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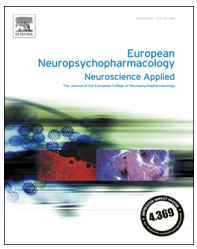

Modeling the development of panic disorder with interoceptive conditioning

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Abstract
Panic disorder is characterized by the paroxysmal occurrence and fear of bodily symptoms. In recent years it has been proposed that patients "learn" to fear cardiorespiratory sensations through interoceptive conditioning. This study sought to model the initial stage of this process in healthy volunteers ($N=44$) using mild cardiac sensations. An additional aim was to explore whether anxiety sensitivity – a known risk factor for panic disorder – modulates such interoceptive learning. Infusions of pentagastrin and saline were used to manipulate the presence versus absence of cardiac sensations, respectively, and served as conditioned stimuli in a differential interoceptive conditioning paradigm. Inhalation of 35% CO_2-enriched air served as the panicogenic, unconditioned stimulus (UCS). In half of the participants ("prepared" condition), cardiac sensations caused by pentagastrin were followed by inhalation of CO_2-enriched air (penta CS+), whereas the absence of such sensations (saline) was followed by room air (saline CS−). The reversed combination ("unprepared" condition) was used in the other half of the participants. Conditioning effects showed up for self-reported UCS-expectancy, but not for skin conductance and anxiety ratings. Only participants from the prepared group learned to expect the UCS, and differential learning was impaired with higher scores on anxiety sensitivity. Expectancy learning was more easily established towards the presence compared to the absence of cardiac sensations, whereas the reverse effect was observed for safety

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learning. Modeling impaired discriminatory learning and the moderating effect of anxiety sensitivity provides new insight in the development of panic disorder.

1. Introduction

Panic disorder (PD) is characterized by the paroxysmal occurrence and fear of bodily symptoms. During panic attacks, patients experience symptoms such as palpitations, dyspnea and chest pain, often associated with the fear of losing control or of dying. As isolated phenomenon, panic attacks are highly frequent: about 22% of the general population experience a panic attack, fulfilling DSM IV criteria, once in their lives (Norton et al., 2008). However, only in a subset of these people this evolves into PD, with frequently occurring panic attacks, both unexpected and linked to certain situations. Genetic factors are estimated to account for about 40% of the risk to develop PD (Hettema et al., 2001). This implies that the majority of the risk is of environmental nature. Human fear conditioning has been proposed to be an important mechanism in the etiology and maintenance of panic disorder as it could explain the transition from relatively isolated panic attacks towards PD (Bouton et al., 2001). In fear conditioning, an initially neutral conditioned stimulus (CS) is paired with an intrinsically aversive stimulus (unconditioned stimulus, UCS) and through associative learning the CS–UCS pairing results in the CS becoming a predictive signal for imminent threat, typically eliciting conditioned fear responses (CR). In recent years, substantial experimental evidence has accrued to support this hypothesis (Grillon, 2002; Lissek et al., 2005, 2009). In most of these studies, environmental stimuli were used as conditioned (CS, e.g. a picture) and unconditioned (UCS, e.g. an electric shock) stimuli. This considerably enhanced insight into the underlying potential learning mechanisms involved in PD. Nevertheless, these studies fall short in modeling the bodily sensations that lead to a panic attack, as panic triggers are often of internal bodily origin (e.g., cardiorespiratory sensations).

To meet this shortcoming, interoceptive fear conditioning (IFC) has been proposed. IFC occurs when a bodily sensation (e.g. palpitations, sweating, heart pounding, minor breathing discomfort) becomes a CS based on the contingency with a UCS (e.g. a panic attack). IFC has been hypothesized to play a key role in the development of PD (Acheson et al., 2011; Bouton et al., 2001; De Cort et al., 2012; Pappens et al., 2012). Specifically, mild interoceptive sensations (for instance, minor heart pounding) that typically emerge in the onset phase of a panic attack are thought to become a CS for a full blown panic attack, thereby reinforcing the learned association and promoting the transition into PD (Pappens et al., 2015).

Previous studies found evidence for IFC in the framework of PD (Acheson et al., 2007; Pappens et al., 2012, 2013, 2014, 2015; Schroijen et al., 2015). Those studies focused on mild respiratory stimuli as conditioned stimuli (CS), and found that fear to a benign respiratory sensation is easily learned when it predicts a more aversive respiratory event (UCS: e.g., an episode of intense dyspnea caused by inhaling CO_2 or being unable to breathe). Interestingly, when the same mild respiratory sensation predicted a "safe" period without aversive respiratory event (UCS), persons still displayed fearful expectations towards the mild respiratory sensation, suggesting that safety learning to interoceptive CSs is hard to establish when they involve the same response system and – therefore – show some resemblance to the initial moments of the UCS (Pappens et al., 2012, 2013; Schroijen et al., 2015). Panic disorder patients show a similar phenomenon, as they typically fear cardiorespiratory sensations that are in essence continuously present and accessible to conscious perception. Such cardiorespiratory sensations are only rarely followed by a panic attack, making them poor predictors thereof. In other words, similar to the experimental findings described above, panic patients seem to remain "blind" for the safety value of mild cardiorespiratory sensations that their body produces continuously, and consequently overestimate the contingency between benign cardiorespiratory sensations and panic attacks.

This is in line with a "preparedness view", positing that evolutionary-prepared, fear relevant stimuli are easier to condition than fear irrelevant, or "unprepared" cues (Mineka and Öhman, 2002). Within a PD framework, previous fear conditioning research has confirmed this using script-based imagery (De Cort et al., 2012; Stegen et al., 1999), or video clips (Forsyth et al., 1996). For example, conditioning is facilitated when using a claustrophobic compared to an emotionally neutral mental image as the CS (Stegen et al., 1999). With the present study, we sought to explore this phenomenon with a truly interoceptive, cardiac sensation as the CS. From a preparedness point of view, it can be hypothesized that anticipatory, panic-related fear is easier established to the presence than to the absence of mild cardiac sensations of arousal. Conversely, safety learning can be expected to be established more easily to the absence than to the presence of such cardiac sensations.

