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INTEROCEPTIVE CUES PREDICTING EXTEROCEPTIVE EVENTS

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Abstract

The growing body of research on interoceptive conditioning has predominantly focused on associative learning paradigms that investigated the formation of intero-interoceptive or extero-interoceptive associations. Yet, little research has explored whether interoceptive sensations can enter an intero-exteroceptive association. Therefore, in an interoceptive conditioning paradigm, healthy participants experienced a respiratory resistance for 8 seconds, causing mild dyspnea (interoceptive conditioned stimulus, CS), that was either paired to an aversive electrocutaneous stimulus (unconditioned stimulus, US) (experimental condition, n = 25), or presented in an unpaired fashion (control condition, n = 25) during the acquisition phase. In a subsequent extinction phase, the US was not delivered anymore. US-expectancy, skin conductance responses (SCR), and eyeblink startle EMG were used as indices of associative learning. During acquisition, we observed stronger US expectancies during the CS as compared to the intertrial interval in the experimental group, but not in the control group, nor during extinction. In line, only in the experimental group did skin conductance responses to the CS increase across acquisition. The pattern of the eyeblink startle data did not reach statistical significance. In sum, interoceptive sensations can become associated with exteroceptive events.

Keywords: Associative learning, Conditioning, Interoception, Extinction

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1. **Introduction**

Associative learning processes involving interoceptive sensations have been implicated in breathing disorders, panic disorders as well as in patients suffering from chronic visceral discomfort and pain (Bouton, Mineka, & Barlow, 2001; Janssens, Verleden, De Peuter, Van Diest, & Van den Bergh, 2009; Zaman, Vlaeyen, Van Oudenhove, Wiech, & Van Diest, 2015) and are an important determinant of the degree of disability (Vlaeyen & Linton, 2012; Zale, Lange, Fields, & Ditre, 2013). For example, interoceptive states or sensations (e.g., state of intoxication) experienced prior to and during a traumatic event (e.g., car accident), may start to act as a trigger for future panic attacks. In the laboratory a prevailing approach to study associative learning processes is through Pavlovian conditioning paradigms: where a relatively 'neutral' stimulus or event (the *conditioned stimulus* or CS) becomes endowed with the capacity to elicit a (anticipatory) response (the *conditioned response* or CR) after it has been paired with another stimulus or event (the *unconditioned stimulus* or US). A growing number of experimental studies have demonstrated the ability of interoceptive sensations to enter such CS-US associations, both as a CS and as an US (Benson et al., 2014; De Peuter et al., 2005; Gramsch et al., 2014; Icenhour et al., 2015; Kattoor et al., 2013; Pappens et al., 2013, 2014; Pappens, Smets, Vansteenwegen, Van Den Bergh, & Van Diest, 2012; Schroijen et al., 2015; Zaman, Van den Bergh, Fannes, & Van Diest, 2014; Zaman, Weltens, et al., 2015). Most of these studies either used a breathing stimulus (i.e., respiratory resistance or occlusion) or a rectal balloon distention as interoceptive stimuli and predominantly focused on the formation of intero-intero (both CS and US are interoceptive) and extero-intero (exteroceptive CS, interoceptive US) associations. To the best of our knowledge, only one study has used an interoceptive stimulus as CS (i.e., esophageal balloon distention) and an exteroceptive stimulus as US (i.e., electrocutaneous stimulus at the wrist) to investigate the acquisition and generalization of interoceptive fear (Zaman, Weltens, et al., 2015). However, as no extinction phase was included it remains unclear to which extend fear responses extinguished after the establishment of an intero-extero association. Therefore, the aim of the current study was to further explore whether interoceptive sensations can elicit a

conditioned response after they have been associated with an exteroceptive event and whether such responses disappear when the CS is no longer predictive of the US (i.e., extinction).

To this end, an interoceptive conditioning paradigm with a mild respiratory resistance as CS and an aversive electrocutaneous stimulus as US was adopted. We measured US-expectancy, skin conductance responses (SCR) and the eyeblink EMG startle reflex. We expected that the CS will trigger the expectation of the US and starts to elicit a fear response (i.e., conditioned responses) as participants learn their association. More precisely, higher US-expectancies, stronger SCRs, and stronger eyeblink startle reflexes during the CS compared to during the ITI in the experimental group at the end of acquisition, but not after extinction nor in the control group. In the control group where the US was presented during the ITI, stronger eyeblink responses during the ITI than during the CS were expected after acquisition, as the CS predicted the absence of the US.

