Anxious anticipation and pain: the influence of instructed *vs* conditioned threat on pain

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Abstract

Negative emotions such as anxiety enhance pain perception. However, certain threat characteristics are discussed to have different or even divergent effects on pain (hypoalgesia *vs* hyperalgesia). In order to investigate the neurobiological basis of different threats, we compared the impact of conditioned threat (CT) *vs* instructed threat (IT) on pain using fMRI. In two groups, participants underwent either Pavlovian threat conditioning or an instructed threat procedure. Afterwards, in an identical test phase participants watched the same visual cues from the previous phase indicating potential threat or safety, and received painful thermal stimulation. In the test phase, pain ratings were increased in both groups under threat. Group comparisons show elevated responses in amygdala and hippocampus for pain under threat in the CT group, and higher activation of the mid-cingulate gyrus (MCC) in the IT group. Psychophysiological interaction analyses in CT demonstrated elevated connectivity of the amygdala and the insula for the comparison of pain under threat *vs* safety. In IT, the same comparison revealed elevated functional connectivity of the MCC and the insula. The results suggest a similar pain augmenting effect of CT and IT, which, however, seems to rely on different networks mediating the impact of threat on pain.

Key words: anxiety; threat; pain

Introduction

Emotions have tremendous influence on the perception of pain. In general, pain is diminished by positive while it is increased by negative emotions (Kenntner-Mabiala and Pauli, 2005; Kenntner-Mabiala *et al.*, 2008; Bushnell *et al.*, 2013; Reicherts *et al.*, 2013). In particular, the anticipation of an aversive event is supposed to cause heightened pain perception (Tracey and Mantyh, 2007). The predictability of the aversive event seems to be a crucial factor, as it was shown that the more unpredictable an upcoming aversive event the greater its pain augmenting effect (Ploghaus *et al.*, 2003). In contrast, negative emotions can also result in a decrease of pain. This seemingly paradox effect was first established in animal research, showing that high levels of stress lead to so-called stress-induced analgesia (Butler

and Finn, 2009). In human studies, decreased pain sensitivity was observed when participants were anticipating an aversive electrical shock, which they had experienced before (predictable threat). However, participants reported more pain when they were expecting an electrical shock they had never experienced before (unpredictable threat) (Rhudy and Meagher, 2000).

The affective modulation of pain supposedly relies on the activation of the descending pain control system, which affects the afferent transmission of spinal nociceptive signals to many brain regions (including the thalamus, amygdala, insula and somatosensory cortex), and consequently is able to either increase or decrease neural activation in response to pain (Wiech and Tracey, 2009; Bushnell *et al.*, 2013). Studies on the pain increasing effect of anticipatory threat suggest the involvement

of regions such as the entorhinal complex, amygdalae, anterior insula and prefrontal cortices (Hsieh *et al.*, 1999; Ploghaus *et al.*, 1999, 2000; Porro *et al.*, 2003; Koyama *et al.*, 2005; Song *et al.*, 2006). In a seminal study, it was demonstrated that the level of expected aversiveness of upcoming heat pain modulates the actual experience and neural processing of this stimulus (Ploghaus *et al.*, 2001). The perception of physically identical heat pain stimuli was increased during trials, which were anticipated as highly compared with moderately painful. This exacerbation of pain co-occurred with elevated responses in hippocampus and entorhinal cortex, which are known to share connections to the amygdalae and were found to be activated in anxiety and threat (McNaughton and Gray, 2000). In light of the potentially contrasting effects of different types of threat on pain (Rhudy and Meagher, 2000), the aim of the present study was to compare two threat induction methods which vary with regard to the predictability of an aversive outcome. Therefore, one group of participants underwent classical fear conditioning in which one cue was always followed by an electrical shock while another cue was never paired with a shock [=conditioned threat (CT)] (Pavlov, 1927). The second group was only instructed that one cue predicts the potential delivery of an electrical shock in contrast to another cue which would indicate safety (=instructed threat, IT) (Phelps *et al.*, 2001; Olsson and Phelps, 2004). Instructed threat was found to elicit elevated physiological, behavioral and neuronal responses (amygdala and insula) following CS+ presentation (Phelps *et al.*, 2001). Although a recent meta-analysis suggests a huge overlap regarding the involved brain areas in conditioned and instructed threat, the rostral dmPFC seems to play a pivotal role in conscious threat appraisal resulting from instructed fear (Mechias *et al.*, 2010). After threat induction, participants of both groups proceeded to an identical test phase in which they watched the respective threat and safety cues again while receiving painful or non-painful heat stimulation. Based on the findings as reviewed above we assumed that both conditioned and instructed threat would result in an increase of pain which should be accompanied by elevated brain responses in sensory and affective pain-related areas. However, according to the findings by (Rhudy and Meagher 2000), one may also assume that predictable threat might lead to a decrease of pain and a co-varying reduction of activity in pain-associated brain regions (Vachon-Presseau *et al.*, 2013) in line with the concept of stress-induced analgesia.

Materials and methods

Participants

Forty-five participants were recruited from the University of Würzburg and received course credit or €20 as compensation. None of them had taken any analgesic medication or alcohol for at least 12 h prior to the test session (self-report). Participants were randomly assigned to one of the two experimental groups: instructed threat (IT, $n = 23$, 11 females) or CT ($n = 22$, 12 females). All subjects had normal or corrected-to-normal vision, were right-handed (Oldfield, 1971), and reported no current or prior history of chronic pain, neurological or psychiatric disorders. Participants were given detailed instructions about the experiment and signed a written informed consent before participating in the study. The experimental procedure was approved by the institutional review board of the medical faculty of the University of Würzburg. Before the actual experimental session in the fMRI scanner, participants also filled out

Table 1. Mean scores in pain threshold and questionnaires in the two experimental groups

Measure	CT ($n = 22$)		IT ($n = 23$)		$F(1,44)$	P
	M	s.d.	M	s.d.		
Pain threshold (°C)	45.53	2.33	45.57	1.76	0.00	0.99
Age	23.13	2.18	24.18	2.77	2.01	0.16
STAI_State	34.74	6.90	36.50	6.22	0.81	0.66
STAI_State_II	34.61	6.83	31.86	5.41	2.22	0.14
STAI_Trait_	35.70	9.44	35.14	7.17	0.05	0.82
PANAS_Positive	30.09	5.90	31.09	4.40	0.42	0.52
PANAS_Negative	12.26	2.80	13.18	5.83	0.46	0.50
PSQ_Total	3.48	1.23	2.97	1.42	1.65	0.21
FPQ	73.17	30.44	70.00	24.82	0.15	0.70
PVAQ	34.87	10.00	29.18	10.14	3.59	0.06
PCS_sum	14.83	8.18	15.73	7.13	0.15	0.70

