

Renewal of conditioned fear in a novel context is associated with hippocampal activation and connectivity

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Abstract

Return of fear is a serious problem in exposure-based treatments of anxiety disorders. Renewal of the fear response may occur when re-encountering the conditioned stimulus within a novel context. Findings in rodents underpin the hippocampus' role in conditioned fear renewal in novel contexts, but it has yet to be investigated in humans. Forty-six healthy men took part in a 2-day, context-dependent, cued fear conditioning paradigm with fear acquisition, extinction learning (day 1) and extinction recall in the acquisition, extinction and a novel context one day later. Conditioned evaluative, skin conductance responses (SCRs) and blood-oxygen-level-dependent responses served as dependent variables. Context-dependent fear renewal was reflected in stronger conditioned SCRs. In the acquisition context, individuals with a higher renewal of conditioned SCRs showed stronger activation of the fear circuit. Hippocampal activation distinguished conditioned responding in the novel compared with the extinction context. Individuals with a stronger renewal of conditioned SCRs in the novel context showed increased effective connectivity of hippocampal activation foci with structures in the fear and extinction network. These results outline the pivotal role of the hippocampus and its connectivity in conditioned fear renewal in a novel context in humans and might have important implications for exposure therapy in anxiety disorders.

Key words: amygdala; extinction; fear conditioning; fMRI; hippocampus

Introduction

Anxiety disorders are among the most common psychiatric disorders (Kessler et al., 2005), leading to high individual suffering and socioeconomic costs. Despite the general effectiveness of cognitive-behavioral therapy (CBT), relapses following successful CBT constitute a serious problem (Bouton, 2002; Boschen et al., 2009). Extinction of conditioned fear is assumed to be a main mechanism of action in CBT of anxiety disorders (Mineka and Zinbarg, 2006; Graham and Milad, 2011). Difficulties

recalling the extinction memory in new contexts might impede the long-lasting transfer of therapy effects into daily life (Boschen et al., 2009; Vervliet et al., 2013). Retrieval of extinction memories in a context distinct from the safe extinction context generally leads to enhanced reoccurrence of conditioned fear in laboratory studies ('renewal effect'; Vervliet et al., 2013). This indicates that conditioned fear is not 'erased' during extinction, but rather that new inhibitory learning takes place (Bouton, 2004; Vervliet et al., 2013).

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The amygdala acts as the key brain region for the acquisition and storage of fear and extinction memories (Quirk and Mueller, 2008). Excitatory projections from the dorsal anterior cingulate cortex (dACC) and inhibitory projections from the ventromedial prefrontal cortex (vmPFC) are assumed to modulate the expression of conditioned fear and extinction memories via the amygdala. In rodents, contextual gating of conditioned fear expression is mediated by projections from the hippocampus to the amygdala (Orsini *et al.*, 2011; Knapska *et al.*, 2012; Orsini and Maren, 2012; Maren *et al.*, 2013).

Neuroimaging studies in humans also demonstrate a crucial role of the hippocampus for context-dependent extinction recall, with findings of hippocampal activation in the safe extinction context (Kalisch *et al.*, 2006; Milad *et al.*, 2007), as well as right-hemispheric hippocampal activation in the acquisition context (Kalisch *et al.*, 2006). These results also point to hippocampal subregional specificity (i.e. anterior-posterior) and/or lateralization effects.

Despite its high clinical relevance, there are no neuroimaging studies exploring the role of the hippocampus for conditioned fear renewal in a novel context in humans. Therefore, the main goal of this functional magnetic resonance imaging (fMRI) study was to investigate the neural basis of conditioned fear renewal in a novel as well as in the acquisition context compared with the safe extinction context, using a 2-day cued fear conditioning paradigm with contextual manipulations (adapted from Milad *et al.*, 2007). We investigated the role of hippocampal activation as well as its co-activation and connectivity with brain regions involved in the recall of conditioned fear and extinction memories. Regarding the importance of predicting relapses after successful exposure therapy, we additionally investigated individual differences in conditioned fear renewal.

Methods

Participants

Forty-eight healthy male students recruited at the local university participated in this study. Since prominent and complex sex differences have been observed in fear conditioning processes (especially due to fluctuating concentrations of sex hormones over the menstrual cycle and the intake of oral contraceptives; for a review see Lebron-Milad and Milad, 2012), we decided to explore fear renewal in a novel context in men exclusively in this study. Students reporting MRI exclusion criteria, chronic or acute illnesses, color blindness, regular intake of medicine, current medical or psychological treatment, drug use, age <18 or >35 years were not eligible for participation. All participants had to be right-handed as assessed by the Edinburgh Inventory of Handedness (Oldfield, 1971) and had normal or corrected-to-normal vision. Two men were excluded because of insufficient quality of the normalization results during preprocessing of the fMRI data, leaving a final sample of 46 men (age: $M = 23.4$ years; $SD = 2.3$ years). Participants were reimbursed with 10 €/h for their participation. All procedures were in accordance with the Declaration of Helsinki and approved by the local ethical review board of the Faculty of Psychology and Sports Science at the Justus Liebig University Giessen.

Stimulus material

Stimuli and procedure were adopted from Milad *et al.* (2007, 2009). Pictures of an office room and a room with a shelf served as contexts A and B, respectively. Additionally, a third context C

was prepared and included, depicting a conference room similar to the other two contexts. Each of the contexts contained the same desk lamp lighting up in either red, blue or yellow, which served as three conditioned stimuli (CS). An LCD projector (model EPSON EMP-7250) projected the pictures onto a screen at the end of the scanner. A mirror mounted to the head coil allowed the subjects to look at the screen.

Electrical stimulation was applied as the unconditioned stimulus (UCS). A Coulbourn Transcutaneous Aversive Finger Stimulator (E13-22) provided the electrical stimulation (1 ms pulses with 50 Hz for a duration of 500 ms) via electrodes (surface size: 1 cm²) attached to the fingertips of the second and third fingers of the right hand. The fingers were not fastened together. The intensity of the electrical stimulation was set individually to be 'unpleasant but not painful' using a gradually increasing rating procedure (for ratings of UCS unpleasantness see Supplementary Materials).

After arrival, participants gave written informed consent, filled out questionnaires on demographic variables, and were tested for red-green color blindness using five Ishihara plates (selected from Ishihara, 1990).