2. Material and methods

2.1. Participants

Of the recruited sixty-five healthy participants, aged between 18 and 60, fifty participants completed the entire experiment (see procedure) (experimental condition: N = 25 (19 females), age: 19.1 (range: 18 - 27), control condition: N = 25 (19 females), age : 19.3 (range: 18 - 24)). Exclusion criteria were: chronic pain, cardiovascular disorder, respiratory disorder, epilepsy, muscular disease, diabetes, pregnancy, and recent surgery of which one had not completely recovered. Participants received instructions not to use any caffeine, analgesics or sedatives on the day of the study. The experiment was approved by the KU Leuven University Medical Ethical Committee.

2.2. Breathing apparatus and respiratory resistance

Participants breathed through a mouthpiece and wore a noseclip. The mouthpiece was connected to a microbial filter (MicroGard, VIASYS) mounted on a heated pneumotachograph (Fleish no. 2, Epalinges, Switzerland). The signal from the pneumotachograph was displayed online and used by

the experimenter to determine the start of an inspiration (cf. infra). The pneumotachograph was connected to a non-rebreathing valve ensuring the separation of inspired and expired air. A vinyl tube (inner diameter: 3.5 cm; length 100 cm) connected the inspiratory and expiratory sides of the non-rebreathing valve with 3-way Y-valves (stopcock type) in the experimenter room enabling easy switching between loaded (with load) and unloaded breathing (without load). The respiratory resistance was created by means of linear Hans Rudolph loads of 10 $cmH_2O/l/s$, one connected to the inspiratory side, and another one to the expiratory side of the breathing circuit. This created a mild sensation of loaded breathing, like breathing through a straw or the feeling of 'having to work harder to catch one's breath'.

2.3. Electrocutaneous stimulation

Electrocutaneous stimulation (US) was delivered by a commercial stimulator (DS5, Digitimer, Welwyn Garden City, England) through surface electrodes attached approximately 2 cm apart on the back of the lower left rib cage (approximately on the 10^{th} rib, 10cm lateral from the spine). The DS5 was controlled by a computer through a National Instruments PCI-6221 16-Bit data acquisition card with analog output (National Instruments, Austin, Texas) and Affect4 software (Spruyt, Clarysse, Vansteenwegen, Baeyens, & Hermans, 2010). A train of square waves with alternating positive/negative stimulation at 10 Hz was presented during 1 s to create an aversive sensation. The intensity of the US was individually calibrated using the ascending method of limits (Yarnitsky, Sprecher, Zaslansky, & Hemli, 1995) and was not necessarily painful but needing some effort to tolerate.

2.4. US-expectancy

US-expectancy was measured continuously by means of an electronic dial that participants could turn across a scale from 0 to 100 with numerical labels at every 10^{th} step and verbal labels at 0 ("no electrocutaneous stimulus"), 50 ("I don't know"), and 100 ("most certainly electrocutaneous

stimulus") with their dominant hand. The dial was used to control a DC output current between 0 and 4.5 V that was digitized at 10 Hz into data ranging from 0 ("no electrocutaneous stimulus") to 100 ("most certainly electrocutaneous stimulus").

2.5. Retrospective ratings and manipulation check

Participants rated the stimuli on the following characteristics: the experienced *intensity* of the respiratory resistance, the electrocutaneous stimulus, and the acoustic startle probe (1 = "*mild*", 2 = "*moderate*", 3 = "*intense*", 4 = "*strong*", 5 = "*unbearable*"); the experienced *unpleasantness* of the respiratory resistance, the electrocutaneous stimulus, and the acoustic startle probe (1 = "*not at all unpleasant*"; 5 = "*extremely unpleasant*"); how painful the US was experienced (1 = "*not at all painful*"; 5 = "*extremely painful*") (Table 1).

2.6. Skin conductance response

Electrodermal activity was recorded with LabLinc V AgCl electrodes (8 mm diameter, well depth 2 mm) filled with a K-Y gel (Johnson & Johnson) and attached to the hypothenar palm of the non-dominant hand, which was cleaned with tap water before the start of the procedure. The inter-electrode distance was 2.5 cm. A Coulbourn skin conductance coupler (LabLinc v71-23) provided a constant 0.5 V across electrodes. The signal was digitized at 100 Hz throughout the study.

