◆ Human Brain Mapping 36:3179–3193 (2015) ◆

Neural Circuitry Underlying Effects of Context on Human Pain-Related Fear Extinction in a Renewal Paradigm

Adriane Icenhour,[1] Joswin Kattoor,[1] Sven Benson,[1] Armgard Boekstegers,[1] Marc Schlamann,[2] Christian J. Merz,[3] Michael Forsting,[2] and Sigrid Elsenbruch[1]*

[1]*Institute of Medical Psychology & Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany*
[2]*Institute of Diagnostic and Interventional Radiology and Neuroradiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany*
[3]*Department of Biological and Clinical Psychology, University of Trier, Trier, Germany*

Abstract: *Objectives:* The role of context in pain-related extinction learning remains poorly understood. We analyzed the neural mechanisms underlying context-dependent extinction and renewal in a clinically relevant model of conditioned abdominal pain-related fear. *Experimental design*: In this functional magnetic resonance imaging study, two groups of healthy volunteers underwent differential fear conditioning with painful rectal distensions as unconditioned stimuli (US) and visual conditioned stimuli (CS^+; CS^-). The extinction context was changed in an experimental group (context group), which was subsequently returned into the original learning context to test for renewal. No context changes occurred in the control group. Group differences in CS-induced differential neural activation were analyzed along with skin conductance responses (SCR), CS valence and CS-US contingency ratings. *Principal observations*: During extinction, group differences in differential neural activation were observed in dorsolateral (dlPFC) and ventromedial (vmPFC) prefrontal cortex and amygdala, mainly driven by enhanced activation in response to the CS^- in the control group. During renewal, observed group differences in activation of dlPFC and orbitofrontal cortex (OFC) resulted primarily from differential modulation of the CS^- in the absence of group differences in response to CS^+ or SCR. *Conclusion:* The extinction context affects the neural processing of nonpain predictive safety

Additional Supporting Information may be found in the online version of this article.

Author contributions: A.I., J.K., A.B., M.S. performed the research; S.E., S.B., M.F. designed the research study; A.I., S.B., C.M. analyzed the data; A.I. and S.E. wrote the paper; S.E. acquired funding; all authors contributed to the interpretation of the data, revised the manuscript, and approved the final version of the manuscript.
Contract grant sponsor: German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), grant number: FOR 1581.

*Correspondence to: Prof. Dr. S. Elsenbruch; Institute of Medical Psychology & Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55, 45147 Essen, Germany. E-mail: sigrid.elsenbruch@uk-essen.de

Received for publication 14 October 2014; Revised 20 April 2015; Accepted 27 April 2015.
DOI: 10.1002/hbm.22837
Published online 9 June 2015 in Wiley Online Library (wileyonlinelibrary.com).

cues, supporting a role of safety learning in pain-related memory processes. *Hum Brain Mapp 36:3179–3193, 2015.*

Key words: fear conditioning; extinction learning; renewal effect; visceral pain; brain imaging; fMRI

INTRODUCTION

Pavlovian fear conditioning, as a translational model in the behavioral neurosciences, has not only provided important insight into the neural mechanisms underlying the formation of fear memories, but has also pointed to both the complexity and clinical relevance of extinction learning [Milad and Quirk, 2012]. Extinction is not simply the erasure of a learned association, but a complex process involving the acquisition of a new, inhibitory memory trace which is mediated by a network of brain areas encompassing prefrontal cortex, amygdala, and hippocampus [Quirk and Mueller 2008]. The context-dependency of extinction learning has been demonstrated in animal and human studies [Bouton, 2004; Maren et al., 2013; Quirk and Mueller, 2008]. One of the most impressive examples from the field of fear conditioning is the return of previously extinguished fear due to a context change after extinction [Bouton, 2004]. This phenomenon, which has been termed renewal effect, has sparked mechanistic work within the behavioral neurosciences, as it provides important insight into the mechanisms mediating human fear extinction [Bouton, 2004; LaBar and Phelps, 2005; Milad et al., 2005; Vansteenwegen et al., 2005]. At the same time, the clinical relevance of renewal is increasingly appreciated as a putative mechanism contributing both to the chronicity of symptoms and to relapse following extinction-based treatments such as exposure therapy in anxiety disorders [Bouton, 2002]. The neural basis underlying the contextual influences on extinction and renewal is only beginning to be understood in humans. First brain imaging studies have emerged which have implemented fear conditioning with contextual manipulations in healthy volunteers [Kalisch et al., 2006; Milad et al., 2007] and patients with anxiety-related psychiatric conditions [Milad et al., 2008; Rougemont-Bucking et al., 2011]. Nevertheless, further insight into the neural circuitry involved in context-dependent extinction and renewal is needed and likely relevant beyond anxiety disorders. Indeed, anxiety symptoms are not only highly comorbid with chronic pain, but both anxiety and pain-related fear likely contribute to the development and maintenance of chronic pain states [Asmundson and Katz, 2009; Asmundson and Taylor, 1996]. Pain-related fear reportedly constitutes a strong predictor of disability in various chronic pain conditions, well in line with fear avoidance models of chronic pain [Crombez et al., 1999]. These models suggest that particularly the threat value of pain as well as the tendency to catastrophize painful experiences are closely associated with conditioned pain-related fear and comprise key factors in the vicious circle that ultimately leads to chronic pain and disability [De Peuter et al., 2011]. Consistently, alterations in Pavlovian fear conditioning and extinction have repeatedly been reported in several chronic pain conditions [Icenhour et al., 2015; Klinger et al., 2010; Labus et al., 2013; Meulders et al., 2015; Nees et al., 2010; Schneider et al., 2004]. Importantly, fear conditioning studies addressing pain perception and processing could demonstrate that conditioned fear of pain does not only impact anticipatory responses, but that these learned emotional responses may substantially alter pain processing itself [Flor et al., 2002; Miguez et al., 2014; Williams and Rhudy, 2007]. Furthermore, individual differences in pain-related fear appear to mediate neural responses to painful stimuli, indicating its crucial involvement in alterations of central pain processing [Ochsner et al., 2006]. Finally, cognitive-behavioral treatment approaches encompassing extinction-based interventions aiming to reduce pain-related fear have proven effective also in chronic pain conditions, underscoring the relevance of pain-related fear learning and extinction in chronic pain [Craske et al., 2011; De Peuter et al., 2011; den Hollander et al., 2010; Ljotsson et al., 2014]. Therefore, investigating the neural underpinnings and the specificity of pain-related fear learning and memory processes may substantially extend existing knowledge from classic fear conditioning paradigms.

Abbreviations

ANOVA	analysis of variance
BA	Brodman area
BMI	body mass index
BOLD	blood oxygen-level dependent
CS	conditioned stimulus
dlPFC	dorsolateral prefrontal cortex
EIR	entire interval response
fMRI	functional magnetic resonance imaging
FPC	frontopolar cortex
FWE correction	family-wise error correction
IBS	irritable bowel syndrome
MCC	midcingulate cortex
OFC	orbitofrontal cortex
ROI	region-of-interest
SCR	skin conductance response
SEM	standard error of the mean
US	unconditioned stimulus
VAS	visual analogue scale
vlPFC	ventrolateral prefrontal cortex
vmPFC	ventromedial prefrontal cortex

In this line of emerging knowledge regarding pain-related fear and its extinction, the putative role of the extinction context remains incompletely understood. Recent data addressing fear of movement-related pain support that the motivational quality of the extinction context impacts extinction of learned pain-related fear, indicating potential contextual influences on extinction-based treatment efficacy also in chronic pain patients [Volders et al., 2014]. Evidence from placebo research also underscores contextual factors in shaping pain processing and central pain modulation [Carlino et al., 2014]. However, the neural mechanisms underlying the sensitivity of extinction to context changes are essentially unknown in the field of visceral pain.

Our line of experimental work focusses on the putative role of conditioned abdominal pain-related fear in the pathophysiology of chronic visceral pain such as in irritable bowel syndrome (IBS). To address the neural mechanisms mediating fear learning and extinction in a clinically relevant model of visceral pain [Keszthelyi et al., 2012; Mayer et al., 2008], we have established differential fear conditioning with rectal distensions as interoceptive unconditioned stimuli (US) and predictive visual cues as conditioned stimuli (CS^+; CS^-) [Kattoor et al., 2013]. As a result of conditioning, the CS^+ as a formerly neutral stimulus comes to elicit negative emotions and activates fear-arousal circuitry, consistent with its threat value. In parallel, the CS^- acquires a positive valence, indicative of its property to signal safety from pain [Kattoor et al., 2013]. In the present functional magnetic resonance imaging (fMRI) study, we aimed to address if extinction of conditioned threat and safety cue properties is sensitive to the extinction context. In addition, we tested renewal of extinguished pain-related memories. To do so, healthy volunteers initially underwent differential delay conditioning. During subsequent extinction, CSs were presented in the absence of US in a new context in an experimental group, whereas no context change occurred in a control group. Renewal in response to continued CS presentations was then tested by a return of the experimental group to the original learning context. We hypothesized that a context change affects learning processes of new predictive properties during extinction, centrally involving ventromedial prefrontal cortex, hippocampus and amygdala. We explored renewal by testing a return of previously extinguished pain-related memories, evidenced by differential skin conductance responses and differential activation of brain structures mediating the formation and reactivation of conditioned fear, especially amygdala and hippocampus.

