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Modulation of the startle reflex by heat pain: Does threat play a role?

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Abstract

Background: Previous studies have indicated that the startle reflex is potentiated by phasic, but not by tonic, heat pain, although the latter is seen as more strongly associated with emotional responses and more similar to clinical pain. The threat value of pain might be a decisive variable, which is not influenced alone by stimulus duration.
Objective: This study aimed at comparing startle responses to tonic heat pain stimulation with varying degrees of threat. We hypothesized that the expectation of unpredictable temperature increases would evoke higher threat and thereby potentiate startle compared with the expectation of constant stimulation.
Methods: Healthy, pain-free subjects ($n = 40$) underwent painful stimulation in two conditions (low/high threat) in balanced order. The only difference between the two conditions was that in the high-threat condition 50% of the trials were announced to include a short further noxious temperature increase at the end. Startle tones were presented prior to this temperature increase still in the phase of anticipation.
Results: We observed startle potentiation in the high-threat compared with the low-threat condition, but only in those participants who took part first in the high-threat condition. Habituation could not account for these findings, as we detected no significant decline of startle responses in the course of both conditions.
Conclusions: Our results suggest that subjective threat might indeed be decisive for the action of pain on startle; the threat level appears not only influenced by actual expectations but also by previous experiences with pain as threatening or not.

1. Introduction

The experience of pain is often functionally associated with actual or impending injury. For this reason, pain is thought to be a particularly salient indicator for threat to our body's integrity. Threatening stimuli activate the motivational defence system (Lang et al., 1998; Bradley et al., 1999, 2005), which corresponds to a neural subcortical circuit organized around the amygdala preparing the organism to engage in protective behaviour (Lang et al., 2000).

According to the view of motivational priming, activation of the motivational defence system can be measured by recording defensive reflexes, as these reflexes should be enhanced in contexts when the defence system is activated (Lang et al., 1997). This assumption has been corroborated by research using the eyeblink component of the startle reflex (Lang, 1995). It is consistently shown that fear-evoking stimuli potentiate the magnitude of the blink reflex (Lang et al., 1990; Grillon et al., 1991; Grillon and Davis, 1997; Bradley et al., 1999; Grillon and Baas, 2003).

However, studies investigating startle potentiation by pain have yet yielded inconclusive results. We found no potentiation of startle amplitude by tonic heat pain in three consecutive experiments, regardless

What's already known about this topic?
- In recent studies, startle was not potentiated by tonic heat pain although this form of pain is especially known for its emotional impact, and startle potentiation by phasic heat pain has already been demonstrated.

What does this study add?
- The results of our study suggest that the subjective threat level associated with a painful stimulus is critical for triggering startle potentiation, and that this threat level is also determined by previous experiences with similar stimuli.

of stimulation intensity and subjects' sensitivity to startle modulation (Horn et al., 2012a,b), whereas Crombez et al. (1997) obtained startle potentiation in response to phasic heat pain. Besides the distinction between tonic and phasic pain, one major difference between these studies was the degree of stimulus predictability: Crombez and colleagues applied short painful and non-painful stimuli in random order, whereas our stimuli consisted in constant stimulation for several minutes. It might be that constant and predictable tonic stimulation is not threatening enough to elicit defensive responding although tonic pain was designed to mimic clinical pain also in its stronger emotional impact. This assumption is in line with research indicating that pain is associated with higher threat when applied in an unpredictable fashion (Arntz et al., 1992; Carlsson et al., 2006; Oka et al., 2010; Lin et al., 2013).

Based on these considerations, we aimed at investigating the effects of tonic heat pain on startle with varying levels of predictability to produce varying threat levels. Experimental manipulations were varying the expectation of the course of pain (predictable vs. unpredictable) and inducing the apprehension that it may get even worse (only in the 'unpredictability' condition), which seemed close to concerns relating to pain in real life.

For that purpose, we applied two tonic stimulation conditions, which were identical in stimulus intensity, but differed regarding the expectations induced: In the high-threat condition, the temperature course was announced as variable with short temperature increases occurring late in half of the trials, whereas in the low-threat condition temperature course was announced as stable. We hypothesized that only high threat would activate the defensive system and potentiate startle. A positive finding would corroborate the assumption that affective–motivational reactions during pain are pain inherent but influenced by the associated threat, which might improve the understanding of emotional inconsistencies during pain especially in clinical settings.

2. Materials and methods

2.1 Subjects

Forty healthy volunteers (female: $n = 20$) between the ages of 31 and 59 [mean age 44.85 years; standard deviation (SD) = 8.82] were recruited by advertisement at the University of Bamberg and in the local newspaper. We chose this age range on purpose because we were interested in age groups not being naive to everyday pain.

No participant had taken any analgesic medication or alcohol at least 24 h prior to the test session. Exclusion criteria included all acute or chronic diseases. Ten women took oral contraceptives; of the remaining 10, three were in the first, six in the second and one in the third phase of their menstrual cycle. All subjects provided written informed consent and received monetary compensation for their participation. The experimental procedure was approved by the ethics committee of the University of Bamberg.

2.2 Materials and procedures

All experimental sessions were conducted in the afternoon. During the whole session, which lasted for approximately 1 h, subjects sat upright in a comfortable chair. Subjects were carefully familiarized with all the methods to be used before the start of the experiment.