Procedure

Before the start of each experimental phase, they were instructed to watch the on-screen presentation with the goal of observing any possible regularity in the occurrence of lamplight colors and electrical stimulation. They were informed that should they discover such a relationship, it would remain stable in all experimental phases: if a lamplight color was safe, it would always be safe; if a lamplight color was followed by electrical stimulation, this might or might not occur again. These instructions were used to facilitate learning of contingencies (a prerequisite for studying extinction memory retrieval) and to avoid participants expecting a complete reversal of contingencies in the extinction phase (i.e. expecting stimulation to occur after CS-presentations). However, note that participants were not informed about the actual CS-UCS contingencies.

The trial structure was identical for all CS types during all experimental phases (except for trials with UCS presentation). After an initial presentation of a black screen with a white fixation cross (duration jittered between 0 and 1.875 s), the context without a CS (turned-off lamp) was presented for a duration of 3 s. This was followed by presentation of the CS (lamp within the context picture lighting up in red, blue or yellow for the three CS types, respectively) for 6 s. During reinforced CS+ trials, the UCS (electrical stimulation) was delivered immediately after the offset of the CS for a duration of 500 ms. A white fixation cross on a black background was shown from CS offset until the start of the next context presentation for 9.125–11 s (total trial duration: 20 s; Figure 1A).

Participants were exposed to fear acquisition in context A as well as extinction learning in context B on day 1. Fear and extinction recall was tested on day 2 for each participant in the acquisition context A, the extinction context B, and in a new context C (Figure 1B and C).

During fear acquisition in context A, two separate CS+ (CS+E and CS+U (see below); e.g. red and yellow light) were shown eight times each, and both CS+ were paired with the UCS in five out of eight trials (62.5% partial reinforcement rate). A third CS (CS-; e.g. blue light) was never paired with the UCS and shown 16 times. The two CS+ were presented in blocks: eight CS+E (or CS+U) trials intermixed with eight CS- trials were shown first, followed by eight CS+U (or CS+E) trials

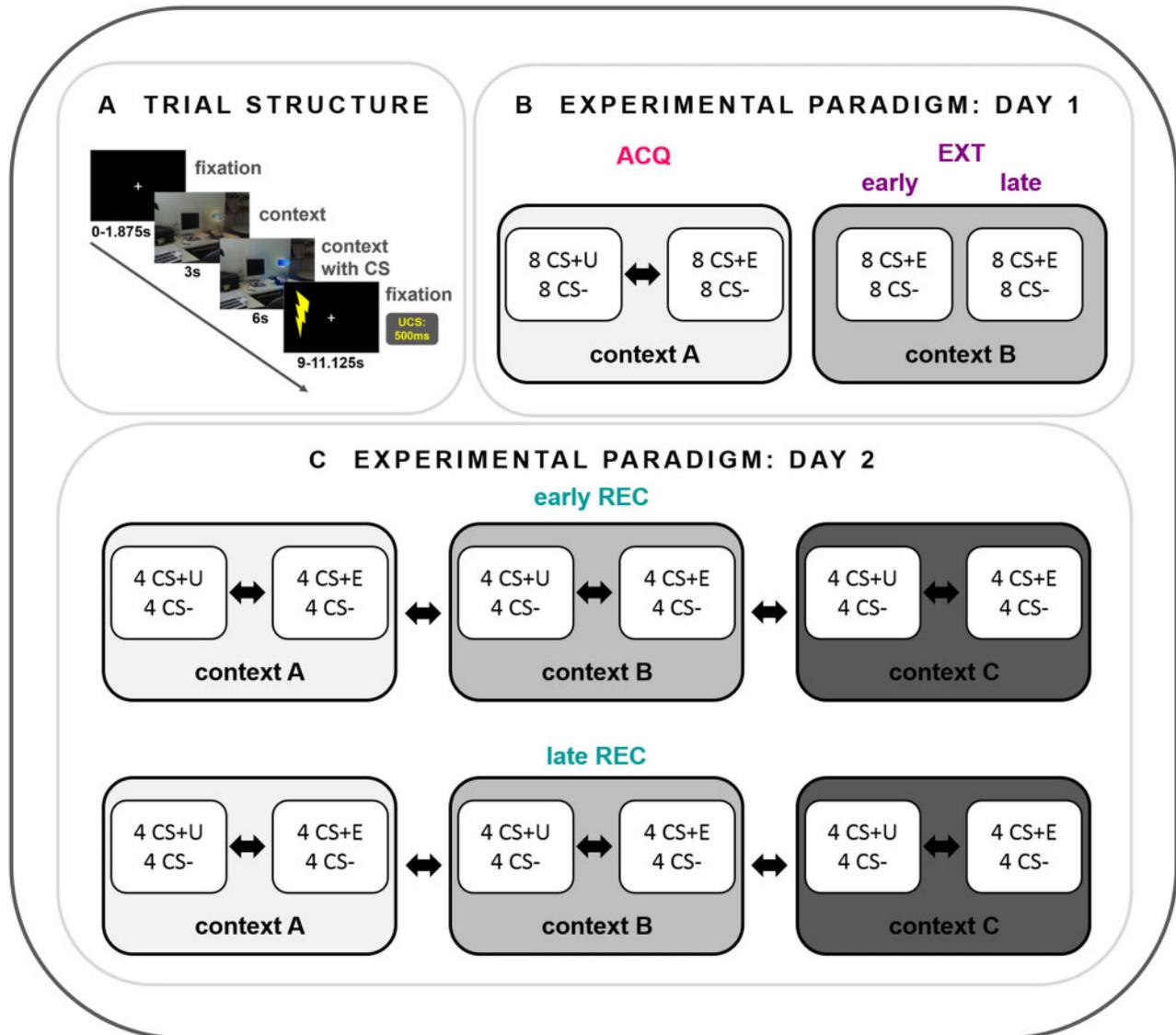


Fig. 1. (A) Description and timing of a typical trial structure (CS+ trial with UCS; CS- trial (not shown) without UCS). (B) Experimental paradigm on the first day (ACQ, fear acquisition; EXT, extinction). (C) Experimental paradigm on the second day (REC, recall). CS+E, CS+ extinguished; CS+U, CS+ unextinguished; CS-, CS minus; UCS, unconditioned stimulus; CS+U and CS+E block order as well as context order during recall was counterbalanced across subjects as indicated by double arrows.

intermixed with 8 CS- trials. The order of CS+E and CS+U block presentation was counterbalanced across subjects.

After a short break, extinction learning took place in context B. The CS+E (extinguished) was shown 16 times without subsequent UCS presentation in order to extinguish the conditioned fear response. The CS+U (unextinguished) was not shown during extinction. Sixteen CS- trials were presented intermixed with the 16 CS+E trials.