2.7. Eyeblink startle response

Orbicularis Oculi electromyographic activity (EMG) was recorded with three LabLinc V AgCl electrodes (4 mm diameter, well depth 1 mm) filled with a TECA electrolyte gel. After peeling the skin to reduce inter-electrode resistance, electrodes were placed on the left side of the face according to the site specifications proposed by Blumenthal et al. (2005). The raw signal was amplified by a Coulbourn isolated bioamplifier with bandpass filter (LabLinc v75–04). The recording bandwidth of

the EMG signal was between 13 Hz and 1 kHz. The signal was rectified online and smoothed by a Coulbourn multifunction integrator (LabLinc v76–23A) with a time constant of 20 ms. Data were digitized at 1 KHz for 1500 ms, starting 500 ms before the onset of the acoustic startle probe (a 100 dBA burst of white noise with instantaneous rise time presented binaurally for 50 ms through headphones; Hoher, Stereo Headphones HF92).

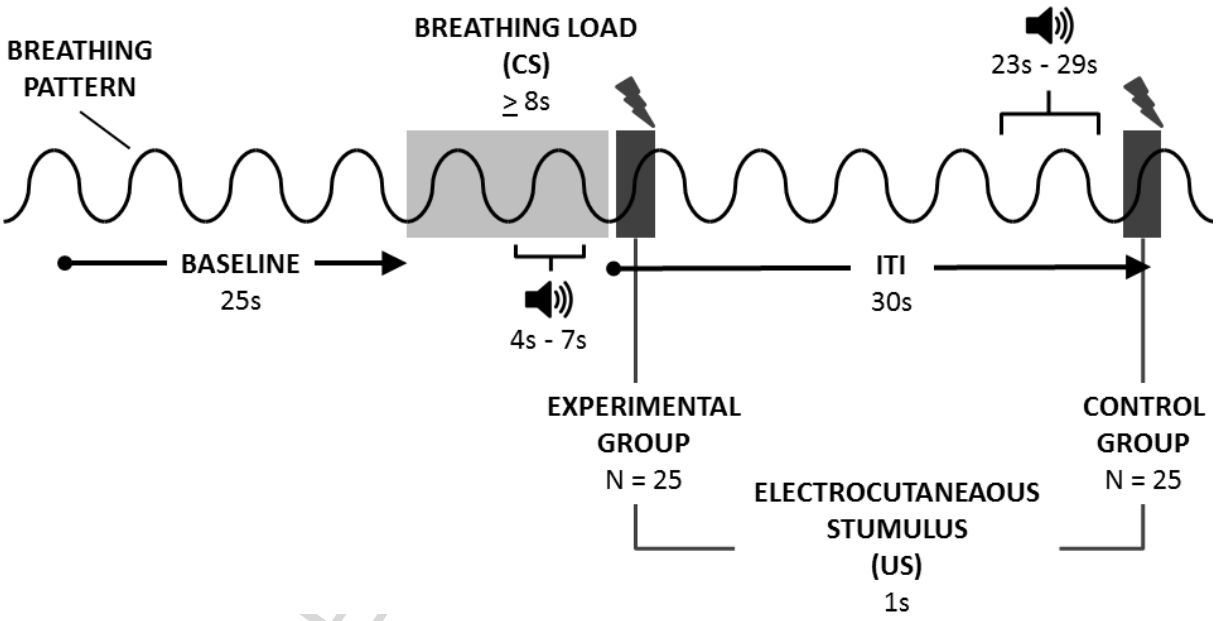

Figure. 1. A schematic representation of the experimental trials for the experimental and the control group, respectively. The electrocutaneous stimulus was not delivered during the extinction phase. The acoustic startle probe was randomly presented in the 4 – 7 s interval post CS onset or in the 23-29 s interval post CS offset.

2.8. Procedure

Upon arrival, participants read and signed the informed consent form. A pseudo-random alternating assignment controlling for participants' gender to the experimental and control conditions was used.

After US calibration, 10 acoustic startle probes were presented with a variable interval (*M* = 30 s, range 17-43 s), after which the respiratory resistance (CS) was presented once for 8 s and

participants rated its experienced intensity and unpleasantness. Participants who did not perceive the respiratory resistance were excluded (N = 15).

