PTSD = 3; NPTSD =1), hospital (PTSD = 1; NPTSD = 2), war (PTSD = 2; NPTSD = 2), rape (PTSD = 7; NPTSD = 4), suicide (PTSD = 1; NPTSD = 3), and aggression (PTSD = 2; NPTSD = 2).

The participants reported to have slept seven or more hours. Exclusion criteria for PTSD patients were comorbid borderline personality disorder, history of schizophrenia-spectrum psychosis, bipolar type I affective disorder and current substance abuse. Further exclusion criteria for all participants were neurological

disorders, traumatic head injuries, mental retardation and lack of German language skills.

Because of technical reasons and artefacts (less than <75% good epochs) we had to exclude 4 subjects per group in the EEG data, 2 subjects in the memory and 3 in the eye-tracker-data.

Table 1: Demographic and clinical variables

	PTSD (20)	**NPTSD (20)**	**Statistic**
AGE (years ± sd, Range)	41.50 ± 8.84 (30–55)	44.35 ± 12.55 (19–62)	t(38) = .461
SEX (f/m)	11/9	13/7	$\chi 2$ =- .745
CAPS (mean ± sd)	68.05 ± 26.52	10.86 ± 11.92	t(35) = 8.37**
ADS (mean ± sd)	1.52 ± 0.35	0.63 ± 0.42	t(37) = 7.10**
STAI (mean ± sd)	57.94 ± 8.65	37.13 ± 9.59	t(37) = 9,22**

CAPS, Clinician-Administered PTSD Scale ($p < .01$ (statistically significant difference between PTSD and trauma control).

ADS-L, General Depression Scale, (P < .01 (statistically significant difference between the PTSD group and the other groups).

STAI-T, State-Trait Anxiety Inventory Trait (P < .01 (statistically significant difference between the PTSD group and the other groups). sd= standard deviation.

Stimulus design

Tailored traumatic cues were mostly taken from the internet and individually rated.

There was no significant difference in valence, arousal, personal relevance or perceived item/background prevalence of the images between the two groups (see Table 3). There was no significant difference in any of the low-level image properties between the two groups (see Table 2). The traumatic cues were inserted in different sort of contexts taken from the internet which were rated as neutral (landscapes, interiors of houses, buildings) in an independent validation study. The contexts were kept stable across the seven trauma picture categories and the cues were mainly placed in the lower part of the picture but alternated with central and upper field

positions to avoid predictability: lower part 81.46%; upper part 11.46% and central part 7.08%.

Neutral cues[3] and contexts[4] were taken from different databases, IAPS (Lang, 2008), GAPED (Dan-Glauser & Scherer, 2011) and EmoPics (Wessa et al., 2010).

EEG data acquisition

EEG was continuously recorded with a high-density array of 128 silver-silver chloride electrodes (Electrical Geodesics, Eugene, Oregon). Impedances were kept below 50 KΩ, as suggested from the manufacturer. The signal was amplified (x1000), filtered online with a band-pass of .01–100 Hz, then digitized at a sampling rate of 1 KHz. The electro-oculogram was recorded from bipolar electrode pairs located at the outer canthi and above and below the left and right eyes.

Eye-tracking data acquisition

The participants were comfortably seated in front of a table-mounted eye-tracking system which was approximately 70 cm away from participants' faces. In order to standardize the distance from the screen and to reduce head movements, which would have led to de-calibration issues, participants were asked to sit their chin in a headrest put in front of the screen. After successful calibration of the eye-tracking system to participants' eye movement patterns, participants were instructed to watch the visual presentation and try to avoid moving or blinking as much as possible. In the end of the calibration procedure, the stimulus presentation started (total duration ~ 20 minutes). Gaze position and fixations measures were recorded continuously during the entire length of the experimental task. We used an EAS binocular remote system complemented by two eye cameras and an IR light source from LC Technologies, Inc., USA (sample rate of 120 Hz, Gaze Position Accuracy <0.45 °; Spatial Resolution 0.2°).

