CORTEX 63 (2015) 352–363

Available online at www.sciencedirect.com

ScienceDirect

Journal homepage: www.elsevier.com/locate/cortex

Research report

# Initial and sustained brain responses to contextual conditioned anxiety in humans

*Marta Andreatta* [a,*,1], *Evelyn Glotzbach-Schoon* [a,1], *Andreas Mühlberger* [a,b], *Stefan M. Schulz* [a,c], *Julian Wiemer* [a] *and Paul Pauli* [a]

[a] *Department of Psychology (Biological Psychology, Clinical Psychology, and Psychotherapy), University of Würzburg, Würzburg, Germany*
[b] *Department of Clinical Psychology and Psychotherapy, University of Regensburg, Regensburg, Germany*
[c] *Comprehensive Heart Failure Center, University of Würzburg, Germany*

ARTICLE INFO

*Article history:*
Received 10 February 2014
Reviewed 14 April 2014
Revised 11 June 2014
Accepted 16 September 2014
Action editor Ahmad Hariri
Published online 2 October 2014

*Keywords:*
Context conditioning
Initial and sustained anxiety
fMRI

ABSTRACT

Contextual fear conditioning takes place if the occurrence of threat cannot be predicted by specific cues. As a consequence the context becomes the best predictor of the threat and later induces anxiety (sustained fear response). Previous studies suggest that both the amygdala and the hippocampus are crucial for contextual fear conditioning. First, we wanted to further elucidate the neuronal correlates of long-lasting contextual threat within a highly ecologically setting created in virtual reality (VR). Second, we wanted to distinguish between initial and sustained components of the anxiety response to a threatening situation. Twenty-four participants were guided through two virtual offices for 30s each. They received unpredictable electric stimuli (unconditioned stimulus, US) in one office (anxiety context, CXT+), but never in the second office (safety context, CXT−). Successful contextual fear conditioning was indexed by higher anxiety and enhanced US-expectancy ratings for CXT+ versus CXT−. Initial neural activity was assessed by modeling the onsets of both contexts, and sustained neural activity by considering the entire context duration (contrasts: CXT+ > CXT−). Amygdala and hippocampus revealed sustained activity. Initial *and* sustained activities were found in the middle temporal gyrus, and primary motor cortex (M1). Additional initial activity was obvious in orbitofrontal (OFC), dorsomedial (dmPFC), and dorsolateral prefrontal cortex (dlPFC). These results suggest that entering a threatening context initially induces conditioned fear reactions (M1), recall of contingency awareness (dlPFC), and explicit threat appraisal (dmPFC, OFC). While remaining in the threatening context might involve anxiety-like conditioned responses (amygdala, M1) and the generation of a spatial map to predict where and when a threatening event may occur (hippocampus). We conclude that in humans initial versus sustained anxiety responses triggered by a threat associated context are associated with distinguishable brain activation patterns involving a fear network and a "contingency-cognitive" network, respectively.


* *Corresponding author*. Department of Psychology, University of Würzburg, Marcusstrasse 9-11, D-97070 Würzburg, Germany.
E-mail address: marta.andreatta@mail.uni-wuerzburg.de (M. Andreatta).
[1] These authors contributed equally.
http://dx.doi.org/10.1016/j.cortex.2014.09.014

## 1. Introduction

Despite several similarities, fear and anxiety differ in certain key dimensions (Davis, Walker, Miles, & Grillon, 2010). Fear is a phasic and specific response prompted by imminent and real threats, while anxiety is a less specific response alerting the organism towards a potential and distal threat. Fear begins and terminates rapidly, while anxiety is characterized by a long-lasting state of apprehension (sustained fear). From a clinical point of view, panic disorder (PD) or posttraumatic stress disorder (PTSD) are characterized by a sensitivity to unpredictable and uncontrollable threats resulting in enhanced anxiety in very different situations (Grillon et al., 2009). In other words, symptoms of PD and PTSD seem better modeled by sustained fear (Mineka & Oehlberg, 2008).

In the same vein, cued and contextual fear conditioning reflect the essential features of phasic and sustained fear, respectively (Davis et al., 2010). In a cue conditioning paradigm, the conditioned stimulus (CS+, e.g., a geometrical shape) reliably signals an aversive unconditioned stimulus (US, e.g., pain, Pavlov, 1927). Subsequently, individuals show fear responses to this cue, e.g., potentiated startle responses (Alvarez, Johnson, & Grillon, 2007; Andreatta, Mühlberger, Yarali, Gerber, & Pauli, 2010; Glenn, Lieberman, & Hajcak, 2012; Hamm and Weike, 2005) and amygdala activity (Andreatta et al., 2012; Büchel, Morris, Dolan, & Friston, 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; for a recent review see Mechias, Etkin, & Kalisch, 2010). In a context conditioning paradigm, the aversive USs are presented while the individual is within a specific context (CXT+ or anxiety context, e.g., a room), but such aversive USs are not time-bounded with a specific cue and the individual is unable to predict the exact delivery of the USs (Maren, Phan, & Liberzon, 2013; Rudy, 2009). Subsequently, this context elicits anxiety as reflected in startle potentiation (Grillon, Baas, Cornwell, & Johnson, 2006; Tröger, Ewald, Glotzbach, Pauli, & Mühlberger, 2012) and amygdala activation (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Lang et al., 2009; Marschner, Kalisch, Vervliet, Vansteenwegen, & Büchel, 2008; Pohlack, Nees, Ruttorf, Schad, & Flor, 2012). Importantly, the anxiety triggered by the context differs from the fear elicited by a cue. In fact, the bed nucleus of the stria terminalis (BNST, Alvarez, Chen, Bodurka, Kaplan, & Grillon, 2011) and the hippocampus (Alvarez et al., 2011; Marschner et al., 2008; Pohlack et al., 2012) have been found to be crucial brain structures involved in contextual fear learning only (for a recent review see Maren et al., 2013). Specifically, the BNST seems to mediate threat-monitoring and hyper-vigilance (Davis et al., 2010; Somerville, Whalen, & Kelley, 2010), while the hippocampus is important for both spatial and temporal mapping of events and objects within the context (Pohlack et al., 2012; Rudy, 2009). Supportively, lesions of the BNST disrupted freezing and the potentiation of the startle responses in a conditioned context in rats (Luyten, van Kuyck, Vansteenwegen, & Nuttin, 2011), and in humans the BNST was specifically active in a context where the US was unpredictable (Alvarez et al., 2011).

While most animal studies used spatial contexts, e.g., different cages, most previous human studies created contextual stimuli (CXT) by presenting long-lasting cues (Marschner et al., 2008) or by changing the light color in the experimental room (Pohlack et al., 2012). These latter CXT are defined by their temporal characteristics and do not require any spatial representation. However, in real-life a context is defined by both temporal and spatial characteristics. Furthermore, context stimuli are characterized by two kinds of representations (Maren et al., 2013; Rudy, 2009). On the one hand, organisms establish representation of the single features of the context (i.e., elemental associative representation). On the other hand, the single features are bound together in order to experience the context as a particular place or unit (i.e., hierarchical or configural representation).

We use virtual reality (VR) to create ecologically valid contexts meeting these criteria (Glotzbach-Schoon et al., 2013; Tröger et al., 2012). Participants are immersed in these contexts, which they can explore in order to form a spatial representation. In a VR paradigm it is possible to create such enriched and diverse situations in a fully controlled fashion as well as in contingency with observed human responses thus closely imitating real situations (Sanchez-Vives & Slater, 2005). In fact, participants may completely immerse in the virtual world and even forget the real environment, thus they feel present in the virtual world (defined as presence). Given a high level of subjective presence, the individual's responses in the VR are very likely comparable to real-world behavior. For this reason, VR has been proposed as an elegant and innovative tool bridging animal models to real-world human behaviors (Huff et al., 2011; Sanchez-Vives & Slater, 2005).

As mentioned above, the heterogeneous symptomatology of anxiety disorders has been modeled with cue and contextual fear. The anxiety response induced by a threating context after contextual fear conditioning very likely is characterized by both an initial (i.e., at the onset of the context, when entering) and a sustained (i.e., throughout the visit of the context) component. There are only few studies in humans which investigated the neuronal mechanisms underlying contextual fear learning (Alvarez et al., 2008; Maren et al., 2013; Marschner et al., 2008; Pohlack et al., 2012) and even less studies focused on a clear distinction between initial and sustained responses to the threatening context (Alvarez et al., 2011; Somerville et al., 2013). We assume that a better understanding of the brain areas mediating initial versus sustained anxiety induced by contexts would allow a better understanding of the mechanisms behind anxiety disorders like PD and PTSD (Mineka & Oehlberg, 2008). Furthermore, this may allow the development of more efficient therapy for these disorders (Graham & Milad, 2011). Hence, the first goal of this study was to disentangle the initial and the sustained components of the anxiety response to a threatening context by using an ecological valid technique like VR.

