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Atypical modulation of startle in women in face of aversive bodily sensations

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ABSTRACT

Eye blink startle magnitude is assumed to be higher in threatening contexts. A scarce amount of studies suggest that this does not hold true when startle is measured during perceived threats to homeostatic integrity. The present study was set up to describe the startle response pattern to a selection of interoceptive stimuli. Female subjects (N = 36) were exposed once to 90 s of continued (1) cold pain, (2) inhalation of a gas mixture of 7.5% CO_2, and (3) breathing against an inspiratory and expiratory resistive load. Each stimulus was preceded and followed by a 90 second period of rest, respectively labeled baseline and recovery. Even after correcting eye blink startle responses for habituation, a decreased startle amplitude was evident during these stimuli. Results suggest that startle amplitude during aversive stimulation is inversely correlated with perceived fearfulness for women, although further studies are necessary to corroborate this interpretation.

1. Introduction

Interoception, the perception of the state of the body, serves to maintain homeostasis and is closely linked to the experience of emotions (Craig, 2002). Interoceptive fear is the apprehension of bodily sensations (Shear et al., 1997) and can manifest itself following the perceived disruption of homeostasis or in the anticipation thereof (Furst and Cooper, 1970). The anticipated or perceived disruption of homeostasis that lies at the heart of interoceptive fear, can potentially relate to any part of the organisms' functioning, including gas-exchange and thermoregulation. Interoceptive fear includes fear of pain, as pain is a perception related to the body state, processed in a neural network that largely overlaps with processing of non-painful interoceptive sensations (Legrain et al., 2011; Moseley et al., 2012), and in that painful stimulation is relayed through a central homeostatic pathway along with other visceral and somatic afferents signaling the disruption of homeostasis (Craig, 2003).

From an evolutionary perspective, fears promote an animal's chances of survival by helping to select a response appropriate for counteracting a perceived or anticipated threat (Ohman and Mineka, 2001). In this line of logic, interoceptive fear can have an adaptive advantage in urging a behavioral response to restore homeostasis or prevent its disruption. However, interoceptive fear in the absence of a real threat may paradoxically lead to over-perception of bodily sensations and to excessive physical symptom reports.

Functional disorders, anxiety disorders, and pain related disorders, affect a significantly large part of the population. In all of these disorders interoceptive fears play a key role, implying that the advancement of both clinical and fundamental knowledge on interoceptive fear is of utmost importance. A body of literature as well as a number of laboratory studies imply that the etiology and maintenance of such disorders is due to associative learning processes (Acheson et al., 2007; Bouton et al., 2001; De Peuter et al., 2011; Mayer, 2000; Meulders et al., 2011; Pappens et al., 2013). Because of interoceptive fear conditioning, originally benign sensations can elicit fear responses, when in the past these benign sensations have preceded an aversive interoceptive sensation.

Although interoceptive fear conditioning has a strong pedigree in the understanding of the aforementioned disorders, relatively little research has elaborated on the basic fear response topography to interoceptive stimulations used in the laboratory. Therefore, the major aim of the current study was to document unconditioned fear responding to such interoceptive stimulations. We made a selection of stimuli frequently used in experimental paradigms on pain (e.g., Helsen et al., 2011) and dyspnea (Acheson et al., 2007; Pappens et al., 2011), namely cold pain, inhalation of CO_2-enriched air, and loaded breathing. We selected these particular stimuli because a limited body of literature on startle in response to these stimuli is already available, although as yet no design has presented these three stimuli in comparable manners within subjects. In this initial study, we limited ourselves to women: we justify this choice given that psychosomatic complaints and disorders

Abbreviations: CP, cold pain; CPT, cold pressor test; EMG, electromyography; IAPS, International Affective Picture System; SAM, self-assessment manikin; VAS, visual analog scale.

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have a higher prevalence among women (Kroenke and Spitzer, 1998; Şar, 2010).