Neutral cues numbers: i_92, i_100, i_101; IAPS: 7233, 7009, 7090, 7705, 7950, 7025, 7192, 7038, 7040, 2870, 7211, 7175, 1450, 7034, 7185; EmoPics: EmoPics 363, EmoPics 311, EmoPics 284, EmoPics 301, EmoPics 95, EmoPics 319, EmoPics 153, EmoPics 159, EmoPics 164.1,,EmoPics 178, EmoPics 158, EmoPics 162.2;

Contexts numbers: wo_02, wue_05, rau_15, tu_02, ge_19, tu_11; GAPED: N089, N093, N099, N101, N104, N105, N098, N086; IAPS: 5120, 5130, 5390, 5711, 7547.

NYAN 2.0 software from Interactive Minds Dresden (IMD) was used for both the picture presentation and for registering, recording, and analyzing participants' eye-tracker data, using the table-mounted Eyegaze Analysis System from LC Technologies Inc. On the second day images and memory responses were administered by Presentation™ software (Neurobehavioral Systems Inc., Albany, CA, USA).

Magnetic resonance imaging (MRI) data acquisition

Magnetic resonance imaging data were acquired on a 3*T* MAGNETOM Trio whole body scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a standard 12-channel head coil using a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence (TR 2300 ms, TE 2.98 ms, field of view 240 x 256 mm2, 160 sagittal slices, voxel size 1.0 x 1.0 x 1.1 mm, parallel imaging (GRAPPA) factor 2).

ERP analyses

In order to obtain a clean independent component (IC) decomposition, data were initially band-pass filtered (1 Hz - 30 Hz), resampled at 256 Hz, cleaned with ASR (Artifact Subspace Reconstruction) toolbox (Mullen et al., 2015), segmented into epochs of 3 sec (-1–2 sec around stimulus onset) and re-referenced to average reference. The remaining data were decomposed by Infomax independent component analysis (ICA) with the algorithm "runica" (Makeig, Jung, Bell, Ghahremani, & Sejnowski, 1997) as implemented in EEGLAB. The ICA matrix was then copied to the original less strongly cleaned data. This allowed obtaining at the same time a cleaned ICA decomposition while preserving the signal from possible distortion due to high frequency filtering effects. Moreover, ICA allowed retaining as much information as possible by allowing cancellation instead of rejection of artifacts from the EEG signal.

The original data were band-pass filtered (0.1 Hz - 30 Hz) using a causal finite impulse response filter with half amplitude, resampled at 250 Hz, cleaned from bad channels, segmented into epochs of 600 msec (-100–500 msec around stimulus onset) on the basis of stimulus type (picture category) and re-referenced to average reference. At this stage we copied to each subject their previously ICA computed matrix (as suggested on the developers website,

https://sccn.ucsd.edu/wiki/Makoto's_preprocessing_pipeline) calculated with 1Hz high-pass filtering (Winkler, Debener, Muller, & Tangermann, 2015).

Any components that reflected muscle activity, electrocardiogram, or eye movements, on the basis of their dipole location, spectra and scalp maps were considered artefacts and excluded from further analysis. Data were baseline corrected by subtracting the mean of the 100-ms pre-stimulus interval.

Components were grouped into several clusters with a joint distance measure, on the basis of dipole locations, power, average ERPs, mean Event Related Spectral Perturbation (ERSP) and Inter-Trial phase Coherence (ITC) measures. These data from each subject were initially decomposed by Principal Component Analysis (PCA), and the resulting component distances were clustered with a k-means algorithm (for further details of this method see (Rissling et al., 2014) .