MATERIALS AND METHODS

Participants

Forty-eight healthy volunteers (24 male, 24 females, mean age 29.87 ± 10.84 years) were recruited by local advertisement. Recruitment procedures included a structured telephone screening followed by a personal interview during which standardized study-related information was provided, screening questionnaires were completed, and informed consent was acquired. Participants were informed that the study goal was to investigate the neural mechanisms of visceral pain-related fear learning and memory processes. They were told that they would see visual signals and experience rectal distensions, but no information was given about experimental phases, changes of CS-US contingencies or contextual manipulations. Exclusion criteria included age <18 or >60 years, body mass index (BMI) <18 or >30 the usual MRI-related criteria (e.g., claustrophobia, ferromagnetic implants), any known medical condition including gastrointestinal, neurological, psychiatric, or endocrinological conditions, or chronic medication use (except hormonal contraceptives, hormone replacement therapy, thyroid medications, or occasional use of over-the-counter allergy or pain medications). The German version of the Hospital Anxiety and Depression Inventory [HADS; Herrmann-Lingen et al., 2005] was implemented as a screening tool for current anxiety or depression symptoms. Additionally, trait anxiety was assessed utilizing the trait version of the State Trait Anxiety Inventory [STAI-T; Laux et al., 1981]. Symptoms suggestive of any functional or organic gastrointestinal condition were ruled out based on a standardized in-house questionnaire [Lacourt et al., 2014]. All participants were right-handed, assessed with a validated questionnaire on motor asymmetries [Reiss and Reiss, 2000]. Pregnancy was ruled out with a commercially available urinary test on the day of the fMRI study. Any previous participation in a conditioning study was also exclusionary. Evidence for structural brain abnormalities from structural MRI led to exclusion. All participants were evaluated digitally for perianal tissue damage (i.e., painful haemorrhoids) which could interfere with balloon placement. The study protocol was approved by the local ethics committee (protocol number 10-4493). All participants gave informed written consent and received 150 €as expense allowance for their participation.

Rectal Distensions

Painful rectal distensions, which served as clinically relevant visceral US herein, constitute a valid and reliable experimental model for the investigation of visceral pain processing [Keszthelyi et al., 2012; Mayer et al., 2008]. These were accomplished with a pressure-controlled barostat system (modified ISOBAR 3 device, G & J Electronics, ON, Canada), as previously described [Benson et al., 2014; Elsenbruch et al., 2010a, 2010b, 2012; Icenhour et al., 2015; Kattoor et al., 2013]. Given high interindividual variations in rectal pain sensitivity in healthy volunteers [Elsenbruch et al., 2014], individualized distension pressures were chosen for US presentation during acquisition. For this, just prior to the initiation of scanning, double-random staircase

distensions with random pressure increments of 2–8 mm Hg and 30-s durations with a maximal distension pressure of 50 mm Hg were delivered. Participants were asked to rate each sensation on a Likert-type scale labelled 1 = no perception, 2 = doubtful perception, 3 = sure perception, 4 = little discomfort, 5 = severe discomfort, still tolerable distension and 6 = pain, not tolerable distension. Pain thresholds were defined as pressures when ratings changed from 5 to 6. Subsequently, participants were prompted to rate pain intensities of pressures just below individual thresholds on a 0–100 mm visual analogue scale (VAS) with endpoints labelled "not painful at all" and "very painful". Pressures corresponding to US intensities between 60 and 70 were chosen for US presentation during acquisition and VAS ratings of pain intensity were assessed at the conclusion of acquisition to confirm moderately painful US.

Experimental Design and Study Procedures

All testings with an overall duration of 90 min were conducted between 16:00 and 19:00 h to control for possible circadian rhythm effects. For feasibility reasons, scheduling of the fMRI study did not control for menstrual cycle phase in naturally cycling female participants ($N = 7$). Following a structural MRI, blood oxygen level dependent (BOLD) responses were acquired using event-related fMRI during three consecutive scanning phases, separated by VAS ratings, assessing (1) visceral pain-related fear conditioning (i.e., acquisition), (2) extinction, and (3) renewal test (Supporting Information Fig. S1). Volunteers were randomly assigned to either an experimental group (context group) or a control group while matching the groups for equal number of males and females. (1) Both groups initially underwent an identical acquisition phase (S1 A and B). Herein, one visual cue (CS^+) was repeatedly followed by a painful rectal distension (US; duration 14 s) while a second cue (CS^-) was presented unpaired (differential delay conditioning). A total of 32 CSs were shown (16 CS^+; 16 CS^-) in pseudo-randomized order and 12 out of the 16 CS^+ were paired with a US (i.e., 75% reinforcement schedule). The US onset varied randomly between eight and twelve seconds after CS^+ onset and both stimuli coterminated. A variable jittering image acquisition technique was implemented to improve temporal resolution [Amaro and Barker, 2006]. Based on our previous work [Kattoor et al., 2013; Benson et al., 2014; Gramsch et al., 2014], varying delays between CS^+ and US presentation as well as intermittent reinforcement were chosen to induce uncertainty and generate more robust conditioned responses [Kalisch et al., 2006; Sehlmeyer et al., 2009]. Intertrial intervals (ITI) were 20 s. (2) During the extinction phase, only visual cues (6 CS^+; 6 CS^-) were presented in the absence of US. To assess context effects on extinction, the extinction context was manipulated in the context group (Supporting Information Fig. S1 B), operationalized by changed CS background color and corresponding room illumination (Supporting Information Fig. S1 C and D). A context manipulation utilizing background colors has previously been implemented in fMRI studies to investigate contextual learning and memory [Kalisch et al., 2006; Lang et al., 2009]. In the control group, the extinction context remained unchanged (Supporting Information Fig. S1 A). (3) During the final test phase, only CSs (6 CS^+; 6 CS^-) were presented to both groups while no US were delivered. To assess the reactivation of extinguished fear memories (i.e., renewal effect), the context group was returned to the original learning context and compared to the control group who remained in the same learning context throughout all phases. Background and room colors as well as visual CS^+ and CS^- were counterbalanced across subjects.

At different time points, online VAS ratings of CS valence, CS-US contingencies and US painfulness were accomplished using an MRI-compatible hand-held fiber optic response system (LUMItouch™, Photon Control Inc., Burnaby, BC, Canada). At baseline and at the conclusion of each phase, participants responded to the question "How do you perceive the circle/square?" on a VAS with "neutral" indicated in the middle of the scale and endpoints labelled "very pleasant" and "very unpleasant" to address CS valence. In addition, contingency awareness was assessed following each phase by prompting participants to respond to the question "How often was the circle/square followed by a rectal distension?" on a VAS with the endpoints "never" and "always". To ensure that all participants had acquired pain-related fear as a prerequisite for investigating subsequent extinction and fear memory reactivation processes, differentially acquired aversion was defined as an inclusion criterion for further analyses. Therefore, valence ratings as indicators of learned emotional aversion in response to CS^+ were critically inspected in an initial blinded analysis. Ratings from nine participants indicated a lack of fear memory formation (i.e. CS^+ being perceived as more pleasant after acquisition compared to baseline) which led to exclusion for further analyses. This resulted in a final sample of 16 participants in the control group (eight males, eight females) and 23 participants in the context group (twelve males, eleven females). Of note, supplementary analyses were carried out to (a) address SCR in excluded subjects and (b) show all BOLD analyses in the whole sample without excluded participants (see result section for details).

Skin Conductance Responses

Online skin conductance responses (SCR) were recorded from electrodes placed on the thenar and hypothenar of the nondominant hand using an MR-compatible recording system (Biopac Systems, Inc., Goleta, CA, USA). After the raw data was high-pass filtered at 0.05 Hz, analysis of CS-specific SCR was accomplished using AcqKnowledge

Software (Biopac). Analyses included the highest amplitude during the anticipation phase during which CS only were presented (entire interval response; EIR) as previously recommended for long duration CS [Pineles et al., 2009] with a latency of 1 second, interval lengths between 7 and 11 seconds and an SCR threshold of 0.01 microsiemens (μS) [Boucsein et al., 2012; Pineles et al., 2009]. Although there may be other conventions of equal validity for scoring SCR, e.g. separating first and second interval responses [Tabbert et al., 2011; Vansteenwegen et al., 2005], we chose the EIR as an approach making ideal use of the data acquired and reducing the vulnerability for type II errors by falsely omitting valid responses when limiting data analyses to a predefined time window [Milad et al., 2007]. Based on this rationale, variable time windows according to the actual CS presentation length were analyzed instead of fixed intervals of 7 s when based on the shortest CS duration. These time windows would have likely rather encompassed orientating responses while conditioned SCR reportedly occurring at later phases of anticipation would have not met criteria for SCR scoring. To extract SCR to CS^+ and CS^-, inflexion points were automatically detected and manually controlled utilizing the software EDA-Bio (1.98; Schäfer, unpublished data). The skin conductance level immediately preceding the inflexion point served as a baseline as previously described [Tabbert et al., 2011]. Amplitudes with peaks exceeding threshold and exhibiting half-time recovery within the defined time window were considered SCR. Before conducting statistical analyses, log-transformation was performed in order to normalize data [Boucsein et al., 2012]. Note that skin conductance data from one participant of the context group had to be excluded due to technical difficulties resulting in a final sample of $N = 22$ participants in the context and $N = 16$ in the control group for SCR analyses.