Psychological therapies for anxiety disorders focus on exposing the patient to the feared object or the feared situation. Exposure treatment, which can be realized effectively both in-vivo and VR (e.g., Mühlberger, Weik, Pauli, & Wiedemann, 2006; Shiban, Pauli, & Mühlberger, 2013), allows learning a new association between the fear triggering events and safety. This new learning is called extinction learning and has been defined as the decrease of defensive conditioned

response to a stimulus (CS+) previously associated with an aversive event (US) (Herry et al., 2010; Milad & Quirk, 2012). These newly formed association between the CS+ and no US inhibits the formerly learned fear response to a stimulus (Milad & Quirk, 2012). Thus, extinction learning does not erase the fear memory but the two memories coexist. Such coexistence has been confirmed by the recovery of conditioned fear responses by means of change of context (renewal), passage of time (spontaneous recovery), and re-exposure to the aversive US (reinstatement) (Bouton, 2004). The context works as occasion setter, which gates either fear or extinction memories (Bouton, 2004; Milad & Quirk, 2012). In line with this view, Kalisch et al. (2006) found an association between contextual information and the recall of extinction or fear memory. According to their findings, the hippocampus processes the context in which the CS+ is presented, and depending on the information activated in the hippocampus individuals respond to the CS+ with greater amygdala (fear memory) or vmPFC (extinction memory) activation. Such coexistence of fear and extinction memories can explain relapses after exposure therapies in anxiety patients (Graham & Milad, 2011). Similar to contextual conditioning, contextual fear extinction is considerably less investigated in animals (Maren et al., 2013; Milad & Quirk, 2012) and even less in humans (Lang et al., 2009; Pohlack et al., 2012). Hence, the second goal of this study was to investigate the mechanisms underlying extinction learning of contextual fear in humans.

To test these assumptions, we developed a VR paradigm where participants learned to associate one virtual office (CXT+, i.e., the anxiety context) with the occurrence of aversive events (US, i.e., painful electric shock), but not another virtual office (CXT−, i.e., the safety context). Importantly, participants could not reliably predict when the delivery of the US occurred during the CXT+ presentation. In a following extinction phase, participants learned that the CXT+ was no longer associated with the aversive US.

During the contextual conditioning phase, we hypothesized greater amygdala as well as hippocampus activation to the CXT+ as compared to CXT− indicating conditioned anxiety (Lang et al., 2009; Marschner et al., 2008; Pohlack et al., 2012). In order to disentangle initial and sustained components of the conditioned anxiety response, we evaluated the onset of the context, i.e., when participants entered the context, to examine the initial anxiety response, and the enduring context to examine the sustained anxiety response. We expected amygdala activation especially when entering (i.e., onset) the CXT+ in line with the results of Alvarez et al. (2011), while we expected hippocampus activation throughout the visit of the CXT+, both as compared to the CXT−. During the extinction phase, we hypothesized greater activation of the vmPFC to the CXT+ as compared to CXT−.

## 2. Material and methods

### 2.1. Participants

A total of 30 participants were recruited via advertisements on a public website. All participants signed the informed consent before the experiment, which was approved by the Ethics Committee of the Medical Faculty of the Julius-Maximilians University of Würzburg. All participants were right-handed and free of any neurological or psychiatric disorders indicated by self-report. Participants received 25 € for their participation.

For the analysis we considered 24 participants (13 females; mean age = 23.17 years; SD = 3.67; range = 19–34 years). Three participants were excluded because they interrupted the recording, one because the US electrode detached, and two because they were unaware of contingencies (see 2.3 Procedure).

### 2.2. Stimulus material

The aversive *unconditioned stimulus* (US) consisted of an electric pulse stimulation at 50 Hz lasting 200 msec. The electric shocks were applied on the left calf via surface bar electrodes consisting of two durable gold-plated stainless-steel disks with 9 mm diameter, 30 mm spacing, and an impedance of 5 Ω. The electric stimulation was generated by a constant-current stimulator (Digitmer DS7A, Digitimer Ltd., Welwyn Garden City, UK) supplying a maximum of 400 V and 10 mA. The intensity of the electric shock was adjusted individually. The pain threshold procedure consisted of two ascending and descending series. Starting from 0 mA, shock intensity was increased or decreased in .5 mA steps (Andreatta et al., 2010). Participants evaluated US intensities on a scale ranging from 0 (*no pain at all*) to 10 (*unbearable pain*). The individual US intensity for the experiment consisted of the mean value of the four intensities (two from the ascending series, and two from the descending series) rated as "*just noticeable pain*" (i.e., 4) and was increased by 30% to avoid habituation resulting in a mean intensity of 2.86 (*SD* = 1.78 mA; mean of the subjective painfulness before conditioning started: 5.13, *SD* = 1.23).

The VR environments were created with Source Engine (Valve Corporation, Bellevue, USA) and consisted of two office rooms which served as CXT. The office rooms had the same square footage, but differed in their layout (wider *vs* longer), floor color (green *vs* red), window view (village *vs* city), and the arrangement of the furniture (see Glotzbach-Schoon et al., 2013; Tröger et al., 2012). The floor colors were exchanged between the two office rooms and counter balanced across participants. The two rooms were separated by a corridor which had a gray floor color and except for a few pictures on the wall it was empty. In one office room (CXT+ or anxiety context), participants could receive 0 to 2 USs in an unpredictable manner (see 2.3 Procedure); while in the other office room (CXT− or safety context) no US was delivered. The assignment of office rooms to conditions (CXT+ *vs* CXT−) and the order of the office rooms were counter balanced across participants. The software CyberSession (version 5.3.38), developed in house, served as controller for the VR. The virtual environments were displayed by a Z800 3D Visor head-mounted display (HMD; eMagin, Hopewell Junction, USA) outside the scanner in our VR-laboratory and via MRI-compatible goggles (VisuaStim; Magnetic Resonance Technologies, Northridge, CA, USA) inside the scanner.

### 2.3. Procedure

The experiment took place on two consecutive days. On Day 1 participants were familiarized with the VR and its equipment in our VR-lab outside the scanner. On Day 2 they underwent contextual fear conditioning and extinction in the fMRI scanner.

*Day 1*: After the arrival in the laboratory, participants filled out a socio-demographic questionnaire, the German version of the Positive And Negative Affect Schedule (PANAS, Krohne, Egloff, Kohmann, & Tausch, 1996) and both the trait and the state part of the German version of the State-Trait Anxiety Inventory (STAI, Laux, Glanzmann, Schaffner, & Spielberger, 1981). Afterwards, participants were situated into the VR by means of the HMD and underwent two phases. During Phase 1, participants could freely navigate in the VR with a joystick. They were instructed to explore the two offices for 2 min each. This was realized to allow the formation of a spatial map of the contexts (O'Keefe & Dostrovsky, 1971). During Phase 2, participants were guided along a pre-recorded pathway through each office once for 30 sec each. No aversive US was delivered. During both phases participant's field of view of the virtual environments was constantly adapted according to their head orientation by means of a Patriot electromagnetic tracking device (Polhemus Corp., Colchester, USA).

Finally, participants rated the anxiety level they had experienced in the two virtual rooms on a visual analog scale (VAS) ranging from 0 (*no anxiety at all*) to 100 (very *high anxiety*), and filled out the trait part of the German STAI (Laux et al., 1981) ($M = 39.42$, $SD = 9.84$), German version of the Behavioral Inhibition and Behavioral Activation Scale (BIS-BAS, Strobel, Beauducel, Debener, & Brocke, 2001) (BIS: $M = 2.91$, $SD = .56$; BAS: $M = 3.10$, $SD = .38$), and the German version of the Anxiety Sensitivity Index (ASI, Alpers and Pauli, 2001) ($M = 17.25$, $SD = 7.84$). Finally, the Igroup Presence Questionnaire (IPQ, Schubert, Friedmann, & Regenbrecht, 2001) was applied to verify the individuals' capacity to feel present in the virtual environment ($M = 5.37$, $SD = 10.20$).