The testing procedure included (1) filling out a set of questionnaires assessing general and pain-related anxiety; (2) the predetermination of stimulation intensity (using pain threshold assessment and a subsequent rating procedure); and (3) the assessment of startle responses in two thermal stimulation conditions (low threat and high threat).

Pain was induced experimentally by the use of a Peltier-based, computerized thermal stimulator with a 3×3 cm^2 contact probe (Medoc TSA-2001; Medoc Ltd., Ramat Yishai, Israel). The contact probe was attached to the left forearm; the probe position was changed after predetermination of stimulation intensity and again between the two stimulation conditions in order to avoid local sensitization.

2.2.1 Questionnaire measures

As participants might differ in their reactions to pain-related threat depending on their anxiety level, we used a set of questionnaires to assess variables relating to general or pain-related anxiety. This set consisted of the German versions of the Pain Catastrophizing Scale (PCS) (Sullivan et al., 1995), the Pain Anxiety Symptoms Scale (PASS) (McCracken et al., 1992; German version: Walter et al., 2002), the Pain Vigi-

lance and Awareness Questionnaire (PVAQ) (McCracken, 1997) and the Anxiety Sensitivity Index (ASI) (Reiss et al., 1986).

2.2.2 Predetermination of stimulus intensity

In order to ensure comparable levels of pain sensation in all subjects, the individual stimulus intensity for later testing was determined in a two-step procedure.

First, heat pain threshold was assessed using the method of adjustment, which is a particularly reliable method as it does not depend on reaction time (Yarnitsky and Ochoa, 1990). Subjects were asked to adjust a temperature starting from 35 °C, using heating and cooling buttons, until they obtained a level which was barely painful. A constant press of the buttons resulted in the heating or cooling of the probe at a rate of 0.5 °C/s. Following a familiarization trial, there were five trials and the average was used to constitute the threshold estimate.

In a second step, we determined two stimulation temperatures [corresponding to a visual analogue scale (VAS) rating of 60 and 70] in a psychophysical rating procedure that has been established as standard in our laboratory (Kunz et al., 2008, 2011a,b). Eight 5-s heat stimuli with plateaus at temperatures of ±0, +1, +2 and +3 °C relative to the individual pain threshold (PT) were applied in a predetermined pseudorandom order [two stimuli per temperature; rate of change to and from plateau: 2 °C/s; interstimulus interval (ISI): 8–10 s]. After each stimulus, subjects were asked to provide intensity ratings on a horizontal VAS of 100 mm. Subjects were told that the left and right ends of the scale corresponded to no pain and extreme pain, respectively. If the most intense stimulus (PT +3 °C) was rated below 50, the series was repeated with an increase by 1 °C for all temperature levels.

Finally, a power function was fitted to the psychophysical data (TableCurve 2D, Scientific Solutions; Systat Software, Inc., San Jose, CA, USA), which allowed for determining the temperatures for VAS rating of 60 and 70.

2.2.3 Assessment of startle responses to painful stimulation with varying threat level

We applied two conditions with similar tonic heat stimulation, which were designed to differ specifically regarding the experience of pain-associated threat. In the low-threat condition, we applied tonic heat stimuli with constant intensity, which were announced as such. In contrast, in the high-threat condition, participants were informed that a further increase in intensity could take place, which in fact occurred in half of the trials. Startle probes were always presented in the same time window (18–28 s after stimulus onset), which was in the high-threat condition always prior to the further temperature increase. Each condition was preceded by a corresponding practice trial to inform the subjects already about the nature of the upcoming threat condition. The sequence of the two threat conditions was randomized

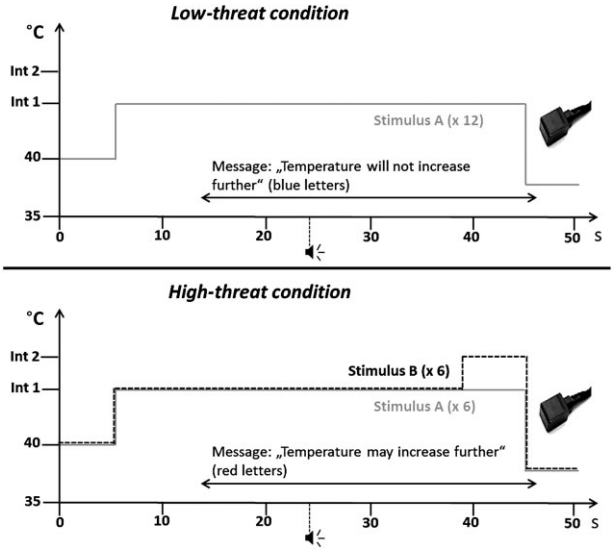

Figure 1 Trial design of both treat conditions (low-threat and high-threat condition).

across subjects. The design of both conditions is described in more detail in the following paragraphs and is also illustrated in Fig. 1.