The present study sought to model IFC to the presence/absence of mild cardiac sensations in a group of healthy volunteers without any personal or familial history of panic (to avoid the possibility of inducing actual panic disorder), however taking into account interpersonal differences in vulnerability by measuring participants' anxiety sensitivity (AS). Susceptibility for IFC seems to differ importantly between persons, and only in anxiety sensitive persons this is expected to lead to the subsequent occurrence of panic attacks (Pappens et al., 2014). The construct of AS refers to individual differences in the fear of anxiety related sensations and the expectation that such sensations can have harmful consequences. It has been proposed as a risk factor for the development of PD in particular (Naragon-Gainey, 2010).

In the present study, 35% CO_2 enriched air was used as the UCS, as it is known to cause panic symptoms in healthy volunteers (Griez et al., 2007; Schruers et al., 2011). Cardiac sensations were induced with an injection of minimal doses of pentagastrin. A very low dose of .2 μg/kg pentagastrin in 1 ml saline selectively evokes minor sensations of palpitations and increased heart rate (Abelson and Liberzon, 1999; Radu et al., 2002). Saline-only injections were used as a placebo stimulus, controlling for the exteroceptive cues that may come along with an injection. Thus, all participants received two types of injections: one inducing cardiac sensations (pentagastrin) and another one associated with the absence of such sensations (saline). In the "prepared" condition (half of the participants), the cardiac sensations caused by pentagastrine served as CS+ (100% reinforcement with the UCS), whereas the absence of such sensations (saline-injections) served as the safety cue (CS−, 0% reinforcement with the UCS). This was reversed in the "unprepared" condition (other half of the participants). In the latter group, the absence of cardiac sensations (placebo injections with saline) served as CS+ (100% reinforcement with the UCS), whereas cardiac sensations caused by pentagastrine served as CS− and were never paired with the UCS. As such, the presence or the absence of mild cardiac sensations occurring after an injection were the best predictor of the panicogenic UCS in the prepared and the unprepared group, respectively. The studied outcomes included skin conductance responses to the CSs, as well as self-reported anxiety and expectancy of the UCS.

We hypothesized greater conditioning to occur in the prepared compared to the unprepared group (hypothesis 1). Furthermore, we hypothesized a facilitated fear learning to the presence compared to the absence of cardiac sensations, and, a facilitated safety learning to the absence compared to the presence of cardiac sensations (hypothesis 2). We explored also whether high-risk participants (indexed by high anxiety sensitivity scores) would exhibit different learning effects as compared to participants at "low" risk.

2. Experimental procedure

2.1. Participants

Forty-four healthy volunteers (all Caucasian, 27 women, mean age = 27.86 ± 10.49; range = 19–56 years) participated in this study. Because of the use of 35% CO_2-enriched air and of pentagastrin, exclusion criteria were the following: history of cardiovascular or pulmonary disease, mental disorder, hypertension, body mass index > 27, personal/ familial history of cerebral aneurysm, pregnancy, epilepsy, current use of psychotropic medication, anxiety- and/or mood disorders in first degree relatives. Sixty individuals were screened; however some of them did meet the exclusion criteria or did not show up (N = 14). Due to equipment failure two subjects were excluded from the analysis of the skin conductance. In return for participation all participants received a voucher of 30 €. The study protocol was approved by the Medical Ethics Committee of Maastricht University, the Netherlands.

2.2. Conditioned stimuli

Pentagastrin (.2 μg/kg) was used as CS. An injection of a small dosage of pentagastrin in 1 ml saline was used to provoke minor sensations of increased heart rate. Research in healthy volunteers showed that an infusion of .2 μg/kg pentagastrin will produce a selective increase in heart rate compared to placebo (Abelson and Liberzon, 1999; Radu et al., 2002). This effect is short-lived and disappears completely after 4 min. Following the injection of pentagastrin, the IV line was flushed with 1 ml normal saline. As placebo stimulus, an injection of 1 ml saline was administered. A physician applied all the infusions.

2.3. Unconditioned stimuli

A single vital capacity inhalation of 35% CO_2 or room air were used as UCS and control inhalation, respectively. Participants had to inhale through a mouthpiece and received a gas mixture of 35% CO_2 and 65% O_2 or a mixture with concentrations identical to room air (medical dry air; 21% 0_2 and 78% N_2). Both mixtures were stored in standard gas cylinders and, after decompression, mixtures were led through a wide vinyl tube with a demand valve at the end. Participants were instructed to exhale as deeply as possible and subsequently to inhale deeply and to hold their breath for 4 s before exhaling again.

2.4. Self-report measurements

During the first 45 s after each injection (CS presentation), participants continuously rated their anxiety with a custom-built dial (Vansteenwegen et al., 2008) on a scale ranging from 0 ("no anxiety at all") to 100 ("worst anxiety ever experienced").

After the 45th second and throughout the last 15 s of each CS presentation, participants were asked to what extent they *expected* to inhale CO_2-enriched air on a scale ranging from 0 ("no CO_2-enriched air") to 100 ("for sure CO_2-enriched air"), further referred to as "expectancy" (Devriese et al., 2006). The expectancy rating was completed on paper, participants noted down a single value on the scale that corresponded to their expectancy.

Panic symptoms were assessed with the Panic Symptom List (PSL). This list consists of the 13 DSM IV symptoms of a panic attack, assessing intensity ratings ranging from "0 – not at all" to "4 – very much". The PSL was administrated on paper before and after each trial. The amount of panic symptoms was calculated by subtracting the pre from the post-measurement (= delta PSL).

The *mental health* of the participants was evaluated diagnostically by a trained clinician using the Mini International Neuropsychiatric Interview (MINI; Lecrubier et al., 1997; Overbeek et al., 1999). In addition, the Montgomery-Asberg Depression Scale (MADRS; Montgomery and Asberg, 1979; Hartong and Goekoop, 1985), the Self-rating Depression Scale (SDS; Zung, 1965; Mook et al., 1990), the Spielberger State/trait Anxiety Scale (Spielberger et al., 1983; van der Ploeg et al., 1980), the Fear Questionnaire (FQ; Marks and Matthews, 1979; Arrindell et al., 1984) and the Anxiety Sensitivity Index (ASI; Reiss et al., 1986; Vancleef et al., 2006) were administered.