The potentiation – i.e. the relative increase in magnitude – of the eye blink component of startle is a well-validated and widely accepted measure of fear responding. An important question relating to the aim of the current study is whether the eye blink component of startle can provide a reliable indication of fear during aversive interoceptive stimulation. The startle reflex is modulated by the motivational system (Lang et al., 2000), and shows an increased amplitude when experiencing fear (Globisch et al., 1999; Hamm et al., 1997) or something which is otherwise unpleasant (Vrana et al., 1988). Affective modulation of the startle reflex magnitude results from activation of a variety of structures in which the amygdala plays a pivotal role. This modulatory effect of the motivational neurocircuitry on the eye blink motor reflex is described in more detail in the literature (e.g., Davis, 2006; Lang et al., 1998; Misslin, 2003). Although potentiation of startle following manipulations that induce fear or unpleasantness is a robust finding, it has predominantly been tested using visual and auditory stimuli. In contrast, the few studies on startle in response to aversive interoceptive stimulation present a more complicated and as yet inconclusive picture of findings.

With regard to thermal pain stimulation, findings are somewhat equivocal. For phasic heat pain, it appears that stimulation of short duration evokes startle potentiation (Crombez et al., 1997), whereas stimulation of a longer duration does not (Horn et al., 2012, in press). For cold pain, there is an overall reduction when averaging startle amplitudes delivered at different times during a prolonged stimulation (Tavernor et al., 2000), whereas such reduction may not be evident at individual time points (De Peuter et al., 2009). Lovallo (1975) describes that pain in response to the cold pressor test (CPT) does not keep rising progressively as time of immersion increases, a finding which may explain why startle probes at particular time intervals are not reduced.

Regarding dyspnea, findings from several studies conducted in our research group strongly suggest that dyspnea induced by the inhalation of CO_2-enriched air is associated with an inhibition of the startle reflex (De Peuter et al., 2009; Pappens et al., 2012; Van Diest et al., 2009b). Paradoxically, when dyspnea is induced by loaded breathing – a mechanical stimulus creating respiratory resistance – startle potentiation is evident when the stimulus is light (near perceptual threshold level), but absent when a respiratory load of higher (moderate) intensity is administered (Pappens et al., 2010). This is paradoxical, because self-report measures as well as skin conductance indicated that the higher load was more aversive and arousing than the light load.

Possible mechanisms for these findings have been suggested by their respective authors, and will be reviewed in the Discussion section. Regardless of the mechanism responsible for the apparently atypical startle pattern found in earlier studies documenting startle responding to the CPT, inhalation of CO_2-enriched air, and loaded breathing, it seems that startle within one type of stimulus is inversely correlated with unpleasantness (Pappens et al., 2010). The following parsimonious conclusions could be made: (a) these types of aversive interoceptive stimulation are associated with a reduction in startle rather than potentiation. (b) As dyspneic stimuli become more aversive as time progresses, it could be expected that startle responsivity decreases overall as the duration of dyspneic interoceptive stimulation increases. However, (c) startle in response to painful peripheral hypothermic stimulation may be an exception in that pain fluctuates over the course of time, and accordingly, startle may not necessarily decrease linearly over time.

To test these hypotheses, in the current study we subjected these earlier findings to a novel experimental paradigm, allowing for a within-subject comparison of unconditioned defensive responding to these three types of sustained, aversive interoceptive stimulation. The primary aim of this study was to shed light on the startle response over time to three types of stimulation. Eye blink startle responses were studied during 90 s periods of cold pain, inhalation of CO_2-enriched air, and loaded breathing. In contrast to the studies of Pappens et al. (2010, 2011), which applied loads for only one inspiration, the continued stimulation allowed for testing our assumption that startle declines linearly during the course of dyspneic stimulation. Since we did not expect potentiation but rather a reduction in startle, it was important to make sure any reduction in startle wouldn't be due to habituation. Therefore it was important to have a design which would allow us to statistically correct for habituation-bound decrease in startle. For this reason, startles were measured during a baseline phase prior to the stimulus phase, and during a recovery phase following the stimulus phase, so that a best fit line could be calculated which would filter out the effects of habituation. Another new element in the current experiment was that respiratory loads were applied both during inspiration and expiration, so that startle eliciting probes would always be administered during actual stimulation.