Statistical Analysis of non-fMRI Data

Statistical analyses of non-fMRI data were computed with IBM SPSS Statistics 21.0 (IBM Corporation, Armonk, NY). Initially, normal distribution of the data was tested using Kolmogorov–Smirnov test. Repeated measures analyses of variance (RM-ANOVA) were computed with Greenhouse-Geisser correction where indicated, followed by post hoc *t*-tests with Bonferroni correction for multiple comparisons. The alpha level for accepting statistical significance was set at $P < 0.05$. All non-fMRI data are shown as mean ± standard error of the mean (SEM) unless indicated otherwise.

Brain Imaging and Analyses

Structural and functional MRI data were acquired on a 3 Tesla scanner with a 32-channel head coil (Skyra, Siemens Healthcare, Erlangen, Germany). For structural images, a 3D-MPRage T_1-weighted sequence (TR 1900 ms, TE 2.13 ms, flip angle 9°, FOV 239 × 239 mm^2, 192 slices, slice-thickness 0.9 mm, voxel size 0.9 × 0.9 × 0.9 mm^3, matrix 256 × 256 mm^2, GRAPPA r=2) was acquired. Blood oxygen level-dependent (BOLD) contrast images were recorded using Multiecho echo-planar imaging (ME-EPI) including three echoes (TE1 13.0 ms, TE2 28.9 ms, TE3 44.8 ms, TR 2000 ms, Flip angle 90°, FOV 220 × 220 mm^2 and matrix 80 × 80 mm^2, GRAPPA $r = 3$) with 36 transversal slices angulated in direction of the corpus callosum with a thickness of 3 mm, voxel-size of 2.8 × 2.8 × 3 mm^3 and a 0.6 mm slice gap [Poser et al., 2006]. Voxel-based analysis of functional MRI data was accomplished with Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, UCL, London, UK) implemented in Matlab R2012a (Mathworks, Sherborn, MA). Initially, functional images were combined, motion and slice-time corrected, normalized to the Montreal Neurological Institute brain (MNI-brain) and spatially smoothed with an isotropic Gaussian kernel of 8 mm. To correct for low frequency drifts in the data, a temporal high-pass filter of 128s was used and serial autocorrelations were accommodated by means of an autoregressive model first-order correction. For statistical first-level analyses, a general linear model (GLM) was applied to the EPI images. The time series of each voxel was fitted with a corresponding task regressor that modeled a box car convolved with a canonical haemodynamic response function (hrf). The first level model included the following regressors: For the acquisition phase: CS^+ (16 trials with a variable duration of 8–12 s); CS^- (16 trials with a variable duration of 8–12 s); US (12 trials with a duration of 14 s); for the extinction phase: CS^+ (6 trials with a variable duration of 8–12 s); CS^- (6 trials with a variable duration of 8–12 s); for the renewal test phase: CS^+ (6 trials with a variable duration of 8–12 s); CS^- (6 trials with a variable duration of 8–12 s). Additionally, six realignment parameters for translation (x, y, z) and for rotation (pitch, roll, yaw) to describe the rigid body transformation between each image and a reference image were implemented as multiple regressors within the model estimation. BOLD responses to pain-predictive cues (CS^+) compared to nonpain-predictive cues (CS^-) were computed and the first-level contrast images ($CS^+ > CS^-$; $CS^- > CS^+$) were used for voxelwise second-level (i.e., group) analyses treating individual subjects as a random factor and including nonsphericity correction. Initially, two sample *t*-tests were conducted for the acquisition phase to confirm the absence of group differences between the context and the control group, treated equally during acquisition. Consequently, acquisition data was analyzed in one-sample *t*-tests in the pooled sample. Group differences in neural activation during extinction and renewal test phases were assessed in two-sample *t*-tests on the first-level differential contrasts ([Context $group_{(CS+>CS-)}$ > Control $group_{(CS+>CS-)}$] and [Control $group_{(CS+>CS-)}$ > Context $group_{(CS+>CS-)}$]). Based on our previous work on pain-related fear

TABLE I. Differential neural activation during acquisition

Phase	Brain region	H	Coordinates			*t*-value	P^a
			x	*y*	*z*		
(A) [CS$^+$ > CS$^-$]							
Early acquisition							
	vmPFC (BA 47)	R	32	26	2	4.53	.019
	Insula	R	34	30	−2	4.83	.006
	Insula	L	−34	20	0	4.58	.011
	Putamen	R	30	16	6	3.68	.050
Late acquisition							
	OFC (BA 11)	R	18	54	−8	4.10	.016
	vmPFC (BA 25)	R	12	26	−20	3.88	.018
	Insula	R	34	22	10	6.14	.000
	Caudate	R	12	10	2	3.83	.027
	Pallidum	R	14	8	0	3.71	.034
	Pallidum	L	−18	6	−4	3.35	.028
	Putamen	R	22	14	0	4.11	.014
	Putamen	L	−18	10	−6	3.71	.034
(B) [CS$^-$ > CS$^+$]							
Early acquisition							
	FPC (BA 10)	R	10	66	12	4.63	.010
	Parahippocampus	L	−28	−14	−28	4.54	.007
	Parahippocampus	R	22	−42	−10	6.38	.000
	Thalamus	R	18	−22	6	3.76	.038
Late acquisition							
	Hippocampus	L	−28	−22	−18	4.89	.002
	Hippocampus	R	24	−10	−20	4.44	.006
	Parahippocampus	R	24	0	−34	4.55	.006

Within-group analyses of differential CS-induced BOLD responses by one-sample *t*-tests with valence ratings as covariate.
[a]Only results of region-of-interest-analyses at P_{FWE}-corrected < 0.05 are shown and exact unilateral *P*-values are given. H = hemisphere; vmPFC = ventromedial prefrontal cortex, OFC = orbitofrontal cortex; FPC = frontopolar cortex; BA = brodman area.

conditioning [Benson et al., 2014; Icenhour et al., 2015; Kattoor et al., 2013], all phases were separated into an early and a late phase, and CS$^+$ valence ratings as behavioral indicators of learned emotional aversion were included as a covariate throughout. ROI were a priori defined based on existing fMRI data addressing contextual influences on fear extinction [Milad et al., 2007] as well as anticipatory pain modulation and pain processing, particularly from conditioning studies with aversive visceral US [Benson et al., 2014; Icenhour et al., 2015; Kattoor et al., 2013; Labus et al., 2013; Schmid et al., 2014; Yaguez et al., 2005]. ROI included amygdala, hippocampus and parahippocampus, insula, thalamus, basal ganglia, somatosensory cortex, cingulate cortex, and prefrontal cortex [dorsolateral prefrontal cortex (dlPFC), ventrolateral prefrontal cortex (vlPFC), ventromedial prefrontal cortex (vmPFC), orbitofrontal cortex (OFC)]. ROI analyses were carried out using anatomical templates constructed from the WFU Pick Atlas (Version 2.5.2) with familywise error (FWE) correction for multiple comparisons set at $P_{FWE} < 0.05$. All results are given as MNI coordinates.

RESULTS

Acquisition

Data from the acquisition phase were initially analyzed to confirm successful differential learning in both groups. Between-group analyses expectedly revealed no significant group differences in behavioral measures, SCR or BOLD responses during acquisition, consistent with identical group treatment during this phase (data not shown). Psychological measures of anxiety and depression indicated no evidence of group differences and overall low to moderate levels of depression and anxiety symptoms in this sample of healthy volunteers (HADS depression scores, mean ± SEM: control group: 1.81 ± .49; context group: .96 ± 0.20; $P = 0.809$; HADS anxiety scores: control group: 2.88 ± 0.63; context group: 2.52 ± .45; $P = 0.450$; STAI-T scores: control group: 29.13 ± 1.09; context group: 32.08 ± 1.57; $P = 0.136$). Therefore, acquisition phase results for the pooled sample are provided to improve clarity and conciseness. Analyses of differential neural activation revealed enhanced responses to pain-predictive CS$^+$ when compared to CS$^-$ in vmPFC, insula and putamen during early acquisition and in vmPFC, OFC, basal ganglia (caudate, pallidum and putamen) and insula during the late acquisition phase ([CS$^+$ > CS$^-$]; all $P_{FWE} < 0.05$; Table IA). Differential neural activation in response to non-pain predictive CS$^-$ was observed in frontopolar cortex, parahippocampus, hippocampus and thalamus ([CS$^-$ > CS$^+$]; all $P_{FWE} < 0.05$; Table IB).

SCR in the pooled sample supported significantly greater electrodermal responses to the CS$^+$ when compared to the CS$^-$ ($t = 2.53$; $P = 0.016$; Fig. 1A). Analyses of behavioral data indicated cognitive awareness of CS-US contingencies (in reality 75% CS$^+$-US reinforcement; 0% CS$^-$-US reinforcement). While CS$^+$-US contingencies were rated rather accurately (72.74 ± 3.97%), CS$^-$-US contingency ratings were less accurate (16.46 ± 3.71%). The differentiation between perceived CS$^+$-US and CS$^-$-US contingencies was highly significant ($t = 9.48$; $P < 0.001$). Valence ratings showed significantly increased aversion of the CS$^+$ following the acquisition phase ($t = 11.34$; $P < 0.001$). This was paralleled by a significant increase in pleasantness of the CS$^-$ ($t = 3.99$; $P < 0.001$; Fig. 2). Average US painfulness, assessed with VAS ratings following acquisition, supported moderately painful stimuli (mean ± SEM: 63.25 ± 5.04 mm).