STAIT/S, State/Trait Anxiety Inventory; PANAS, Positive Affect/Negative Affect Schedule; PSQ, Pain Sensitivity Questionnaire; FPQ, Fear of Pain Questionnaire; PVAQ, Pain Vigilance and Avoidance Questionnaire; PCS, Pain Catastrophizing Scale.

questionnaires on current positive or negative affect (PANAS) (Krohne *et al.*, 1996), and state and trait anxiety (STAI) (Laux *et al.*, 1981). After the experiment, participants filled out questionnaires on pain catastrophizing (PCS) (Sullivan *et al.*, 1995) pain-related fears (FPQ) (Roelofs *et al.*, 2005), sensitivity for pain (Ruscheweyh *et al.*, 2009) vigilance for pain (PVAQ) (McCracken, 1997). The groups did not differ in these variables (Table 1).

Instructed (IT) vs conditioned (CT) threat

To elicit unpredictable threat, one group underwent an instructed fear paradigm (Phelps *et al.*, 2001; Olsson and Phelps, 2004). In this group (IT), participants were instructed that they would receive at least one and up to three shocks throughout the experiment during threat cue presentation, while being assured that they would never receive any shock during the safety cue. No shock work-up procedure was performed and participants actually never received any shocks. In the CT group, the amplitude of the shock was determined individually by a work-up procedure. 1 mA was added to the respective shock amplitude resulting in the unconditioned stimulus (UCS) intensity, which was used during conditioning. Participants were given no information about the stimulus contingency between cues and shocks before or during the experiment. In the conditioning phase, participants received a shock at the offset of each threat cue, while they never received any shock paired with the safety cue (Figure 1).

Thermal stimulation

Thermal stimuli were delivered using a Somedic MSA thermal stimulator (Somedic Sales AB, Hörby, Sweden) and a MRI-compatible Peltier thermode with an active surface of 25 × 50 mm^2. The thermode was attached to the volar forearm of the non-dominant hand. The individual pain threshold was assessed by applying 10 trials of gradually increasing temperature (1 °C/s) from a baseline of 32 °C; participants were asked to stop the stimulus delivery by a button press as soon as they felt pain. The average pain threshold temperature was $M = 42.48$ °C, s.d. $= 2.87$, and did not differ between groups (see Table 1). The individual pain threshold was used as painful stimulus, whereas the same temperature minus 2 °C was used as non-
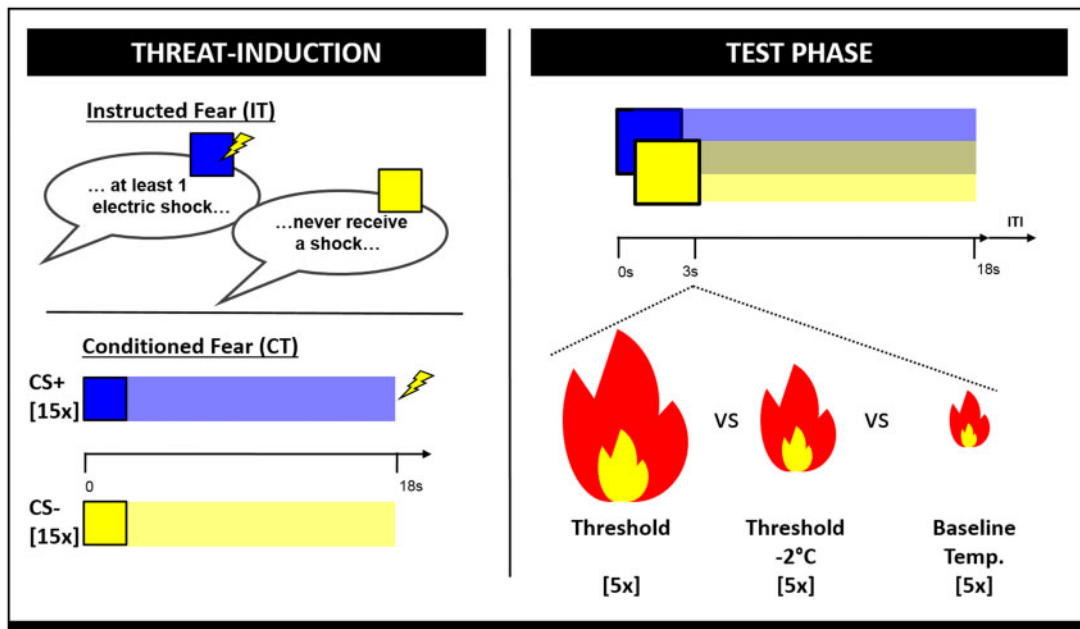
Fig. 1. Experimental paradigm. Participants underwent threat induction (CT *vs* instructed threat, IT) according to their group assignment before entering the test phase, which was identical in both groups. Pain stimuli (five painful = pain threshold, five non-painful = pain threshold – 2 °C) during the test phase were delivered 3 s after visual cue (threat *vs* safety) onset (cue duration = 18 s), and remained at the target temperature (painful *vs* non-painful) for 3 s. Participants rated thermal pain intensity and unpleasantness after each trial. During five trials per condition no thermal pain stimulation was administered.

painful stimulus in the experimental session. In no-pain trials, the thermode remained on the baseline level. During the experiment, heat (pain) stimuli were applied at a rise time of 5 °C/s starting from a baseline that was defined as 10 °C lower than the individual pain threshold temperature and remained for 3 s on the target level.

Skin conductance level

Skin conductance level (SCL) was continuously recorded (500 Hz) using a V-Amp amplifier (Brainproducts, Munich, Germany). MRI-compatible electrodes were placed on the medial phalanges of the second and third digit of the non-dominant hand. The V-Amp system constantly delivered 0.5 V across the two Ag/AgCl standard electrodes (8 mm diameter) filled with a 0.05 molar sodium chloride electrolyte paste. Changes in skin conductance were determined by subtracting the activity in the 1 s before cue onset. Cue-evoked changes were determined by scoring the average level from 1 to 7 s after visual cue onset. Pain-evoked changes were determined by scoring the mean amplitude from 8 to 18 s after visual cue-onset (=time point thermal stimulus reached target temperature until end of trial). Before conducting statistical analyses, logarithms of all values (SCL + 1) were computed to normalize the distribution (Venables and Christie, 1980).