*Day 2*: Twenty-four hours later, participants were invited for fMRI scanning. Before starting the experiment as well as after the experiment, participants completed the state part of the STAI and the PANAS. The pain threshold assessment was conducted in the scanner as descried above. Afterwards, participants first underwent an anatomical scan in absence of any stimulation; second we applied a GRE field mapping in order to verify the homogeneity of the magnetic field, and then the experiment started. The experiment consisted of two phases: the conditioning phase and the extinction phase. After each phase, participants reported their anxiety level on the same VAS as described on Day 1 as well as their contingency awareness on a VAS ranging from 0 (*no US expectation*) to 100 (US *surely expected*). Additionally, participants were asked to recall in which office the US had been delivered. The contingency awareness indicates the verbal ability of participants to indicate CXT-US associations. Two participants were not able to recall the correct room; they were considered as unaware and excluded from the analysis.

During *conditioning* (Fig. 1) each learning trial had the following sequence. Participants stood in front of the door of one office room. As soon as the door opened, they were guided through it on pre-recorded paths. There were six different paths per room, and each path was completed twice per each room. Participants entered CXT+ and CXT− 12 times each and spent 30 sec in each virtual office (all together the conditioning phase consisted of 24 trials). Order of CXT+ and CXT− visits were counter balanced across participants. In four out of 12 CXT+ trials, no US was delivered ($CXT+_{unpaired}$); in four CXT+ trials one US was delivered, and in the remaining four CXT+ trials two USs were delivered. The US was randomly and unpredictably delivered between 8 sec and 23 sec after context onset. Importantly, the first CXT+ trial was always paired with a US.

During *extinction*, the trial sequence was the same but no US was delivered. Participants were guided into the two offices again (CXT+, CXT−). The paths leading through the virtual rooms were the same as during conditioning, lasting 30 sec each. Participants entered CXT+ and CXT− 8 times each.

The inter-trial interval (ITI), defined as the time between the exit of one context and the entry of the next one, lasted 10 sec on average (i.e., between 7.5 sec and 12.5 sec) for both phases.

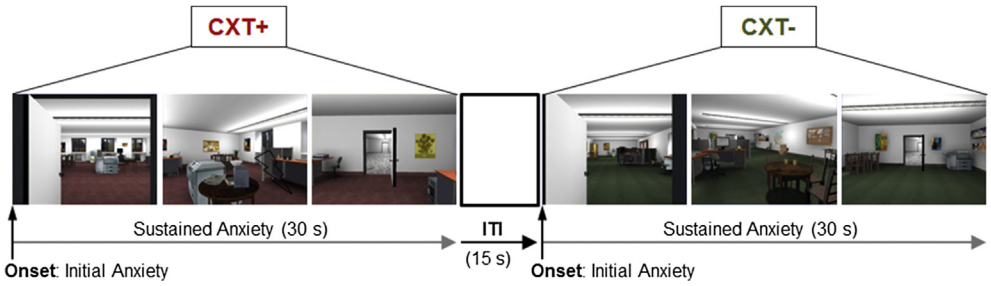


**Fig. 1 – Experimental design. Participants entered two virtual rooms, which differed in the color of the carpet (red, green), furniture disposition and view from the windows. In one room (CXT+ or anxiety context), 0 to 2 painful electric shocks (aversive US) could have been delivered unpredictably, while in the other room (CXT− or safety context) no shock was delivered. The visit of each room lasted 30 sec. Each trial started with a 15 sec inter-trial interval (ITI) during which the VR screen was black. Room entry starts with the opening of the room's door and ended with leaving the room through this door. The extinction phase was similar to the conditioning phase, except that no US was delivered. During conditioning, each context was entered 12 times, while during extinction 8 times.**

### 2.4. Magnetic resonance imaging

Brain images were acquired using a 1.5T MR scanner (Avanto 1.5T, Siemens, Erlangen, Germany) with a standard head coil. The structural-image acquisition consisted of 160 T1-weighted sagittal magnetization-prepared rapid gradient-echo imaging (MP-RAGE) 3D MRI sequences (MPRAGE, 1 mm slice thickness, TR = 2250 msec, TE = 3.93 msec, 8° flip angle, FOV = 250 mm, matrix = 256 × 256, voxel size = 1 × 1 × 1 mm). For functional imaging, a total of 420 volumes for the conditioning phase, and a total of 280 volumes for the extinction phase were registered using a $T^*_2$-weighted gradient echo-planar imaging sequence (EPI) with 25 axial slices tilted approx. 30° to the AC-PC line and covering the whole brain (5 mm slice thickness; 1 mm gap, descending order, TA = 100 msec; TE = 40 msec, TR = 2500 msec, flip angle = 90°, field of view = 240 × 240 mm, matrix size = 64 × 64, voxel size = 3.1 × 3.1 × 5 mm). The first eight volumes of each session were discarded to allow for T1 equilibration.

### 2.5. Data reduction and statistical analysis

FMRI data were analyzed using Statistical Parametric Mapping (SPM8, Wellcome Department of Cognitive Neurology, London, UK) in MatLab R2008b (MathWorks Inc., Sherborn, MA, USA). Realignment (b-spline interpolation) and slice time corrections were performed (Ashburner & Friston, 2005). To allow localization of functional activation on the participants' structural MRIs, T1-scans were co-registered to each participant's mean image of the realigned functional images. Co-registered T1 images were then segmented, and in the next step, EPI images were spatially normalized into the Montreal Neurological Institute (MNI) space using the normalization parameters obtained from the segmentation procedure (voxel size 2 × 2 × 2 $mm^3$) and spatially smoothed with an 8 mm full-width-half-maximum (FWHM) Gaussian kernel. The voxel-based time series were filtered with a high pass filter (128 sec time constant).

Conditioning and extinction phases were analyzed separately. In order to distinguish initial anxiety responses from sustained anxiety responses, we separately analyzed the hemodynamic response for the onset of the two events (event-related analysis) and for the whole duration of the two contexts (30 sec each, block-wise analysis), respectively. Initial anxiety responses were modeled by convolving stick functions with the canonical hemodynamic response function (HRF) at the moment of door opening (see also Fig. 1). For the block-wise analysis of the conditioning phase, we considered all the CXT− trials, but only the $CXT+_{unpaired}$ trials in which no US was delivered to prevent contamination of the sustained anxiety response with US-evoked responses. In both the block-wise and the event-related analyses, the six movement parameters of the rigid body transformation were introduced as covariates. For each participant, we computed the following one-sample *t*-contrasts separately for the conditioning and the extinction phases: $_{unpaired}CXT+$ versus $_{all}CXT-$ (sustained) and $_{all}CXT+$ versus $_{all}CXT-$ (initial).

For *a priori* expected activations, Region of Interest (ROI) analyses were carried out for amygdala, hippocampus, striatum (caudate and putamen), insula, anterior cingulate cortex (dACC, BA 24), ventromedial PFC (vmPFC, BA 10) using masks from WFU Pickatlas software (Version 2.4, Wake Forest University, School of Medicine, NC, USA) separately for the two hemispheres. We expected effects especially in the right hemisphere since the right amygdala has been reported to be particularly and more strongly involved in conditioning involving sensorial aversive stimuli (Mechias et al., 2010; Phelps, Delgado, Nearine, & LeDoux, 2004). Based on this, we performed all ROI analyses separately for the left and the right hemispheres. Because our scanner was not appropriate to identify small structural differences, no ROI analysis was carried out for the BNST. For all ROI analyses, a minimum cluster size of 5 voxels was required (Tabbert, Stark, Kirsch, & Vaitl, 2006). The statistical threshold for the activation was set to $p < .05$ (corrected for family-wise error, FWE). Next, to reveal extended activations outside the ROIs, a whole brain (WB) analysis was conducted by setting the statistical threshold for activation to $p < .001$ (uncorrected) with a minimum cluster size of 10 voxels and then corrected with Monte Carlo simulation (the re-sampled cluster size resulted in 55 voxels, which is equivalent to a whole brain false discovery rate of $p = .05$, Slotnick, Moo, Segal, & Hart, 2003).

We further conducted an exploratory finite impulse response analysis (FIR) to reveal the time course of the brain activity during the context presentations (30 sec). The fMRI signal was re-estimated for significantly activated ROIs in the block-wise analysis (sustained fear) for the contrast $_{unpaired}CXT+ > _{all}CXT-$ during conditioning. Based on the supra-threshold voxels ($p < .05$, FWE-corrected, $k \geq 5$ voxels) the percent signal change was calculated and then this signal was extracted using the rfxplot toolbox (Gläscher, 2009) for each repetition time from context onset to context offset. Given that our goal was to disentangle initial and sustained anxiety response,[2] we additionally verified with separated ANOVAs for each of the significant cluster whether the BOLD signal was specifically greater at the context's onset or throughout the context's visit (see Supplemental Material).