2.5. Physiological measurements

Skin conductance (electrodermal activity) was recorded with two Fukuda standard Ag/AgCl electrodes (1 cm in diameter) filled with KY jelly and attached to the hypothenar palm of the left hand, which was first cleaned with tap water. The inter-electrode distance was 2.5 cm. The Coulbourn skin conductance coupler (V71 – 23) provided a constant .5 V across the electrodes. The analog signal was passed through a 12-bit AD-converter and digitized at 10 Hz.

The Electrocardiogram (ECG) was measured using three standard Ag/AgCl electrodes filled with KY jelly; one was attached to the left side of the body between the third and the fourth rib starting from below and two were attached just beneath the right and left clavicle (Pappens et al., 2011; Van Diest et al., 2009). The signal was sampled

at 1000 Hz and transduced, amplified and filtered through a Coulbourn S75 - 05 isolated bioamplifier.

Participants breathed through a mouthpiece and wore a nose clip. The mouthpiece was connected to a microbialfilter (MicroGard, VIASYS) that was connected to a non-rebreathing valve ensuring the separation of inspiratory and expiratory air. A vinyl tube (inner diameter: 3.5 cm; length 100 cm) connected the inspiratory side of the non-rebreathing valve with a 3-way Y-valve (stop cock type) enabling easy switching between 35% CO_2 and normal air.

The ECG and skin conductance signals were recorded using Affect 4.0 software (Hermans et al., 2005). The signals were treated off-line with a custom made software program PSychoPHysiological Analysis (PSPHA; DeClerck et al., 2006); a modular script-based program to generate and apply calibration factors and to extract parameters from each of the recorded signals. All waveforms were visually inspected off-line and technical abnormalities as well as movement artifacts were eliminated using the PSPHA software.

2.6. Procedure

The study was conducted at the Academic Anxiety Center of Mondriaan Mental Health and Maastricht University, the Netherlands. The actual aim of the study was concealed and therefore, before screening, the participants were told that the goal of the study was to study the effect of infusions on physiological parameters. Participants were also told that all information would be given at the end of the experiment. The procedure was then explained in detail. After informed consent was obtained, participants were screened and the questionnaires were administered. Participants were randomly assigned to the prepared or unprepared group and both groups consisted of 22 participants each.

The experiment consisted of four phases; habituation, pre-exposure, acquisition and test. During the *habituation phase* participants became familiar with the test environment. He/she sat in a chair wearing headphones, the intravenous catheter was inserted and electrodes were attached. After the instructions, participants took the mouthpiece in and put on the nose-clip.

After the habituation phase, the *pre-exposure phase* started which included two trials. In the first trial an injection with pentagastrin and in the second trial an injection with saline were given. In this stage, participants were informed about the content of the injection. During the subsequent *acquisition phase*, the "prepared" group received pentagastrin (penta CS+) followed by the UCS in three trials, and saline (saline CS−) followed by room air inhalation in three other trials. In the "unprepared" group this was reversed (saline CS+/ penta CS−). The CS+ and CS− were presented in a semi-randomized order and the intertrial intervals lasted 2 min.

Each trial lasted 250 s and consisted of five main time windows: (1) baseline between 0 and 20 s, (2) infusion of saline or pentagastrin between 20 s and 40 s (completed by the 40th second), (3) CS period between 40 and 100 s, (4) one inhalation of either 35% CO_2-enriched air or normal room air and (5) an intertrial interval of 150 s. The CS period on itself had a duration of 60 s during which anxiety was continuously measured online throughout the first 45 s and US expectancy was measured on paper during the last 15 s of the CS. The total CS period lasted for 60 s during which the pentagastrin induced effects that peak around 40–45 s. With an ITI of 150 s in combination with 2 sets of PSL questions, we assured an appropriate time window larger than 240 s for the pentagastrin effects to vanish completely. Figure 1 shows a summary of the global trial structure. Prior to as well as after each trial, the Panic Symptom List (PSL) was assessed on paper.

After the acquisition phase, the *test phase* started. It included 6 trials, in which both the CS+ and the CS− were presented 3 times. Both CSs were followed by an inhalation of room air.

Four fixed presentation orders were used with the restriction that a specific CS never occurred more than two times in a row and the orders were counterbalanced with half of the orders starting with CS+/−, and the other half starting with CS−/+ trials respectively. Participants were randomized across these four orders.

2.7. Parameter extraction and statistical design

Nine participants who did not respond with a greater heart rate increase (difference score between CS and baseline period) to pentagastrin relative to saline during the pre-exposure phase were excluded from further analyses.

For skin conductance responses and anxiety ratings, we focused on the 40–45 s time window after CS onset, corresponding to the time window during which participants displayed a peak in anxiety and heart rate during the pre-exposure pentagastrin infusion. For skin conductance, we calculated difference scores between this 40–45 s time window after CS onset and the baseline (20 s). To correct for individual differences in skin conductance, a Rose's range correction (SC-MinSC/MaxSC-MinSC) was applied (Lykken and Venables, 1971).

Pre-exposure data for anxiety and skin conductance were analyzed separately in a mixed repeated measures ANOVA with