Self-report ratings

After each thermal stimulus, subjects rated its intensity and unpleasantness on a visual analog scale (VAS), converted offline to values between 0 and 100. The scale for pain intensity ratings was labeled 'no pain' at the left and 'unbearable pain' at the right lower end of the scale, for pain unpleasantness ratings 'not unpleasant at all' and 'extremely unpleasant', respectively. The ratings were obtained by moving a cursor using the index and middle finger of the right hand on a keypad (LUMItouch™;

Photon Control, Inc., Burnaby, BC, Canada). After the test phase participants were asked to rate the visual cues with regard to valence (−4 = very negative, +4 = very positive), arousal (1 = not arousing at all, 9 = very arousing) and threat (1 = not threatening at all; 9 = very threatening). In addition, participants should give shock expectancy ratings on a 100-point VAS (0 = not at all; 100 = for sure) for threat and safety cues.

Procedure

The experiment had a 2 (group: IT *vs* CT) × 2 (cue: threat *vs* safety) mixed design. Visual cues (blue *vs* yellow squares, 200 × 200 pixels) served as both threat and safety cues, counterbalanced across subjects. Visual stimuli were presented via MRI-compatible goggles (VisuaStim, Magnetic Resonance Technologies, Northridge, CA, USA, 800 × 600 screen resolution) controlled by Presentation (Neurobehavioral Systems, Albany, CA, USA). The experiment comprised two phases: a threat-induction phase and a subsequent test phase (Figure 1). During conditioning in the CT group (30 trials), visual cues were presented for 18 s, and reinforced threat trials terminated with an electrical shock (100% contingency). The inter-trial interval (ITI) varied between 15 and 17.5 s.

The test phase was identical for both groups: the same visual cues as in the threat induction phase were presented for 18 s, intermitted by ratings and an additional ITI varying between 5.5 and 7.5 s. In addition to the presentation of threat and safety cues, thermal stimuli (five painful, five non-painful per cue condition) were administered during the trials starting 3 s after visual cue onset. In order to assess brain activity evoked by cues alone, there were also five trials per condition in which no thermal stimulation was delivered. After each trial, participants were asked to rate intensity and unpleasantness of the thermal stimulus. After the test phase, participants were asked to rate the cues with regard to affective valence, arousal, threat level and shock probability (manipulation check).

fMRI data acquisition and analysis

Scanning parameters. The MR scanning was performed on a 1.5 Tesla whole-body tomograph (Siemens Avanto, Germany). Functional data included whole-brain T2*-weighted single-shot gradient echo-planar images (EPIs) recorded with a repetition time of 2.5 s (echo time = 30 ms, flip angle = 90°, field-of-view = 200 mm, acquisition matrix = 64 × 64, voxel-size = 3.1 × 3.1 × 5 mm^3). Each volume contained 25 axial slices parallel to the AC-PC-line (from the anterior to the posterior commissure) that were acquired in interleaved order. Slices were 5 mm wide with a 1-mm gap. A total of 500 EPIs were recorded in every participant during the test phase. A high-resolution structural image of the brain was created via T1-weighted magnetization-prepared rapid gradient-echo imaging (MP-RAGE; repetition time = 2250 ms, echo time = 3.93 ms, flip angle = 8°, field-of-view = 256 mm, acquisition matrix = 256 × 256, voxel size = 1 × 1 × 1 mm^3). If the magnetic field is inhomogeneous, EPI images are often spatially distorted (Hutton *et al.*, 2002). Therefore, a gradient echo (GRE) field mapping (TR: 1000 ms, TE: 10 ms, slices: 25, slice thickness: 5 mm, FOV: 240 mm, matrix size: 64 × 64) was performed prior to the acquisition of the functional MRI data to compensate for inhomogeneity of the magnetic field.

fMRI preprocessing. Standard preprocessing of fMRI data using Statistical Parametric Mapping software (SPM8; Wellcome Department of Imaging Neuroscience, London, UK) included slice-time correction, realignment (using an individual voxel displacement map on the basis of GRE field-mapping), co-registration and segmentation. In the next step, functional images were spatially normalized into standard Montreal Neurological Institute (MNI) space using a voxel size of 2 × 2 × 2 mm^3, and smoothed with an 8-mm full-width-half-maximum (FWHM) Gaussian kernel. A high-pass temporal filter (cut-off 128 s) and correction for auto-correlation between successive scans were applied to the time series (AR1).

First level analysis. At the first level of the analysis, regressors in the design matrix included the two thermal stimuli and the no-stimulation condition, and their respective sub-events, which is the ramp-up of the thermal stimulation and the actual target temperature (LowPain$_{ramp-up}$, HighPain$_{ramp-up}$; LowPain, HighPain, NoPain), as well as the two visual cue-onsets (Threat, Safe), resulting in fourteen regressors, eight regressors for pain-related activity: Safe_LowPain$_{ramp-up}$, Threat_LowPain$_{ramp-up}$, Safe_HighPain$_{ramp-up}$, Threat_HighPain$_{ramp-up}$, Threat_LowPain, Threat_HighPain, Safe_LowPain, Safe_HighPain, and 6 regressors for visual cue-related activity: ThreatCue_NoPain, ThreatCue_LowPain, ThreatCue_HighPain, SafeCue_NoPain, SafeCue_LowPain and SafeCue_HighPain. The cue-onset was defined at visual cue onset, the start of thermal stimulation ($_{ramp\,up}$) was defined as 3 s after cue onset, and the peak of thermal stimulation (target temperature) was defined to occur at 5 s after cue onset. In addition, realignment parameters were included as nuisance regressors accounting for movement artifacts during scanning. Event-related brain activation was modeled by convolving stick functions with the canonical hemodynamic response function.

Second level analysis. The effect of threat on pain processing in the brain was examined by computing the interaction contrast [(Threat_HighPain > Threat_LowPain) > (Safe_HighPain > Safe_LowxPain)] for each participant. This approach ensured that the obtained activity was specific to painful heat stimulation under threat. A general effect of threat on pain was examined across groups (CT + IT). The main focus of the study—the potential difference between instructed *vs* CT—was further examined by calculating a between-groups *t*-contrast of the aforementioned interaction. In addition, we were interested in neural activity correlating with the impact of threat on self-reported pain ratings. Therefore, we calculated for each group the difference in pain ratings for threat and safety trials (threat—safety). This difference was calculated separately for pain intensity and pain unpleasantness ratings and entered as a covariate in the second level analyses of the contrast [Threat_HighPain > Safe_HighPain].