2.2.3.1 Low-threat condition

The low-threat condition consisted of only one stimulus type (stimulus A), which was repeated 12 times (see Fig. 1) and looked like this: Temperature increased from the 35 °C baseline to 40 °C (rate of rise: 5 °C/s) and remained constant for 5 s; after that, it increased to the temperature intensity corresponding to a VAS rating of 60 (rate of rise: 2 °C/s) and remained there for 40 s. This two-step approach to the destination temperature should lower disturbing perceptual dynamics at stimulus onset, which result often when using steep stimulus ramps from temperature overshoots and strong perceptual responses to temperature changes. A startle probe was presented between 18 and 28 s after stimulus onset. After 40 s, temperature decreased to baseline and subjects provided ratings regarding the startle noise and the temperature (ratings are described in more detail below), followed by the next stimulus (ISI: 5 s). During each stimulus, the message 'Temperature will not increase further' appeared on the computer screen in front of the subject, written in blue letters. This message appeared 13 s after onset of the painful temperature and remained on the screen until stimulus offset.

2.2.3.2 High-threat condition

The high-threat condition consisted of two types of thermal stimuli (stimulus A, stimulus B), which were presented in random order; both types were repeated six times (see Fig. 1).

Stimulus A was identical to the stimuli used in the low-threat condition (constant stimulation with VAS 60 for 40 s). Stimulus B looked like this: Temperature increased from the 35 °C baseline to 40 °C (rate of rise: 5 °C/s) and remained constant for 5 s; after that, it increased to VAS 60 (rate of rise: 2 °C/s) and remained there for 35 s, followed by a second temperature increase to VAS 70, which lasted for 5 s. Accordingly, the course of stimulus B was identical to stimulus A during the first phase until the further increase to VAS 70. Again, a startle probe was presented between 18 and 28 s after stimulus onset and prior to the second temperature increase. After the second increase, temperature decreased to baseline and subjects provided ratings regarding the startle noise and the temperature, followed by the next stimulus (ISI: 5 s). In the high-threat condition during both stimuli A and B, the message 'Temperature may increase further' appeared on the computer screen in front of the subject, written in red letters. Again, this message was displayed 13 s after onset of the painful temperature and remained on the screen until stimulus offset. The sequence of stimulus A and stimulus B was randomized once and then set for all subjects.

In order to avoid potential tissue damage, the maximum values to be used for tonic stimulation were set to 48.5 °C for VAS 60 and to 49.5 °C for VAS 70, respectively. Temperature intensity had to be restricted to these maximum values for seven participants; however, subjective ratings indicated that these participants perceived the resulting stimulation temperature as equally aversive as the remaining 33.

2.2.3.3 Startle noise presentation

To elicit the blink reflex, we applied brief acoustic stimuli (white noise bursts), 50 ms in duration, with an intensity of 105 dB binaurally over headphones. Subjects wore headphones, over which they heard constant white noise of 68 dB as masking background noise. During each pain stimulus, one startle noise burst was presented in the time interval between 18 and 28 s after stimulus onset, in order that subjects had already read the message on the screen and were either expecting a second temperature rise (high-threat condition) or no second temperature rise (low-threat condition).

2.2.3.4 Ratings

After each heat stimulus, subjects were required to provide three ratings regarding the startle noise (one rating) and the temperature (two ratings) within the next 22 s. The intensity of the startle noise was rated on a computerized numerical rating scale ranging from 0 (labelled as 'no noise') to 100 (labelled as 'extremely loud noise'). For the two temperature ratings, we used a computerized version of the Self-Assessment Manikin (SAM) (Lang, 1980) as the gold standard for the ratings of emotional responses. Subjects were advised to choose via mouse click one of five manikins or a box in between as the ratings of valence and arousal. Thus, ratings ranged from 1 to 9; higher ratings corresponded to positive valence and high arousal, respectively.

2.2.3.5 Electromyographic recording and analysis

Startle blinks were measured by recording surface electromyography (EMG) activity on the M. orbicularis oculi beneath the right eye. For that purpose, Ag/AgCl electrodes filled with electrode paste were used. Prior to application of the electrodes, skin was cleaned with an alcoholic skin detergent to reduce electrode resistance.

EMG raw signals were recorded using the device SIGMA Plpro/Type Databox DB 36 (SIGMA Medizin-Technik GmbH, Gelenau, Germany) including a 16-bit AD convertor with a dynamic range from 0.5 μV to 2 mV. The recording bandwidth of the EMG signal was between 0.2 and 300 Hz; input resistance was above 20 mOhm. The signal was sampled at 512 Hz. After recording, the raw signal was analysed offline using the program 'Vision Analyzer' (Brain Products, Munich, Germany). First, the signal was cut into segments, containing the EMG responses to the startle probes. In each segment the raw signal was smoothed, using a 50 Hz notch filter, 20 Hz high-pass filter and 256 Hz low-pass filter, rectified and integrated. The integration procedure was executed over a time interval from 0 to 250 ms after startle noise onset. Finally, responses were considered as invalid and excluded from analysis if considerable fluctuations in the baseline EMG activity were detected and/or if the peak of activity did not occur in the predefined time window (30–100 ms) after stimulus onset. Overall, 4.3% of responses had to be excluded.