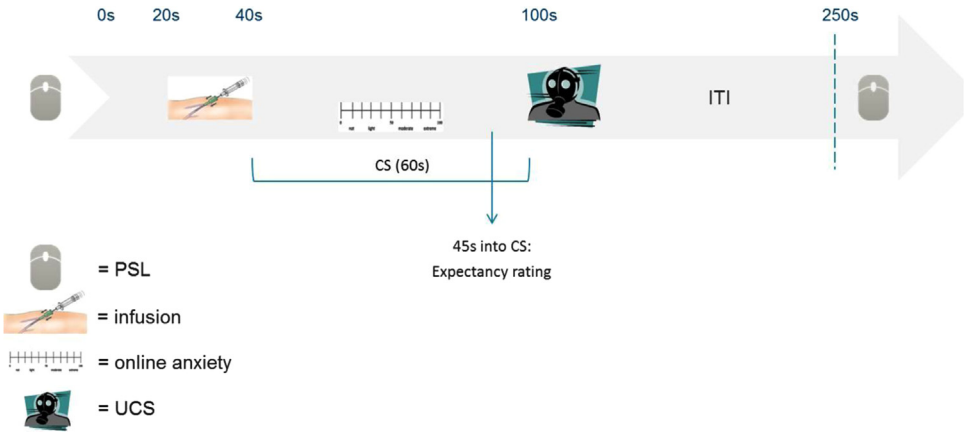

Figure 1 A representation of the global trial structure, consisting of 5 phases: (1) baseline from 0 to 20 s, (2) infusion between 20 and 40 s (completed by the 40th s), (3) CS period of 60 s during which anxiety was continuously rated during the first 45 s and US expectancy was rated during the final 15 s, (4) one inhalation of 35% CO_2-enriched air or normal room air and (5) an intertrial interval of 150 s after which the PSL was assessed.

3 factors: (1) AS as a continuous inter-individual variable (predictor), (2) group (prepared, unprepared) and (3) "Injection" (pentagastrin or saline).

The change scores in panic symptoms in the conditioning phase were analyzed with non-parametric tests.

Conditioning data for expectancy, self-reported anxiety and skin conductance were analyzed in mixed repeated measures ANOVAs with 4 factors: (1) AS as a continuous inter-individual variable (predictor), (2) group (prepared, unprepared), (3) CS-type (+/−), and (4) trial (1-6). Both latter variables were within subject variables. When the crucial 3-way interaction (group * trial * CS-type) was significant, contrasts were run to further test our first hypothesis. First, to know whether conditioning was present in both groups, we calculated an index for differential learning between CS + and CS− (CS+ minus CS−; referred to as learning index "Li") for the first and third trial of each group. In order to conclude that conditioning was present, the difference score at the end of acquisition (third trial; Li_3) should exceed the one at the beginning of acquisition (first trial; Li_1). As such, we calculated a change index (Ci) by subtracting the learning index at the first trial from the third trial (Li_3 - Li_1) for each group. Between group differences were then tested by contrasting the change indices of both groups.

To test whether fear and safety learning are more easily established to pentagastrin and saline, respectively (second hypothesis), we first calculated difference scores between the third and the first acquisition trial for each CS-type ("$CS+_3$ minus $CS+_1$" and "$CS-_3$ minus $CS-_1$" for respectively fear learning and safety learning). To test for between group differences, ANOVA's were run with injection-type (pentagastrin, saline) as a categorical and AS as a continuous between subject factor for both fear (CS+) and safety (CS−) learning separately.

Alpha was set at a significance level of .05. Greenhouse-Geisser corrections were applied for main effects and interactions involving more than two within subject levels. Uncorrected degrees of freedom and corrected *p*'s will be reported together with η_p^2. All analyses were performed with Statistica Version 12.

3. Results

3.1. Sample characteristics

Depression, general anxiety and anxiety severity scores were well below clinical values without significant differences between groups ($p > .2$, see Table 1).

Table 1 Mean scores and standard deviations between brackets of the Questionnaires for the Prepared and Unprepared group. Score ranges are mentioned between brackets.

	Prepared	Unprepared
MADRS (0-60)	.4 (1,4)	.36 (.9)
SDS (20-80)	27.2 (5.1)	29.9 (7.3)
STAI-state (20-80)	26.9 (6.2)	26.4 (6.1)
STAI-trait (20-80)	28.8 (7.4)	29.1 (6.8)
FQ (0-120)	9.9 (12.3)	9.2 (7.4)
ASI (0-64)	15.7 (8.8)	14.6 (6.7)

Note: MADRS = Montgomery-Asberg Depression Scale; SDS = Self-rating Depression Scale; STAI-state = State Trait Anxiety Inventory-state; STAI-2 = State Trait Anxiety Inventory-trait; FQ = Fear Questionnaire Total; ASI = Anxiety Sensitivity Index.

3.2. Panic symptoms to the CO_2 and room air inhalations

After an inhalation of 35% CO_2, panic symptoms increased more compared to after an inhalation of room air ($x^2(5, N = 35) = 67.6$, $p < .01$, see Figure 2). There were no significant correlations between the PSL difference scores and AS ($p > .2$). Differences between the groups were noted only for the first 35% CO_2 trial (prepared > unprepared) and for all of the three room air trials (CS−) (Figure 3, CS_1+, $x^2(1, N = 35) = 6.2$, $p < .02$; CS_1-, $x^2(1, N = 35) = 13.9$, $p < .01$; CS_2-, $x^2(1, N = 35) = 9.6$, $p < .01$; CS_3-, $x^2(1, N = 35) = 8.3$, $p < .01$). Following the room air trials in the acquisition phase, participants reported more panic symptoms when the CS− involved an injection with pentagastrin (unprepared group) as compared to saline (prepared group).

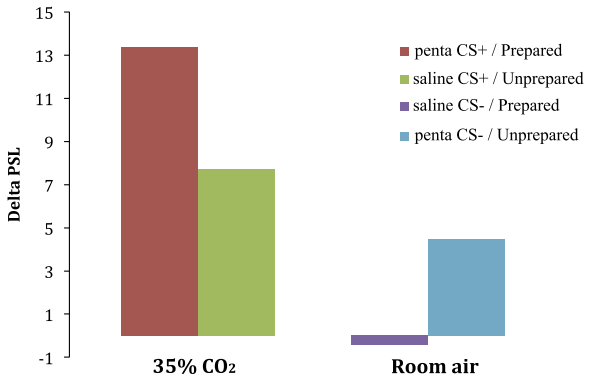

Figure 2 Mean change in panic symptoms after inhalation of 35% CO_2 (CS+) or after room air (CS−) in the Prepared and Unprepared group during the first trial.