We applied two different statistical procedures for (A) the analysis of pain-associated activity and (B) responses following the presentation of threat *vs* safety cues (manipulation check). For (A), we followed the approach of previous fMRI studies on pain modulation (Geuter *et al.*, 2013; Freeman *et al.*, 2015; Schmid *et al.*, 2015) and explored pain-related activity within predefined regions of interest (ROIs) based on previous fMRI studies including thalamus, insula, somatosensory cortex (Koyama *et al.*, 2005), amygdala (Schmid *et al.*, 2015), hippocampus (Ploghaus *et al.*, 2001), mid-cingulate gyrus (MCC) (Wiech *et al.*, 2010) and dorsolateral prefrontal cortex (Lorenz *et al.*, 2003). ROIs were based on anatomical templates, constructed from the automatic anatomic labeling and the Talairach Daemon database included in the Wake Forest University (WFU) PickAtlas (Maldjian *et al.*, 2003, 2004). Results were corrected for multiple comparison using family wise error (FWE) correction at a level of $P < 0.05$, separately or each ROI. Regarding B), in order to investigate the effect of the threat-induction procedure, we analyzed cue effects calculating the contrast of ThreatCue_NoPain > SafeCue_NoPain. Here, instead of using predefined ROIs, we explored activity at whole-brain level, applying a-priori significance threshold of $P < 0.001$, and a cluster threshold of $k \geq 61$. This is equivalent to a whole brain false discovery rate of $P < 0.05$, according to a Monte Carlo simulation with respect to the applied imaging parameters. To determine this cluster threshold, an MATLAB-based script was used to simulate patterns of activated voxels (10 000 repetitions) within the used field of view (Slotnick *et al.*, 2003). All analyses used a random-effects model for contrast maps of *t*-scores. Regions with significant activations are reported according to the automatic anatomic labeling included in the WFU PickAtlas (AAL).

Psychophysiological interaction (PPI) analysis. Functional connectivity was investigated by conducting separate PPI analyses for the CT and IT group (Friston *et al.*, 1997). Based on the results of the between-group analyses, we chose the *left amygdala* as the seed region in the CT group, and the *right MCC* in the IT group. Thus, the activation pattern of the left amygdala and the right MCC served as the physiological variable, and the contrast [Threat_HighPain > Safe_HighPain] was included as the psychological variable of interest. The activation of the seed region was obtained by extracting the principal Eigenvariate within a sphere of 5 mm radius around the individual peak voxel within the seed region. The amygdala seed region was defined on the basis of the AAL. The MCC seed region was defined as a sphere of 15 mm radius around the peak voxel derived from the group comparison analysis ($x = 12$, $y = 6$, $z = 30$; see Results section). Activity was explored within the predefined ROIs as specified in the previous section. Results were corrected for multiple comparison using FWE correction at a level of $P < 0.05$ separately for each ROI. The significance threshold was set to $P < 0.05$, FWE-corrected.

Table 2. Affective ratings for threat and safety cues

| Cue | Rating | CT (n = 23) | | IT (n = 22) | | t-test |
		M	s.d.	M	s.d.	P
Threat	Threat (1 to 9)	5.26	2.47	5.18	2.26	0.91
	Valence (−4 to 4)	−1.65	1.27	−1.45	1.06	0.57
	Arousal	5.26	2.38	4.86	2.55	0.59
	Shock Probability	72.35	32.58	63.14	25.97	0.91
Safety	Threat (1 to 9)	4.09	1.88	2.95	2.01	0.06
	Valence (−4 to 4)	−0.26	1.60	1.59	1.56	<0.01
	Arousal	4.09	1.93	3.32	1.89	0.18
	Shock Probability	38.35	26.34	15.09	23.40	<0.01

Results

Manipulation check

Affective ratings of the cues after the test phase (Table 2) were analyzed with repeated-measures ANOVAs including the within-subjects factor CUE (safety *vs* threat) and the between-subjects factor GROUP (IT *vs* CT). Threat compared with safety cues were rated as more arousing, $F(1,43) = 25.40$, $P < 0.001$, $\eta_p^2 = 0.37$, more threatening, $F(1,43) = 24.62$, $P < 0.001$, $\eta_p^2 = 0.36$, and more likely to be followed by a shock, $F(1,43) = 54.44$, $P < 0.001$, $\eta_p^2 = 0.56$. In the CT group, shock probability was rated generally higher as in the IT group $F(1,43) = 7.41$, $P = 0.009$, $\eta_p^2 = 0.15$. In both groups, threat cues were rated as more negative than safety cues $F(1,43) = 57.88$, $P < 0.001$, $\eta_p^2 = 0.57$. This effect was further qualified by a significant interaction of GROUP and CUE, $F(1,43) = 8.05$, $P = 0.007$, $\eta_p^2 = 0.16$, which was due to a more pronounced difference in the IT group $F(1,21) = 54.27$, $P < 0.001$, $\eta_p^2 = 0.72$, than in the CT group, $F(1,22) = 11.46$, $P = 0.003$, $\eta_p^2 = 0.34$.

Cue-related SCL changes were analyzed by a repeated-measures ANOVA containing the within-subjects factor CUE (Threat *vs* Safe), TIME (trials 1–5) and the between-subjects factor GROUP (IT *vs* CT). Due to recording failure, SCL data were only available for 42 participants (21 in each group). In line with the cue ratings, SCL change [Ln (SCL + 1)µS] in response to threat (M = 0.001, s.d. = 0.028) was larger compared with safety cues (M = −0.010, s.d. = 0.026), $F(1,40) = 6.01$, $P = 0.020$, $\eta_p^2 = 0.13$, without any differences between groups. The factor time was marginally significant, $F(1,40) = 2.81$, $P = 0.054$, $\eta_p^2 = 0.07$, pointing at habituation over time.

In order to explore cue-related brain responses, we analyzed the contrast [ThreatCue_NoPain > SafeCue_NoPain]. In both groups combined, threat *vs* safety cues evoked more pronounced activity in the superior medial frontal cortex ($x = 0$, $y = 28$, $z = 50$; $P < 0.001$, $k \geq 61$). The between-group comparison revealed no significant clusters.