During the recall phase on day 2, all three CS (CS+E, CS+U, CS-) were presented to each participant in context A, B and C (within-subjects design). The recall phase was divided into two halves, each comprising half of the CS trials and each including all contexts. Again, presentation occurred in blocks, the first including four CS+U trials intermixed with four CS- trials within one context, followed by a block of four CS+E trials intermixed with four CS- trials within the same context (e.g. context A). The same was done for contexts B and C. The order of CS+U and CS+E blocks within the contexts as well as the

order of the contexts A, B and C (ABC, ACB, BAC, BCA, CAB, CBA) was counterbalanced across subjects. The second half of the recall phase comprised the same context and CS orders as the first. A pseudo-randomized stimulus order, in which no more than two consecutive presentations of the same CS were allowed, was used for all phases. During the extinction learning and recall phases, the electrodes for delivery of the electrical stimulation stayed attached to the fingers but did not provide electrical stimulation.

After recall on day 2, participants got out of the scanner and retrospectively indicated arousal, valence, fear and UCS expectancy for each of the context-CS combinations and the contexts without a CS (for details see Supplementary Materials).

Skin conductance responses and analyses

Skin conductance responses (SCRs) were sampled (sampling rate: 100 Hz) with an optical fiber SCR coupler built in-house.

Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium were placed on the hypothenar eminence of the left hand. No online filter was applied, raw SCR data were low-pass filtered afterwards with a cutoff frequency of 10 Hz. SCRs were defined as maximum amplitudes (using a foot-to-peak analysis) starting within a window of 1–6.5 s after CS onset. Range-correction of SCRs was accomplished by dividing the raw data by the largest response to the UCS (acquisition phase) to account for individual variability. One participant's electrodermal data had to be discarded because of an SCR coupler malfunction (this participant's data were not excluded from the main fMRI data analyses).

Statistical comparisons of mean SCRs were conducted separately for fear acquisition, extinction and recall via analysis of variance. For fear acquisition, the within-subjects factor CS (CS + E, CS + U, CS-) was entered; additionally, the factor time was introduced for extinction learning (early vs. late; each comprising eight trials for CS + E and CS-). For the recall phase, the within-subjects factors CS as well as context (A, B, C) and the between-subjects factor context order (ABC, ACB, BAC, BCA, CAB, CBA; possible confounding factor) were entered in order to test for differences in conditioned responding between contexts. Analysis of fear and extinction recall on day 2 was restricted to the first half of the recall phase (early recall) in order to capture actual fear and extinction 'recall' rather than re-extinction processes most likely to occur in the long run (first and second halves combined) of the recall phase.

All statistical analyses were performed in IBM SPSS Statistics for Windows 22.0 with Greenhouse-Geisser correction if needed (in this case adjusted degrees of freedom are reported in form of decimals), and the statistical significance level was set to $P \leq 0.05$. Significant main or interaction effects were followed by appropriate *post-hoc* tests. Results with a trend towards significance are reported up to $P \leq 0.10$.

FMRI data acquisition and analyses

Brain images were acquired using a 1.5 T whole-body tomograph (Siemens Symphony with a quantum gradient system) with a standard head coil. Structural image acquisition encompassed 160 T1-weighted sagittal images (MPRAGE; 1 mm slice thickness). For functional imaging, 287 volumes each for fear acquisition and extinction learning as well as 822 volumes for recall were registered using a T2*-weighted gradient echoplanar imaging sequence with 25 slices covering the whole brain (slice thickness = 5 mm; 1 mm gap; descending slice order; TA = 100 ms; TE = 55 ms; TR = 2.5 s; flip angle = 90°; field of view = 192 × 192 mm²; matrix size = 64 × 64 pixel). The first three volumes of each session were discarded because of an incomplete steady state of magnetization. The axial slices were oriented parallel to the orbitofrontal cortex-bone-transition to minimize susceptibility artifacts in prefrontal areas. A gradient echo field map sequence was measured before all functional runs to get information for the unwarping of B₀ distortions.

All imaging data were analyzed using Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK, 2009) implemented in MatLab R2012a (Mathworks Inc., Sherborn, MA). We included the following preprocessing steps for all three sessions separately: unwarping and realignment, slice time correction, co-registration of functional data to each participant's anatomical image, segmentation into gray and white matter, normalization to the standard space of the Montreal Neurological Institute (MNI) brain, and spatial smoothing (isotropic 3D Gaussian filter; FWHM: 9 mm).

Fear acquisition, extinction learning and recall were integrated as separate sessions in one first-level model in SPM8 including the following experimental conditions (for the respective phase when applicable): context alone, blocks of eight trials (acquisition, extinction) and four trials (recall) for CS + E and CS + U as well as eight trials of CS- (separately for each context during recall), UCS, UCS omission (after CS+ presentation), and non-UCS (after CS- presentation). All regressors were modeled based on a stick function convolved with the canonical hemodynamic response function in the general linear model, without specifically modeling the durations of the different events (i.e. event-related design). Covariates in the model comprised the six movement parameters from the realignment step. Furthermore, a high-pass filter (time constant = 128 s) was implemented. On the second level, random effects group analyses were conducted in SPM8 (one- and two-sample t-tests, full factorial design). The factor context order was implemented in the main comparisons of conditioned responding between contexts in the entire group to control for order effects.

We analyzed functional coupling of the fear and extinction network by conducting psychophysiological interaction (PPI) analyses for each subject. These explore the effective connectivity between a seed region and other brain areas in interaction with an experimental task (CS + E vs CS-). In our case, the hippocampus was entered as the seed region (volume of interest; 5 mm sphere around the peak voxel; see 'Results' section).

For all statistical analyses, we used exploratory whole brain as well as region of interest (ROI) analyses targeting the main structures of the fear and extinction circuitry (cf. Graham and Milad, 2011; Milad and Quirk, 2012; Maren et al., 2013): amygdala, dACC, hippocampus, insula and vmPFC (maximum probability masks; probability threshold set to 0.50; Harvard-Oxford Cortical and Subcortical Structural Atlases, Harvard Center for Morphometric Analysis; http://www.cma.mgh.harvard.edu/fsl_atlas.html). The left and right dACC masks were created with the MARINA software package (Walter, 2002), and the vmPFC masks consisted of 8 mm spheres surrounding the two peak voxels for extinction-related neural responses in the vmPFC (MNI coordinates $x=2, y=40, z=-16$ and $x=6, y=50, z=-12$), as indicated in a meta-analysis of extinction studies (Diekhof et al., 2011). Regarding exploratory whole brain analyses, the significance threshold was set to $P \leq 0.05$ on voxel-level corrected for multiple testing [family-wise error (FWE) correction]; the minimal cluster size (k) was 10 voxels. For the ROI analyses, the significance threshold was set to $P \leq 0.05$ on voxel-level, corrected for multiple testing within each ROI (FWE-corrected; using the small volume correction option of SPM8). Results with a trend towards significance are reported up to $P_{\text{corr.}} \leq 0.10$.