Together, and in line with our previous reports [Benson et al., 2014; Gramsch et al., 2014; Icenhour et al., 2015; Kattoor et al., 2013], these data confirm successful differential learning of abdominal pain-related signal properties resulting from specific valence changes and neural responses to both pain-predictive CS$^+$ as well as non-pain predictive CS$^-$.

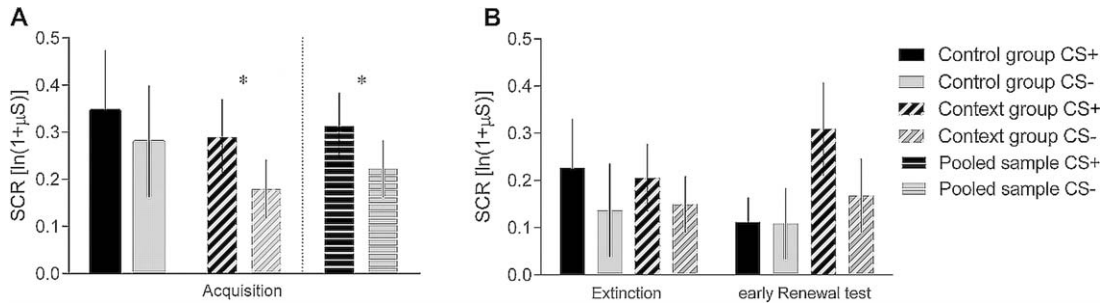

Figure 1.

Mean skin conductance responses to predictive CS^+ and CS^- during acquisition separately for context and control groups and in the pooled sample (**A**) and group comparisons during late extinction and early renewal test phases (**B**). During acquisition, greater electrodermal responses to CS^+ compared to CS^- were observed, supporting differential fear learning. While no significant group differences were observed during late extinction, a return to the original learning context led to greater SCR to the CS^+ when compared to the CS^- during the early renewal test phase. However, group differences did not reach statistical significance. Data are shown as mean ± SEM. For statistical details, see text. *$P < 0.05$.

Separate analyses of SCR and valence ratings within each group were additionally conducted to confirm differential learning in both groups. SCR results revealed differentiation between CS^+ and CS^-, although mean SCR to CS^+ were significantly higher compared to CS^- in the context group only ($t = 2.33$; $P = 0.030$; Fig. 1A). Analyses of valence ratings conducted separately for context and control group confirmed differential changes in aversion ratings of predictive cues in both groups, indicated by significantly higher aversion to CS^+ compared to CS^- following acquisition (control group: $t = 7.10$; $P < 0.001$; context group: $t = 9.53$; $P < 0.001$) as well as a significant increase of aversion to pain-predictive CS^+ after acquisition when compared to baseline ratings (control group: $t = 6.59$; $P < 0.001$; context group: $t = 9.54$; $P < 0.001$; Fig. 2).

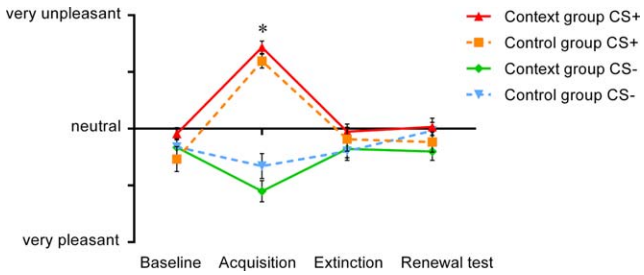

Figure 2.

CS valence ratings assessed using visual analogue scales (VAS). Both groups showed a significant increase in negative valence in response to the CS^+, paralleled by significantly higher perceived pleasantness of the CS^- following acquisition. By the end of extinction, valence ratings for both CS^+ and CS^- had returned to baseline levels with no detectable group differences and no effect of a context change in the context group. No significant group differences were observed after the renewal test phase. For statistical details, see text. *$P < 0.05$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Context Effects on Extinction Learning

To address the context-dependency of extinction learning, the context group experienced extinction in a new context, whereas no context change occurred in the control group. For analyses of group differences in differential neural modulation during extinction, two-sample t-tests on the first-level differential contrasts were computed. Results revealed significant group differences in dlPFC, vmPFC and amygdala for the differential contrast [Context group $_{CS+>CS-}$ > Control group $_{CS+>CS-}$] during the early phase of extinction, whereas no significant group differences were observed during late extinction (all $P_{FWE} < 0.05$; Table II; Fig. 3). Parameter estimates revealed that differences observed were driven by reduced differential modulation in the context when compared to the control group (Fig. 3). In other words, significant group differences in two-sample t-tests resulted from greater CS^+-CS^- differentiation in the control group, whereas virtually no such differentiation was seen in the context group. Results from supplementary analyses on differential neural activation during early and late extinction within context and control groups separately are provided in Supporting Information Tables S2 and S3.

Behavioral analyses revealed comparable CS^+-US and CS^--US contingency awareness (in reality 0% CS-US contingency) with no significant group differences and no significant differentiation between CS^+ (context group: 8.61 ± 3.76%; control group: 13.31 ± 5.22%) and CS^- (context group: 6.04 ± 3.05%; control group: 10.31 ± 4.09%). This was paralleled by a return of CS^+ as well as CS^- valence ratings to baseline levels, without evidence of significant group differences (context group: $t = 10.64$;

TABLE II. Group differences in differential neural activation during extinction [$CS^+ > CS^-$]

Phase	Brain region	H	Coordinates			*t*-value	P^a
			x	*y*	*Z*		
[Context group $_{(CS+>CS-)}$ > Control group $_{(CS+>CS-)}$]							
Early extinction							
	dlPFC (BA 8)	L	−36	10	58	4.85	0.011
	vmPFC (BA 11)	R	6	60	−12	3.85	0.024
	Amygdala	L	−12	0	−16	3.38	0.025
Late extinction							
	–	–	–	–	–	–	–
[Control group $_{(CS+>CS-)}$ > Context group $_{(CS+>CS-)}$]							
	–	–	–	–	–	–	–

Between-group analyses of differential CS-induced BOLD responses by two-sample *t*-tests with valence ratings as covariate.
[a]Only results of regions-of-interest analyses at P_{FWE}-corrected < 0.05 are shown and exact unilateral *P*-values are given. H = hemisphere; dlPFC = dorsolateral prefrontal cortex; vmPFC = ventromedial prefrontal cortex; BA = Brodman area. For visualization, see Figure 3.

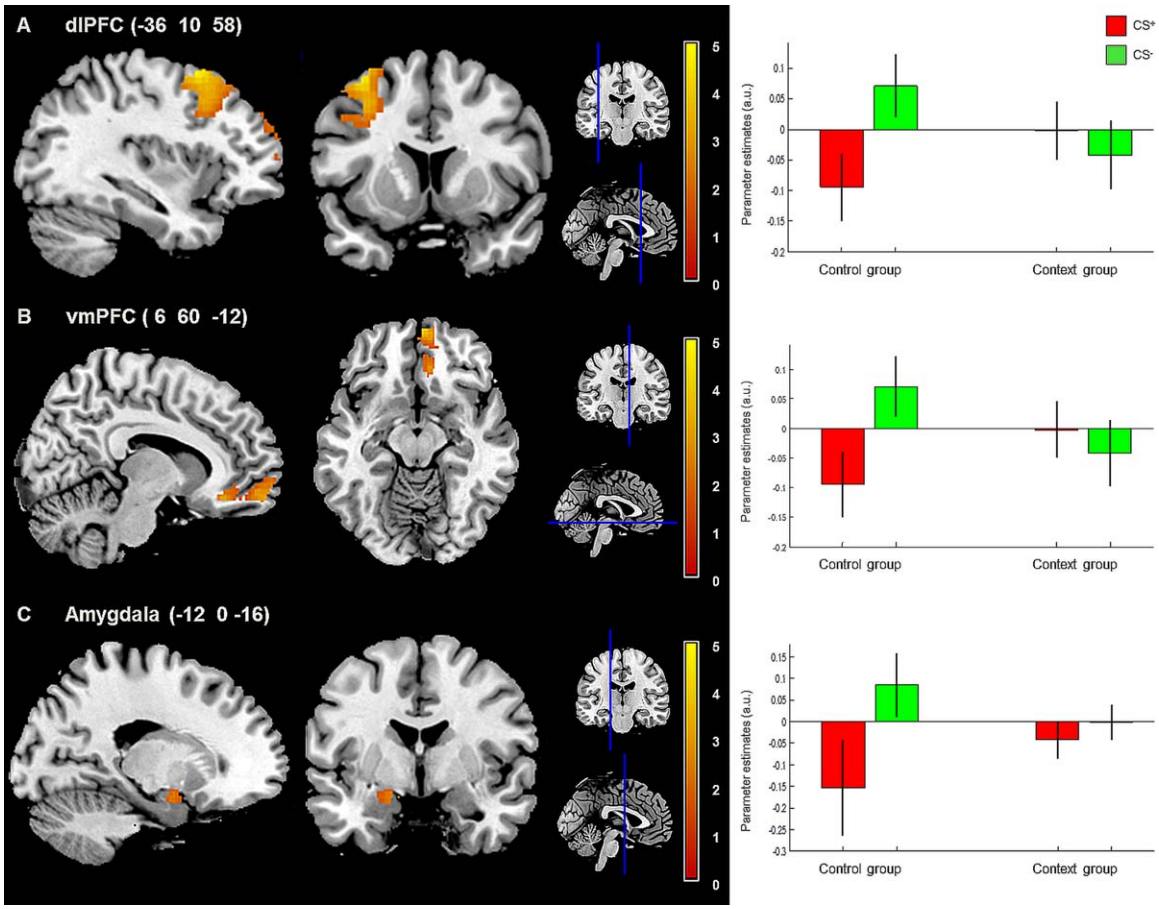

Figure 3.