Eye-tracker analysis

The eye movement parameters total time to first fixation (TTtFF), Total fixation count (TFC), mean fixation duration (in ms), total mean fixation duration (TMFD) and dwelling time or total gaze duration (TGD: sum of all fixation durations in ms) were sampled with the pupil center corneal reflection method and extracted after manually tracing the main object called area of interest (AOI) aka CUE, in each image. This procedure allowed us to obtain separated values for cues and contexts.

MRI data analysis

The processing briefly involved applying a Talairach transformation (Reuter, Rosas, & Fischl, 2010; Reuter, Schmansky, Rosas, & Fischl, 2012), motion correction and averaging, removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Segonne et al., 2004), segmentation of the subcortical white matter and deep gray matter volumetric structures (Fischl et al., 2002), intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction. Once the cortical models were complete, a surface inflation and registration to a spherical atlas was performed, which utilized individual cortical folding patterns to match cortical geometry across subjects. Individual volumes were adjusted for total subcortical volume.

RESULTS

Ratings of the stimuli (picture ratings)

The self-assessment manikin (SAM; (Bradley & Lang, 1994)) was employed to assess arousal and valence of the pictures, and transformed to a 9-point scale (ranging from 1 = very calm to 9 = very arousing, 1 = very pleasant to 9 = very unpleasant).This was followed by a full neuropsychological and clinical assessment (see Table 2).

Table 2: Picture ratings

	PTSD	**NPTSD**	**Statistic**
Arousal	5,41	4,74	t(36) = 1.14
Valence	6,3	6,14	t(36) = 0.476
Relevance	4,79	5,68	t(36) = 0.334
Figure Ground	3,88	4,07	t(36) = 0.168

No significant difference in image hue, saturation, brightness, red-green-blue (RGB), luminance ($p > 0.05$) across picture category was found. Regarding the traumatic category, ANOVA showed no differences in arousal, valence, figure-ground balance scores and relevance between groups (see Table 3).

Table 3

	Trauma (accident)	Trauma (aggression)	Trauma (suicide)	Trauma (rape)	Trauma (hospital)	Trauma (war)	Trauma (fire)
Hue (sd)	0,35 (0,08)	0,35 (0,09)	0,33 (0,10)	0,35 (0,10)	0,36 (0,08)	0,34 (0,10)	0,33 (0,11)
Saturation (sd)	0,28 (0,13)	0,28 (0,13)	0,29 (0,13)	0,29 (0,12)	0,28 (0,11)	0,28 (0,13)	0,29 (0,11)
Brightness (sd)	0,57 (0,09)	0,56 (0,09)	0,58 (0,09)	0,56 (0,10)	0,58 (0,09)	0,56 (0,09)	0,59 (0,09)

R (sd)	126,56 (21,73)	124,59 (19,29)	131,13 (20,83)	126,44 (21,30)	127,28 (17,56)	124,50 (21,24)	134,51 (20,69)
G (sd)	130,98 (20,96)	128,72 (19,81)	132,27 (21,38)	128,57 (21,51)	133,19 (18,70)	128,35 (19,04)	131,99 (18,26)
B (sd)	122,95 (26,38)	121,31 (26,27)	122,76 (27,78)	120,95 (27,71)	127,28 (17,56)	120,23 (25,20)	122,72 (24,96)
Luminance (sd)	128,75 (20,53)	126,64 (19,04)	130,85 (20,52)	127,07 (21,01)	130,80 (18,11)	126,28 (18,84)	131,69 (18,47)

KB= kilobytes; sd= standard deviation

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3 GENERAL DISCUSSION

3.1 Summary of the aims and main results

We conducted investigations on the role of conditioning and encoding mechanisms in the pathogenesis and pathophysiology of anxiety and stress/trauma related disorders with a special focus on PTSD.

Study 1

Cued and contextual conditionings are crucial learning mechanisms and recognized experimental models of anxiety disorders. Because cued conditioning may better model discrete phobic aspects (Indovina et al., 2011) while context conditioning better resemble aspects of sustained anxiety (Grillon, 2002a) it has been suggested that these two mechanisms might be involved in the development of different disorders along the anxiety spectrum (Grillon, 2002b, 2008).