The acquisition phase comprised 8 trials in which the CS was presented at the start of the succeeding inspiration after a baseline of 25 seconds of unloaded breathing. The CS was presented for at least 8 s and was maintained until the end of a respiratory cycle. In the *experimental condition*, at the start of the first inspiration after these 8 s, the US was delivered followed by 30 s of unloaded breathing. In the *control condition* the participants breathed freely for 30 s after CS offset, and received the US at the end of the trial. Acoustic startle probes were presented randomly in the 4-7 s interval after CS onset on 50% of the trials and in the 23-29 s interval after CS offset (i.e., in the inter-trial-interval; ITI) on the other 50% of the trials. Trials with a startle probe during the CS and during the ITI were semi-randomized within phases such that no more than 2 consecutive trials of the same type were allowed. Trials are schematically presented in Fig. 1.

Prior to the extinction phase, participants completed the retrospective ratings during a short break. The *extinction phase* was identical to the acquisition phase except that the US was never delivered. Participants were not informed about the absence of the US during this phase. At the end of the extinction phase, the retrospective ratings were reassessed.

2.9. Response definition, data reduction, and statistical analysis

Data from the online US-expectancy data (electronic dial) were averaged across two intervals: US-expectancy during the CS and ITI was calculated as the average US-expectancy in the 0-8 s interval post CS onset and in the 17-23 s interval post CS offset, respectively. The resulting averages were then entered into a 2 (Group: experimental *vs.* control) x 2 (Phase: acquisition *vs.* extinction) x 2 (Stimulus: CS *vs.* ITI) x 8 (Trial) repeated measures ANOVA with Phase, Stimulus, and Trial as within-subject variables. Planned contrasts were created to test for group differences on the change in US-expectancy during the CS relative to the ITI across acquisition trials and across

extinction trials, as we expected higher US-expectancies during the CS compared to during the ITI throughout acquisition in the experimental group but in the control group. During extinction no group differences were expected.

All psychophysiological waveforms were visually inspected off-line and technical abnormalities and movement artifacts were eliminated. Skin conductance responses (SCR) were calculated by subtracting the average skin conductance during the 1 s interval preceding CS onset from the maximum skin conductance in the 8 s interval post CS onset using PSPHA (de Clercq, Verschuere, de Vlieger, & Crombez, 2006). Resulting raw SCR data (expressed in µS) were entered into a 2 (Group: experimental *vs.* control) x 2 (Phase: acquisition *vs.* extinction) x 8 (Trial) repeated measures ANOVA with Phase and Trial as within-subject variables. Two planned contrasts were created to test for group differences in the pattern of SCR across acquisition trials and across extinction trials, as we expected higher SCRs to the CS+ throughout acquisition for the experimental group only. During extinction no group differences were expected.

Eyeblink startle (EMG) responses were calculated by subtracting the mean value from the 0 - 20 ms time window following probe onset from the peak value in the 21 - 175 ms time window post probe onset using PSPHA (de Clercq et al., 2006). To reduce interindividual variation, startle amplitudes were transformed into T-scores and mean startle amplitudes were calculated. Data from 'non-responders' – i.e., participants with no responses on more than 30% of the experimental probes (excluding habituation probes) – were excluded (N = 5). In order to assure reliable estimates of the startle responses, data were averaged across trials per phase and entered into a 2 (Group: experimental *vs*. control) x 2 (Phase: acquisition *vs*. extinction) x 2 (Stimulus: CS *vs*. ITI) repeated measures ANOVA with Phase and Stimulus as within-subject variables. Two planned contrasts were created to test for group differences in the startle amplitudes during the CS compared to the ITI during acquisition and extinction respectively. Throughout acquisition stronger eyeblink responses during the CS than during the ITI were expected in the experimental group whereas in the control group stronger eyeblink responses during the ITI than during the CS were expected. During extinction

no group differences were expected.

An α-level of .05 was set for statistical significance. Greenhouse-Geisser (G-G) corrections were applied for violations of sphericity. Uncorrected degrees of freedom and G-G corrected *p*'s are reported together with ε. Partial squared éta (η_p^2) effect sizes are reported. Post hoc test are corrected for multiple testing by a Bonferroni correction. All contrasts were tested two-tailed. Data analyses were performed using SPSS 20 ©.

3. Results

3.1. Participants and manipulation checks

The final sample included fifty participants. At the start of the study, there were no differences between the groups regarding demographic characteristics as well as retrospective ratings (all p's > .05) (see Table 1).