Group differences in differential neural activation during extinction. Group comparisons revealed significant differential neural activation in dlPFC (**A**), vmPFC (**B**), and amygdala (**C**) during early extinction, resulting from greater CS^+-CS^- differentiation in the control when compared to the context group in all regions, as indicated by parameter estimates (all $P_{FWE} < 0.05$). Activations were superimposed on a structural T_1-weighted MRI used for spatial normalization, masks for relevant ROI were applied and activations were thresholded at $P < 0.001$ uncorrected for visualization purposes; color bars indicate *t*-scores. For statistical details, see Table II. dlPFC = dorsolateral prefrontal cortex; vmPFC = ventromedial prefrontal cortex; a.u. = arbitrary units.

TABLE III. Group differences in differential neural activation during renewal test [$CS^+ > CS^-$]

Phase	Brain region	H	Coordinates			*t*-value	P^a
			x	*y*	*z*		
[Context group $_{(CS+>CS-)}$ > Control group $_{(CS+>CS-)}$]							
Early renewal							
	OFC (BA 11)	L	−18	22	−16	4.27	.013
Late renewal							
	–	–	–	–	–	–	–
[Control group $_{(CS+>CS-)}$ > Context group $_{(CS+>CS-)}$]							
Early renewal							
	dlPFC (BA 44)	R	64	10	18	3.92	.040
Late renewal							
	–	–	–	–	–	–	–

Between-group analyses of differential CS-induced BOLD responses by two-sample *t*-tests with valence ratings as covariate.
[a]Only results of regions-of-interest analyses at P_{FWE}-corrected < 0.05 are shown and exact unilateral *P*-values are given. H = hemisphere; OFC = orbitofrontal cortex; dlPFC = dorsolateral prefrontal cortex; BA = Brodman area. For visualization, see Figure 4.

$P < 0.001$; control group: $t = 3.33$; $P = 0.002$; Fig. 2). Finally, CS^+-CS^- differentiation in SCR observed during early extinction ($t = 2.63$; $P = 0.014$) was abolished by the late extinction phase (Fig. 1B). No significant group differences were observed in electrodermal responses during extinction. To exclude that observed context-effects during extinction were merely due to a generalization decrement in the context group, SCR data were critically tested regarding a fear generalization decrement across contexts as suggested for human renewal research [Vervliet et al., 2013]. Comparisons of CS^+-induced SCR to the last acquisition trial(s) and to the first extinction trial(s) in the context group revealed no significant differences, indicating generalization of conditioned responses across contexts (last acquisition vs. first extinction trial $t = 1.02$; $P = 0.319$; last two acquisition trials vs. first two extinction trials $t = 0.81$; $P = 0.428$).

Renewal Effects

We tested the hypothesis of a return of previously extinguished fear, evidenced by differential skin conductance responses and differential activation of brain structures mediating the formation and reactivation of conditioned fear, especially amygdala and hippocampus. Analysis of BOLD responses revealed no effects for either amygdala or hippocampus. However, group comparisons showed significant differential activation in OFC [Context group $_{CS+>CS-}$ > Control group $_{CS+>CS-}$] and dlPFC [Control group $_{CS+>CS-}$ > Context group $_{CS+>CS-}$] in the early renewal test phase (all $P_{FWE} < 0.05$; Table III; Fig. 4), which resulted from differential modulation of the CS^- in both regions, as indicated by parameter estimates. No significant group differences were detected on BOLD-level in the late renewal test phase. Although SCR analyses suggested greater electrodermal responses to the CS^+ when compared to the CS^- in the context group during the early renewal test phase (Fig. 1B), differences between groups did not reach significance ($t = 1.77$; $P = 0.087$) and no significant differentiation was observed during the late renewal test phase. No significant group differences in CS valence ratings were detected at the conclusion of the renewal test phase (Fig. 2).

Additional supplementary analyses

To confirm the exclusion of nine individuals indicating a lack of differential fear acquisition, SCR data in this subgroup were inspected in a supplementary analysis. Results indicated insufficient CS^+-CS^- differentiation during initial learning ($t = 0.903$; $P = 0.393$), especially a lack of learned CS^+-related SCR over trials ($F = 1.13$; $P = 0.352$) in this subgroup.

Additionally, supplementary analyses of imaging data were conducted for all experimental phases (a) including the full sample, that is, $N = 48$ without exclusion based on a lack of learned CS^+ aversion (Supporting Information Tables S4 and S5) and (b) without CS^+ valence as covariate (Supporting Information Tables S6, S7, and S8). Results confirmed essentially similar albeit in parts weaker findings (i.e., lower *t*-values), leading to partly nonsignificant results.

DISCUSSION

While the neural mechanisms mediating the context-dependency of extinction and renewal in conditioned fear paradigms are relatively well-characterized in animal models [Maren et al., 2013], human brain imaging studies are scarce. With the exception of a single study [Milad et al., 2007], effects of a change of context conducted following the formation of differential fear in response to predictive CS has not been addressed. Therefore, the goal of this study was to address the context-dependency of extinction and renewal in a differential fear conditioning

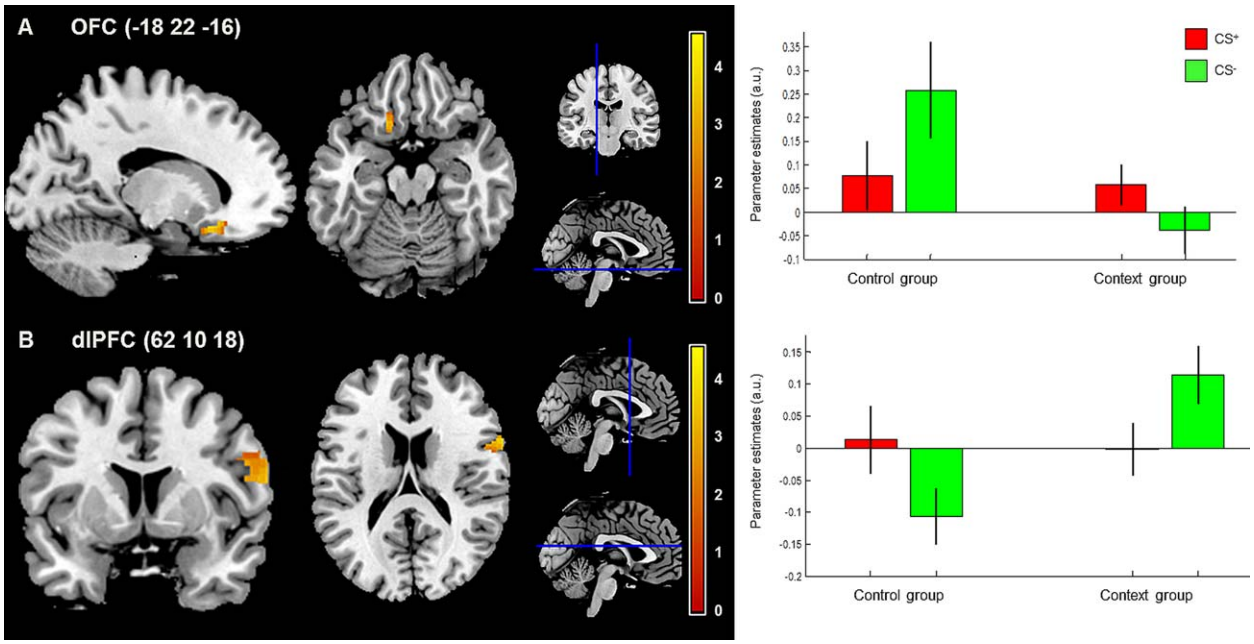

Figure 4.