Moreover, the DMN has been described as facilitating a state of endogenous and exogenous monitoring of stimuli, orchestrating shifts to other resting state networks (salience or attentional control) depending on necessity (Bar, 2007; Gusnard, Akbudak, Shulman, & Raichle, 2001) and possibly also an ongoing processing of past events (Miall & Robertson, 2006). Therefore, investigating neurobiological individual changes in the pattern of the DMN along with physiological indicators of these two associative learning mechanisms can be helpful in elucidating potentially pathogenic patterns associated with different anxiety disorders.

We found that individual differences in DMN network connectivity are associated with different psychophysiological patterns during fear and anxiety learning. Interestingly, the amygdala and the prefrontal cortex (PFC) are associated with cue conditioning, mediating phasic fear learning. The modulatory role of the PFC on the amygdala in conditioned fear has been already described in several other fMRI studies, either in its dorsolateral (Eippert et al., 2007; Kim & Hamann, 2007), or medial subdivisions (Ochsner & Gross, 2005). Moreover it has also been shown that increased resting state connectivity of amygdala, ACC and PFC is significantly higher in PTSD compared to NPSTD (Brown et al., 2014). In contrast, connectivity of hippocampus and sensory-motor regions at rest are associated with contextual conditioning

indicators, possibly mediating anxiety learning. Altered hippocampal connectivity with frontal, temporal and parietal control regions have been previously associated with symptom severity in PTSD (Dunkley et al., 2014; Spielberg, McGlinchey, Milberg, & Salat, 2015).The emergence of an interaction within this network with relevant node of the well-known an previously described “fear network” (Holzschneider & Mulert, 2011) opens interesting scenarios towards the role of individual differences in brain connectivity at rest, its continuously at play processes interacting with the mechanisms altered in anxiety or stress related disorders. In fact, reduced connectivity between the DMN and regions relevant for aversive learning at rest has proven to be a good discriminability index for both cues and contexts. Something similar has been shown before, for dangerous and safe cues (Marstaller, Burianova, & Reutens, 2017).

Further, both, resting state connectivity of amygdala and hippocampus show a negative association with trait anxiety in the discovery sample. This results, needs further investigation but show a certain potential. Even at rest relevant biological hubs and physiological indicators relevant for different fear learning mechanisms (cue and context conditioning) are associated with proneness to anxiety and support the idea that these mechanisms might differently relate to different anxiety disorders (Mineka & Oehlberg, 2008; Nees, Heinrich, & Flor, 2015).

Connectivity in brain networks at rest has been shown to predict reactivity during specific tasks (T. Feng, Feng, & Chen, 2013; Schultz et al., 2012) and in this perspective might become a useful tool and target for understanding resilience and vulnerability to anxiety and trauma-related disorders.

Study 2

Encoding mechanisms (responsible for extracting, selecting and organizing internal and external information) regarding the traumatic cues and its contexts, have not yet been investigated in PTSD. We also know little about the interaction of these mechanisms with the well reported dual representation memory impairment (Brewin, 1996; Brewin & Burgess, 2014) in PTSD.

EEG recordings and ERP analysis are well suited to study real-time information processing, perceptual and attentional patterns at the neural level and the C1 is the first visual component that also responds to different stimulus locations (Clark et al., 1994; Jeffreys & Axford, 1972). Furthermore, eye-tracking methodologies have been fruitfully used to highlight behavioral attention profiles towards traumatic contents in PTSD (Felmingham et al., 2011), in a more spontaneous and ecological way than requiring verbal or motor responses.

Therefore, we investigated encoding mechanisms in PTSD compared to NPTSD traumatized controls, combining eye-tracking and EEG recordings during free viewing of ecologically valid material (traumatic cues embedded in neutral ecological backgrounds). Moreover, in order to test for the specificity of this effect towards trauma-related material we also included neutral cues.