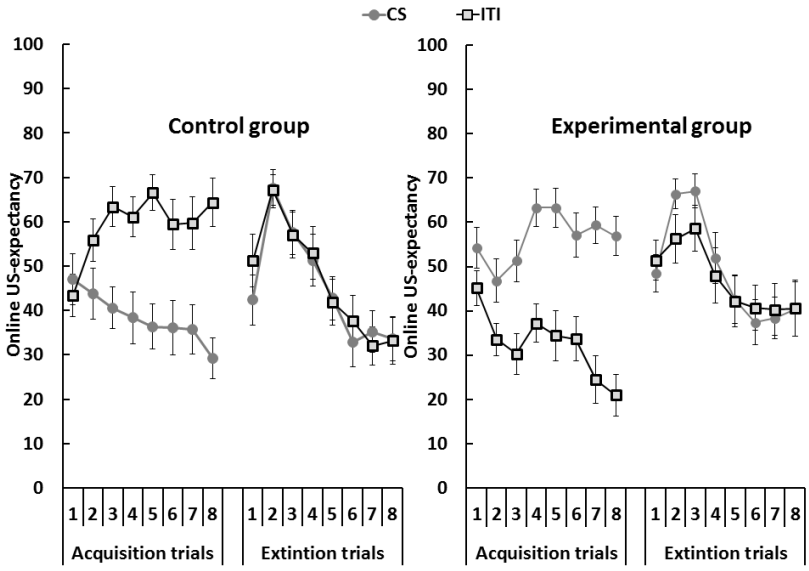

Figure. 2. Online US-Expectancy data during the CS and the ITI across the acquisition and the extinction phase for the experimental (right panel) and the control group (left panel) separately. US-expectancy was rated on a 0-100 scale with labels at 0 ('*certainly no electrocutaneous stimulus*'), 50 ('*I don't know*'), and 100 ('*certainly electrocutaneous stimulus*'). Vertical bars denote standard errors.

3.2. Online US-expectancy

There was a four-way Group x Phase x Stimulus x Trial interaction ($F(7, 336) = 8.41$, $p < .001$, $\eta_p^2 = 0.15$, $\varepsilon = .74$). Planned contrasts revealed group differences in the pattern of US-expectancy throughout acquisition for the CS relative to the ITI ($F(1,48) = 40.10$, $p < .001$), whereas groups showed an identical pattern of US-expectancy during extinction trials ($F(1,48) = 2.37$, $p = .13$) (see Fig.2). In the experimental group, the expectation of the US during the ITI decreased linear throughout acquisition ($F(1,48) = 13.74$, $p = .004$[1], $\eta_p^2 = 0.23$) whereas it did not change for the CS ($F(1,48) = 1.43$, $p > .99$[1]). In the control group a stronger expectation of the US during the ITI developed across acquisition trials ($F(1,48) = 14.74$, $p = .002$[1], $\eta_p^2 = 0.24$) whereas during the CS the decrease in US-expectations failed to reach significance after multiple testing correction ($F(1,48) = 4.78$, $p = .14$[1], $\eta_p^2 = 0.091$). Furthermore, in the experimental group the expectation of the US increased from the last acquisition trial to the first extinction trial for the ITI ($F(1,48) = 17.20$, $p < .001$[1], $\eta_p^2 = 0.26$) but did not change for the CS ($F(1,48) = 2.33$, $p = .52$[1]) whereas in the control group, a trend towards an increase in US-expectancy during the CS ($F(1,48) = 5.7$, $p = .084$[1], $\eta_p^2 = 0.11$) was observed but no change for the ITI ($F(1,48) = 3.16$, $p = .37$[1], $\eta_p^2 = 0.06$). During extinction, regardless of group, an overall increase in US-expectancy was observed from the 1st to the 2nd extinction trial $F(1,48) = 20.03$, $p < .001$[1], $\eta_p^2 = 0.29$. After the 2nd extinction trials the expectation of the US decreased across groups and stimuli ($F(1,48) = 65.69$, $p < .001$[2], $\eta_p^2 = 0.58$) (Fig.2).

Other significant effects were a main effect of trial, $F(7, 336) = 12.15$, $p < .001$, $\eta_p^2 = 0.20$, $\varepsilon = .67$; Group x Interval interaction effect, $F(1, 48) = 45.24$, $p < .001$, $\eta_p^2 = 0.49$; Phase x Trial interaction effect, $F(7, 336) = 11.40$, $p < .001$, $\eta_p^2 = 0.19$, $\varepsilon = .52$; Group x Phase x Interval interaction effect, $F(1, 48) = 45.02$, $p < .001$, $\eta_p^2 = 0.48$; the Group x Interval x Trial interaction effect, $F(7, 336) = 4.08$, $p < .001$, $\eta_p^2 = 0.08$, $\varepsilon = .79$. The other main and interaction effects were not significant.