The critical variables were peak latency and amplitude of blink responses. Peak latency was defined as time from startle noise burst onset to the maximum value of voltage. Amplitude was defined as voltage difference between the averaged baseline and voltage peak within a time frame of 30–100 ms after startle noise burst onset. Peak latency and amplitude were calculated separately for each response, resulting in a total of 24 values (12 values per condition) per subject. Missing values – resulting from the exclusion procedure described above – were substituted by means, which were calculated from the available data for each individual.

2.2.4 Statistical analysis

2.2.4.1 Subjective ratings

To evaluate the effects of the experimental threat conditions on valence and arousal ratings of the temperature as well as on startle noise ratings, we conducted three repeated measurement analyses of variance (ANOVAs) with 'condition' (low threat vs. high threat) as within-subject factor and 'sequence of condition' (low threat – high threat vs. high threat – low threat) as between-subject factor.

2.2.4.2 Startle reflex

To evaluate the effects of the experimental threat conditions on startle peak latency and amplitude, we conducted two

Table 1 Descriptive statistics of questionnaire measures.

	M (SD) Our sample	M (SD) Comparable sample	Author; sample characteristics
PCS	13.25 (7.86)	12.5 (8.3)	Lee et al., 2010 Healthy adults
PASS	59.70 (28.00)	65.04 (29.07)	Osman et al., 1994 Community sample
PVAQ	34.38 (10.14)	34.4 (11.8)	Roelofs et al., 2002 College students
ASI	14.20 (6.10)	17.95 (9.15)	Reiss et al., 1986 College students

ASI, Anxiety Sensitivity Index; PASS, Pain Anxiety Symptoms Scale; PCS, Pain Catastrophizing Scale; PVAQ, Pain Vigilance and Awareness Questionnaire; SD, standard deviation.

repeated measurement ANOVAs with 'condition' (low threat vs. high threat) and 'time course' (startles 1–12) as within-subject factors and 'sequence of condition' (low threat – high threat vs. high threat – low threat) as between-subject factor. For *F*-tests, partial eta squared (η^2) is reported as an estimate of effect size. For detailed analyses, one-sided paired samples *t*-tests were computed (directed hypotheses were available for the effect of threat); Cohen's *d* is reported to describe effect size for paired comparisons.

To explore the relationship between individual differences and startle reactions during both conditions, we computed correlations between questionnaire scores and startle in both threat conditions.

SPSS 19 (IBM, Armonk, NY, USA) was used for all calculations; significant effects were assumed at $\alpha = 0.05$.

3. Results

3.1 Participant characteristics

Descriptive statistics of questionnaire scores are presented in Table 1. For each of the questionnaires, mean scores and SDs were very similar to those reported by other authors in non-clinical samples, which is not surprising given that we excluded participants with mental disorders and pain problems. Thus, there was no evidence for heightened general or pain-related anxiety in our sample (see Table 1).

3.2 Pain threshold and stimulation intensities

Mean values were 44.4 °C (SD: 2.2 °C) for pain threshold, 46.8 °C (SD: 1.9 °C) for stimulation intensity 1 (corresponding to a VAS rating of 60) and 47.6 °C (SD: 1.9 °C) for stimulation intensity 2 (corresponding to a VAS rating of 70). Thus, stimulation intensities were in the range typically used for inducing moderately to strongly painful sensations (Granot et al., 2003, 2008; Kunz et al., 2008, 2011a,b).

3.2 Effects of threat conditions on temperature and noise ratings

3.2.1 Temperature valence ratings

The ANOVA yielded no significant effect of threat condition on valence ratings [$F(1,38) = 0.464$; $p = 0.500$; $\eta^2 = 0.012$]. Additionally, there was no interaction between condition and sequence of conditions [$F(1,38) = 1.000$; $p = 0.324$; $\eta^2 = 0.026$]. Valence ratings were clearly at the negative pole of the SAM scale (1 = extremely negative, 9 = extremely positive) (high-threat condition: M = 2.6; SD = 1.0; low-threat condition: M = 2.4; SD = 1.1).

3.2.2 Temperature arousal ratings

For temperature arousal ratings, we detected no significant main effect of threat condition either [$F(1,38) = 0.031$; $p = 0.862$; $\eta^2 = 0.001$]. However, there was a trend towards an interaction between condition and sequence of conditions [$F(1,38) = 3.472$; $p = 0.070$; $\eta^2 = 0.084$]. Participants who started with the high-threat condition provided significantly higher arousal ratings for the temperature in the high-threat than in the low-threat condition (high-threat condition: M = 6.2; SD = 1.7; low-threat condition: M = 5.7; SD = 2.1; T(19) = 2.001; $p = 0.030$; $d = 1.32$). In contrast, there was no significant difference between threat conditions in participants, who started with the low-threat condition (high-threat condition: M = 5.5; SD = 2.1; low-threat condition: M = 5.9; SD = 1.5; T(19) = 0.981; $p = 0.170$; $d = 0.36$).

3.2.3 Startle noise ratings

The ANOVA yielded no significant effect of threat condition on valence ratings [$F(1,38) = 2.550$; $p = 0.119$; $\eta^2 = 0.063$]. Additionally, there was no interaction between condition and sequence of conditions [$F(1,38) = 1.495$; $p = 0.229$; $\eta^2 = 0.038$]. Overall, ratings for the noises burst were – as expected – in the medium range (high-threat condition: M = 45.4; SD = 17.3; low-threat condition: M = 43.3; SD = 17.8).