To test the general conclusions we made earlier, we respectively expected to observe:

(a) A reduced startle blink magnitude during aversive interoceptive stimulation, as compared to prior and following an aversive interoceptive stimulus. Given our design, this would correspond to a reduction of startle during stimulus phase as compared to baseline and recovery phase.
(b) For both dyspneic stimuli, we hypothesized a progressive reduction of the startle magnitude during the stimulus phase, as unpleasant dyspneic stimuli have been shown earlier to be associated with reduced startle responding, and as these stimuli are thought to become progressively more unpleasant as time since the onset increases.
(c) For the CPT, we expected a quadratic response pattern during the stimulus phase, given that the overall average of multiple startle responses is associated with a reduction in amplitude (Tavernor et al., 2000) while no such reduction has been evident during the 30 to 60 second period following stimulus onset (De Peuter et al., 2009), the latter which is perhaps due to the fluctuations in pain sensations during cold stimulation.

In line with earlier findings, it was expected that all stimuli would be scored as unpleasant rather than pleasant, that these stimuli would induce some self-perceived arousal as opposed to complete calm, leading to sub-maximal levels of feelings of dominance, and to induce some fear.

2. Materials and methods

2.1. Participants

Thirty-six female psychology freshmen (mean age: 19 y/old) participated in return for course credit. Exclusion criteria were pregnancy, presence or history of cardiovascular disease, pain-related conditions, or respiratory disease. Participants were randomly assigned to one of six orders of stimulus presentation — stimulus presentation orders were counterbalanced. The study protocol was approved by the Ethics Committee of the Department of Psychology in accordance with the Declaration of Helsinki (World Medical Association, 1997); prior to participation, all subjects read and signed an informed consent with information about the sensations that could possibly follow from exposure to the stimuli, a guarantee about anonymity, and that participation was voluntary and could be terminated at any point in time without loss of the promised course credit.

2.2. Stimuli and apparatuses

2.2.1. Cold pressor

The cold pressor test (CPT) was used as a cold pain (CP) stimulus. The CPT consisted of a Plexiglas water basin (Julabo®, Seelbach,

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Germany), model 19A, containing a type FT200 cooler and type ED water circulator. During the CPT, participants were requested to immerse their right hand to the wrist in this water-filled basin positioned on the right-hand side of their seat. The water with a constant temperature of 6 °C was circulated to prevent buildup of warmer water around the hand; the hand was to be held in the cold water for a duration of 90 s. Pain at this temperature is experienced as intense, very cold and deep, and produces sympathetic autonomous responses (Casey et al., 1996). Participants were explicitly told beforehand that this was not a pain tolerance test and were informed about the duration the hand had to be held in the cold water; this information was provided with the purpose of discouraging participants to withdraw their hand prematurely, although they were free to withdraw their hand at any time. In the 90 s prior to and the 90 s following the CPT, participants immersed their hand in a stainless steel water basin, model FBATH18 (Techne®, Staffordshire, United Kingdom), with the water having a constant temperature of 30 °C and circulating by means of the TE-10D Tempette® thermo regulator and circulator. Immersing the hand in water of 30 °C prior and following the CPT was intended to create equal conditions for everyone during the experiment. The two approximately equally sized water basins – one cold and one lukewarm – were purposefully chosen for their visual distinctiveness as to prevent subjects from immersing their hand in the wrong basin at the wrong time.

2.2.2. CO_2

A gas mixture of CO_2 enriched air, with a proportion of 7.5% CO_2, 21% O_2 and 71.5% N_2 was administered for a duration of 90 s to induce sensations of dyspnea. The decompressed gas mixture was contained in a meteorological balloon and connected to the inspiratory port. Apart from dyspneic sensations and altered respiratory behavior, 7.5% CO_2 enriched air can elicit (transient) sweating, feelings of warmth, and dizziness (Devriese et al., 2006; Stegen et al., 1998). Effects of CO_2 inhalation are thought to be cumulative, with less effect on the first few breaths. Similarly, after termination of CO_2 administration, the blood pH level gradually – not instantaneously – returns to its normal level, an effect which is referred to as washout.