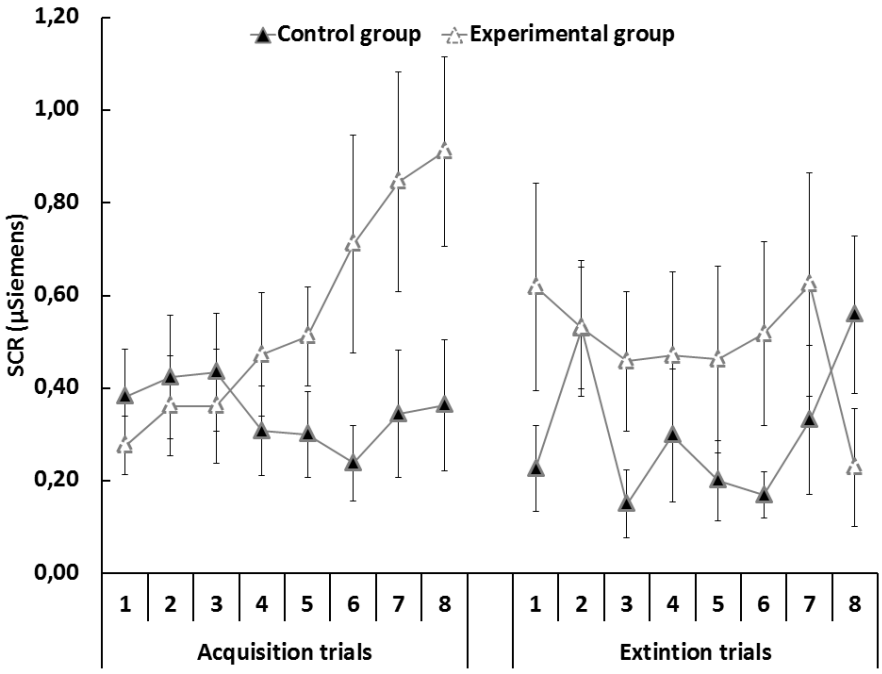

Figure. 3. Skin conductance responses across the acquisition and the extinction phase for the experimental and the control group separately. Vertical bars denote standard errors.

3.3. Skin conductance response

Data from one participant in the experimental condition were unavailable due to technical failure, resulting in n = 24 for the experimental and n = 25 for the control group. There was a significant Group x Phase x Trial interaction (F(7, 329) = 3.21, p < .01, η_p^2 = .06, ε = .69), originating from different SCR patterns across trials between groups during acquisition (F(1,47) = 11.22, p = .002, η_p^2 = .19) but not during extinction (F(1,47) = 2.11, p = .15) (see Fig. 3). The experimental group developed an elevated SCR to the CS across acquisition trials (F(1,47) 16.89, p < .001[2], η_p^2 = .26), whereas the control group did not (F(1,47) = .63, p = .86[2]). No increase or decrease in SCR across extinction trials was observed regardless of group (F(1,47) = .11, p = .74). None of the other main or interaction effects reached significance.

3.4. Eyeblink startle

Exclusion of non-responders resulted in *n* = 21 for the experimental group and *n* = 24 for the control group. The results are presented in Fig. 4. The three-way Group x Phase x Stimulus interaction effect was not significant. Planned contrast revealed marginally significant group differences both during acquisition and during extinction for startle responses to the CS relative to the ITI ($F(1,43) = 2.91$, $p = .095$, $\eta_p^2 = .63$; $F(1,43) = 3.8$, $p = .058$, $\eta_p^2 = .81$. Furthermore, there was a significant main effect of Phase, $F(1, 43) = 10.15$, $p = .003$, $\eta_p^2 = .19$, with stronger eyeblink startle responses in the acquisition phase than in the extinction phase. Furthermore, there was no main effect of Stimulus $F(1, 43) = .11$, $p = .74$, but there was a significant Group x Stimulus effect $F(1,43) = 5.16$, $p = .028$, driven by a tendency for larger responses across phases for the ITI compared to the CS in the control group $F(1,43) = 3.63$, $p = .13^2$, $\eta_p^2 = .078$, and an opposite tendency in the experimental group $F(1,43) = 1.76$, $p = .4^2$. A significant Phase x Stimulus interaction effect revealed stronger eyeblink startle responses during the ITI than during the CS in the acquisition phase, and the opposite pattern during the extinction phase, $F(1, 43) = 8.45$, $p < .01$, $\eta_p^2 = .16$. The Group x Phase interaction effect was not significant $F < 1$.