Lastly, after the block-wise and the event-related analyses we exported the beta values of the significant clusters for the respective contrasts CXT+ > CXT− and computed Pearson correlation analyses with the trait version of the STAI (Laux et al., 1981).

The anxiety and the contingency awareness ratings were analyzed separately with ANOVAs with the within-subjects factors context (anxiety, safety) and phase which had three levels (pre-conditioning, after-conditioning, after-extinction) for the anxiety ratings and two levels (after-conditioning, after-extinction) for the contingency ratings. When necessary, the Greenhouse-Geisser correction (GG-ε) of the degrees of freedom was applied and the partial $\eta^2$ values are reported as

[2] To this purpose, we additionally modeled the initial and the sustained neural anxiety responses in the same analysis applying a mixed block/event-related analysis (Petersen & Dubis, 2012. The mixed block/event-related design. *NeuroImage* 62: 1177–1184). The results after this analysis are in line with those indicated in the manuscript. For further information, please contact the authors.

measures of effect size. The alpha ($\alpha$) level for all statistical tests was set to $p < .05$ (two-tailed).

## 3. Results

### 3.1. Questionnaires

Analysis reveals a significant change in participants' positive mood [$F(2,40) = 6.17$, GG-$\varepsilon = .752$, $p = .010$, $\eta_p^2 = .236$], but not in their negative mood [$F(2,40) = 2.21$, GG-$\varepsilon = .674$, $p = .143$, $\eta_p^2 = .099$] or their momentary anxiety [$F(2,40) = .25$, $p = .777$, $\eta_p^2 = .013$]. Participants' positive mood do not differed significantly from Day 1 ($M = 29.57$, $SD = 5.46$) to Day 2 [$M = 28.95$, $SD = 5.99$; $t(20) = .73$, $p = .475$], but participants report a decrease in their positive mood from the beginning to the end ($M = 26.00$, $SD = 7.48$) of the experiment [$t(20) = 2.99$, $p = .007$].

### 3.2. Ratings

#### 3.2.1. Anxiety (Fig. 2)

Analysis reveals a significant main effect of context [$F(1,23) = 27.95$, $p < .001$, $\eta_p^2 = .549$] and phase [$F(2,46) = 16.45$, $p < .001$, $\eta_p^2 = .418$]. The main effect of context indicates that the anxiety context induces a general higher level of anxiety compared to the safety context. Participants' anxiety is significantly higher after the conditioning phase on Day 2 compared to both the ratings after the familiarization phase on Day 1 [$F(1,23) = 33.94$, $p < .001$, $\eta_p^2 = .596$] and those after the extinction phase on Day 2 [$F(1,23) = 5.24$, $p = .032$, $\eta_p^2 = .185$]. Moreover, the anxiety level after the extinction is still higher than after the familiarization phase on Day 1 [$F(1,23) = 10.82$, $p = .003$, $\eta_p^2 = .320$].

The interaction effect between context and phase turn out significant [$F(2,46) = 25.25$, GG-$\varepsilon = .8001$, $p < .001$, $\eta_p^2 = .523$], see Fig. 2. Post-hoc $t$-tests indicate that before the conditioning phase on Day 1, the self-reported anxiety level does not differ between the two rooms [$t(23) = .07$, $p = .948$]. As expected, after the conditioning phase on Day 2 participants report a significantly higher anxiety level in the anxiety context than in the safety context [$t(23) = 7.54$, $p < .001$]. Supportively, participants' anxiety ratings for the anxiety context significantly increase from before to after conditioning [$t(23) = 7.04$, $p < .001$], but not for the safety context [$t(23) = .62$, $p = .54$]. After extinction participants still report a higher level of anxiety for the anxiety context compared to the safety context [$t(23) = 2.83$, $p = .009$]. However, their anxiety ratings for the anxiety context significantly decrease from after conditioning to after extinction [$t(23) = 4.09$, $p < .001$], whereas the anxiety ratings for the safety context do not change [$t(23) = 1.47$, $p = .156$].

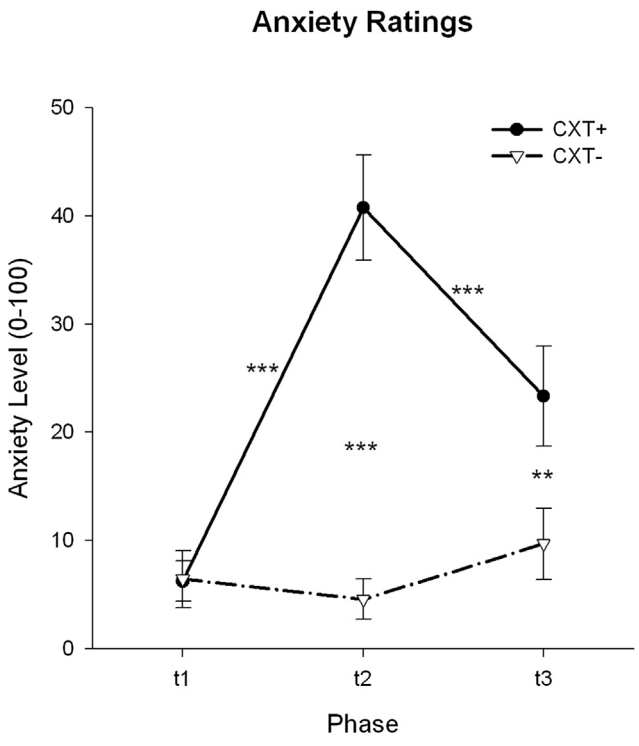


**Fig. 2 – Anxiety ratings. The lines (with standard errors) depict the anxiety ratings for the anxiety context (CXT+, solid line) and the safety context (CXT−, dash-dot line), respectively. On Day 1 (t1) before conditioning both context elicited similar anxiety levels. After conditioning (t2), participants reported significant higher anxiety levels in CXT+ compared to CXT−, and this difference was still present after extinction (t3). Notably, participants' anxiety levels in CXT+ significantly increased from before to after conditioning and then significantly decreased to after extinction. ***$p < .001$, **$p < .01$.**

#### 3.2.2. Contingency awareness

Analysis reveals significant main effects of context [$F(1,22) = 119.04$, $p < .001$, $\eta_p^2 = .844$] and phase [$F(1,22) = 23.46$, $p < .001$, $\eta_p^2 = .516$]. Furthermore, we find a significant interaction effect between context and phase [$F(1,22) = 27.93$, $p < .001$, $\eta_p^2 = .559$]. Follow-up $t$-tests reveal that after conditioning participants could correctly indicate in which context the aversive US was delivered. Namely, contingency ratings are significantly higher for the anxiety context ($M = 85.22$, $SD = 2.80$) compared to the safety context [$M = 13.22$, $SD = 4.78$; $t(22) = 12.10$, $p < .001$]. Participants still report after extinction a higher association between the anxiety context and the US ($M = 37.52$, $SD = 5.32$) than between the safety context and the US [$M = 20.96$, $SD = 5.93$; $t(22) = 2.50$, $p = .020$]. However, participants' contingency ratings decrease significantly from after conditioning to after extinction, but only for the anxiety context [$t(22) = 8.87$, $p < .001$] and not for the safety context [$t(22) = 1.0$, $p = .329$].

### 3.3. Neural activities

The significant activations for the event-related (initial response) and the block-wise (sustained response) analyses are reported in Fig. 3 and Table 1 for the conditioning phase and in Table 2 for the extinction phase.