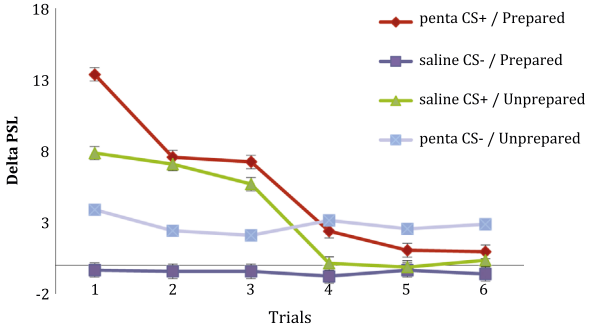

Figure 3 Mean panic symptoms after inhalation of 35% CO_2 (CS+) or after room air (CS−) in the Prepared and Unprepared group during the acquisition (trial 1-3) and test phase (trial 4-6). Standard errors are presented in bars.

3.3. Conditioned stimuli

3.3.1. Expectancy

We observed higher UCS expectancies for CS+ than CS− (main effect of CS-type: $F(1,31) = 12.31$, $p < .002$, *partial* $\eta^2 = .28$), but this effect was significantly modulated by trial and group

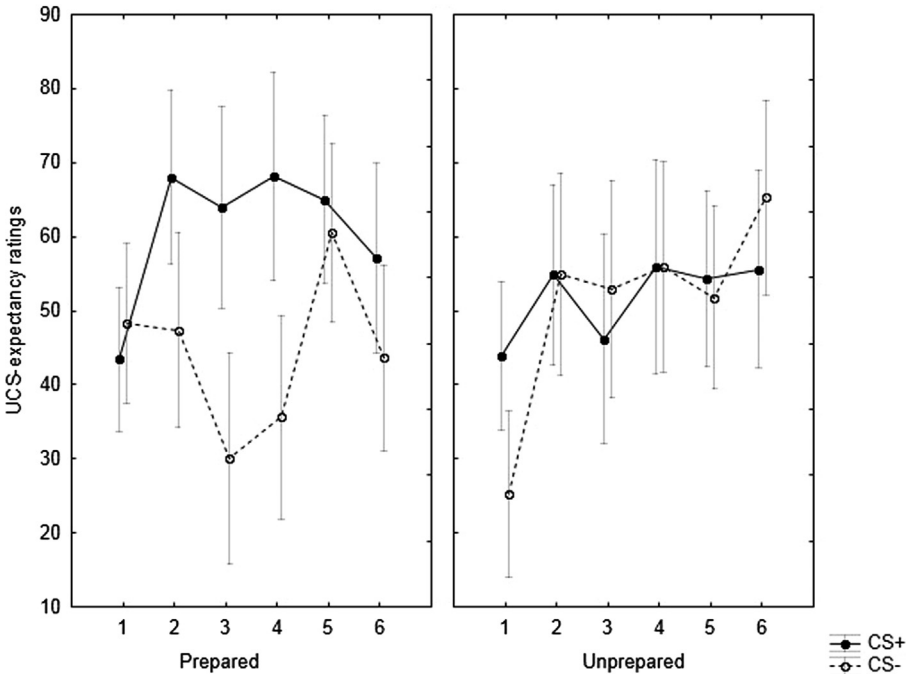

Figure 4 Expectancy ratings for CS+ and CS− in the Prepared and Unprepared group during the acquisition (trial 1–3) and test (trial 4–6) trials. Confidence intervals are presented in bars.

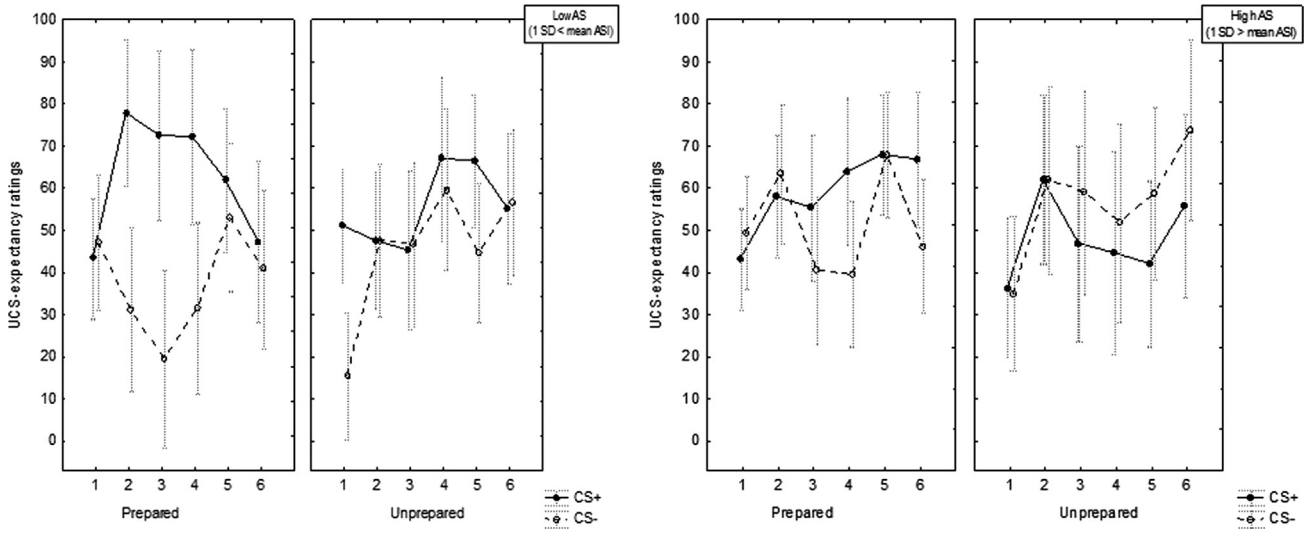

Figure 5 Expectancy ratings for CS+ and CS− in the Prepared and Unprepared group, separately for the Low (1 SD below mean ASI; $N = 5$) and High (1 SD above mean ASI; $N = 6$) anxiety sensitivity (AS) group, on the left and right respectively. Confidence intervals are presented in bars.