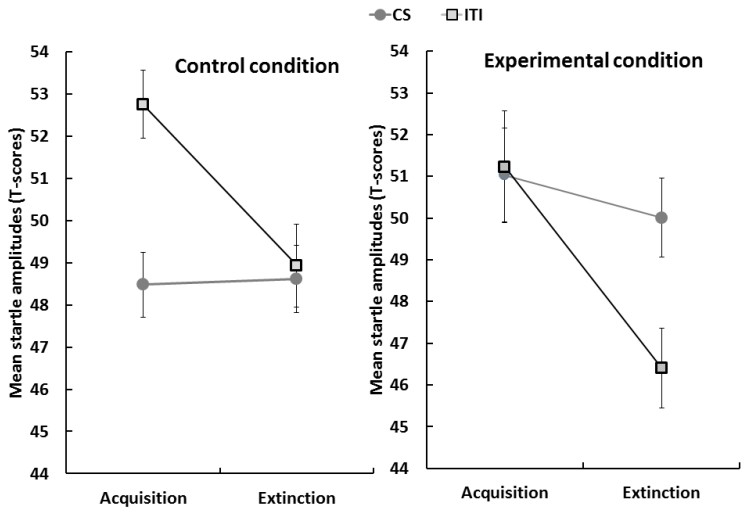

Figure. 4. Eyeblink startle responses during the CS and the ITI for the acquisition and the extinction phase for the experimental (right panel) and the control group (left panel) separately. Acoustic startle probes were presented in 50% of the trials during the CS and in 50% of the trials during the

ITI. The order of the trials with a probe during the CS or the ITI was semi-randomized within the acquisition and the extinction phase separately. Vertical bars denote standard errors.

4. Discussion

The aim of the current study was to explore whether interoceptive sensations can elicit a conditioned response after they have been associated with an exteroceptive event. In a simple conditioning paradigm, a respiratory stimulus (CS) was paired with an exteroceptive aversive electrocutaneous stimulus (US) in the experimental but not in the control group where it was followed by a safety period. Higher US-expectancy, stronger SCR, and stronger eyeblink startle reflexes during the CS compared to during the ITI in the experimental group at the end of acquisition, but not after extinction nor in the control group were expected. In the control group, stronger eyeblink responses during the ITI than during the CS were expected after acquisition.

The increase in SCR across acquisition trials in the experimental condition but not in the control conditions suggests that participants learned to associate the CS with the onset of the US. However, the observed pattern differs from most conditioning studies where normally a gradual decline in response strength is observed across trials although this decline is less profound in the group where the CS was associated with the US (Pappens et al., 2012). Interestingly, our observation suggests that interoceptive sensations can be endowed with the capacity to elicit strong sympathetically-driven arousal responses after they have been linked to an aversive exteroceptive event. As such, interoceptive sensations can acquire the potential to elicit sudden increases in arousal thereby facilitating the onset of a panic attack (Bouton et al., 2001). In line with the SCR data, participants in the experimental condition reported stronger US-expectancy during the CS than during the ITI across acquisition, whereas the control group showed the opposite pattern. A similar pattern has been found in previous studies using both interoceptive and exteroceptive conditioning protocols (Pappens et al., 2012).

Next, in the US-expectancy data, a sudden loss of differentiation between the CS and the ITI was observed on the first extinction trial. This emerged from an increase in US-expectancy for the ITI in the experimental group and an increase for the CS in the control group. Furthermore, both groups displayed a general increase in US-expectancy during the first extinction trials. The data suggest that two (control group) or three (experimental group) unreinforced CS presentations may be necessary before US-expectancy decreases, similar to findings of Pappens et al. (2012). However, contrary to their findings, it seems here that the initial presentation of the CS without the US violates the expectation of the US and hence boosts uncertainty levels about the occurrence of the US as reflected by the general increase in US-expectancy. Furthermore, in line with the absence of group differences for US-expectancy, we did neither observe any differences between groups in SCR during extinction corroborating previous findings from our group (Pappens et al., 2012).