#### 3.3.1. Conditioning

The event-related ROI analyses ($p < .05$, $k \geq 5$, FWE-corrected) focusing on **initial neural response** related to the contexts' onset (contrast $_{all}$CXT+ > $_{all}$CXT−) do not reveal significant activations. The WB analysis ($p < .001$, uncorrected, $k \geq 55$,

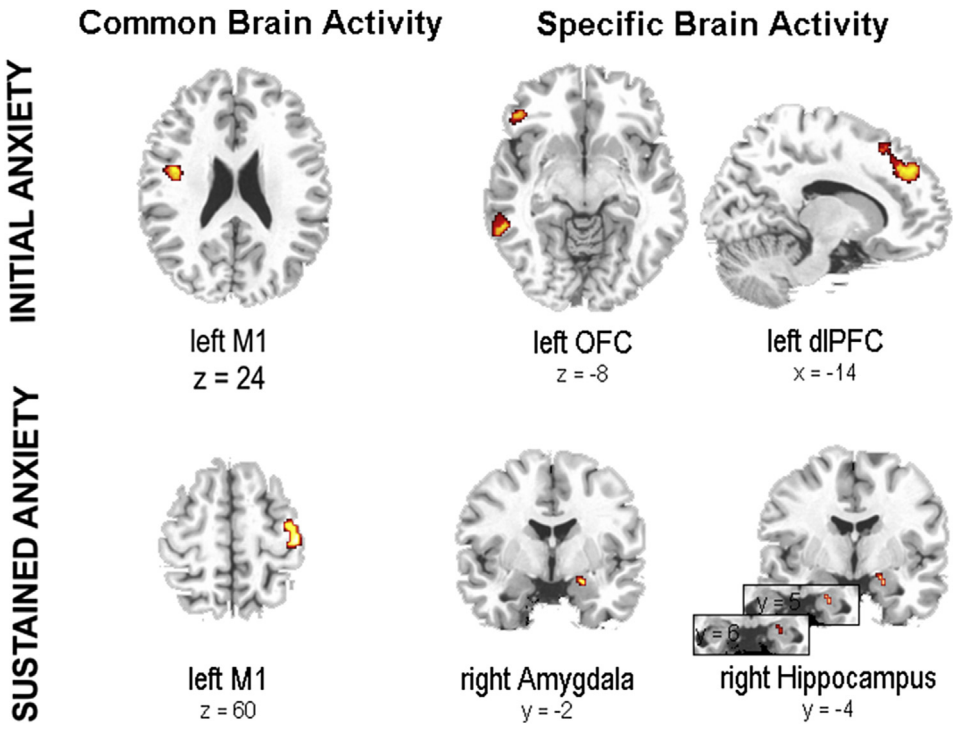


**Fig. 3 – Initial and sustained brain activity during contextual fear conditioning. Initial anxiety responses (upon panel) were detected at the onset of the anxiety context contrasted to the safety context; sustained anxiety responses (bottom panel) were revealed as contrasts between the anxiety and the safety contexts throughout the 30 sec of context presentation. Motor area was activated by both initial and sustained anxiety (left panel). OFC and dlPFC were specifically active at the onset of the threatening context (upon right panel). Amygdala and hippocampus activities were peculiar for sustained anxiety responses (right bottom panel).**

after Monte Carlo simulation) reveals increased activation in a distributed prefrontal network which consisted of orbitofrontal cortex (OFC; BA 11, 47), dorsolateral prefrontal cortex (dlPFC: superior frontal gyrus), dorsomedial prefrontal cortex (dmPFC: middle temporal gyrus), and primary motor cortex (M1: precentral gyrus). The reversed contrast ($_{all}$CXT− > $_{all}$CXT+) yields no significant activations. Lastly, no significant correlation between the above mentioned brain activities and the anxiety trait is found (*ps* > .079).

The ROI analyses for the **sustained neural response** (contrast $_{unpaired}$CXT+ > $_{all}$CXT−) indicate enhanced activity in the right amygdala and the right hippocampus (*ps* < .05, FWE-corrected).[3] The subsequent FIR analyses confirms that both the amygdala (Fig. 4) and the hippocampus activations are higher for CXT+ compared to CXT− when analyzed over the entire context presentation (for further FIR analyses of the significant clusters see Supplemental Material). Amygdala ($r = -.401$, $p = .052$), but not hippocampus ($r = -.334$, $p = .111$) activation negatively correlates with the participants' trait anxiety. Extended neural activation (WB analysis, $p < .001$, uncorrected, $k \geq 55$, after Monte Carlo simulation) is found in the primary motor cortex (M1; precentral gyrus). For the reversed contrast ($_{all}$CXT− > $_{unpaired}$CXT+) we find greater activation in the left supplementary motor area (SMA).

#### 3.3.2. *Extinction*

Both the ROI and the WB analyses of **initial** and **sustained** conditioned neural responses reveals no significant activations for the contrast $_{all}$CXT+ > $_{all}$CXT−. Sustained activations for the reversed contrast $_{all}$CXT− > $_{all}$CXT+ are revealed in the right frontal network (inferior and middle frontal gyrus; WB: $p < .001$, uncorrected, $k \geq 55$, after Monte Carlo simulation).

## 4. Discussion

This study was designed to investigate the neural mechanisms involved in the acquisition and extinction of initial and sustained components of the anxiety response triggered by a threat associated context. Using VR as an ecologically valid paradigm to implement contextual fear conditioning in a highly controlled yet nearly realistic setting, participants learned an association between an aversive event (the US, a painful electric shock) and a long-lasting stimulus comprised of visiting a virtual room (the CXT+ or anxiety context).

[3] Notably, we also performed an analysis contrasting the 4 $_{unpaired}$CXT+ trials and the 4 matched CXT− trials only which revealed comparable results. In fact, ROI analysis revealed for both the amygdala and the hippocampus enhanced activity to the CXT+ compared to the CXT−. While, the WB analyses confirmed the activation in the right precentral gyrus (BA 3, M1) triggered by the CXT+ compared to the CTX-, although this cluster did not survive after Monte Carlo simulation ($x = 46$, $x = 22$, $z = 60$; $k = 16$).

**Table 1 – Coordinates and statistics for the analysis of the conditioning phase.**

| Contrast | Brain structure | x | y | z | Z | Cluster size | p |
|---|---|---|---|---|---|---|---|
| **Initial** | | | | | | | |
| $_{all}$CXT+ > $_{all}$CXT− | Middle temporal gyrus L (BA 21) | −56 | −44 | −4 | 4.80 | 287 | <.001 |
| | Precentral gyrus L (BA 6) | −42 | −4 | 24 | 4.38 | 105 | <.001 |
| | Superior frontal gyrus L (BA 9) | −14 | 44 | 36 | 4.12 | 222 | <.001 |
| | OFC L (BA 11, BA 47) | −46 | 34 | −8 | 3.83 | 73 | <.001 |
| $_{all}$CXT− > $_{all}$CXT+ | No significant activations | | | | | | |
| **Sustained** | | | | | | | |
| $_{unpaired}$CXT+ > $_{all}$CXT− | Precentral gyrus R (BA 3, BA 4) | 46 | −22 | 60 | 3.80 | 192 | <.001 |
| | *Hippocampus R | 18 | −4 | −16 | 3.66 | 8 | .020 |
| | *Amygdala R | 18 | −2 | −16 | 3.62 | 14 | .007 |
| $_{all}$CXT− > $_{unpaired}$CXT+ | Supplementary motor area L (BA 8) | 0 | 24 | 54 | 4.14 | 94 | <.001 |

Threshold at $p \leq .001$ uncorrected for whole brain analysis with a minimum cluster size of $k = 55$ (after Monte Carlo simulation) unless asterisked; *$p < .05$ FWE-corrected for ROI analysis ($k = 5$); L = left, R = right hemisphere. Coordinates x, y, and z of the peak voxels are given in Montreal Neurological Institute (MNI) space. BA = Brodmann area. OFC = orbitofrontal cortex.

Importantly, the aversive US was delivered in an uncontrollable and unpredictable manner so that we induced a feeling of sustained fear (Davis et al., 2010). A second and different virtual room comprised a safety context (CXT−), in which no US was delivered. Compared to the safety context, the anxiety context elicited enhanced anxiety as revealed by ratings and stronger activations in amygdala, hippocampus, primary motor cortex (M1). These findings clearly suggest that our VR paradigm successfully induced contextual fear learning. The amygdala and the hippocampus have been proposed as crucial brain areas for the acquisition and expression of conditioned fear responses, not only to a cue (Büchel et al., 1998; LaBar et al., 1998; Mechias et al., 2010), but also to a context (Alvarez et al., 2008, 2011; Maren et al., 2013; Marschner et al., 2008; Pohlack et al., 2012). The activation of motor areas suggests the intention to behaviorally respond to the threatening context, which might involve avoidance behaviors (Büchel et al., 1998).

The study's goals were to disentangle initial and sustained components of the conditioned anxiety response and to reveal a common brain network involved in both initial and sustained anxiety responses. Specifically, the M1 was found to be activated to both the entry into the threatening context (i.e., initial response) and throughout its visit (i.e., sustained response), while the amygdala and the hippocampus were strongly active during the long-lasting visit of CXT+ only and not at its onset. Considering that the amygdala is crucial for encoding, storing, and retrieving fear memories (Mechias et al., 2010), these findings suggest that the amygdala is important for processing sustained fear (Alvarez et al., 2008, 2011; Pohlack et al., 2012). Furthermore, this study is the first revealing that amygdala activation is negatively correlated with the participants' trait anxiety, i.e., the more anxious the participant, the less discriminative activation between CXT+ and CXT− was found. Interestingly this result is in line with previous studies, which suggested that anxiety patients are less able to distinguish between threat and safety (Lissek et al., 2005; Mineka & Oehlberg, 2008). Notably, the missing initial amygdala activation in response to the CXT+ onset does not exclude its crucial role in coordinating initial anxiety response. In fact, in the classical cue conditioning literature amygdala activation is sometimes found at CS+ onset (Büchel et al., 1998; LaBar et al., 1998; Marschner et al., 2008), but sometimes not (Knight, Cheng, Smith, Stein, & Helmstetter, 2004; Merz, Hermann, Stark, & Wolf, 2013). Possibly, this inconsistency might be due to differences in signal-to-noise ratio or to methodological differences between studies.