We found an early encoding bias in PTSD. Both, at the perceptual level, in the morphology of the first visual ERP component, the C1, (positive in polarity for PTSD and negative for NPTSD), and at the behavioral attentional level, in the time to first fixation biased to cues at the expense of the contexts to a higher extent in PTSD than NPTSD.

Due to its retinotopic properties, polarity inversion of the C1 has been found to reflect upper/lower visual field sensory processing (Clark et al., 1994; Jeffreys & Axford, 1972) also in recent studies (Bayer et al., 2017; Di Russo et al., 2012). This studies involved the use of simple stimuli displayed in different hemifields and/or quadrants but it has been proposed that a polarity reversal for complex stimuli with adaptive relevance would refer to the processing of competing information in the visual field with associated neural representations (West et al., 2011). Thus, while individuals with PTSD start to atypically process cues in the lower part of the picture penalize the contexts, NPTSD do the opposite and this already at sensorial stages of the information processing. Previous studies already showed top-down modulations of the C1 in aversive and perceptual learning (Stolarova et al., 2006), anxiety states and emotional processing (Rossi & Pourtois, 2012) as well as reward/motivation (Bayer et al., 2017), but not in PTSD. Importantly this perceptual biased was only present for cues of the traumatic category and not of the control neutral category. The fact that we could not identify a C1 while using neutral cues points towards a specific role of

emotionally relevant material in affecting PTSD patients at first stages of information processing (Braeutigam, Bailey, & Swithenby, 2001). Perceptual priming studies have supported the hypothesis of a perceptual advantage for trauma-related stimuli, increasing readiness towards possible trauma reminders and possibly biasing consequent behavior (Ehlers et al., 2002; Kleim et al., 2012). Our results are in line with this hypothesis, better clarifying an associated lack in processing of contextual information that might be responsible also for a previously reported lack in conceptualization (Lyttle et al., 2010). Indeed, this perceptual bias continues with a behavioral one, PTSD subjects are orienting their attention later at the contexts, and this deficit is predictive of the memory for the association between cues and contexts, while it is not for unitary representations of the same material. Again, we could not find this effect for neutral cues neither in our eye-tracking nor in the memory data. Several studies have described a threat-related attentional bias in PTSD (Bar-Haim et al., 2007; Cisler & Koster, 2010; Felmingham et al., 2011; Thomas, Goegan, Newman, Arndt, & Sears, 2013), but our results show that even though both groups look first at cue, the difference is in the contextual processing as previously suggested (Flor & Wessa, 2010; Liberzon & Abelson, 2016; Maren et al., 2013).

In line with the neuroimaging literature on PTSD, we could show the involvement of limbic cortical (ACC) and subcortical (hippocampus) regions as explaining a proportion of the variance in our ERP window.

Moreover, we also tested differences in subcortical amygdalar and hippocampal volume in a limited sub-group of our sample, and we were able to show a significant reduction of the latter in PTSD patients. The volume of the hippocampus was also significantly negatively correlated with the memory performance in the item category, which required mainly a unitary representation. Individuals with a smaller hippocampus are were able to better remember elemental information. This is in line with previous theoretical accounts that highlights a dissociation between amygdalar and hippocampal volumes in processing of cues and contexts (Cacciaglia et al., 2015).

The memory results show both enhanced unitary representations and impaired associative processing in PTSD in accordance with previously proposed theories (Acheson et al., 2012) as well as dual representation accounts (Brewin et al., 1996).

Moreover, the associative memory performance was significantly predicted from the encoding profile.