Group differences in differential neural activation during renewal test phase. Group comparisons revealed significant CS-induced differential neural activation in OFC (**A**) and dlPFC (**B**) in the early renewal test phase, resulting from differential modulation of the CS^- rather than CS^+, as indicated by parameter estimates (all $P_{FWE} < 0.05$). Activations were superimposed on a structural T_1-weighted MRI used for spatial normalization, masks for relevant ROI were applied and activations were thresholded at $P < 0.001$ uncorrected for visualization purposes; color bars indicate *t*-scores. For statistical details, see Table III. OFC = orbitofrontal cortex; dlPFC = dorsolateral prefrontal cortex; a.u. = arbitrary units.

paradigm with clinically relevant visceral pain as US. Initial analysis of the acquisition phase essentially confirmed successful differential learning, as indicated by significant SCR differentiation, CS valence changes, and differential neural modulation in response to the CSs, in line with our previous work [Kattoor et al., 2013]. As a result of conditioning, pain-predictive CS^+ acquired a negative emotional valence and resulted in activation of pain and pain-regulatory brain regions encompassing insula, prefrontal regions and basal ganglia. At the same time, the CS^- acquired positive emotional valence, and led to significant differential activation of thalamus, hippocampal regions and frontopolar cortex, areas previously reported to encode and process reward-related cue-outcome associations [Krawczyk, 2002; Wolosin et al., 2013]. Hence, differential conditioning with rectal pain as US involves distinct and specific learning in response to both the CS^+ as a threat signal and CS^- as a predictor of safety from pain.

Extinction

A change in extinction context resulted in altered neural activation in dlPFC, vmPFC and amygdala, consistent with our hypothesis. The role of vmPFC and amygdala in extinction learning has been well-established [Quirk and Mueller, 2008]. The vmPFC is critically involved in the processing and inhibitory control of emotions [Etkin et al., 2011; Roy et al., 2012; Schiller and Delgado, 2010]. Within the amygdala, inhibitory interneurons are activated by a prefrontal-hippocampal network during extinction, mediating a suppression of previously conditioned fear [Maren et al., 2013; Quirk and Mueller, 2008]. In line with previous data addressing contextual effects on extinction following differential fear conditioning [Milad et al., 2007], our findings support that this network is sensitive to the extinction context and also involves the dlPFC as an established pain-modulatory region mediating top-down cortico-limbic inhibition [Lorenz et al., 2003]. Interestingly, differences between groups were driven by *reduced* differentiation of neural activation to predictive cues in the group with a context change (context group). Unlike the context group, during extinction the control group showed marked neural activation to CS^- relative to CS^+ in all these brain regions, presumably reflecting the formation of a new, inhibitory memory trace. Hence, the context change apparently suppressed re-learning of safety and danger signal properties normally occurring during extinction. Herein, and in sharp contrast to context conditioning studies [Kalisch et al., 2006; Lang et al., 2009], the new

extinction context unequivocally signals safety given the absence of USs, which may activate mechanisms associated with safety learning irrespective of previously learned cue properties. The lack of differential neural activation to CSs in the context group could therefore be explained with a loss of salience of the CSs in favor of the context as a new, more salient safety signal.

Differences at the neural level occurred in the absence of group differences in SCR, which is consistent with previous reports addressing contextual modulation of extinction [Effting and Kindt, 2007; Lang et al., 2009; Milad et al., 2007]. Therefore, it is difficult to judge if the observed lack of neural differentiation reflects suppression or facilitation of new inhibitory learning in the context group. It is important to consider that in parallel with the initiation of new inhibitory learning, other neural processes presumably take place during early extinction, including prediction error processing and recall of residual conditioning memory [Herry et al., 2010; Milad et al., 2007; Quirk and Mueller 2008], which are difficult to disentangle, given our relatively short extinction phase. In contrast to the directionality of differential activation observed herein, previous findings on neural mechanisms involved in extinction learning following a context change reported enhanced CS^+-induced neural activation and reduced responses to CS^- in vmPFC and amygdala [Milad et al., 2007]. These discrepancies could be attributed to differences in methodological approaches. Specifically, Milad et al., [2007] reported results from later trials of a long extinction phase following a context-change within one group, while observations reported herein are based on group differences during the early phase of an overall short extinction phase.

Behavioral measures, assessed at the conclusion of the extinction phase, revealed accurate contingency awareness in both groups as well as a full reversal of emotional valence changes induced during acquisition. Hence, the context change we conducted did clearly not affect behavioral outcomes of extinction.

Against our hypothesis, our analysis did not reveal group differences in differential hippocampal activation during extinction. This could be explained in light of previous evidence showing hippocampal involvement during encoding of an association between context, CS and aversive US [Alvarez et al., 2008; Lang et al., 2009] as well as in contextual conditioning [Kalisch et al., 2006]. Unlike our study implementing a context-change in a cue conditioning paradigm, these paradigms combined contextual manipulations with US presentations, which may explain a lack of hippocampal involvement observed herein. Others have emphasized the involvement of hippocampus especially during extinction retrieval following consolidation [Quirk and Mueller, 2008; Sehlmeyer et al., 2009], supporting a role of hippocampus in extinction recall rather than extinction learning, well in line with observations by Milad et al [2007]. Finally, US omission especially during the early phase of extinction may by itself represent a context change of equal salience in both groups, precluding significant group differences in hippocampal activation.

Renewal

Our analyses of group differences during the renewal test phase do not support the hypothesis that a return into the original learning context elicits a reactivation of the previously extinguished fear memory trace. We neither observed significantly greater differential SCR in response to the CS^+ in the context group nor did we observe group differences in the activation of hippocampus or amygdala. Instead, we found significant group differences in differential neural activation within dlPFC and OFC, which were attributable to modulation of the CS^- rather than CS^+. These activation patterns observed particularly in response to CS^- are well in line with the role of dlPFC and OFC in learned safety cue processing [Christianson et al., 2012; Pollak et al., 2010], but may also reflect emotion regulation through reappraisal processes involving selective attention and re-evaluation of CS properties [Golkar et al., 2012; Ochsner and Gross 2005], especially of CS^-.

Of note, we herein did not observe differential amygdala activation in response to pain-predictive CS^+ in any experimental phase. This is at odds with our hypothesis and earlier findings from our group showing CS^+-related amygdala activation during the late phase of acquisition [Kattoor et al., 2013]. In light of the considerable variability in fear conditioning neuroimaging findings in general, including inconsistent results of amygdala activation [Sehlmeyer et al., 2009], more work is needed to address the reproducibility of amygdala activation and its putative role in pain-related fear conditioning.

Limitations and Perspectives

Our experimental design differs from previous human studies on contextual learning and extinction in three distinct ways: (i) We herein implemented acquisition, extinction and a renewal test phase within one scanning session, while others have included an explicit consolidation phase and then tested for extinction recall [Kalisch et al., 2006; Milad et al., 2007]. Until more knowledge about the consolidation of pain-related fear extinction becomes available, it is difficult to discern if and to what extent the lack of a dedicated consolidation phase affected our results observed in the renewal phase. (ii) In contrast to electric shock as most commonly used US in fear conditioning, we employed rectal distensions as clinically-relevant visceral US [Keszthelyi et al., 2012; Mayer et al., 2008]. This interoceptive stimulation differs with respect to its stimulation properties, neural processing and possibly ecological validity [Aziz et al., 2000; De Peuter et al., 2011]. Indeed, from an evolutionary standpoint, the ability to learn and remember signals predicting danger or safety regarding visceral pain allows effective survival strategies. This idea

is well in line with principles of preparedness or belongingness, illustrating that certain CS-US associations are more easily learned and more resistant to extinction than others, based on their biological significance or the conceptual closeness of CS and US [Hamm et al., 1989; Ohman and Mineka, 2001]. Preparedness may not only influence the acquisition and extinction of pain-related fear, but has also previously been shown to alter pain perception [Miguez et al., 2014; Williams and Rhudy, 2007]. Besides interoceptive US application, homoreflexive conditioning approaches emphasize the implementation of interoceptive CS as more clinically-relevant models to investigate pain-related fear learning and memory [De Peuter et al., 2011; Pappens et al., 2013]. Although pain has been demonstrated to be more readily associated with visual compared to for example gustatory cues [Rachman, 1991], if and to which extent the neural circuitry mediating pain-related learning and extinction is in fact US-, or in this respect also CS-modality-specific requires further clarification. (iii) We implemented a relatively short number of trials during extinction learning, consistent with our previous work on visceral pain-related fear learning [Benson et al., 2014; Icenhour et al., 2015; Kattoor et al., 2013]. Behavioral and SCR data clearly supported full extinction in both groups, but as specified above, especially neural responses observed may have mirrored processes occurring in parallel to early extinction learning. Phases were separated by online ratings, which likely indicated the beginning of a new experimental phase following acquisition in both groups and may have facilitated extinction learning. Additionally, previous reports on learning involving interoceptive US from the gastrointestinal tract indicate rapid acquisition processes [Gramsch et al., 2014; Stockhorst et al., 2007], which may also apply to extinction. Future research should aim to address these arising open questions, especially focusing on the role of CS and US modalities in pain-related learning and memory processes. Finally, we have previously observed sex differences in the neural processing of visceral pain-related fear learning and memory reactivation [Benson et al., 2014], which may play a key role in the higher female preponderance for several chronic pain conditions, including IBS [Mogil, 2012]. Sex-related differences may indeed also contribute to contextual effects on extinction and future studies including sufficient sample sizes will be needed to address the role of sex and gender in context effects on pain-related extinction.

Our results strongly support the sensitivity of learned safety cue properties to contextual changes in visceral pain-related fear extinction and extend existing data reporting safety signals to affect learning and extinction of movement-related fear [Meulders et al., 2014; Meulders and Vlaeyen, 2012]. Given evidence suggesting that the processing of learned safety and reward share common neural pathways [Christianson et al., 2012] and that pain-relief may indeed be perceived as rewarding (den Hollander et al., 2010; Navratilova and Porreca, 2014], the neural processing of learned safety as well as reward could play a role in chronic pain. Specifically, learned safety may contribute to the development and maintenance of safety-seeking and avoidance behavior aiming at relieving pain, which, according to fear-avoidance models of chronic pain, crucially impacts pain chronification [De Peuter et al., 2011; den Hollander et al., 2010] and likely hampers extinction-based treatment efficacy in chronic pain patients [Volders et al., 2012, 2014].