In our study, participants were immersed in the VR without seeing the real experimental room or the scanner while the actual experiment was going on. It is possible that they might have been "present" in the virtual rooms and somehow forgot the external real room[4] (Riva et al., 2007; Sanchez-Vives & Slater, 2005). In contrast, previous studies have used fluctuation of colors lasting several seconds (Pohlack et al., 2012) or presented the rooms on a computer screen (Alvarez et al., 2008, 2011). Hence, in these cases participants could see the real experimental room for the whole duration of the experiment and consequently they could not experience complete immersion or full presence in the experimentally varied contexts. As previous studies proposed (Huff et al., 2011; Sanchez-Vives & Slater, 2005), presence may be a function of consciousness within the VR. Consequently, the more presence the individual feels, the more realistic or reality-connected its reactions are. Therefore, it is tempting to interpret the brain activations triggered by the virtual contexts, especially those we observed in the motor areas, as correlates of fear behavior, which might reflect a reality-related response. Thus, immersion into a threatening virtual context, as realized in this study, might be a promising and reliably tool for studying real-world context effects. Although participants reported presence in the VR, we have to acknowledge that playing back a VR path as realized in the present study is like watching a movie from the first person perspective. Therefore, we cannot be sure whether our results can be generalized to real VR environment allowing participants to freely move within the virtual rooms. Unfortunately,

[4] Interestingly, the presence scores of the IPQ significantly and positively correlated with the differential anxiety ratings (anxiety ratings in anxiety context − anxiety ratings in safety context) both after conditioning ($r = .451$, $p = .027$) and after extinction ($r = .478$, $p = .018$). These results support the idea that the more presence the participant reported, the stronger the conditioning effect was.

**Table 2 – Coordinates and statistics for the analysis of the extinction phase.**

| Contrast | Brain structure | x | y | z | Z | Cluster size | p |
|---|---|---|---|---|---|---|---|
| **Initial** | | | | | | | |
| CXT+ > CXT− | No significant activations | | | | | | |
| CXT− > CXT+ | No significant activation | | | | | | |
| **Sustained** | | | | | | | |
| CXT+ > CXT− | No significant activations | | | | | | |
| CXT− > CXT+ | Inferior frontal gyrus R | 34 | 28 | 24 | 4.23 | 97 | <.001 |
| | Middle frontal gyrus R (BA 6,8) | 34 | 6 | 56 | 3.95 | 71 | <.001 |

Threshold at $p \leq .001$ uncorrected for whole brain analysis with a minimum cluster size of $k = 55$ unless asterisked (after Monte Carlo simulation); L = left, R = right hemisphere. Coordinates x, y, and z of the peak voxels are given in Montreal Neurological Institute (MNI) space. BA = Brodmann area. ACC = anterior cingulate cortex. vmPFC = ventromedial prefrontal cortex.

this is very complicated to realize in the scanner environment due to movement restrictions.

Despite participants were not free to move in the virtual world during conditioning, the opening of the virtual door may have been the moment when they realized whether they were about to enter the threat or the safety context. Supportively, all participants included in our analysis were aware and could correctly report the association between the CXT+ and the US. Additionally, the view of the threatening context might have induced distress as the anxiety ratings indicate, and the individuals might have looked for the most appropriate reaction, which is avoidance. We think that the observed activity in M1 very likely reflects avoidance-like responses. Our participants learned that they could not actively avoid entering the threatening context, and this might have determined a greater desire to avoid this context. This conclusion is supported by a previous study of us in which we asked participants to enter one out of three virtual rooms again (CXT+, CXT− and a novel context) after contextual fear conditioning. In one condition, participants could actively enter (or avoid) the room by means of a joystick, whereas in the other condition they choose the room and then they were passively guided into it. Participants showed stronger avoidance responses, i.e., more participants avoided the anxiety context, when they were passively guided into the threatening context as compared to when they could actively enter the contexts (Glotzbach, Ewald, Andreatta, Pauli, & Mühlberger, 2012). Notably, M1 activation is commonly reported in

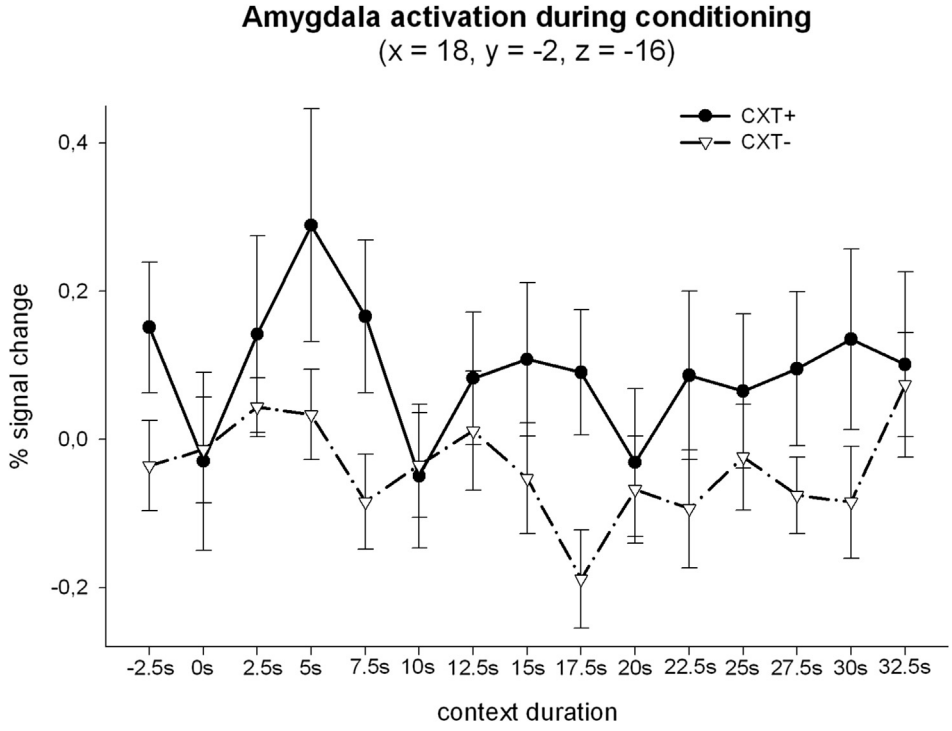


**Fig. 4 – FIR of the right amygdala during context presentations (30 sec ± 2.5 sec). The lines (with standard errors) depict the sustained activation (on the y-axis, % signal change) in the amygdala during conditioning throughout the 30 sec (on the x-axis) of the anxiety context (solid line) and the safety context (dash-dot line), respectively. Amygdala showed greater activation in response to the CXT+ than to CXT−, and importantly this activation was evident throughout the visit of the threatening context.**

studies using discrete stimuli signaling threats (i.e., the classical cue fear conditioning, Büchel et al., 1998; Tabbert et al., 2006) suggesting that the onset of a threatening context might work as a cue signaling a potentially dangerous situation. Since we observed these responses also during the whole visit of the threatening room, it seems possible that participants have felt a strong and continuous desire to leave the threatening room throughout their visit. Hence, the forced stay in a threatening situation together with the strong desire to get away from this distressing situation might have determined the activation of amygdala and M1, respectively. Notably, the involvement of the motor cortex is often reported in associative aversive learning (Büchel et al., 1998; Mechias et al., 2010; Tabbert et al., 2006), but it is likewise put to the background (Butler et al., 2007). From a clinical point of view, it would be interesting to verify participants' neural and behavioral responses if they would have the possibility to freely avoid the threatening room. In fact, the possibility to avoid a threatening situation induces a feeling of relief and consequently this positive feeling works as a negative reinforcer (Kim, Shimojo, & ÓDoherty, 2006; Mowrer, 1951, 1956).