It has also been proposed that a dissociation between perceptual and episodic memory would account for flashbacks and intrusions stating that contextualization processes would alter selective attention and recoding of the sensory input, thus pointing to a more organized and integrated information representation (more easily consciously accessible and reducing involuntary intrusions) (Brewin, 2014). Although episodic memory does not seem to cause PTSD symptoms (Wessa, Jatzko, & Flor, 2006), the problems with contextual processing seem to be relevant. Altogether, our results are favoring these hypotheses suggesting a hippocampal processing impairment as responsible for the memory deficits and instigated by a strongly biased encoding strategy of the cues versus contexts.

3.2 Limitations

First study: In cue conditioning, during early acquisition, the association between the DMN and differential SCRs was observed at an uncorrected threshold, but it was part of our a priori hypotheses and supported from previous literature (Cheng, Richards, & Helmstetter, 2007; Linnman, Zeidan, Pitman, & Milad, 2012).
The participants in this study were predominantly male. We did not find significant differences in the ROIs in the connectivity maps as shown from an unpaired t-test analysis and running the same analysis with covariates in the general linear models. Nevertheless, further studies should investigate possible sex effects in DMN connectivity and conditioning.

Second study: A paradigm with shorter and multiple repetitions of the same stimuli would have been better suited for studying early perceptual ERP components but would have precluded our chance to assess effects of encoding on memory performance. Further studies with a purely perceptual focus on traumatic cues/contexts could replicate this finding more robustly. Also, we believe further analyses comparing traumatic cues of different emotional valences would reveal a trauma (or lack of) specificity bias.
Lastly, even though several studies have profited from the usage of the MNI template for source locations analyses (Milne, Scope, Pascalis, Buckley, & Makeig, 2009; Rissling et al., 2014), we suggest the use of MRI structural data for future

investigations with clearly defined anatomical hypotheses. This was not possible for the limited structural data sample of this study.

3.3 Outlook

Complementing existing cognitive and neurobiological models of PTSD with the findings of the present study, two factors clearly emerged in explaining pathogenesis, pathophysiology and even prognosis of the disorder. The first factor points towards individual differences during the encoding of the traumatic experience, that can lead to biased unstructured and poorly integrated contextual representations of the event with “hyper-represented” traumatic details. The second factor instead refers to individual differences after the traumatic experience, while encoding stimuli in the environment and monitoring for possible new threats and trauma reminders leading to an accumulation of unstructured representations, in long-term perceptual memory (Brewin, 2014), that possibly contribute to generalization phenomena.

Both, factors might rely on the well reported neurobiological alterations mostly with a focus in hippocampal volume reduction, insufficient prefrontal inhibition and a hyper-responsive amygdala.

The first study highlighted the possible importance of the predisposition of the brain networks at rest (in the DMN), different patterns could already represent a vulnerability factor in healthy controls for dually encoding and representing the information during the traumatic event.
We could speculate that individuals showing reduced connectivity with both amygdala and hippocampus already at rest would be those more likely to develop PTSD if exposed to a traumatic event, because of their tendency to make fast fear and sustained anxiety associations. This speculation is supported by our findings that reduced connectivity of amygdala and hippocampus also separately predicted trait anxiety scores, with individuals high in trait anxiety generally showing both connectivity reduction patterns already at rest.

The second study highlighted the relevance of intervening in the perceptual and attentional processes at play after the trauma in order to flexibly restructure already existing memories and future memories.
Individuals that keep not being able to attend to contextual details and to make new associations with potential trauma reminders, which ultimately affects general cognitions, thoughts and mood, could be those who develop and maintain PTSD.

A recent “working event model” proposed by Richmond and Zacks (2017) posits the hippocampus as a key structure not only in creating and storing but also updating event representations, and highlights the importance to take into account event perception and memory as accessible processes in their interaction with action control. Mental representations that are multimodal in nature and built under adaptive pressure, can be updated through segmentation, analysis of each event with its contextual features (Richmond & Zacks, 2017), enabling deeper awareness.
Such reconstruction could have the potential to break maladaptive processing and learning loops and finally predict and direct newly “constructed” adaptive behaviors.