(CS-type * trial * group interaction: $F(5,155) = 5.42$, $p < .001$, *partial* $\eta^2 = .15$, $\varepsilon = .77$, see Figure 4). Follow-up analyses of the latter interaction showed that differential learning between CS+ and CS− occurred in the prepared group, but not in the unprepared group. In the prepared group, the learning index (Li) was significantly greater at the third acquisition trial (Li_3) compared to the first acquisition trial ($Li_1 = -5.06 < Li_3 = 30.56$; $F(1,17) = 8.35$, $p < .02$, *partial* $\eta^2 = .33$). In the unprepared group, Li_3 was significantly lower compared to Li_1, indicating the absence of differential learning effects ($Li_1 = 21.88 > Li_3 = -5.76$; $F(1,16) = 9.05$, $p < .01$, *partial* $\eta^2 = .36$). Between group comparisons of the change index (Li_3 minus Li_1 for each group) showed that differential learning effects were significantly stronger and exclusively occurred for the prepared as compared to the unprepared group ($Ci_{prepared} = 33.24 > Ci_{unprepared} = -27.65$; $F(1,16) = 10.37$, $p < .01$, *partial* $\eta^2 = .39$), confirming our first hypothesis.

Regarding our second hypothesis, findings confirmed that fear learning was greater to pentagastrin compared to saline (diff score $CS+_{pentagastrin}$ (= 20.60) > diff score $CS+_{saline}$ (= 2.31); $F(1,31) = 5.38$, $p < .05$, *partial* $\eta^2 = .15$). Safety learning in turn was more easily established to saline

compared to pentagastrin (diff score $CS-_{saline}$ (= −18.15) < diff score $CS-_{pentagastrin}$ (= 27.74); $F(1,31) = 6.51$, $p < .05$, *partial* $\eta^2 = .17$).

Learning was modulated by inter-individual differences in AS, as shown by a CS-type * AS interaction ($F(1,31) = 6.90$, $p < .05$, *partial* $\eta^2 = .18$) and a significant CS-type * trial * group * AS interaction ($F(5,155) = 3.07$, $p < .05$, *partial* $\eta^2 = .09$). Figure 5 shows the 3-way interaction, respectively for the low and high AS subgroups and suggests that greatest and fastest learning occurred in participants with low anxiety sensitivity, particularly in the prepared group. This was confirmed by follow-up comparisons showing that the differential learning index was significant from the second trial on in participants from the prepared group scoring low on anxiety sensitivity ($F(1,31) = 15.17$, $p < .001$), but not in those scoring high ($F(1,31) = 2.61$, $p = .12$). In addition, only participants low in anxiety sensitivity (prepared group) displayed clear extinction learning, as the learning index was no longer significant in the 6th trial for the participants scoring low in anxiety sensitivity ($F(1,31) = .37$, $p = .55$), whereas it was for those high in anxiety sensitivity ($F(1,31) = 5.48$, $p < .05$).

Also for the unprepared group, Figure 5 suggests a trend towards better learning in the low compared to the high anxiety sensitivity group. Following acquisition (trials 4-5), the expectancies of participants scoring lower on anxiety sensitivity are, although not significant, more or less consistent with a differential learning effect (Mean $CS+_{4-6}$ = 62,97, Mean $CS-_{4-6}$ = 53.76; $F(1,31) = .12$, $p = .73$). For participants scoring high on anxiety sensitivity, the average expectancies for CS+ remain below those for CS− (see Figure 5; Mean $CS+_{4-6}$ = 47.60, Mean $CS-_{4-6}$ = 61.50; $F(1,31) = 1.54$, $p = .22$), which is opposite to a differential learning effect.

3.3.2. Self-reported anxiety

3.3.2.1. Pre-exposure. Participants felt significantly more anxious when they were injected with pentagastrin than when they were injected with saline (main effect of Injection: $F(1, 31 = 16.53$, $p < .001$, *partial* $\eta^2 = .35$)), with no differences between groups ($p > .16$).

3.3.2.2. Conditioning. Pentagastrin elevated anxiety, irrespective of whether it served as a CS+ or CS− during acquisition (penta CS+ in the prepared group; penta CS− in the unprepared group; CS-type * group interaction: $F(1, 31) = 4.52$, $p < .05$, *partial* $\eta^2 = .13$). However, the crucial CS-type * trial * group interaction was not significant ($F(5, 155) = 1.18$, $p = .32$, *partial* $\eta^2 = .04$, $\varepsilon = .72$, Figure 6).

3.3.3. Skin conductance

3.3.3.1. Pre-exposure. Skin conductance increased significantly stronger when participants were injected with pentagastrin as compared to placebo (main effect of Injection: $F(1, 28) = 4.65$, $p < .05$, *partial* $\eta^2 = .14$, Pentagastrin = 35.91 and Saline = 2.03).

3.3.3.2. Conditioning. The CS-type * trial * group (see Figure 7) was not significant ($F(5, 150) = .30$, $p = .88$, *partial* $\eta^2 = .01$, $\varepsilon = .81$). Other main and interaction effects were also not significant.

4. Discussion

The present study sought to model interoceptive fear conditioning (IFC) to the presence/absence of cardiac sensations in a group of healthy volunteers, taking into account and exploring the contribution of interpersonal differences in anxiety sensitivity (AS). The presence versus absence of cardiac