3.3 Effects of threat conditions on startle peak latency and amplitude

3.3.1 Startle peak latency

There was no effect of threat condition on startle reflex peak latency [$F(1,38) = 2.442$; $p = 0.126$; $\eta^2 = 0.060$].

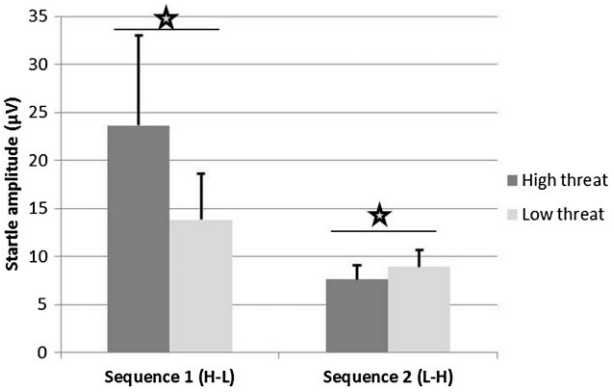

Figure 2 Mean (+standard error) of startle amplitude for both threat conditions and sequence conditions. (Note: Star symbol indicates significant differences between means $\alpha < 0.05$.)

Additionally, the interaction between condition and sequence of conditions failed to reach significance [$F(1,38) = 0.008$; $p = 0.927$; $\eta^2 < 0.001$]. However, we found a significant main effect of time course [$F(11,418) = 1.894$; $p = 0.038$; $\eta^2 = 0.047$], indicating a slight decline of peak latency in the course of each of the two conditions [difference between mean values in the first half (trial 1–6) and the second half (trial 7–12): 1.0 ms in the high-threat condition, 4.0 ms in the low-threat condition]. Overall, mean values of peak latency lay in the expected range (high-threat condition: M = 80.9 ms; SD = 16.7; low-threat condition: M = 78.0, SD = 18.7).

3.3.2 Startle amplitude

The main effect of threat condition on startle amplitude did not reach significance [$F(1,38) = 3.025$; $p = 0.090$; $\eta^2 = 0.074$]; however, there was a significant interaction between threat condition and sequence of conditions [$F(1,38) = 5.049$; $p = 0.031$; $\eta^2 = 0.117$]. Participants who ran first through the high-threat condition displayed significant potentiation of startle amplitude in the high-threat compared with the low-threat condition [$T(19) = 2.007$; $p = 0.030$; $d = 2.63$; see Fig. 2]. In contrast, in participants who started with the low-threat condition, amplitude was even diminished in the high-threat condition [$T(19) = 2.197$; $p = 0.021$; $d = 2.16$].

Although these findings suggest substantial time effects, we observed no significant main effect of time course on startle amplitude [$F(11,418) = 1.524$; $p = 0.120$; $\eta^2 = 0.039$; see Fig. 3]; the interaction effects of time course with condition and sequence of conditions also failed to reach significance [time course × condition: $F(11,418) = 1.454$; $p = 0.146$; $\eta^2 = 0.037$; time course × sequence of conditions: $F(11,418) = 1.417$; $p = 0.162$; $\eta^2 = 0.036$]. Accordingly, no pronounced habituation of startle amplitude became obvious in the course of both threat conditions.

3.4 Relationship between anxiety and startle responses

As inter-individual differences in anxiety have been found to be related to startle reactivity and to reactions to pain-associated threat, we conducted a correlation analysis to explore the relationship between questionnaire scores (PCS, PASS, PVAQ, ASI; see 3.1) and startle responses to both threat conditions. However, we detected no significant correlations (correlation coefficients ranging from 0.002 to 0.29; all ps > 0.05), indicating that inter-individual differences in these variables were of minor importance in our experiment.

4. Discussion

As pain is an important warning signal for potential damage to our health, one would expect that defen-

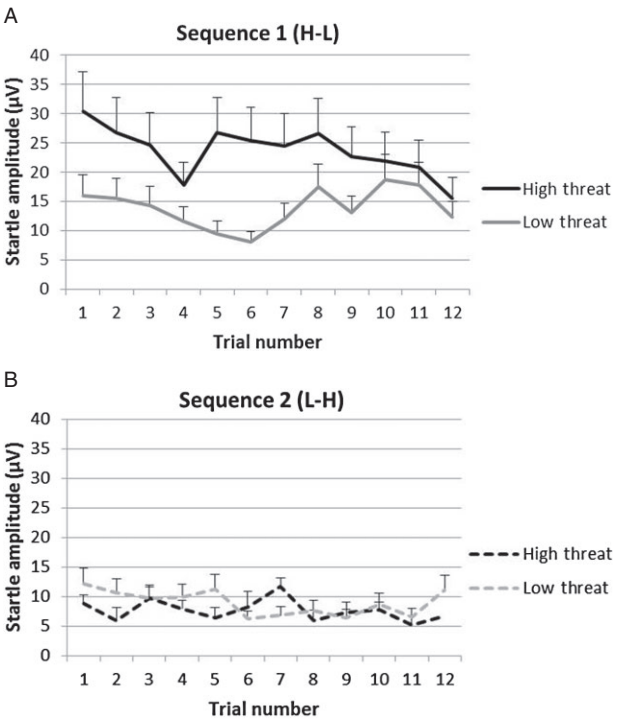

Figure 3 (A) Time course of startle amplitude means [+standard error (SE)] from trial 1 to 12 for sequence 1 (high threat – low threat). (B) Time course of startle amplitude means (+SE) from trial 1 to 12 for sequence 2 (low threat – high threat).