Ultimately, more knowledge regarding the putative role of contextual effects on pain-related danger and safety learning and memory processes in patients with chronic pain is needed. Thus far, the clinical implications of context-related effects have been established in anxiety and addictive disorders, and are beginning to be appreciated in pain-related fear of movement [Meulders and Vlaeyen, 2013; Volders et al., 2014]. Therefore, more mechanistic insight regarding the role of context in pain-related fear and safety learning may contribute to optimizing emerging extinction-based treatment approaches for chronic pain [Boersma et al., 2004; de Jong et al., 2005; Vlaeyen et al., 2002] including IBS [Craske et al., 2011; Ljotsson et al., 2011, 2014].

ACKNOWLEDGMENTS

The authors thank Sarah Hampel for excellent support in conducting this project and Alex Luft and Dr. Marcel Gratz for technical support. The authors would further like to express their gratitude to Dr. Benedikt Poser and the Donders Institute for providing the multi-echo EPI sequence used in this study. The funding agency had no role in the conception, analysis or interpretation of the data.

REFERENCES

Alvarez RP, Biggs A, Chen G, Pine DS, Grillon C (2008): Contextual fear conditioning in humans: Cortical-hippocampal and amygdala contributions. J Neurosci 28:6211–6219.

Amaro E, Barker GJ (2006): Study design in fMRI: basic principles. Brain Cogn 60:220–232.

Asmundson GJ, Katz J (2009): Understanding the co-occurrence of anxiety disorders and chronic pain: State-of-the-art. Depress Anxiety 26:888–901.

Asmundson GJ, Taylor S (1996): Role of anxiety sensitivity in pain-related fear and avoidance. J Behav Med 19:577–586.

Aziz Q, Thompson DG, Ng VW, Hamdy S, Sarkar S, Brammer MJ, Bullmore ET, Hobson A, Tracey I, Gregory L, Simmons A, Williams SC (2000): Cortical processing of human somatic and visceral sensation. J Neurosci 20:2657–2663.

Benson S, Kattoor J, Kullmann JS, Hofmann S, Engler H, Forsting M, Gizewski ER, Elsenbruch S (2014): Towards understanding sex differences in visceral pain: Enhanced reactivation of classically-conditioned fear in healthy women. Neurobiol Learn Mem 109:113–121.

Boersma K, Linton S, Overmeer T, Jansson M, Vlaeyen J, de Jong J (2004): Lowering fear-avoidance and enhancing function through exposure in vivo. A multiple baseline study across six patients with back pain. Pain 108:8–16.

Boucsein W, Fowles DC, Grimnes S, Ben-Shakhar G, roth WT, Dawson ME, Filion DL (2012): Publication recommendations for electrodermal measurements. Psychophysiology, 49:1017–1034.

Bouton ME (2002): Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. Biol Psychiatry 52:976−986.

Bouton ME (2004): Context and behavioral processes in extinction. Learn Mem 11:485–494.

Carlino E, Frisaldi E, Benedetti F (2014): Pain and the context. Nat Rev Rheumatol 10:348–355.

Christianson JP, Fernando AB, Kazama AM, Jovanovic T, Ostroff LE, Sangha S (2012): Inhibition of fear by learned safety signals: A mini-symposium review. J Neurosci 32:14118–14124.

Craske MG, Wolitzky-Taylor KB, Labus J, Wu S, Frese M, Mayer EA, Naliboff BD (2011): A cognitive-behavioral treatment for irritable bowel syndrome using interoceptive exposure to visceral sensations. Behav Res Ther 49:413–421.

Crombez G, Vlaeyen JW, Heuts PH, Lysens R (1999): Pain-related fear is more disabling than pain itself: Evidence on the role of pain-related fear in chronic back pain disability. Pain 80:329–339.

de Jong JR, Vlaeyen JW, Onghena P, Cuypers C, den Hollander M, Ruijgrok J (2005): Reduction of pain-related fear in complex regional pain syndrome type I: The application of graded exposure in vivo. Pain 116:264–275.

De Peuter S, Van Diest I, Vansteenwegen D, Van den Bergh O, Vlaeyen JW (2011): Understanding fear of pain in chronic pain: Interoceptive fear conditioning as a novel approach. Eur J Pain 15:889–894.

den Hollander M, de Jong JR, Volders S, Goossens ME, Smeets RJ, Vlaeyen JW (2010): Fear reduction in patients with chronic pain: A learning theory perspective. Expert Rev Neurother 10: 1733–1745.

Effting M, Kindt M (2007): Contextual control of human fear associations in a renewal paradigm. Behav Res Ther 45:2002–2018.

Elsenbruch S, Rosenberger C, Bingel U, Forsting M, Schedlowski M, Gizewski ER (2010a): Patients with irritable bowel syndrome have altered emotional modulation of neural responses to visceral stimuli. Gastroenterology 139:1310–1319.

Elsenbruch S, Rosenberger C, Enck P, Forsting M, Schedlowski M, Gizewski ER (2010b): Affective disturbances modulate the neural processing of visceral pain stimuli in irritable bowel syndrome: An fMRI study. Gut 59:489–495.

Elsenbruch S, Schmid J, Basler M, Cesko E, Schedlowski M, Benson S (2012): How positive and negative expectations shape the experience of visceral pain: An experimental pilot study in healthy women. Neurogastroenterol Motil 24:914–e460.

Elsenbruch S, Schmid J, Kullmann JS, Kattoor J, Theysohn N, Forsting M, Kotsis V (2014): Visceral sensitivity correlates with decreased regional gray matter volume in healthy volunteers: A voxel-based morphometry study. Pain 155:244–249.

Etkin A, Egner T, Kalisch R (2011): Emotional processing in anterior cingulate and medial prefrontal cortex. Trends Cogn. Sci 15:85–93.

Flor H, Birbaumer N, Schulz R, Grusser SM, Mucha RF (2002): Pavlovian conditioning of opioid and nonopioid pain inhibitory mechanisms in humans. Eur J Pain 6:395–402.

Golkar A, Lonsdorf T, Olsson A, Lindstrom KM, Berrebi J, Fransson P, Schalling M, Ingvar M, Öhman A (2012): Distinct Contributions of the Dorsolateral Prefrontal and Orbitofrontal Cortex during Emotion Regulation. PLoS One 7:e48107 .

Gramsch C, Kattoor J, Icenhour A, Forsting M, Schedlowski M, Gizewski ER, Elsenbruch S (2014): Learning pain-related fear: Neural mechanisms mediating rapid differential conditioning, extinction and reinstatement processes in human visceral pain. Neurobiol Learn Mem 116C:36–45.

Hamm AO, Vaitl D, Lang PJ (1989): Fear conditioning, meaning, and belongingness: A selective association analysis. J Abnorm Psychol 98:395–406.

Herrmann-Lingen C, Buss U, Snaith RP (2005): Hospital Anxiety and Depression Scale (HADS-D) - Deutsche Version. Bern: Huber.

Herry C, Ferraguti F, Singewald N, Letzkus JJ, Ehrlich I, Lüthi A (2010): Neuronal circuits of fear extinction. Eur J Neurosci 31: 599–612.

Icenhour A, Langhorst J, Benson S, Schlamann M, Hampel S, Engler H, Forsting M, Elsenbruch S (2015): Neural circuitry of abdominal pain-related fear learning and reinstatement in irritable bowel syndrome. Neurogastroenterol Motil 27:114–127.

Kalisch R, Korenfeld E, Stephan KE, Weiskopf N, Seymour B, Dolan RJ (2006): Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. J Neurosci 26:9503–9511.

Kattoor J, Gizewski ER, Kotsis V, Benson S, Gramsch C, Theysohn N, Maderwald S, Forsting M, Schedlowski M, Elsenbruch S (2013): Fear conditioning in an abdominal pain model: Neural responses during associative learning and extinction in healthy subjects. PLoS One 8:e51149

Keszthelyi D, Troost FJ, Masclee AA (2012): Irritable bowel syndrome: Methods, mechanisms, and pathophysiology. Methods to assess visceral hypersensitivity in irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 303:G141–154.

Klinger R, Matter N, Kothe R, Dahme B, Hofmann UG, Krug F (2010): Unconditioned and conditioned muscular responses in patients with chronic back pain and chronic tension-type headaches and in healthy controls. Pain 150:66–74.

Krawczyk DC (2002): Contributions of the prefrontal cortex to the neural basis of human decision making. Neurosci Biobehav Rev 26:631–664.

LaBar KS, Phelps EA (2005): Reinstatement of conditioned fear in humans is context dependent and impaired in amnesia. Behav Neurosci 119:677–686.

Labus JS, Hubbard CS, Bueller J, Ebrat B, Tillisch K, Chen M, Stains J, Dukes GE, Kelleher DL, Naliboff BD, Fanselow M, Mayer EA (2013): Impaired emotional learning and involvement of the corticotropin-releasing factor signaling system in patients with irritable bowel syndrome. Gastroenterology 145: 1253−1261 e1–e3.