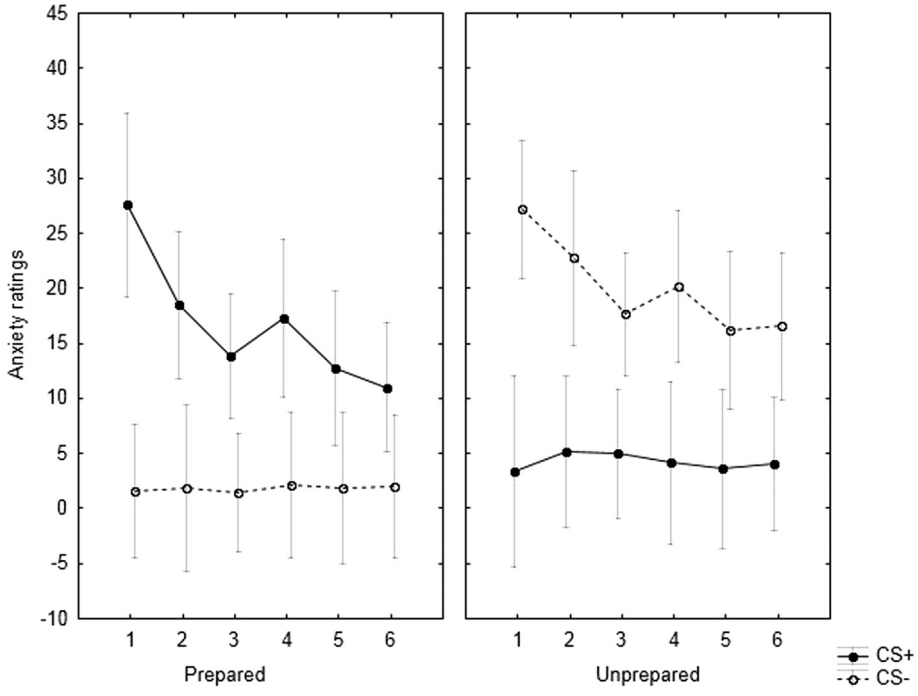

Figure 6 Anxiety ratings for CS+ and CS− in the Prepared and Unprepared group during the acquisition (trial 1-3) and test (trial 4-6) trials. Confidence intervals are presented in bars.

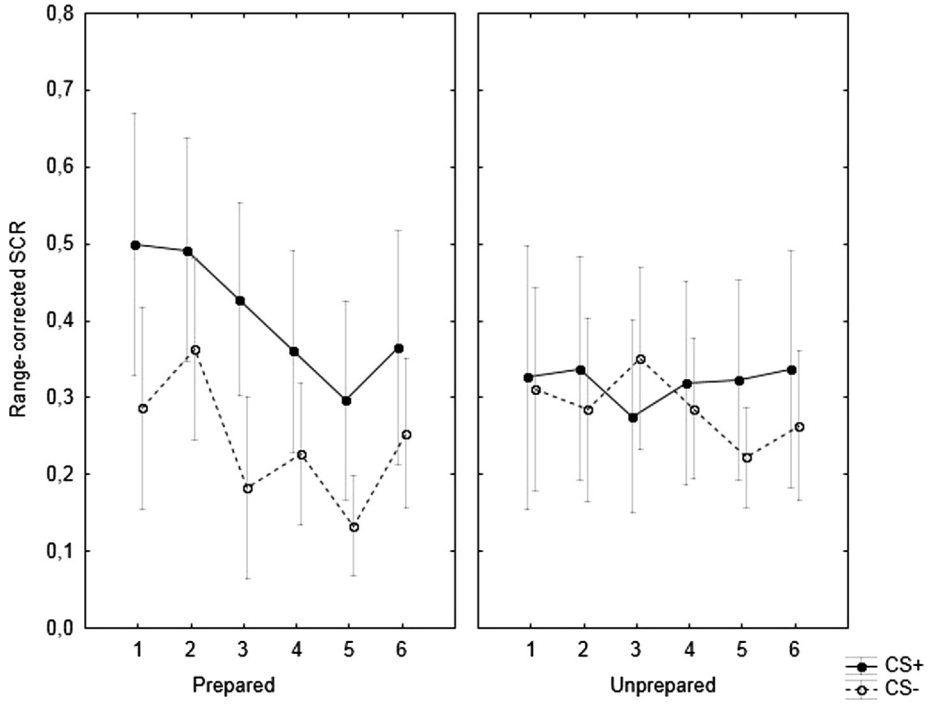

Figure 7 Ranged corrected SCR during acquisition and test for CS+ and CS− in the Prepared and Unprepared group during the acquisition (trial 1-3) and test (trial 4-6) trials. Confidence intervals are presented in bars.

sensations following an injection of pentagastrin or saline, respectively, served as predictors (CSs) of panic symptoms as induced by an inhalation of 35% CO_2 (UCS). Our findings confirm that considerably more panic symptoms were reported following inhalation of 35% CO_2 than after room air, supporting the use of CO_2-enriched air inhalation as an ecologically valid model to study panic symptoms in healthy volunteers (Griez et al., 2007). One group of participants ("prepared" condition) received the panicogenic UCS following the pentagastrin and whereas saline was followed by room air. For a second group ("unprepared" condition), this relation was reversed. Based on previous findings suggesting impaired safety learning to respiratory sensations in a panic-relevant IFC paradigm (Pappens et al., 2012, 2013; Schroijen et al., 2015), and based on the idea that the presence of cardiac sensations is more inherently related to a panic attack than the absence of such sensations, we expected greater learning to occur in the prepared compared to the unprepared condition. We also expected greater fear learning when the CS+ involved the presence compared to the absence of cardiac sensations, and greater safety learning when the CS− involved the absence compared to the presence of cardiac sensations.

Findings indicated clear contingency learning in the prepared, but not the unprepared condition. We interpret these findings within the framework of the proposed role of IFC in the development of PD (Bouton et al., 2001). Specifically, through IFC minor bodily symptoms come to predict future panic symptoms. This was expressed in higher expectancy ratings at the end of acquisition for pentagastrin (CS+) compared to saline (CS−). This differential learning was only present in the prepared condition in which the UCS was linked with the presence of "panic-relevant" cardiac sensations as a predictor (pentagastrin CS+). Moreover, our data show that fear learning, defined as an increase in expectancy to the CS+, could be established more easily to the presence than to absence of cardiac sensations. Conversely, safety learning (decline in expectancy to the CS−) was established more easily to the absence compared to the presence of cardiac sensations. These findings can be interpreted in line with the "preparedness view", holding that cues that are relevant with respect to the UCS are easier to condition than UCS-irrelevant cues (Mineka and Öhman, 2002). Our findings are also in accordance with previous fear conditioning research using script-based imagery (De Cort et al., 2012; Stegen et al., 1999), video clips (Forsyth et al., 1996) and odors (Devriese et al., 2000; Van den Bergh et al., 1995, 1997) as CSs.