As suggested above, our VR setting might have promoted responses to the threatening situation that are closer to real-life in comparison to experimental settings in previous studies. Our findings revealed specific brain areas with sustained activation to the long-lasting context suggesting coordinated neural activity and presumably learning throughout the threatening situation. Notably, this is highly similar to real-life situations when individuals experience traumatic events. For instance, traumatic events during a war typically last from several minutes to hours. Notably, the development of PTSD following such trauma (for a recent review about PTSD see Jovanovic & Norrholm, 2011) has been linked to altered learning and memory processes (Parsons & Ressler, 2013; Trezza & Campolongo, 2013). Although aversive classical conditioning has been proposed as a biomarker for anxiety disorders (Mineka & Oehlberg, 2008) and patients with anxiety disorders, in particular PTSD, seem to be specifically sensitive to threat (Jovanovic et al., 2010), we still may consider that sustained amygdala activation might also play a role in other disorders such as major depression (MDD) (Siegle, Steinhauer, Thase, Stenger, & Carter, 2002).

The observed sustained hippocampal activation to the anxiety context confirms and extends previous studies in animals (Kim & Fanselow, 1992; Rudy, 2009) and humans (Alvarez et al., 2008, 2011; Lang et al., 2009; Marschner et al., 2008; Pohlack et al., 2012). During contextual fear learning it is of crucial importance to form a temporal trace (Knight et al., 2004; Marschner et al., 2008) as well as a spatial (Maguire et al., 1998; O'Keefe & Dostrovsky, 1971) and a configural (Fanselow, 2009; Rudy, 2009) map of the context. These three aspects are crucial for associating the aversive US with a complex and long-lasting stimulus such as a context. The spatial map refers to spatial organization of a context (O'Keefe & Dostrovsky, 1971), the configural map refers to a representation of the context as a whole entity formed by its single components (e.g., single objects of the furniture in the virtual office, Rudy, 2009), and the temporal trace refers to the temporal representation of the (long-lasting) context (Eichenbaum, 2013). Because of the lack of specific and defined cues signaling the aversive US, tracking one's location in a given context and memorizing the temporal gap between the painful events may be the only way to experience some control in foreseeing the US. The hippocampus seems to process the context as a unitary element and may crucial for associating the complete context with the aversive USs.

Additionally, we found activation in prefrontal regions (i.e., dlPFC and OFC) in response to CXT+ onset (for the specificity of dlPFC and OFC in initial anxiety processing see Supplemental Material). According to the dual-system theory proposed by Rudy (2009), a context requires two kinds of processing. On the one hand, a representation of the context as coherent unit is necessary, which is supported by activation in the hippocampal formation as reported above. On the other hand, a representation of the single components of that particular context is necessary. Most likely, this processing is located in the PFC. Possibly, the context is experienced as a unitary stimulus when the individuals are guided through the virtual rooms, probably related to hippocampal representation of the above mentioned maps. In contrast, the moment of entering a context may represent a distinct event involving some sort of binding which implies prefrontal activation. Specifically, the dlPFC has been associated with working memory processes including binding (Baddeley, 2003). Furthermore, the OFC has been implicated in outcome-expectancy determining flexible behaviors (Schoenbaum, Roesch, Stanaker, & Takahashi, 2009). Therefore, it is conceivable that the objects in one room were momentarily maintained in working memory in order to be bound with each other and processed as unitary stimulus. Contemporaneously, while entering the threatening room participants recognized it as the aversive context and expected the painful electric shock. Respectively, the binding of the objects might be provided by the dlPFC and the expectancy of the US might be provided by the OFC.

This study also investigate the brain responses underlying extinction learning of contextual fear in humans. Surprisingly, we did not find significant initial or sustained activation to the CXT+ as referred to the CXT− during extinction. Unexpectedly, we found prefrontal activation in response to the safety context (CXT−) as compared to the anxiety context (CXT+) during extinction. Interestingly, several previous studies also reported greater activations to the safety cue (CS−) as compared to the threat cue (CS+) (Merz et al., 2013; Phelps et al., 2004), but none of these studies have discussed these findings. A plausible explanation may be that during extinction the conditioned stimulus (CS+ or CXT+) is presented without the US and this leads to new inhibitory learning. In our study, we did not mention to our participants any change of contingencies from one phase (i.e., the conditioning) to the next one (i.e., the extinction). It is therefore possible that participants still believed that they would receive the painful shock in the extinction phase as well. Hence, they might have started thinking that the US will come in the other room, i.e., the safety context. This seems to be in line with the sustained activation that we found in the prefrontal areas during the visit of the safety room. Supporting this speculation, we observed that contingency ratings for the CXT− slightly increased after extinction, although this difference did not reach the significance level.

In summary, we found distinguishable patterns of activation for the initial and the sustained component of an anxiety response in humans involving a fear network (i.e., amygdala, hippocampus and M1) and a "contingency-cognitive" network (i.e., dlPFC and OFC), respectively. However, we could not find clear evidence for brain areas involved in extinction of contextual fear. The VR paradigm we used proved to be a promising methodological approach as it reflects several aspects of real contexts and behavior in contexts. Our results are an important step in understanding the mechanisms of learning and changing contextual fear, which might become helpful in planning more efficient and effective therapeutic approaches.

## Conflict of interests

PP and AM are shareholders of a commercial company that develops virtual environment research systems for empirical studies in the field of psychology, psychiatry, and psychotherapy. No further potential conflicting interests exist.

## Acknowledgments

We sincerely thank M. Müller for his technical support and P. Reicherts S. Geuter for his help in the analysis. The work was supported by the Collaborative Research Center "Fear, Anxiety, Anxiety Disorders," SFB-TRR 58 project B1 to PP and AM.

## Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cortex.2014.09.014.

## REFERENCES

Alpers, G., & Pauli, P. (2001). *Angstsensitivitäts-Index*. Würzburg: Julius-Maximailians Universität.

Alvarez, R. P., Biggs, A., Chen, G., Pine, D. S., & Grillon, C. (2008). Contextual fear conditioning in humans: cortical-hippocampal and amygdala contributions. *The Journal of Neuroscience, 28*, 6211–6219.

Alvarez, R. P., Chen, G., Bodurka, J., Kaplan, R., & Grillon, C. (2011). Phasic and sustained fear in humans elicits distinct patterns of brain activity. *NeuroImage, 55*, 389–400.

Alvarez, R. P., Johnson, L., & Grillon, C. (2007). Contextual-specificity of short-delay extinction in humans: renewal of fear-potentiated startle in a virtual environment. *Learning & Memory, 14*, 247–253.

Andreatta, M., Fendt, M., Mühlberger, A., Wieser, M. J., Imobersteg, S., Yarali, A., et al. (2012). Onset and offset of aversive events establish distinct memories requiring fear- and reward network. *Learning & Memory, 19*, 518–526.

Andreatta, M., Mühlberger, A., Yarali, A., Gerber, B., & Pauli, P. (2010). A rift between implicit and explicit conditioned valence after pain-relief learning in humans. *Proceedings of the Royal Society B: Biological Sciences, 277*, 2411–2416.

Ashburner, J., & Friston, K. J. (2005). Unified segmentation. *NeuroImage, 26*, 839–851.

Baddeley, A. (2003). Working memory: looking back and looking forward. *Nature Review Neuroscience, 4*, 829–839.

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning & Memory, 11*, 485–494.

Büchel, C., Morris, J., Dolan, R. J., & Friston, K. J. (1998). Brain systems mediating aversive conditioning: an event-related fRMI study. *Neuron, 20*, 947–957.

Butler, T., Pan, H., Tuescher, O., Engelien, A., Goldstein, M., Epstein, J., et al. (2007). Human fear-related motor neurocircuitry. *Neuroscience, 150*, 1–7.

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology, 35*, 105–135.

Eichenbaum, H. (2013). Memory on time. *TRENDS in Cognitive Sciences, 17*, 81–88.

Fanselow, M. S. (2009). From contextual fear to a dynamic view of memory systems. *TRENDS in Cognitive Sciences, 14*, 7–15.

Gläscher, J. (2009). Visualization of group data in functional neuroimaging. *Neuroinformatics, 7*, 73–82.

Glenn, C. R., Lieberman, L., & Hajcak, G. (2012). Comparing electric shock and a fearful screaming face as unconditioned stimuli for fear learning. *International Journal of Psychophysiology, 86*, 214–219.

Glotzbach-Schoon, E., Tadda, R., Andreatta, M., Tröger, C., Ewald, H., Grillon, C., et al. (2013). Enhanced discrimination between threatening and safe contexts in high-anxious individuals. *Biological Psychology, 93*, 159–166.

Glotzbach, E., Ewald, H., Andreatta, M., Pauli, P., & Mühlberger, A. (2012). Contextual fear conditioning effects predict subsequent avoidance behavior. *Cognition & Emotion, 26*(7), 1256–1272.