Results

Fear acquisition

Fear acquisition was successful, as indicated by a significant differentiation of SCRs between the three CS [main effect CS: $F_{(1.9, 84.8)} = 36.54$; $P < 0.001$; $\eta^2 = 0.454$], due to higher SCRs to the CS + E [$T_{(44)} = 7.17$; $P < 0.001$; $\eta^2 = 0.539$; Figure 2] and CS + U [$T_{(44)} = 7.99$; $P < 0.001$; $\eta^2 = 0.592$] compared with the CS-, while the CS + E and CS + U did not differ [$T_{(44)} = 0.40$; $P > 0.68$].

On the neural level, enhanced differential conditioned activation was found in the bilateral insula, dACC, and at trend-level in the left amygdala, and reduced vmPFC activation was

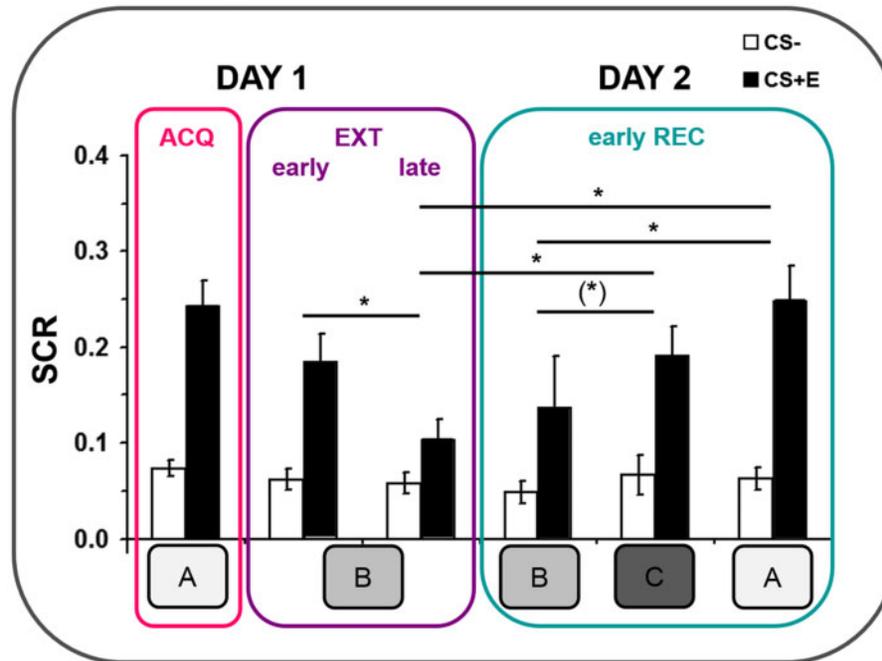


Fig. 2. Mean (\pm SE) SCRs for the CS + E (CS+ extinguished) and CS– during fear acquisition (ACQ) in context A on day 1, early and late extinction learning (EXT) in context B on day 1 as well as early recall (REC) in contexts B, C and A on day 2. CRs to CS+E and CS– differed significantly during each phase as well as in each context during recall. Significant differences ($P \leq 0.05$) in conditioned SCRs for main comparisons (conditioned responding in B during early vs late extinction, in each context during early recall, in each context during early recall compared with late extinction, in contexts C vs B and A vs B during early recall) are marked with *; trends up to $P \leq 0.10$ are marked with (*).

found for the combined CS+ compared with the CS– (Supplementary Table S1A).

Extinction learning

Differential conditioned SCRs (CS + E minus CS–) declined from early to late extinction learning (CS \times time interaction: $F_{(1,44)} = 10.09$; $P = 0.003$; $\eta^2 = 0.187$; main effect CS: $F_{(1,44)} = 19.28$; $P < 0.001$; $\eta^2 = 0.305$; main effect time: $F_{(1,44)} = 6.12$; $P = 0.017$; $\eta^2 = 0.122$), which was due to a significant reduction in responding from early to late extinction regarding the CS + E [$T_{(44)} = 2.98$; $P = 0.005$; $\eta^2 = 0.168$], but not the CS– [$T_{(44)} = 0.32$; $P > 0.75$; Figure 2], indicating successful extinction of conditioned fear.

During early extinction learning, activation of the insula and the dACC still remained significant for CS + E minus CS–, most likely representing fear expression. Activation of the dACC declined from early to late extinction learning. No significant activation was found during late extinction learning (Supplementary Table S1B–E).

Fear and extinction recall

During recall on the next day, the order of context presentations showed interactions with context (context \times context order: $F_{(8,7,67,7)} = 2.81$; $P = 0.008$; $\eta^2 = 0.265$) and with CS and context (CS \times context \times context order: $F_{(11,7,91,1)} = 1.72$; $P = 0.076$; $\eta^2 = 0.181$). These interactions with context order occurred when comparing contexts A and C as well as CS+U and CS– (see Supplementary Materials for more details). Due to these order effects, we restricted further SCR, rating and fMRI analyses to the comparison between CS + E and CS– in contexts B vs C and A vs B, which were not confounded by context order.

Extinction recall in the extinction context. Conditioned SCRs to the CS + E compared with the CS– in context B did not significantly increase from late extinction learning to early extinction recall [$F_{(1,39)} = 0.21$; $P = 0.65$], pointing to successful recall of the extinction memory. However, during early recall in context B (day 2), SCRs were still higher for the CS + E than for the CS– [$T_{(44)} = 2.86$; $P = 0.007$; $\eta^2 = 0.157$; see Figure 2].

Extinction recall in context B (contrast CS + E minus CS–) resulted in marginally significant stronger activation of the left hippocampus and significantly stronger activation of the left insula as well as reduced activation of the right amygdala and right hippocampus (Table 1A, Figure 3A).

In order to test for individual differences in the amount of extinction recall in context B, the total group was divided into two subgroups by means of a median split of conditioned SCRs (CS + E minus CS– in context B; MD = 0.02, SD = 0.16), leading to a high extinction recall group (HighExtRec; $n = 22$, lower than median; mean differential SCRs: $M = -0.03$, SD = 0.09) and a low extinction recall group (LowExtRec; $n = 23$, higher than or equal to median; mean differential SCRs: $M = 0.17$; SD = 0.16) which differed significantly in regard to conditioned SCRs [$T_{(34,3)} = 5.10$; $P < 0.001$]. The HighExtRec group showed significantly stronger activation in the vmPFC and in the left hippocampus during extinction recall (CS + E minus CS–) in context B compared with the LowExtRec group (Table 1B, Figure 3B).