4 SUMMARY

This dissertation aimed at investigating the role of fear learning and encoding mechanisms in the development and maintenance of anxiety and trauma-related disorders in two studies.

In study 1, we used functional resting state connectivity with skin conductance data of cued and contextual fear conditioning, well known experimental models for anxiety disorders in 119 healthy individuals.

In study 2, we combined high-density electroencephalography (EEG) and eye-tracking during free picture viewing of traumatic cues embedded in neutral contexts in 20 patients with post-traumatic stress disorder (PTSD) and 20 trauma controls who did not develop PTSD (NPTSD). A memory test of the same materials followed.

We hypothesized increased functional connectivity of the default mode network (DMN) with the amygdala and frontal control regions relevant for cued and the hippocampus relevant for contextual aversive learning and associated skin conductance responses (SCRs).

The main result of this study showed that two different DMN connectivity patterns were linked to lower differential SCRs during fear and anxiety learning. One involved the amygdala and the medial prefrontal cortex (cue), and one included the hippocampal formation and sensorimotor areas (context).

In the second study, we expected an early perceptual bias for trauma-related cues at the expense of the context in PTSD compared to NPTSD as visible in the modulation of polarity of the visual C1 and in eye tracking early fixation measures. In the memory performance, we expected the PTSD group to better retrieve pictures requiring a more elemental/unitary strategy (where the association between cues and contexts was kept constant) and consequently in being especially worse than NPTSD in retrieving cue-context modified associations.

In the EEG data we found that the PTSD but not the NPTSD group processed mainly traumatic cues at the expense of the context. This outcome was present at the very first stages of information processing as indicated by polarity changes of the event-related potential (ERP) C1. In the eye-tracker we found that, even though both groups oriented initially towards the cue, the PTSD looked significantly later at the context than the NPTSD. ERPs and times to first fixations of the eye-tracker for the

context, but not the cue, predicted significantly the following associative memory performance.

Because of the recognized clinical implications of cue and context learning mechanisms in trauma and anxiety disorders our findings of study 1 highlight the relevance of brain connectivity differences as possible biomarkers already at rest and in healthy individuals. For example, in populations with high exposure to traumatic events these biomarkers could be examined in order to promote resilience. Study 2 shows that a contextual impairment possibly related to lower hippocampal volume may underlie the encoding and memory deficits in PTSD. The memory deficits may relate to the strongly biased encoding strategy of the cues versus contexts.

In conclusion, already at rest there are different neural patterns plausibly associated with individual differences in learning about cues and contexts. In patients with PTSD the disturbed memory of cue-context relationships seems to originate already from disturbed encoding of the context already at very early perceptual stages. The hippocampus seems to be a key structure not only in creating and storing but also updating event representations based on accessible perceptual and attentional information. Interventions that aim at this encoding and memory of contextual association might improve PTSD treatments.

5 ABBREVIATIONS

ACC Anterior cingulate cortex
BOLD blood oxygen level dependent
CR Conditioned response
CS Conditioned stimulus
dACC Dorsal anterior cingulate cortex
DMN Default mode network
EEG Electroencephalography
ERP Event-related potential
fMRI Functional magnetic resonance imaging
Rs-fMRI Resting state functional magnetic resonance imaging
GAD Generalized anxiety disorder
MDE Major depression episode
NIMH National Institute of Mental Health
PD Panic disorder
PTSD Posttraumatic stress disorder
NPTSD traumatized individuals who did not develop PTSD
PFC Prefrontal cortex
mPFC Medial prefrontal cortex
PCC Posterior cingulate cortex
SCR Skin conductance responding
SP Specific phobia
STAI Stait Trait Anxiety Inventory
UR Unconditioned response
US Unconditioned stimulus
vmPFC Ventromedial prefrontal cortex

6 REFERENCES (INTRODUCTION AND GENERAL DISCUSSION)

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