Pain ratings

Sensory and affective pain ratings were analyzed by repeated-measures ANOVAs containing the within-subjects factors PAIN (low *vs* high), CUE (threat *vs* safety) and TIME (trials 1–5), and the between-subjects factor GROUP (IT *vs* CT). For affective pain ratings (Figure 2), a significant modulation of pain by threat was observed in later trials, as the significant three-way interaction PAIN × CUE × TIME indicates, $F(4,172) = 2.55$, $P = 0.041$, $\eta_p^2 = 0.06$. Separate ANOVAs for painful and non-painful trials revealed a significant interaction of CUE and TIME only for the painful trials, $F(4,172) = 3.70$, $P = 0.007$, $\eta_p^2 = 0.08$. Especially in later trials
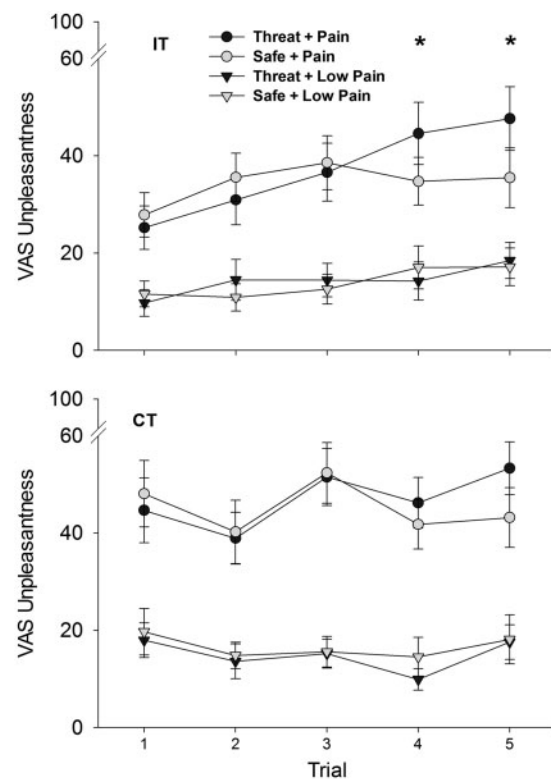

Fig. 2. Mean sensory and affective pain ratings for painful and non-painful thermal stimuli. Ratings are depicted for each trial under threat and safety cues, separated for both groups (IT *vs* CT).

painful thermal stimulation was rated as more unpleasant under threat compared with safety [trial 4: $t(44) = 2.14$, $P = 0.040$; trial 5 = $t(44) = 3.13$, $P = 0.003$], whereas early trials did not differ (Ps > 0.39). Interestingly, the GROUP × TIME interaction was also found to be significant, $F(4,172) = 3.52$, $P = 0.009$, $\eta_p^2 = 0.08$, which points at higher ratings of both painful and non-painful thermal stimuli in the CT compared with the IT group at the beginning of the test phase, especially for trial 1 [CT > IT: $t(43) = 2.86$, $P = 0.007$].

For sensory pain ratings, no modulation by threat was observed in both groups. Pain ratings were higher for painful compared with non-painful heat stimuli, $F(1,43) = 144.45$, $P < 0.001$ $\eta_p^2 = 0.77$. Overall, pain was found to increase during the experiment, as indicated by a marginal significant effect of TIME, $F(4,172) = 2.75$, $P = 0.060$, $\eta_p^2 = 0.06$. Similar to the affective pain ratings, the interaction of GROUP and TIME was significant, $F(4,172) = 5.47$, $P < 0.001$, $\eta_p^2 = 0.11$.
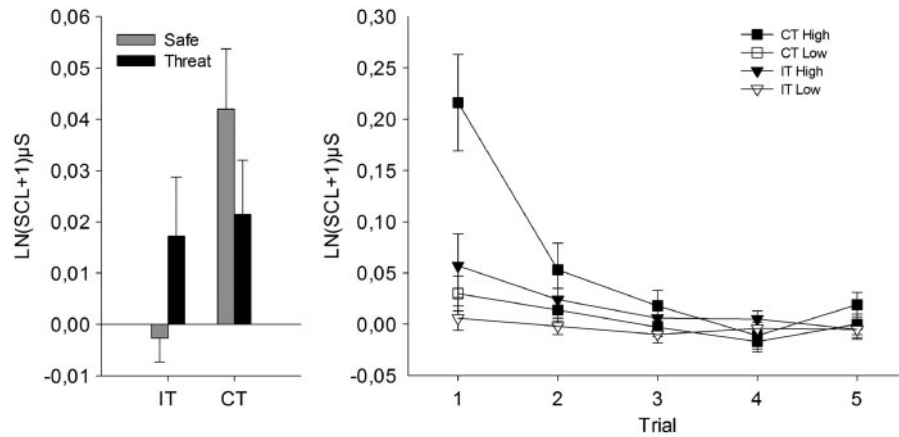
Fig. 3. Mean SCL (+SEM) in response to threat *vs* safety cues (*left*) and in response to the thermal stimulation (painful *vs* non-painful) over time (trials 1–5) separated for IT *vs* CT (*right*).

Pain-related SCL

Pain-related SCL changes were analyzed by repeated-measures ANOVA containing the within-subjects factor CUE (threat *vs* safety), PAIN (low *vs* high), TIME (trials 1–5) and the between-subjects factor GROUP (IT *vs* CT). As expected, painful compared with non-painful thermal stimulation elicited larger SCL responses, $F(1,40) = 18.83$, $P < 0.001$, $\eta_p^2 = 0.32$. This effect was further qualified by a significant interaction of PAIN × TIME × GROUP, $F(4,169) = 4.05$, $P = 0.030$, $\eta_p^2 = 0.09$, as a result of a more pronounced differentiation between early painful and non-painful trials in the CT group [trial 1, high *vs* low, CT group: $t(20) = 3.94$, $P = 0.001$; IT group, $t(20) = 1.76$, $P = 0.09$]. In addition, the interaction of CUE × GROUP was significant, $F(1,40) = 5.81$, $P = 0.021$, $\eta_p^2 = 0.13$ due to higher responses in the IT group during threat relative to safety compared with the CT group, $t(20) = 2.06$, $P = 0.052$, and $t(20) = -1.49$, $P = 0.15$, respectively (Figure 3).

fMRI

Modulation of pain by threat. For both groups combined, the contrast [(Threat_HighPain > Threat_LowPain) > (Safe_HighPain > Safe_LowPain)] resulted in activation of the right postcentral gyrus (see Table 3; $P < 0.05$, FWE-corrected).

Modulation of pain by instructed *vs* CT. Comparing groups, we found enhanced activation in the left amygdala and the right hippocampus under CT compared with IT for the contrast [(Threat_HighPain > Threat_LowPain) > (Safe_HighPain > Safe_LowPain)]. The reverse group comparison revealed stronger activation of the right MCC in the IT group than in the CT group (see Table 3; $P < 0.05$, FWE-corrected).