sive responses like the startle reflex are potentiated during painful stimulation. This has been shown for phasic heat pain (Crombez et al., 1997). In contrast, we found no startle potentiation during constant and predictable tonic heat pain in two preceding studies (Horn et al., 2012a,b), which might be due to insufficient perceived threat evoked by this type of stimulation. The unpredictability of pain intensity has appeared to be a major influence on the level of perceived threat (Price et al., 1980; Carlsson et al., 2006; Oka et al., 2010). Therefore, we hypothesized that tonic pain with expected but unpredictably timed further pain increases elicit stronger defensive responding (indicated by startle potentiation) than constant and predictable tonic pain. In order to test this hypothesis, we recorded startle responses in two tonic heat pain conditions with identical stimulation intensity, one being constant and announced as predictable (low-threat condition) and another one including expected but unpredictably timed further increases in temperature (high-threat condition).

In slight contrast to our hypothesis, we obtained no significant main effect of threat condition: Overall, startle responses in the high-threat condition were not stronger than responses in the low-threat condition. However, we found a significant interaction between threat condition and sequence of condition: Startle potentiation by high threat was present only in those participants who underwent the high-threat stimulation as first condition. In contrast, participants who started with the low-threat condition displayed later even smaller responses in the high-threat condition.

Since these findings suggest that startle responses may have declined from the first to the second condition irrespective of the threat level, they might be simply explained by habituation. However, there is one strong argument contradicting this explanation: There was no significant effect of time within both conditions (from trial 1 to trial 12), which makes substantial habituation effects unlikely. Furthermore, we found this pattern of results not only for startle responses, but also for subjective ratings of stimulus arousal: The high-threat condition was rated as more arousing than the low-threat condition only when it was presented first. This consistency between startle and subjective ratings suggests that our observations reflect time-related changes in the appraisal of pain-associated threat rather than a mere habituation of a physiological variable. It seems that the high-threat condition lost its threatening features when preceded by the low-threat condition.

One factor that might have played a role for this effect is novelty. It might be the case that the threat instruction was highly successful at the beginning of the experiment, when subjects had no experience with the painful stimulation, but lost its efficiency when they were already familiarized with the stimulation. As threat is generally difficult to establish in experimental settings, familiarity with the painful stimulus might have provided enough safety to counteract expectations induced by the threat instruction.

Alternatively, our findings might also be explained by carry-over effects of threat impact: Participants who started with the low-threat condition learned that the painful stimulation was non-threatening, resulting in lower fear responses to the consecutive high-threat condition. A similar effect is known from fear conditioning experiments: Here, it its more difficult to condition fear to a cue which is already known from other contexts prior to conditioning ('latent inhibition'; Lubow and Moore, 1959). This type of inhibition is believed to protect us from developing fear of stimuli that have already been experienced as non-threatening beforehand (Tröger et al., 2012) and is known to play a role not only in cue conditioning but also in context conditioning (Westbrook et al., 1994; Richardson and Elsayed, 1998). Our paradigm might be comparable with context conditioning, as in both conditions, the tonic heat stimuli were identical regarding physical modality, intensity as well as technique of application and could thus be viewed as the context in which participants perceived threat (i.e., expectation of a further pain increase) or did not (no expectation). The fact that this carry-over effect was observed only from the low-threat to the high-threat condition might be explained by two factors. First, conditions for extinction were much better for the sequence high threat – low threat, as all 12 stimuli presented in the low-threat condition were non-threatening and able to extinguish fear responses. In contrast, 50% of the trials in the high-threat condition were still non-threatening, thus probably prolonging extinction of learned safety in the sequence low threat – high threat. Second, as experimental settings have to provide generally a high degree of safety to participants due to ethical considerations, it might be that learning of safety is generally easier to establish in the laboratory than learning of threat.

As it has already been shown that effects of pain-associated threat on performance in a reaction–time task vary due to inter-individual differences in anxiety (Moseley et al., 2003) and there is generally an association between anxiety and startle responsivity (Cook, 1999; Temple and Cook, 2007), we analysed the relationship between startle responses and scores in questionnaires assessing anxiety sensitivity, pain-

related anxiety, pain catastrophizing and pain vigilance. However, there was no association between these questionnaire scores and startle responses, suggesting that inter-individual differences in dispositional anxiety were of minor importance in the range produced by our healthy, pain-free sample. This is in accordance with our previous study reporting no startle potentiation by tonic pain even in startle-sensitive subjects (Horn et al., 2012b).

Taken together, our experiment yielded the interesting result that the sequence of conditions plays an important role when comparing responses to painful stimulation with high- versus low-threat value in a within-subject design. The observation that reactions to the high-threat condition were diminished in participants who had already completed the low-threat condition suggests that pain-associated threat might be determined not only by current threat characteristics of the painful stimulus but also by previous experiences with similar stimuli. Alternatively, a certain degree of novelty might be necessary to induce threat in experimental settings.