Renewal in the novel context. Conditioned SCRs (CS + E minus CS–) tended to be higher in context C compared with B during early recall [$F_{(1,39)} = 3.23$; $P = 0.080$; $\eta^2 = 0.077$], and were significantly higher during early recall in context C compared with late extinction learning in context B [$F_{(1,39)} = 4.16$; $P = 0.048$; $\eta^2 = 0.096$], indicating fear renewal in the novel context C (Figure 2).

Additionally, stronger differential activation (contrast CS + E minus CS-) of the left hippocampus occurred in context B compared with C. The right hippocampus showed stronger activation in the reverse contrast (context C compared with B; Table 2A, Figure 4A). PPI analyses with these two hippocampal activation foci set as seed regions did not reveal any significant results within the entire group. Furthermore, there was no interaction with the factor context order in any ROI.

A comparison of activation in context C minus B during context-only presentations (3s before CS onset) showed enhanced activation in the right occipital cortex, insula, right hippocampus (trend), dACC and vmPFC, whereas marginally significant

Table 1. Activation differences for CS + E minus CS- during early recall in context B in the (A) entire group and (B) high extinction recall (HighExtRec) group compared with the low extinction recall (LowExtRec) group

Structure	x	Y	z	Z _{max}	P _{corr}
(A) Extinction recall in context B					
Activation for CS+E minus CS-					
L hippocampus	-27	-37	-2	3.13	0.056
L insula	-33	23	4	3.51	0.028
Activation for CS- minus CS+E					
R amygdala	30	-7	-17	2.95	0.046
R hippocampus	30	-13	-23	3.33	0.033
(B) Extinction recall in context B in the HighExtRec vs LowExtRec group					
HighExtRec minus LowExtRec group: activation for CS+E minus CS-					
L hippocampus	-27	-19	-23	3.19	0.047
vmPFC	0	44	-11	3.07	0.026
vmPFC	0	53	-11	2.98	0.033
LowExtRec minus HighExtRec group: activation for CS+E minus CS-					
No significant results					

The significance threshold was set to $P \leq 0.05$ (FWE-corrected; small volume correction). Trends up to $P_{\text{corr}} \leq 0.10$ are reported in italics. All coordinates (x, y, z) are given in MNI space. L, left; R, right.

enhanced activation was found in the right amygdala for the opposite contrast (see Supplementary Table S2A).

The median split for conditioned SCRs in contexts C vs B [(CS + E minus CS- in context C) minus (CS + E minus CS- in context B); MD = 0.00, SD = 0.23] revealed a HighREN C group ($n = 22$, higher than median; mean differential SCRs: $M = 0.21$, SD = 0.24) and a LowREN C group ($n = 23$, lower than or equal to median; mean differential SCRs: $M = -0.08$; SD = 0.10) which differed significantly from each other [$T_{(23,3)} = 4.73$, $P < 0.001$; $\eta^2 = 0.501$]. Comparing the HighREN C with the LowREN C group for conditioned responding (CS + E vs CS-) in contexts C vs B did not result in any significant activation differences (see Table 2A). However, the left hippocampus seed region (more strongly activated in B vs C in the entire group) showed stronger connectivity with the right dACC, bilateral insula and vmPFC in the HighREN C compared with the LowREN C group during fear renewal. Furthermore, the right hippocampus seed region (more strongly activated in C vs B in the entire group) exhibited stronger connectivity with the bilateral amygdala and hippocampus, as well as with the right dACC at trend level in the HighREN C compared with the LowREN C group during conditioned fear renewal (Table 2B, Figure 4B and C).

Renewal in the acquisition context. Differential conditioned SCRs were higher in context A during early recall compared with context B during early recall [$F_{(1,39)} = 5.93$; $P = 0.020$; $\eta^2 = 0.132$] and compared with late extinction learning [$F_{(1,39)} = 7.85$; $P = 0.008$; $\eta^2 = 0.168$], indicating fear renewal in context A (Figure 2).

Analyses of conditioned responding between contexts A and B did not result in any significant activation differences (Table 3A). Furthermore, there was no interaction with the factor context order in any ROI. A comparison of activation in context A minus B during context-only presentations (3 s before CS onset) showed stronger activation in the right insula and vmPFC (see Supplementary Table S2B), while an interaction with context order (possible confounding variable) was found for the left insula (MNI: $x = -33$, $y = -25$, $z = 13$; $Z_{\text{max}} = 3.59$; $P_{\text{corr}} = 0.036$).

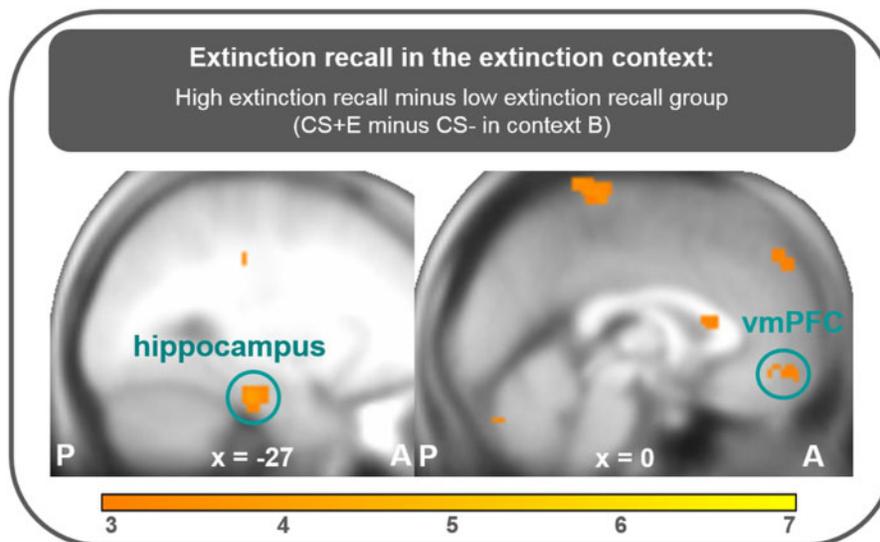


Fig. 3. Neural activation of differential conditioned responding (CS + E minus CS-) in the extinction context B in the high extinction recall ($n = 22$) minus low extinction recall group ($n = 23$) in the left hippocampus and in the vmPFC. The intensity threshold was set to $P = 0.0025$ (uncorrected) for illustration purposes; activations were superimposed on the MNI305 T1 template. All coordinates (x, y, z) are given in MNI space. The color bar depicts T-values. L, left; R, right; A, anterior; P, posterior.