Correlations between BOLD activity and pain ratings. Across both groups, enhanced pain intensity under threat *vs* safety was associated with activity in the right thalamus (contrast: [Threat_HighPain > Safe_HighPain]; see Table 3). In contrast, pain unpleasantness ratings did not correlate significantly with brain activity within the ROIs (see Table 3; $P < 0.05$, FWE-corrected).

Temporal effects on BOLD activity and modulation by anxiety. Potential time effects were explored by incorporating a time modulation regressor (t-mod), however no linear trend of BOLD

activity within the predefined ROIs was found. Furthermore, including STAI-S and STAI-T scores reveled no significant correlation with activation in any of the ROIs.

Functional connectivity. Within the CT group, the PPI analysis revealed increased functional connectivity for the contrast [Threat_HighPain > Safe_HighPain] between the left amygdala (seed region) and the left and right insula. Within the IT group, functional connectivity for the same contrast was enhanced between the right MCC serving as the seed region and left and right insula, and the left postcentral gyrus (see Table 3; $P < 0.05$, FWE-corrected).

Between-group comparisons using the amygdala as a seed region revealed more pronounced connectivity with the left and right dlPFC in IT > CT. The same analysis using the MCC as a seed region indicated stronger connectivity with the amygdala and the anterior insula in CT > IT (see Table 3; $P < 0.05$, FWE-corrected).

Discussion

In this study, we aimed at identifying neural correlates of the modulation of pain by different types of threat. To this end, predictable threat was induced in participants using classical fear conditioning, whereas unpredictable threat was induced using a mere cognitive manipulation (instructed threat). In a test phase identical for both groups, pain processing was investigated when participants watched the respective threat and safety cues. As the ratings and changes in SCL and BOLD activity in response to the cues show, the threat induction was successful in both groups. Elevated activity in the medial PFC in response to the threat cue is in line with previous studies demonstrating its role in fear learning (Gramsch *et al.*, 2014; Fullana *et al.*, 2016), fear memory and fear expression in humans and animals (Milad and Quirk, 2002; Maren and Quirk, 2004; Etkin *et al.*, 2011).

In both groups, threat-related hyperalgesia was observed in elevated affective pain ratings, which was accompanied by augmented SCL changes in response to thermal pain in the IT group. Contradictory to previous studies (Rhudy and Meagher, 2000, 2003), fear conditioning did not lead to hypoalgesia. Elevated pain ratings of the CT group in the beginning of the test phase supposedly reflect the successful induction of threat in response to the conditioning procedure. However, after the

Table 3. Significant brain activity associated with the modulation of pain processing by instructed threat (IT) and CT

Region		H	MNI coordinates			T	P_{FWE}
			x	Y	z		
Contrast [(Threat_HighPain > Threat_Low Pain) > (Safe_HighPain > Safe_LowPain)]							
IT + CT	Postcentral	R	62	2	20	4.62	0.019
CT > IT	Amygdala	L	−30	−4	−14	4.58	0.002
	Hippocampus	R	26	−28	−8	3.96	0.032
IT > CT	Cingulate gyrus (MCC)	R	12	6	30	4.64	0.016
Connectivity (PPI) with left amygdala as seed for the contrast [Threat_HighPain > Safe_HighPain]							
CT	Insula	L	−30	22	−8	4.85	0.019
	Insula	R	32	26	4	5.22	0.009
CT > IT	*No significant clusters*						
IT > CT	DLPFC	R	44	36	18	4.10	0.035
	DLPFC	R	46	40	16	4.04	0.040
	DLPFC	L	−42	14	36	4.46	0.012
Connectivity (PPI) with MCC as seed for the contrast [Threat_HighPain > Safe_HighPain]							
IT	Insula	L	−38	8	−10	4.64	0.034
	Insula	R	38	−10	18	5.36	0.008
	Postcentral	L	−66	−16	22	5.32	0.018
CT > IT	Amygdala	L	−30	2	−22	3.05	0.048
	Insula	R	34	16	−10	3.82	0.049
IT > CT	*No significant clusters*						
Correlations with pain ratings [Threat_HighPain – Safe_HighPain] for the contrast [Threat_HighPain > Safe_HighPain]							
IT + CT	Pain unpleasantness						
	No significant clusters						
	Pain intensity						
	Thalamus	R	8	−6	0	4.21	0.013

The table shows properties of peak voxels within significant clusters. Significance threshold: $P < 0.05$, *FWE-corrected*; H, Hemisphere; PPI, Psychophysiological Interaction; MCC, mid-cingulate gyrus.

CS+ was presented for several times without the US, fear extinction is very likely (Milad and Quirk, 2012; Sperl *et al.*, 2016). In the IT group instead, threat persist over time and even might increase in the course of the experiment. In support for this hypothesis, Bublatzky *et al.* (2014) found that a verbal threat instruction persists over multiple experimental sessions and even longer periods (up to 3 days) without any actual experience of the UCS. On the neural level, the interaction of threat and pain revealed a stronger activation of the amygdala (and to a lesser degree of neighboring hippocampal areas) for the CT group (see Table 3, Figures 4 and 5). This is in line with findings from fear conditioning studies showing stronger amygdala and hippocampus activation in response to threat compared with safety cues (Maren, 2001). In the present study, elevated amygdala activation might indicate the integration of successful threat detection and nociceptive input leading to increased pain perception. In favor of this interpretation, the pivotal role of the amygdala for pain processing and its modulation was underscored in recent years, especially since it is perfectly located to serve as an integrating hub mediating cognitive and emotional aspect of pain (Simons *et al.*, 2014; Neugebauer, 2015). Enhanced hippocampus activation in the CT group fits with previously observed effects of threat-induced hyperalgesia in a comparable paradigm, in which increased pain perception during anticipation of aversive events came along with elevated activation in hippocampal and neighboring entorhinal cortical areas (Ploghaus *et al.*, 2001). Under instructed threat, the interaction of threat and pain revealed stronger activation of the MCC compared with CT. This is in accordance with a previous finding demonstrating a correlation of MCC activity and the likelihood