Graham, B. M., & Milad, M. R. (2011). The study of fear extinction: Implication for anxiety disorders. *The American Journal of Psychiatry, 168*, 1255–1265.

Grillon, C., Baas, J. M., Cornwell, B., & Johnson, L. (2006). Context conditioning and behavioral avoidance in a virtual reality environment: effect of predictability. *Biological Psychiatry, 60*, 752–759.

Grillon, C., Pine, D. S., Lissek, S., Rabin, S., Bonne, O., & Vythilingam, M. (2009). Increased anxiety during anticipation of unpredictable aversive stimuli in posttraumatic stress disorder but not in generalized anxiety disorder. *Biological Psychiatry, 66*, 47–53.

Hamm, A. O., & Weike, A. I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology, 57*, 5–14.

Herry, C., Ferraguti, F., Singewald, N., Letzkus, J. J., Ehrlich, I., & Lüthi, A. (2010). Neuronal circuits of fear extinction. *European Journal of Neuroscience, 31*, 599–612.

Huff, N., Alba Hernandez, J., Fecteau, M., Zielinski, D., Brady, R., & LaBar, K. S. (2011). Revealing context-specific conditioned fear memories with full immersion virtual reality. *Frontiers in Behavioral Neuroscience, 5*.

Jovanovic, T., & Norrholm, S. D. (2011). Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience, 5*.

Jovanovic, T., Norrholm, S. D., Blanding, N. Q., Davis, M., Duncan, E., Bradley, B., et al. (2010). Impaired fear inhibition is a biomarker of PTSD but not depression. *Depression and Anxiety, 27*, 244–251.

Kalisch, R., Korenfeld, E., Stephan, K. E., Weiskopf, N., Seymour, B., & Dolan, R. J. (2006). Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. *The Journal of Neuroscience, 26*, 9503–9511.

Kim, H., Shimojo, S., & ÓDoherty, J. (2006). Is avoiding an aversive outcome rewarding? Neural substrates of avoidance learning in the human brain. *PLOS Biology, 4*, 1453–1461.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*, 675–677.
Knight, D. C., Cheng, D. T., Smith, C. N., Stein, E. A., & Helmstetter, F. J. (2004). Neural substrates mediating human delay and trace fear conditioning. *The Journal of Neuroscience, 24*, 218–228.
Krohne, H. W., Egloff, B., Kohmann, C. W., & Tausch, A. (1996). Untersuchungen mit einer deutschen Version der "Positive and Negative Affect Schedule" (PANAS). *Diagnostica, 42*, 139–156.
LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E., & Phelps, E. A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fRMI study. *Neuron, 20*, 937–945.
Lang, S., Kroll, A., Lipinski, S. J., Wessa, M., Ridder, S., Christmann, C., et al. (2009). Context conditioning and extinction in humans: differential contribution of the hippocampus, amygdala and prefrontal cortex. *European Journal of Neuroscience, 29*, 823–832.
Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. D. (1981). *Das state-trait Angstinventar*. Weinheim: Beltz Test.
Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., et al. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behavior Research and Therapy, 43*, 1391–1424.
Luyten, L., van Kuyck, K., Vansteenwegen, D., & Nuttin, B. (2011). Electrolytic lesions of the bed nucleus of the stria terminalis disrupt freezing and startle potentiation in a conditioned context. *Behavioural Brain Research, 222*, 357–362.
Maguire, E. A., Burgess, N., Donnett, J. G., Frackowiak, R. S. J., Frith, C. D., & O'Keefe, J. (1998). Knowing where and getting there: a human navigation network. *Science, 280*, 921–924.
Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nature Review Neuroscience, 14*, 417–428.
Marschner, A., Kalisch, R., Vervliet, B., Vansteenwegen, D., & Büchel, C. (2008). Dissociable roles for the hippocampus and the amygdala in human cued versus context fear conditioning. *The Journal of Neuroscience, 28*, 9030–9036.
Mechias, M. L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *NeuroImage, 49*, 1760–1768.
Merz, C. J., Hermann, A., Stark, R., & Wolf, O. T. (2013). Cortisol modifies extinction learning of recently acquired fear in men. *Social Cognitive and Affective Neuroscience, 8*.
Milad, M. R., & Quirk, G. (2012). Fear extinction as a model for translational neuroscience: ten years of progress. *Annual Review of Psychology, 63*, 129–151.
Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*, 567–580.
Mowrer, O. H. (1951). Two-factor learning theory: summary and comment. *Psychological Review, 58*, 350–354.
Mowrer, O. H. (1956). Two-factor learning theory reconsidered, with special reference to secondary reinforcement and the concept of habit. *Psychological Review, 63*, 114–128.
Mühlberger, A., Weik, A., Pauli, P., & Wiedemann, G. (2006). One-session virtual reality exposure treatment for fear of flying: 1-Year follow-up and graduation flight accompaniment effects. *Psychotherapy Research, 16*, 26–40.
O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Research, 34*, 171–175.
Parsons, R. G., & Ressler, K. J. (2013). Implications of memory modulation for post-traumatic stress and fear disorders. *Nature Neuroscience, 16*, 146–153.
Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. London: Oxford University Press.
Petersen, S. E., & Dubis, J. W. (2012). The mixed block/event-related design. *NeuroImage, 62*, 1177–1184.
Phelps, E. A., Delgado, M. R., Nearine, K. I., & LeDoux, J. E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron, 43*, 897–905.
Pohlack, S. T., Nees, F., Ruttorf, M., Schad, L. R., & Flor, H. (2012). Activation of the ventral striatum during aversive contextual conditioning in humans. *Biological Psychology, 91*, 74–80.
Riva, G., Mantovani, F., Capideville, C. S., Preziosa, A., Morganti, F., Villani, D., et al. (2007). Affective interactions using virtual reality: the link between presence and emotions. *CyberPsychology & Behavior, 10*, 45–56.
Rudy, J. W. (2009). Context representations, context functions, and the parahippocampal–hippocampal system. *Learning & Memory, 16*, 573–585.
Sanchez-Vives, M. V., & Slater, M. (2005). From presence to consciousness through virtual reality. *Nature Review Neuroscience, 6*, 332–339.
Schoenbaum, G., Roesch, M. R., Stanaker, T. A., & Takahashi, Y. K. (2009). A new perspective on the role of the orbitofrontal cortex in adaptive behaviour. *Nature Reviews Neuroscience, 10*, 885–892.
Schubert, T., Friedmann, F., & Regenbrecht, H. (2001). The experience of presence: factor analytic insights. *Teleoperators and Virtual Environments, 10*, 266–281.
Shiban, Y., Pauli, P., & Mühlberger, A. (2013). Effect of multiple context exposure on renewal in spider phobia. *Behaviour Research and Therapy, 51*, 68–74.
Siegle, G. J., Steinhauer, S. R., Thase, M. E., Stenger, V. A., & Carter, C. S. (2002). Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. *Biological Psychiatry, 51*, 693–707.
Slotnick, S. D., Moo, L. R., Segal, J. B., & Hart, J., Jr. (2003). Distinct prefrontal cortex activity associated with item memory and source memory for visual shapes. *Cognitive Brain Research, 17*, 75–82.
Somerville, L. H., Wagner, D. D., Wig, G. S., Moran, J. M., Whalen, P. J., & Kelley, W. M. (2013). Interactions between transient and sustained neural signals support the generation and regulation of anxious emotion. *Cerebral Cortex, 23*, 49–60.
Somerville, L. H., Whalen, P. J., & Kelley, W. M. (2010). Human bed nucleus of the stria terminalis indexes hypervigilant threat monitoring. *Biological Psychiatry, 68*, 416–424.
Strobel, A., Beauducel, A., Debener, S., & Brocke, B. (2001). Eine deutschsprachige Version des BIS/BAS-Fragebogens von Carver und White. *Zeitschrift für Differentielle und Diagnostische Psychologie, 22*, 216–227.
Tabbert, K., Stark, R., Kirsch, P., & Vaitl, D. (2006). Dissociation of neural responses and skin conductance reactions during fear conditioning with and without awareness of stimulus contingencies. *NeuroImage, 32*, 761–770.
Trezza, V., & Campolongo, P. (2013). The endocannabinoid system as a possible target to treat both the cognitive and emotional features of post-traumatic stress disorder. *Frontiers in Behavioral Neuroscience, 7*.
Tröger, C., Ewald, H., Glotzbach, E., Pauli, P., & Mühlberger, A. (2012). Does pre-exposure inhibit fear context conditioning? A virtual reality study. *Journal of Neural Transmission, 119*, 709–719.