5. Limitations

There are also some limitations worth mentioning. First, we did not include a condition with non-painful stimulation; thus, we are not able to tell whether startle changes due to the threat manipulation were limited to painful stimulation or might be achieved also by use of non-painful stimuli. Since we were mainly interested in the difference between painful stimulation with a low- and high-threat value, we deliberately accepted this limitation.

Second, subjects were required to rate the heat stimuli regarding their valence and arousal, but not regarding their painfulness. This decision was made due to the fact that we were interested in the affective responses elicited by low- versus high-threat stimulation, which are usually characterized by such ratings. The limited amount of time after each trial did not allow for further ratings. The perceived painfulness of the heat stimuli over the time course of the experiment might have been of particular interest, when checking for habituation.

Third, we did also not include a subjective rating of threat or anxiety elicited by our conditions. Based on previous research, we presupposed that tonic pain stimulation with unpredictable course would be more threatening than constant stimulation. Furthermore, subjects rated the arousal elicited by the tonic pain stimulation, which should be closely related to threat appraisal and in fact mirrored the startle results. However, a direct manipulation check regarding the perceived threat should be included in future studies.

6. Conclusions

Currently, the role of pain-associated threat is a much discussed topic in experimental pain research (e.g., Moore et al., 2013; Schoth et al., 2013). To our knowledge, our study is the first to investigate startle modulation by tonic pain stimulation with varying degrees of threat. High threat was induced by letting the participants expect unpredictable increases in stimulus intensity, thus mimicking an important feature of clinical pain states, namely the patient's apprehension that their pain might get worse. Our results show that startle was potentiated by high-threat compared with low-threat stimulation, but only in those participants who started with the high-threat condition and thus had their first experience with the painful stimulus in a threatening context. Based on this observation, we suggest that pain-associated threat is modulated not only by the current experience but also by previous experiences with similar pain stimuli, which is a conclusion of interest for the temporal dynamics of chronic pain.

Author contributions

C.H.-H. and S.L. designed the study together and are responsible for the integrity of the work as a whole. C.H.-H. conducted the study and did the analyses. Both authors discussed the results and wrote the article together.

References

Arntz, A., Van Eck, M., de Jong, P.J. (1992). Unpredictable sudden increases in intensity of pain and acquired fear. *J Psychophysiol* 6, 54–64.

Bradley, M., Cuthbert, B., Lang, P. (1999). Affect and the startle reflex. In *Startle Modification: Implications for Neuroscience, Cognitive Science and Clinical Science*, M. Dawson, A. Schell, A. Boehmelt, eds. (New York: Cambridge University Press) pp. 157–183.

Bradley, M., Moulder, B., Lang, P. (2005). When good things go bad: The reflex physiology of defense. *Psychol Sci* 16, 468–473.

Carlsson, K., Andersson, J., Petrovic, P., Petersson, K., Ohman, A., Ingvar, M. (2006). Predictability modulates the affective and sensory-discriminative neural processing of pain. *Neuroimage* 32, 1804–1814.

Cook, E.W. III (1999). Affective individual differences, psychopathology, and startle reflex modification. In *Startle Modification: Implications for Neuroscience, Cognitive Science and Clinical Science*, M. Dawson, A. Schell, A. Boehmelt, eds. (New York: Cambridge University Press) pp. 187–208.

Crombez, G., Baeyens, F., Vansteeenwegen, D., Eelen, P. (1997). Startle intensification during painful heat. *Eur J Pain* 1, 87–94.

Granot, M., Sprecher, E., Yarnitsky, D. (2003). Psychophysics of phasic and tonic heat pain stimuli by quantitative sensory testing in healthy subjects. *Eur J Pain* 7, 139–143.

Granot, M., Weissman-Fogel, I., Crispel, Y., Pud, D., Granovsky, Y., Sprecher, E., Yarnitsky, D. (2008). Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) para-

digm: Do conditioning stimulus painfulness, gender and personality variables matter? *Pain* 136, 142–149.

Grillon, C., Ameli, R., Woods, S., Merikangas, K., Davis, M. (1991). Fear-potentiated startle in humans: Effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology* 28, 588–595.

Grillon, C., Baas, J. (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clin Neurophysiol* 114, 1557–1579.

Grillon, C., Davis, M. (1997). Fear-potentiated startle conditioning in humans: Explicit and contextual cue conditioning following paired versus unpaired training. *Psychophysiology* 34, 451–458.

Horn, C., Blischke, Y., Kunz, M., Lautenbacher, S. (2012a). Does pain necessarily have an affective component? Negative evidence from blink-reflex experiments. *Pain Res Manag* 17, 1–10.

Horn, C., Schaller, J., Lautenbacher, S. (2012b). Investigating the affective component of pain: No startle modulation by tonic heat pain in startle responsive individuals. *Int J Psychophysiol* 84, 254–259.

Kunz, M., Chatelle, C., Lautenbacher, S., Rainville, P. (2008). The relation between catastrophizing and facial responsiveness to pain. *Pain* 140, 127–134.