to evaluate a laser stimulus as being painful when participants were led to believe that an actually safe stimulation might damage their skin (Wiech *et al.*, 2010). According to the authors, the engagement of MCC ensures that potentially more threatening stimuli also receive more attention and thereby supports their reliable detection. Connectivity analyses revealed for CT elevated connectivity between the amygdala and bilateral insula for pain under threat *vs* safety. For IT, higher connectivity of the MCC with bilateral insula and the somatosensory cortex was observed. Between-group comparisons revealed higher connectivity of amygdala and left and right dlPFC in IT compared with CT. Regarding the connectivity analysis using the MCC as a seed region, CT showed significantly higher connectivity with the amygdala and the anterior insula than IT. This might be interpreted as indicating that in CT, amygdala (and MCC) activity co-varies with the anterior insula encoding especially the more cognitive-affective domain of pain while in IT, functionally connectivity was found for both the anterior, and the mid insula, which is supposed to reflect more the sensory discriminative component of pain (Brooks *et al.*, 2002; Wiech *et al.*, 2014). More pronounced connectivity of amygdala and DLPFC in IT compared with CT might reflect the role of DLPFC in higher order appraisal processes (see Kalisch *et al.*, 2006) when threat is solely instructed by verbal suggestion. Although the MCC and the amygdala may play distinct roles in the modulation of pain by conditioned and instructed threat, the observed hyperalgesia in both conditions might be mediated via similar neural mechanisms. That is, both the ACC in the IT group and the amygdala in the CT group displayed enhanced functional connectivity with the anterior insula. It has been suggested previously that the
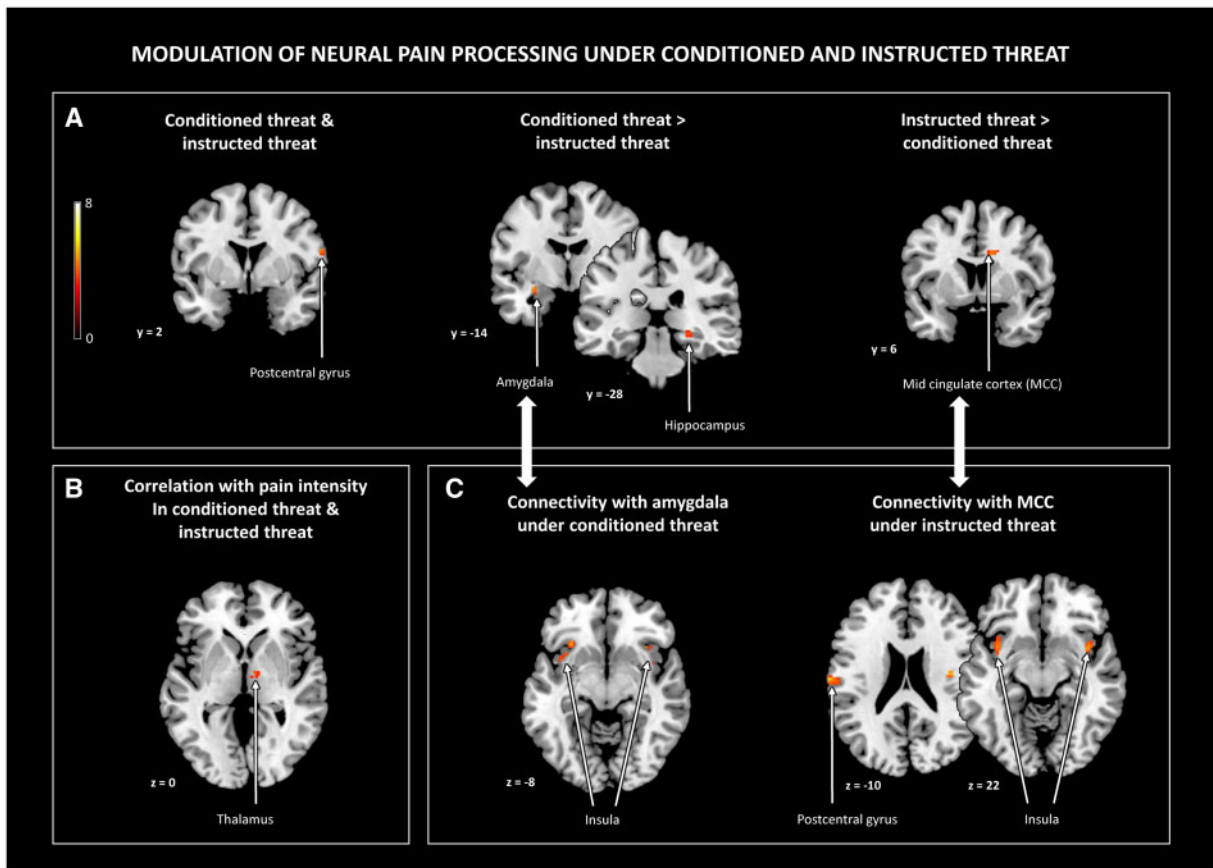
Fig. 4. Neural pain processing under conditioned and instructed threat. (A) In both, the conditioned and the instructed threat group combined (*left*), the right postcentral gyrus responded more to painful stimuli under threat than under safety (contrast: [(Threat_HighPain > Threat_Low Pain) > (Safe_HighPain > Safe_LowPain)]). In the CT group (*middle*), the left amygdala and the right hippocampus were more active in this contrast than in the instructed threat group. In the instructed threat group (*right*), the right MCC was more active than in the CT group. (B) In both threat groups combined, the difference in pain intensity ratings between threat and safety was positively correlated with right thalamus activity (contrast: [Threat_HighPain > Safe_HighPain]). (C) Within the CT group, the amygdala showed enhanced functional connectivity (contrast: [Threat_HighPain > Safe_HighPain]) to bilateral insula (*left*), while the MCC showed enhanced functional connectivity to bilateral insula and left postcentral gyrus within the instructed threat group (*right*). Statistical threshold: $P < 0.05$, FWE-corrected. Display threshold: $P < 0.001$ (uncorrected).

(anterior) insular cortex plays an important role in the awareness of bodily experiences (Craig, 2009). Furthermore, the insula is crucial for the modulation of pain by negative emotions (Roy *et al.*, 2009). Probably the same is true for the effect of threat on pain even though the involvement of additional brain areas depends on specific threat characteristics such as its predictability, cognitive representations and acquisition. The interaction of threat and pain across all participants showed a stronger activation of the somatosensory cortex contralateral to stimulation for painful threat trials in line with previous studies (Roy *et al.*, 2009). Moreover, sensory pain ratings were correlated with activity in the thalamus for painful heat stimuli during threat *vs* safety trials, which corresponds with previous findings of enhanced thalamic activity due to negative expectations modulating pain (Koyama *et al.*, 2005; Atlas *et al.*, 2010). Thus, the present findings add to the literature showing that nociceptive brain regions are also modulated by stimulus expectancies (Tracey, 2010; Atlas and Wager, 2012). Several studies investigating the effect of stimulus/outcome expectancy on pain processing revealed that even short-term expectations (e.g. threat cues) might have strong effects on pain (Phelps *et al.*, 2001; Koyama *et al.*, 2005; Lorenz *et al.*, 2005; Atlas *et al.*, 2010). Furthermore, understanding how anticipatory threat influences pain is crucial for understanding nocebo effects, as nocebo hyperalgesia is probably the result of negative outcome expectations leading to increased pain experiences (Tracey, 2010). Neuroimaging studies of nocebo effects in pain found elevated activation in hippocampus, MCC and the amygdala (Kong *et al.*, 2008; Bingel *et al.*, 2011; Schmid *et al.*, 2015), which were also involved in the present study. These findings might be interpreted as further evidence for the similarities of nocebo hyperalgesia and exacerbated pain by negative affect.