Lacourt TE, Houtveen JH, Doornen LJ, Benson S, Grigoleit JS, Cesko E, Elsenbruch S (2014): Biological and psychological predictors of visceral pain sensitivity in healthy premenopausal women. Eur J Pain 18:567–574.

Lang S, Kroll A, Lipinski SJ, Wessa M, Ridder S, Christmann C, Schad LR, Flor H (2009): Context conditioning and extinction in humans: Differential contribution of the hippocampus, amygdala and prefrontal cortex. Eur J Neurosci 29:823–832.

Laux L, Glanzmann P, Schaffner P, Spielberger CD (1981): STAI. Das State-Trait-Angstinventar. Theoretische Grundlagen und Handanweisung. Weinheim: Beltz.

Ljotsson B, Andersson G, Andersson E, Hedman E, Lindfors P, Andreewitch S, Ruck C, Lindefors N (2011): Acceptability, effectiveness, and cost-effectiveness of internet-based exposure treatment for irritable bowel syndrome in a clinical sample: A randomized controlled trial. BMC Gastroenterol 11:110

Ljotsson B, Hesser H, Andersson E, Lackner JM, El Alaoui S, Falk L, Aspvall K, Fransson J, Hammarlund K, Lofstrom A,

Nowinski S, Lindfors P, Hedman E (2014): Provoking symptoms to relieve symptoms: A randomized controlled dismantling study of exposure therapy in irritable bowel syndrome. Behav Res Ther 55:27–39.

Lorenz J, Minoshima S, Casey KL (2003): Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 126:1079–1091.

Maren S, Phan KL, Liberzon I (2013): The contextual brain: Implications for fear conditioning, extinction and psychopathology. Nat Rev Neurosci 14:417–428.

Mayer EA, Bradesi S, Chang L, Spiegel BMR, Bueller JA, Naliboff BD (2008): Functional GI disorders: From animal models to drug development. Gut 57:384–404.

Meulders A, Jans A, Vlaeyen JWS (2015): Differences in pain-related fear acquisition and generalization: An experimental study comparing patients with fibromyalgia and healthy controls. Pain 156:108–122. 10.1016/j.pain.0000000000000016.

Meulders A, Meulders M, Vlaeyen JWS (2014): Positive affect protects against deficient safety learning during extinction of fear of movement-related pain in healthy individuals scoring relatively high on trait anxiety. J Pain 15:632–644.

Meulders A, Vlaeyen JW (2012): Reduction of fear of movement-related pain and pain-related anxiety: An associative learning approach using a voluntary movement paradigm. Pain 153: 1504–1513.

Meulders A, Vlaeyen JW (2013): The acquisition and generalization of cued and contextual pain-related fear: An experimental study using a voluntary movement paradigm. Pain 154:272–282.

Miguez G, Laborda MA, Miller RR (2014): Classical conditioning and pain: Conditioned analgesia and hyperalgesia. Acta Psychol (Amst.) 145:10–20.

Milad MR, Orr SP, Lasko NB, Chang Y, Rauch SL, Pitman RK (2008): Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. J Psychiatr Res 42: 515–520.

Milad MR, Orr SP, Pitman RK, Rauch SL (2005): Context modulation of memory for fear extinction in humans. Psychophysiology 42:456–464.

Milad MR, Quirk GJ (2012): Fear extinction as a model for translational neuroscience: Ten years of progress. Annu Rev Psychol 63:129–151.

Milad MR, Wright CI, Orr SP, Pitman RK, Quirk GJ, Rauch SL (2007): Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol Psychiatry 62:446–454.

Mogil JS (2012): Sex differences in pain and pain inhibition: Multiple explanations of a controversial phenomenon. Nat Rev Neurosci 13:859–866.

Navratilova E, Porreca F (2014): Reward and motivation in pain and pain relief. Nat Neurosci 17:1304–1312.

Nees F, Ruddel H, Mussgay L, Kuehl LK, Romer S, Schachinger H (2010): Alteration of delay and trace eyeblink conditioning in fibromyalgia patients. Psychosom Med 72:412–418.

Ochsner KN, Gross JJ (2005): The cognitive control of emotion. Trends Cogn Sci 9:242–249.

Ochsner KN, Ludlow DH, Knierim K, Hanelin J, Ramachandran T, Glover GC, Mackey SC (2006): Neural correlates of individual differences in pain-related fear and anxiety. Pain 120:69–77.

Ohman A, Mineka S (2001): Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. Psychol Rev 108:483–522.

Pappens M, Van den Bergh O, Vansteenwegen D, Ceunen E, De Peuter S, Van Diest I (2013): Learning to fear obstructed breathing: Comparing interoceptive and exteroceptive cues. Biol Psychol 92:36–42.

Pineles SL, Orr MR, Orr SP (2009): An alternative scoring method for skin conductance responding in a differential fear conditioning paradigm with a long-duration conditioned stimulus. Psychophysiology 46:984–995.

Pollak DD, Rogan MT, Egner T, Perez DL, Yanagihara TK, Hirsch J (2010): A translational bridge between mouse and human models of learned safety. Ann Med 42:115–122.

Poser BA, Versluis MJ, Hoogduin JM, Norris DG (2006): BOLD contrast sensitivity enhancement and artifact reduction with multiecho EPI: Parallel-acquired inhomogeneity-desensitized fMRI. Magn Reson Med 55:1227–1235.

Quirk GJ, Mueller D (2008): Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33:56–72.

Rachman S (1991): Neo-conditioning and the classical-theory of fear acquisition. Clin Psychol Rev 11:155–173.

Reiss M, Reiss G (2000): Studies on motorial asymmetries. Fortschr Neurol Psyc 68:70–79.

Rougemont-Bucking A, Linnman C, Zeffiro TA, Zeidan MA, Lebron-Milad K, Rodriguez-Romaguera J, Rauch SL, Pitman RK, Milad MR (2011): Altered processing of contextual information during fear extinction in PTSD: An fMRI study. CNS Neurosci Ther 17:227–236.

Roy M, Shohamy D, Wager TD (2012): Ventromedial prefrontal-subcortical systems and the generation of affective meaning. Trends Cogn Sci 16:147–156.

Schiller D, Delgado MR (2010): Overlapping neural systems mediating extinction, reversal and regulation of fear. Trends Cogn Sci 14:268–76.

Schmid J, Langhorst J, Gass F, Theysohn N, Benson S, Engler H, Gizewski ER, Forsting M, Elsenbruch S (2014): Placebo analgesia in patients with functional and organic abdominal pain: A fMRI study in IBS, UC and healthy volunteers. Gut 64:418–427.

Schneider C, Palomba D, Flor H (2004): Pavlovian conditioning of muscular responses in chronic pain patients: Central and peripheral correlates. Pain 112:239–247.

Sehlmeyer C, Schoning S, Zwitserlood P, Pfleiderer B, Kircher T, Arolt V, Konrad C (2009): Human fear conditioning and extinction in neuroimaging: A systematic review. PLoS One 4:e5865

Stockhorst U, Enck P, Klosterhalfen S (2007): Role of classical conditioning in learning gastrointestinal symptoms. World J. Gastroenterol., 13:3430–3437.

Tabbert K, Merz CJ, Klucken T, Schweckendiek J, Vaitl D, Wolf OT, Stark R (2011): Influence of contingency awareness on neural, electrodermal and evaluative responses during fear conditioning. Soc Cogn Affect Neurosci 6:495–506.

Vansteenwegen D, Hermans D, Vervliet B, Francken G, Beckers T, Baeyens F, Eelen P (2005): Return of fear in a human differential conditioning paradigm caused by a return to the original acquistion context. Behav Res Ther 43:323–336.

Vervliet B, Baeyens F, Van den Bergh O, Hermans D (2013): Extinction, generalization, and return of fear: A critical review of renewal research in humans. Biol Psychol 92:51–58.

Vlaeyen JW, de Jong J, Geilen M, Heuts PH, van Breukelen G (2002): The treatment of fear of movement/(re)injury in chronic low back pain: Further evidence on the effectiveness of exposure in vivo. Clin J Pain 18:251−261.

Volders S, Meulders A, De Peuter S, Vervliet B, Vlaeyen JW (2012): Safety behavior can hamper the extinction of fear of

movement-related pain: An experimental investigation in healthy participants. Behav Res Ther 50:735–746.

Volders S, Meulders A, De Peuter S, Vlaeyen JW (2014): The reduction of fear of movement-related pain: Does motivational context matter? Clin J Pain. DOI:10.1097/AJP.0000000000000187. (Epub ahead of print).

Williams AE, Rhudy JL (2007): The influence of conditioned fear on human pain thresholds: Does preparedness play a role?. J Pain 8:598–606.

Wolosin SM, Zeithamova D, Preston AR (2013): Distributed hippocampal patterns that discriminate reward context are associated with enhanced associative binding. J Exp Psychol Gen 124:1264–1276.

Yaguez L, Coen S, Gregory LJ, Amaro E, Jr., Altman C, Brammer MJ, Bullmore ET, Williams SC, Aziz Q (2005): Brain response to visceral aversive conditioning: A functional magnetic resonance imaging study. Gastroenterology 128:1819–1829.