Table 2. (A) Activation differences for CS+ E minus CS- during early recall in context C compared with B in the high renewal C (HighREN C) group compared with the low renewal C (LowREN C) group. Effective connectivity of the left (B) and right (C) hippocampus for CS+E minus CS- during early recall in context C compared with B in the entire group and in the HighREN C compared with the LowREN C group

Structure	x	y	z	Z _{max}	P _{corr}
(A) Activation in context C vs B (CS+E – CS–)					
Entire group: context C – B					
R hippocampus	30	-13	-23	3.29	.039
Entire group: context B – C					
L hippocampus	-33	-22	-14	3.28	.039
HighREN C vs LowREN C group					
no significant results					
(B) Effective left hippocampus connectivity in context C vs B (CS+E – CS–)					
Entire group					
No significant results					
HighREN C minus LowREN C group: context C minus B					
R dACC	12	-13	40	4.26	0.002
L insula	-42	-13	1	4.38	0.001
R insula	42	-13	4	4.16	0.003
vmPFC	0	47	-8	2.97	0.035
vmPFC	0	44	-11	3.31	0.023
LowREN C minus HighREN C group: context C minus B					
No significant results					
(C) Effective right hippocampus connectivity in context C vs B (CS+E – CS–)					
Entire group					
No significant results					
HighREN C minus LowREN C group: context C minus B					
L amygdala	-18	-13	-14	3.00	0.039
R amygdala	18	-10	-14	3.21	0.024
R dACC	15	-13	43	3.12	0.097
L hippocampus	-21	-16	-14	3.18	0.050
R hippocampus	21	-13	-17	3.23	0.045
LowREN C minus HighREN C group: context C minus B					
no significant results					

The significance threshold was set to $P \leq 0.05$ (FWE-corrected; small volume correction). Trends up to $P_{\text{corr}} \leq 0.10$ are reported in italics. All coordinates (x, y, z) are given in MNI space. L, left; R, right.

The median split for CS+E minus CS- in contexts A vs B [(CS+E minus CS- in context A) minus (CS+E minus CS- in context B); MD = 0.02, SD = 0.34] resulted in a HighREN A group ($n = 22$, higher than median; mean differential SCRs: $M = 0.35$, $SD = 0.39$) and a LowREN A group ($n = 23$, lower than or equal to median; mean differential SCRs: $M = -0.07$, $SD = 0.09$). It needs to be mentioned that the results for the different groups based on the median split for extinction recall, fear renewal in the novel context and fear renewal in the acquisition context differed from each other (e.g. participants in the HighREN A group were not necessarily those belonging to the HighREN C group). The HighREN A and LowREN A group significantly differed in conditioned electrodermal responding [CS+E vs CS-; $T_{(27.5)} = 5.26$; $P < 0.001$; $\eta^2 = 0.511$]. Individual differences in fear renewal concerning context A minus B (CS+E vs CS-) became apparent in the form of a stronger activation of the left amygdala, bilateral dACC, left hippocampus, bilateral insula and at trend level in the right amygdala and the right hippocampus in the HighREN A compared with the LowREN A group (Table 3B, Figure 5).

Discussion

The main goal of this study was to elucidate the role of the hippocampus and its interaction with crucial brain areas in the fear and extinction circuit for the renewal of conditioned fear in a novel context and the acquisition context. Along with context-dependent recovery of conditioned SCRs, hippocampal activation differentiated conditioned responding in the novel from that in the extinction context. Additionally, enhanced effective connectivity of the hippocampus with important structures in the fear and extinction network characterized individuals with a stronger renewal of conditioned SCRs in the novel context. Enhanced recovery of conditioned SCRs in the acquisition context was related to enhanced activation of relevant structures in the fear circuit.

In line with previous findings concerning extinction recall in the safe extinction context (Kalisch et al., 2006; Milad et al., 2007), the left hippocampus and the vmPFC also showed stronger activation in this study in individuals with stronger extinction recall as indicated by reduced conditioned SCRs. These findings support the role of the hippocampus in contextual modulation and that of the vmPFC in the inhibition of conditioned fear expression via the central amygdala (Quirk and Mueller, 2008). This reduced fear expression might also be reflected in the diminished activation of the right amygdala during extinction recall in our study; this, however, has not been found in previous studies (Phelps et al., 2004; Kalisch et al., 2006; Milad et al., 2007). Decreased amygdala activation during recall is also in line with differential functions of distinct amygdala subnuclei in the expression and inhibition of conditioned fear during recall (cf. Quirk and Mueller, 2008). Accordingly, this deactivation might reflect suppression of conditioned fear output via the central amygdala.

Enhanced renewal of conditioned SCRs in the novel context compared with extinction recall and late extinction learning in the extinction context is in line with previous findings (Neumann and Kitlertsirivatana, 2010; Balooch et al., 2012; but see Effting and Kindt, 2007). Correspondingly, hippocampal activation differentiated conditioned responding in the novel context from the extinction context, emphasizing the important role of the hippocampus in conditioned fear renewal, as indicated by previous studies in animals (Maren et al., 2013) and humans (Kalisch et al., 2006).

In line with a previous fMRI study investigating conditioned fear renewal in the acquisition context (Kalisch et al., 2006), the left hippocampus showed stronger activation in the extinction compared with the novel context. Right hippocampal activation was diminished in the extinction but not in the novel context, a finding that is in accordance with conditioned right hippocampal activation in the acquisition context observed previously by Kalisch et al. (2006). Altogether, these findings provide first evidence of a hippocampal hemispheric specialization in conditioned fear renewal in humans. Studies in mice have also indicated that a hemispheric specialization of hippocampal subregions exists, showing that left but not right hippocampal CA3-CA1 synapses were involved in associative spatial long-term memory processes (Shipton et al., 2014; for an overview see El-Gaby et al., 2015). This corresponds with our results indicating a prominent role of left hippocampal activation in the contextual modulation of extinction recall in the safe extinction context.

Further findings suggest a different functionality of anterior vs posterior areas of the hippocampus, with a stronger amount of amygdala-projecting neurons in the anterior hippocampus in primates for instance (for an overview see Strange et al., 2014).