Kunz, M., Chen, J.I., Lautenbacher, S., Vachon-Presseau, E., Rainville, P. (2011a). Cerebral regulation of facial expressions of pain. *J Neurosci* 31, 8730–8738.

Kunz, M., Rainville, P., Lautenbacher, S. (2011b). Operant conditioning of facial displays of pain. *Psychosom Med* 73, 422–431.

Lang, P. (1980). *Self-Assessment Manikin* (Gainesville, FL: University of Florida).

Lang, P. (1995). The emotion probe: Studies of motivation and attention. *Am Psychol* 50, 372–385.

Lang, P., Bradley, M., Cuthbert, B. (1990). Emotion, attention, and the startle reflex. *Psychol Rev* 97, 377–395.

Lang, P., Bradley, M., Cuthbert, B. (1997). Motivated attention: Affect, activation, and action. In *Attention and orienting: Sensory and motivational processes*, P. Lang, R. Simons, M. Balaban, eds. (Hillsdale, NJ: Erlbaum) pp. 97–135.

Lang, P., Bradley, M., Cuthbert, B. (1998). Emotion, motivation, and anxiety: Brain mechanisms and psychophysiology. *Biol Psychiatry* 44, 1248–1263.

Lang, P., Davis, M., Öhman, A. (2000). Fear and anxiety: Animal models and human cognitive psychophysiology. *J Affect Disord* 61, 137–159.

Lee, J.E., Watson, D., Frey Law, L.A. (2010). Lower-order pain-related constructs are more predictive of cold pressor pain ratings than higher-order personality traits. *J Pain* 11, 681–691.

Lin, C.S., Niddam, D.M., Hsu, M.L., Hsieh, J.C. (2013). Pain catastrophizing is associated with dental pain in a stressful context. *J Dent Res* 92, 130–135.

Lubow, R., Moore, A. (1959). Latent inhibition: The effect of nonreinforced pre-exposure to the conditional stimulus. *J Comp Physiol Psychol* 52, 415–419.

McCracken, L.M. (1997). Attention' to pain in persons with chronic pain: A behavioral approach. *Behav Ther* 28, 271–284.

McCracken, L.M., Zayfert, C., Gross, R.T. (1992). The Pain Anxiety Symptoms Scale: Development and validation of a scale to measure fear of pain. *Pain* 50, 67–73.

Moore, D.J., Keogh, E., Eccleston, C. (2013). The effect of threat on attentional interruption by pain. *Pain* 154, 82–88.

Moseley, G.L., Brhyn, L., Ilowiecki, M., Solstad, K., Hodges, P.W. (2003). The threat of predictable and unpredictable pain: Differential effects on central nervous system processing? *Aust J Physiother* 49, 263–267.

Oka, S., Chapman, C., Kim, B., Shimizu, O., Noma, N., Takeichi, O., Imamura, Y., Oi, Y. (2010). Predictability of painful stimulation modulates subjective and physiological responses. *J Pain* 11, 239–246.

Osman, A., Barrios, F.X., Osman, J.R., Schneekloth, R., Troutman, J.A. (1994). The Pain Anxiety Symptoms Scale: Psychometric properties in a community sample. *J Behav Med* 17, 511–522.

Price, D., Barrell, J., Gracely, R. (1980). A psychophysical analysis of experiential factors that selectively influence the affective dimension of pain. *Pain* 8, 137–149.

Reiss, S., Peterson, R.A., Gursky, D.M., McNally, R.J. (1986). Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. *Behav Res Ther* 24, 1–8.

Richardson, R., Elsayed, H. (1998). Shock sensitization of startle in rats: The role of contextual conditioning. *Behav Neurosci* 112, 1136–1141.

Roelofs, J., Peters, M.L., Muris, P., Vlaeyen, J.W.S. (2002). Dutch version of the Pain Vigilance and Awareness Questionnaire: Validity and reliability in a pain-free population. *Behav Res Ther* 40, 1081–1090.

Schoth, D., Yu, K., Liossi, C. (2013). The role of threat expectancy in attentional bias and thermal pain perception in healthy individuals. *J Health Psychol* 19, 653–663.

Sullivan, M.J., Bishop, S.R., Pivik, J. (1995). The pain catastrophizing scale: Development and validation. *Psychol Assess* 7, 524–532.

Temple, R.O., Cook, E.W. III (2007). Anxiety and defensiveness: Individual differences in affective startle modulation. *Motiv Emot* 31, 115–123.

Tröger, C., Ewald, H., Glotzbach, E., Pauli, P., Mühlberger, A. (2012). Does pre-exposure inhibit fear context conditioning? A virtual reality study. *J Neural Transm* 119, 709–719.

Walter, B., Hampe, D., Wild, J., Vaitl, D. (2002). Die erfassung der angst vor schmerzen: Eine modifizierte deutsche version der pain anxiety symptom scale (PASS-D). *Der Schmerz* 15 (Suppl. 1), 83.

Westbrook, R., Good, A., Kiernan, M. (1994). Effects of the interval between exposure to a novel environment and the occurrence of shock on the freezing responses of rats. *Q J Exp Psychol B* 47, 427–446.

Yarnitsky, D., Ochoa, J.L. (1990). Studies of heat pain sensation in man: Perception thresholds, rate of stimulus rise and reaction time. *Pain* 40, 85–91.