Furthermore, the crucial interaction effect in startle eyeblink responses did not reach statistical significance. However, the overall higher amplitudes for the CS relative to the ITI in the experimental group and the opposite pattern in the control group provide some support for the established association. One explanation of a lack of effect might be that the averaged startle amplitudes included both the initial as well as the late acquisition trials. Hence, the learning curve across acquisition trials remains unclear. Given that we only had 4 trials with a probe during the CS and four trials with a probe during the ITI, separation into an early and late acquisition phase would have affected the reliability of our data as sufficient data points are required for estimation of startle amplitudes (Blumenthal et al., 2005). As startle eyeblink responses involve the mobilization of subcortical defensive response networks (Lang et al., 2000), one potential alternative explanation for the differences between startle eyeblink responses and SCRs could be that the adopted US was capable of eliciting arousal responses but was insufficiently threatening to activate subcortical defensive networks.

Despite these interesting findings, the current study suffers from some limitations. *First*, on average, the CS was experienced as intense and as unpleasant as the US. For some participants, the

CS may even have been more aversive than the US, leading to 'inverse' conditioning with the more aversive stimulus predicting the less aversive (Mallan, Lipp, & Libera, 2008). Furthermore, in the context of interoceptive conditioning studies, the use of strict neutral CSs has always been counterintuitive as the stimuli used as CS are often inherently more unpleasant compared to exteroceptive ones, for example, a geometrical shape (Bouton et al., 2001). However, interoceptive sensations have been found to elicit conditioned fear responses only after they have been associated with an aversive stimulus (Ceunen et al., 2016; Pappens et al., 2012; Schroijen et al., 2015; Zaman, Weltens, et al., 2015). From both a clinical and theoretical perspective, it would be interesting to further explore whether intero-extero associations (i.e., unpleasant CS and US) only develop in certain contexts (i.e., a negative state) and whether they develop faster and are more reluctant to extinction compared to a strict neutral CS (Seligman, 1971). *Second*, as previously mentioned, presenting only one acoustic startle probe per trial may have reduced the reliability of the eyeblink startle results. It may be more appropriate to present multiple startle probes within one trial using a different pattern of randomization to increase the reliability of the data.

In summary, we demonstrated that interoceptive stimuli can enter a CS-US association with an exteroceptive stimulus and elicit a conditioned response, suggesting that benign breathing sensations are capable of triggering increased arousal when experienced in an aversive context, potentially resulting in a full blow panic attack. Furthermore, it provides a theoretical basis for investigating the extinction of interoceptive associations, which in turn can for example, guide the optimization of interoceptive exposure as a treatment in chronic visceral pain.

5. Acknowledgments

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Footnotes

[1] Bonferonni correction by factor 4.

[2] Bonferonni correction by factor 2.

Table 1. Demographic characteristics, intensity of the electrocutaneous stimulus, and subjective ratings (means and standard deviations) for the experimental and control group.

	Experimental group	Control group
n (gender: f / m)	25 (19 / 6)	25 (19 / 6)
age (range)	19.1 (18 - 27)	19.3 (18 - 24)
US intensity (mA)	3.63 (2.4) $_a$	3.83 (2.2) $_a$
Pre-exposure		
CS intensity	1.8 (0.9) $_a$	2.0 (0.7) $_a$
CS unpleasantness	2.4 (1.0) $_a$	2.6 (0.9) $_a$
Post-acquisition		
CS intensity	2.6 (0.9) $_a$	2.7 (0.9) $_a$
CS unpleasantness	2.8 (1.0) $_a$	3.0 (1.1) $_a$
US intensity	2.4 (0.7) $_a$	2.7 (0.6) $_a$
US unpleasantness	2.7 (1.0) $_a$	3.1 (0.9) $_a$
US painful	2.2 (0.9) $_a$	2.6 (0.8) $_a$
US expectancy	7.4 (2.4) $_a$	6.0 (2.1) $_b$
Post-extinction		
CS intensity	2.8 (0.7) $_a$	3.0 (0.8) $_a$
CS unpleasantness	3.2 (1.1) $_a$	3.3 (1.1) $_a$
SP intensity	1.8 (0.8) $_a$	2.3 (0.9) $_a$
SP unpleasantness	2.0 (1.0) $_a$	2.3 (1.2) $_a$

Note. US = unconditioned stimulus (electrocutaneous stimulus), CS = conditioned stimulus (load), SP = startle probe. Different subscripts indicate a significant differences between groups at the level of p = .05.

Highlights

- *Intero-extero associations can be acquired.*
- *During acquisition skin conductance responses increased in the experimental group only.*
- *Across acquisition US-expectancy ratings during the CS increased in the experimental group only.*
- *During extinction no group differences were observed."*