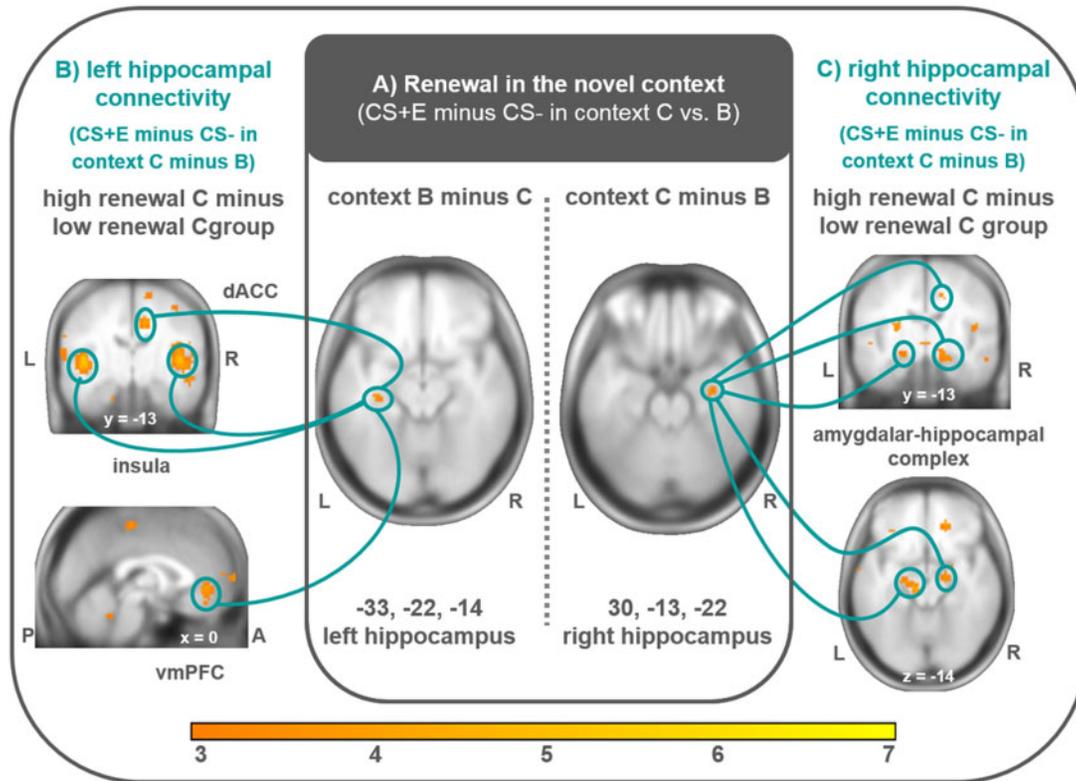


Fig. 4. Neural activation and connectivity for differential conditioned responding [CS + E (extinguished CS+) minus CS–] in the novel context C compared with the extinction context B. **(A)** Significantly stronger left hippocampal activation in context B compared with C and right hippocampal activation in context C compared with B. **(B)** Left hippocampal connectivity with bilateral insula, left vmPFC and right dACC during conditioned fear renewal (CS+E minus CS– in context C compared with B) in the high renewal C vs low renewal C group. **(C)** Right hippocampal connectivity with bilateral amygdala, hippocampus and right dACC (marginally significant) during conditioned fear renewal (CS+E minus CS– in context C compared with B) in the high renewal C vs low renewal C group. The intensity threshold was set to $P = 0.0025$ (uncorrected) for illustration purposes; activations were superimposed on the MNI305 T1 template. All coordinates (x, y, z) are given in MNI space. The color bar depicts T-values. L, left; R, right; A, anterior; P, posterior.

This subregional specificity fits the finding of a more anterior activation of the right hippocampus in response to the novel context and a more posterior activation of the left hippocampus towards the extinction context in the present study. However, the opposite anterior–posterior dissociation during fear renewal in the acquisition context has also been reported in humans (Kalisch *et al.*, 2006). Importantly, the applied anterior–posterior localization of hippocampal areas is somewhat descriptive and should therefore be interpreted with caution. As a result, it might not adequately match the localization of other findings in humans, the anterior–posterior axis in primates, or the ventral–dorsal axis in rats (Strange *et al.*, 2014).

Findings in rodents indicate that renewal in a novel context is mediated by direct projections from the hippocampus to the amygdala as well as by indirect projections to the amygdala via the prelimbic (PL; homologue of the human dACC) and infralimbic (IL; homologue of the human vmPFC; Orsini *et al.*, 2011) cortices. In this study, the left hippocampal area (showing stronger activation in context B compared with C in the entire group) exhibited stronger connectivity with the dACC, insula and vmPFC in individuals with high compared with low renewal of conditioned SCRs. The dACC (PL) most likely exerts its prominent role in the expression of conditioned fear via direct influences on the basolateral amygdala (Milad *et al.*, 2007; Quirk and Mueller, 2008) and is suggested to be involved in the long-term storage and retrieval of contextual memories (Maren *et al.*, 2013). The insula is connected to both the amygdala and the dACC (Augustine, 1996) and might be involved in identifying

Table 3. Activation differences for CS + E minus CS– during early recall in context A compared with B in the entire group and in the high renewal A (HighREN A) group compared with the low renewal A (LowREN A) group

Structure	x	y	z	Z_{\max}	P_{corr}
Activation in context A vs B (CS+E minus CS–)					
Entire group					
No significant results					
HighREN A minus LowREN A group: context A minus B					
L amygdala	–15	–10	–14	3.32	0.015
R amygdala	27	–4	–20	2.89	0.053
L dACC	0	14	28	4.05	0.005
R dACC	3	14	28	4.12	0.004
L hippocampus	–18	–19	–17	3.98	0.004
R hippocampus	27	–34	–2	3.02	0.075
L insula	–39	–7	10	3.80	0.011
R insula	39	–7	4	4.07	0.004
LowREN A minus HighREN A group: context A minus B					
No significant results					

The significance threshold was set to $P \leq 0.05$ (FWE-corrected; small volume correction). Trends up to $P \leq 0.10$ are reported in italics. All coordinates (x, y, z) are given in MNI space. L, left; R, right.

emotionally significant information (Phillips *et al.*, 2003). In contrast, the vmPFC (IL) projects to inhibitory intercalated neurons in the amygdala, thereby diminishing fear output via an inhibition of the central amygdala (Quirk *et al.*, 2003; Milad and Quirk,