A possible alternative a-priori hypothesis was that CT in contrast to IT might actually lead to hypoalgesia, since previously, predictable threat was found to result in decreased, whereas less predictable threat led to increased pain sensitivity (Rhudy and Meagher, 2000). Interestingly, the same authors report a follow-up experiment, in which both types of threat manipulation reduced pain (Rhudy and Meagher, 2003). Over the past decades, it has become increasingly clear that the nature, duration and intensity of the stressor are key determinants of the effects of stress and threat on pain. While exposure to an acute, robust, intense stressor seems to induce a reduction in pain responding, a phenomenon described as stress-induced analgesia (Butler and Finn, 2009), exposure to repeated physical or psychological stressors, which may be more anticipatory and thus anxiogenic in nature, typically results in the phenomenon of stress-induced hyperalgesia in humans (Kuehl *et al.*, 2010;
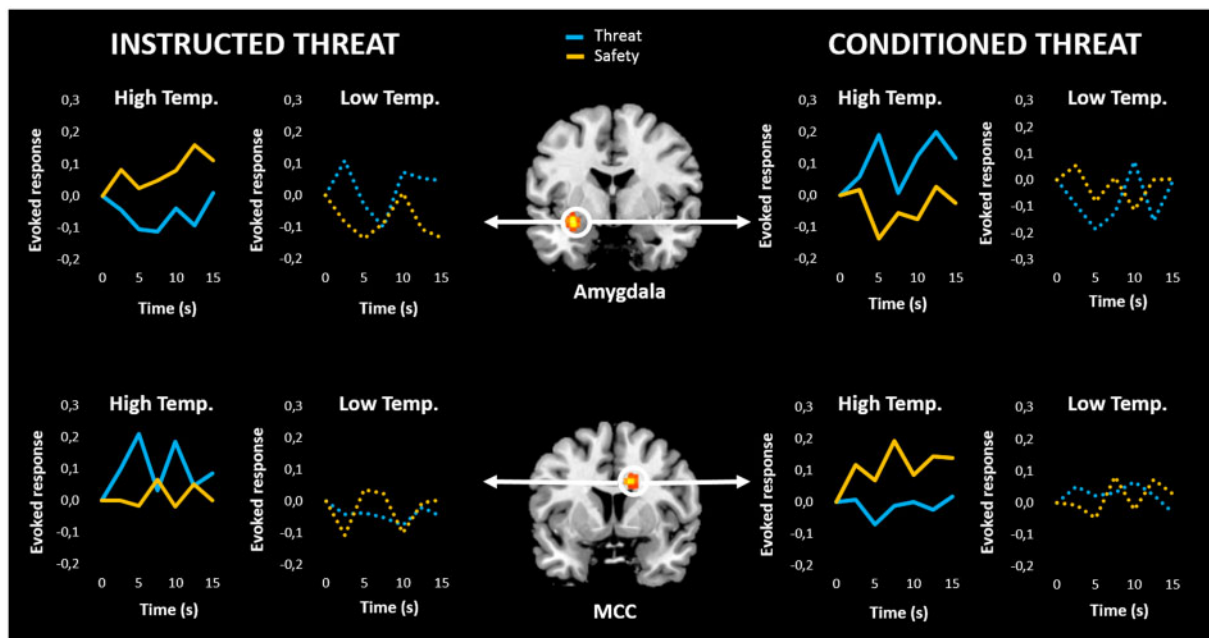
Fig. 5. Peri-stimulus-time-histograms (rfxplot for SPM; Glascher, 2009) of MCC and amygdala activation for the combination of threat *vs* safety and high *vs* low pain trials separately for the instructed threat (*left*) and the CT group (*right*). Statistical threshold: $P < 0.05$, FWE-corrected. Display threshold: $P < 0.001$ (uncorrected).

Gibbons *et al.*, 2012; Crettaz *et al.*, 2013). In a comprehensive review of human and animal models of stress-induced hyperalgesia, it is concluded that psychological stress-based models usually involve assessment of pain responding during or following exposure to a conditioned or unconditioned aversive stimulus (Jennings *et al.*, 2014), which is also the case in the present CT paradigm (CT group). Consequently, one has to conclude that both the cognitive instruction of threat as well as the conditioning of threat may both have induced anticipatory anxiety and thus resulted in hyperalgesia. In line with this interpretation, electric shocks, which were preceded by phobia-relevant pictures, were found to evoke increased pain unpleasantness and sensory-motor activity in fear patients compared with healthy controls (Wiemer *et al.*, 2015). Most likely, hypoalgesia is observed reliably only if the induced threat is very intense, which is rather difficult to achieve in human experiments. For example, Flor *et al.* demonstrated that a conditioning procedure, in which a mental arithmetic task and the presentation of white noise served as UCS, resulted in a decreased pain threshold, when participants were exposed to the conditioned stimulus (CS+; tone or light) without the UCS compared with a control condition (Flor and Grusser, 1999; Flor *et al.*, 2002).

Some limitations of the present study should be considered for future research. Firstly, we applied rather short pain stimuli of moderate intensity. Probably stronger, prolonged pain stimulation might result in more pronounced effects. Secondly, we chose an invariable trial structure (timing of cue onset and pain onset was fixed) in order to omit an additional source of uncertainty besides our experimental manipulation. However, such an approach results in highly correlated regressors and makes it very difficult to disentangle the contribution of each regressor independently.

In conclusion, our data reveal a pain augmenting effect of both predictable (CT) and unpredictable (IT) threat, which in the case of CT seems to rely predominantly on activation of the amygdala whereas instructed threat leads to elevated activation of MCC. However, both regions revealed elevated functional connectivity with the insula, irrespective of the type of threat induced. Further research is needed to elucidate the processes underlying the pain augmenting effects of threat and elaborate conditions under those hypoalgesia may be observed in humans.

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