As AS has been shown to be a risk factor for the pathogenesis of PD, we incorporated it as predictor variable in the present analyses (Naragon-Gainey, 2010). Individuals exhibiting high AS are characterized by enhanced interoceptive sensitivity, in that they are more accurate perceivers of their heart beat (Domschke et al., 2010). In the present study, high AS persons were impaired in learning to predict the appearance of the UCS upon the CS+, compared to persons scoring low on AS. This moderating effect of AS is in line with previous studies showing that PD patients exhibit impaired discriminatory learning and overgeneralization of fear as indicated by enhanced behavioral (Haddad et al., 2012; Lissek et al., 2005, 2010, 2009) and neural (Tuescher et al., 2011) responses to safety cues. It has been proposed that during acquisition and extinction, anxiety patients and high anxious healthy individuals not only exhibit greater excitatory conditioning to the danger cue, but also impaired inhibitory conditioning to the safety cue (Lissek et al., 2005; Gazendam et al., 2013; Kindt and Soeter, 2014). Studies confirmed such impaired responding to the safety cue, especially to perceptually similar cues (Haddad et al., 2012; Lissek et al., 2010, 2009). Therefore,

it can be hypothesized that the initial development of PD may not result from superior conditioning, but rather from an impairment in clear discriminatory conditioning and overgeneralization: highly anxious persons seem to over-predict danger and under-predict safety. This interpretation is in accordance with recent conditioning accounts of anxiety disorders (Beckers et al., 2013).

In contrast to the findings for expectancy ratings, conditioning effects were absent for anxiety ratings and skin conductance responses, which may relate to some specific features of the present paradigm. To allow for an assessment of the UCS expectancy, participants stopped rating their level of anxiety rating 15 s prior to the UCS. However, the time window just prior to the UCS is likely the one in which participant's experienced strongest anticipatory anxiety. Also, an intrinsic effect of pentagastrin on the skin conductance response (SCR) is evident from the pre-exposure phase. Strong effects of pentagastrin on SCR may have overruled and masked potential effects of fear (learning) on SCR in the conditioning phase. An alternative interpretation for the lack of conditioning effects on anxiety and skin conductance could be that the present paradigm in health volunteers merely established "expectation learning", rather than a learned fear response. This is not surprising, keeping in mind that for the goal of the present study it was mandatory to study healthy volunteers, albeit with different levels of AS.

Awareness of the CS–UCS contingency is described as an important issue in the field of conditioning and is considered as necessary by some authors (Lovibond and Shanks, 2002). For example, one study showed that differential conditioning in skin conductance and startle was only established in aware individuals who could verbalize the CS–UCS relationship. The unaware participants were not able to discriminate between CS+ and CS−, displayed higher levels of general anxiety and subsequently exhibited stronger avoidance behaviour (Grillon, 2002). This is understandable, as learning via conditioning promotes predictability and restricts the range of cues that signal danger; failure to learn should therefore enhance overall anxiety. It was therefore proposed that context conditioning caused by unpredictability rather than explicit cue conditioning represents a model for anxiety. In accordance with this, it has also been demonstrated that panic patients develop more generalized anxiety when fearful events are less predictable (Grillon et al., 2008).

We note that in the present study, the participants were not informed about the experimental set-up. Furthermore, the use of both the pentagastrin and saline also promotes relatively higher rates of unawareness of CS–UCS associations (especially in the "ambiguous" unprepared group), making learning more difficult to occur. On the basis of the expectancy ratings there is a striking division between low and high AS. Persons with low AS in the prepared condition learned the contingencies after one learning trial. On the contrary, highly anxious participants were more impaired and/or slow in detecting the contingencies. It is conceivable, in line with the "context conditioning" hypothesis (Grillon, 2002), that high AS participants were more impaired to learn because of their higher anxiety state. Indeed, the high AS participants had significantly higher anxiety scores at the start of the experiment based on the STAI-state compared to low AS.

Psychiatrists and psychologists typically encounter people when they have become "patients". They rarely witness the origin of complaints that mark the transition from "normality" to psychopathology. The present study is an attempt to experimentally model the very first steps towards the development of panic. In the most recent edition of the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-5), is stated that panic attacks can occur in all mental disorders as well as in some medical conditions. Accordingly, DSM-5 adopted the occurrence of panic attacks as a specifier (a prognostic factor to aid in the diagnosis) of all mental disorders (American Psychiatric Association, 2013). This underscores the widespread clinical relevance of the findings in the present study.

Some limitations of the present study should be acknowledged, thereby suggesting directions for future research. First, the sample size is relatively small to investigate higher order interactions with AS, it would therefore be interesting for future research to replicate our findings on AS in a larger sample. Second, a potential and important limitation of the present study is that it lacked a manipulation check to ensure that pentagastrin consistently caused cardiac sensations and that saline did not produce interoceptive sensations. Participants without a strong cardiovascular response to pentagastrin may have been unable to differentiate between both type of CSs. To limit this possibility, we excluded participants ($N = 9$) who failed to show an increase in heart rate to pentagastrin relative to saline in the pre-exposure phase. Future research should consider actively monitoring the perceived bodily sensations induced by both the pentagastrin and saline injections. Third, adding startle reflex measurement could help in disentangling implicit from declarative learning in future studies (Beckers et al., 2013; Soeter and Kindt, 2010). Fourth, in order to make a distinction between healthy persons at risk in developing PD, the construct of anxiety sensitivity was used. Including first degree relatives of PD patients would possibly be even more naturalistic, but poses ethical constraints. Finally, we did not follow up on the long term effects of the conditioning manipulation. It would be interesting to focus on reinstatement or renewal issues in an interoceptive conditioning paradigm.

In sum, the present study provides some support to the hypothesized role of interoceptive conditioning in the development of PD and the dysfunctional nature of pathological anxiety. The initial development of PD seems not to be the result of superior conditioning, but rather of impairment in clear discriminatory conditioning and overgeneralization.

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Contributors

We state that each author contributed substantially to conception and design, or acquisition of data, or analysis and interpretation of data and drafted the article or revised it critically for important intellectual content and gave final approval of the version to be published. All authors contributed to and have approved the final manuscript.

Conflict of interest

There were no conflicts of interest for any of the authors.

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