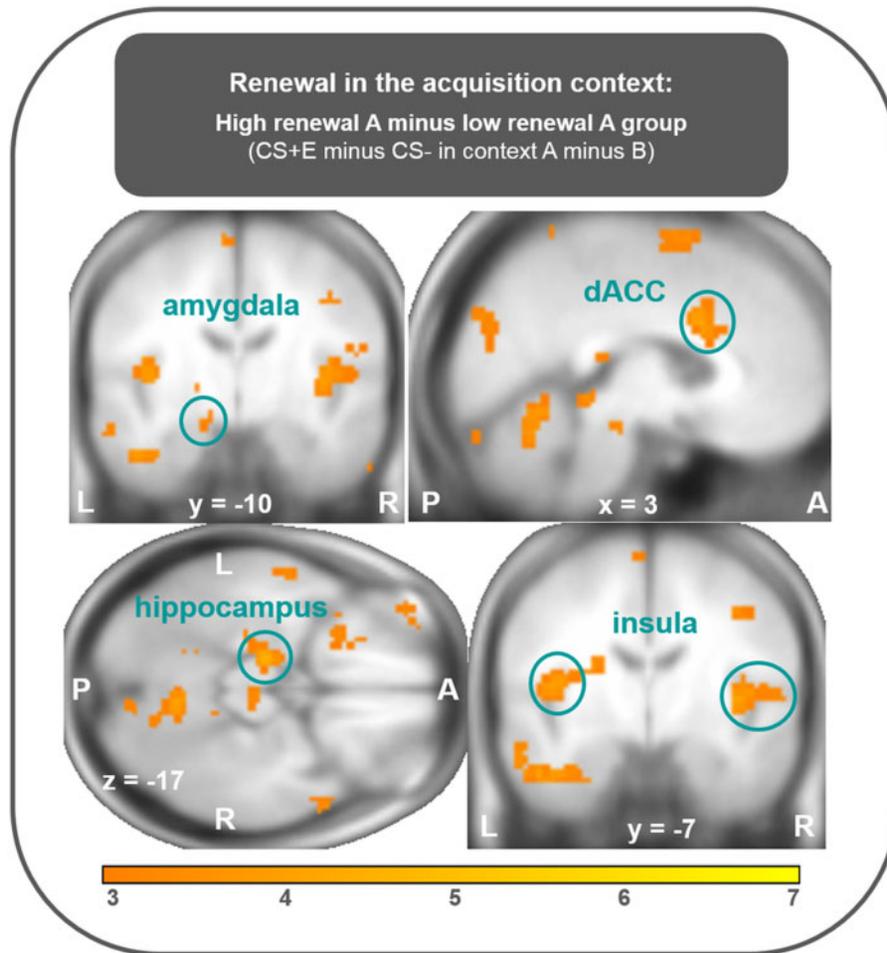


Fig. 5. Neural activation of differential conditioned responding (CS + E minus CS-) in context A compared with context B in the high renewal A vs low renewal A group in the left amygdala, right dACC, left hippocampus and bilateral insula. The intensity threshold was set to $P = 0.0025$ (uncorrected) for illustration purposes; activations were superimposed on the MNI305 T1 template. All coordinates (x, y, z) are given in MNI space. The color bar depicts T-values. L, left; R, right; A, anterior; P, posterior.

2012). The right hippocampus (showing reduced activation in context B compared with C in the entire group) was more strongly connected to the bilateral amygdala and hippocampus in participants with a stronger renewal of conditioned SCRs. This connectivity pattern is also in line with the abovementioned subregional (anterior/posterior) specialization of the hippocampus. Enhanced connectivity of the right anterior but not the left posterior hippocampus with the bilateral amygdala and the left hippocampus is in line with stronger projections from the anterior hippocampus to the amygdala (Strange et al., 2014). In light of the diminished activation of the right anterior hippocampus during recall in the safe extinction context, it could be speculated that a reduced suppression of its activation and connectivity is related to enhanced conditioned fear expression in non-safe contexts. However, it needs to be emphasized that these results are speculative and need to be examined in future studies.

All in all, the connectivity results correspond very well with the aforementioned neurobiological model of fear renewal (Milad and Quirk, 2012; Maren et al., 2013), pointing to the importance of direct hippocampal-amygdalar as well as hippocampal-dACC/insular/vmPFC connections for fear renewal in an unfamiliar context in humans. Overall, the hippocampus seems to be a detector of safe compared with novel contexts during extinction recall, while the strength of renewal in the novel

context is determined by hippocampal connectivity with further important structures in the fear and extinction circuit.

In accordance with previous studies (Neumann and Longbottom, 2008; Zeidan et al., 2011), exposure to the original acquisition context, compared with the extinction context, led to a stronger renewal of conditioned SCRs in fear-relevant structures (amygdala, dACC, hippocampus, insula), but only in individuals which also showed stronger renewal of conditioned SCRs in the acquisition context. This differs critically from the finding that renewal in a novel context resulted in differential hippocampal activation in the entire group, and thus translates into a distinct connectivity in persons exhibiting a stronger renewal in SCRs in the novel context. These results indicate that different mechanisms might underlie renewal in the acquisition context, in which the context is known, as opposed to renewal in a novel context, in which the extinction memory needs to be transferred to an unknown context. Findings in animals and humans suggest that extinction learning leads to the development of a new inhibitory CS-noUCS memory trace which is specific to the extinction context (Bouton, 1994; cf. Vervliet et al., 2013). During recall, this extinction memory is assumed to compete with the excitatory CS-UCS fear memory resulting from fear acquisition which is supposed to be less context-specific. Accordingly, renewal in the acquisition context might result from reduced inhibition of the fear memory (as observed during

recall in the extinction context) and/or from an excitatory effect of the acquisition context on the activation of the fear memory. In a novel context, in contrast, conditioned responding might only be associated with reduced inhibition, and the activation of the fear memory might rely more strongly on further factors such as individual differences, possibly indicated by the connectivity findings in our study.

Apart from CS processing, context processing was gated by the insula and vmPFC for context A minus B, and the insula, dACC and vmPFC for context C minus B, indicating enhanced responding of relevant structures in the fear and extinction network towards contextual cues signaling safety or uncertainty. Enhanced activation of the dACC in the novel context is in line with enhanced dACC activation in the safe extinction context going along with enhanced conditioned fear expression in post-traumatic stress disorder patients (Rougemont-Bücking et al., 2011).

Limitations of this study comprise the questionable transferability of findings to females, especially regarding the impact of sex hormones on (context-dependent) learning processes (Lebron-Milad and Milad, 2012; van Ast et al., 2012). Additionally, the brain circuits involved in longer-term fear renewal remain to be studied.

In summary, the results of the current study demonstrate the involvement of hippocampal activation and connectivity as well as the possible relevance of subregional/hemispheric specificity in conditioned fear renewal towards a novel context compared with extinction recall in the safe extinction context in humans. These findings might contribute to an improved understanding of difficulties in generalizing therapy effects over time and contexts.

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Supplementary data

Supplementary data are available at SCAN online.

Conflict of interest. None declared.

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