2.2.3. Resistive loads

Resistive loads require extra effort from the respiratory muscles – the diaphragm and intercostals – during breathing, in order to maintain flow rate and volume. The accompanying sensation is comparable to breathing through a narrow tube and resembles dyspneic sensations experienced in COPD, asthma and other types of obstructed breathing (Younes, 1995). Unlike CO_2 administration, loaded breathing can be noticed from the first breath. Prolonged loaded breathing may additionally have some cumulative effects, as respiratory muscles can become fatigued. In the current study, two resistive loads were used: one was applied to the inspiratory valve, and one to the expiratory valve. Applying both an inspiratory and expiratory load on breathing ensured that stimulation was continuously unpleasant as it was for the other two stimuli, and that the startle eliciting probe would always fall during actual stimulation. Both loads were of an intensity of 1.96 kPa l^{-1} s, an intensity rated as unpleasant (Pappens et al., 2010).

2.2.4. Breathing apparatus

Throughout the experiment – except during self-report – participants breathed through a mouthpiece while wearing a nose clip. The mouthpiece was fitted on a microbial filter, which in its turn was connected to a non-rebreathing valve to ensure that inspiratory and expiratory air remained separated. The inspiratory and expiratory port of the non-rebreathing valve were both connected to a manual directional control three-way T-shape™ stopcock-type™ valve (Hans Rudolph, Inc., series 2110) by means of a vinyl tube (inner diameter 3.5 cm; length 100 cm). In the loaded breathing trial, the valves allowed easy switching between loaded and unloaded breathing. In the CO_2 inhalation trial, the three-way valve allowed easy switching between breathing room air and CO_2 enriched air on the inspiratory side.

2.2.5. Eye blink startle response

Electromyographic (EMG) activity of the left orbicularis oculi muscle as response to acoustic startle probes (95 dB) was measured by the placement of three electrodes filled with high conductivity Microlyte electrolyte gel. One electrode was placed perpendicular under the pupil when the eye was in forward gaze, the second approximately 1 to 2 cm lateral to the first (center-to-center) following the curvature of the eye, and one signal ground electrode was placed on the center of the forehead. All sites were first cleaned with alcohol to reduce inter-electrode resistance. The raw signal was amplified by a Coulbourn isolated bioamplifier with bandpass filter (LabLinc v75-04) with a 90 Hz high pass filter. This signal was routed to a Coulbourn 4 channel integrator (LabLinc v76-24), which rectified and smoothed the signal online with a time constant of 20 ms. The EMG signal was sampled at 1000 Hz starting 500 ms prior to the onset of the auditory startle probe, until 1000 ms after probe onset.

2.2.6. Software

All signals were transmitted through a 16-Bit National Instruments PCI-6221 data acquisition card (National Instruments, Austin, Texas) to a computer. Affect 4.0 software (Spruyt et al., 2010) was used for running the experiment as well as for data acquisition. A modular script-based program named PSychoPHysiological Analysis and abbreviated as PSPHA (De Clercq et al., 2006) was used to handle the recorded signals offline and to extract the relevant parameters necessary for statistical analysis.

2.2.7. Self-report measures

At the end of each trial a computerized 9-point scale of the language-free self-assessment manikin (SAM-scale, Bradley and Lang, 1994) was administered to retrospectively rate the mean valence (unpleasant = 1; pleasant = 9), arousal (calm = 1; excited = 9), and dominance (lack of control = 1, sense of control = 9) felt during the 90 second stimulus. Before proceeding to the next trial, subjects were requested to indicate their fear as experienced during the stimulus period on a computerized horizontal visual analog scale (VAS; 0 = not at all scared; 100 = extremely scared).

2.3. Procedure

Upon arrival, the experimenter led the participants into the experimental room where he provided them an informed consent. The informed consent briefly mentioned all stimuli, as well as the sensations each stimulus may respectively elicit, and that any sensations felt were without harm and were of a transient nature. Participants were requested to read through the consent before agreeing to sign it. After signing, a brief questionnaire of medical history in relation to exclusion criteria (see Section 2.1) was provided. Next, electrodes were attached — subjects were informed that these were meant for measuring physiological responses, albeit without further specifications. The experimenter verbally went through the experimental procedure, and then placed headphones on the participant. Participants were requested to put on the nose clip and breathe through the mouthpiece, and told to keep their eyes fixated in the direction of the computer screen. Participants sat upright (not reclined) throughout the entire experiment and lights remained on (not dimmed). Prior to initiation of the experimental manipulations, the experimenter left and went to the operator room, adjacent to the room where participants were left alone throughout the entire experiment; the experimenter remained in the operator room until the experiment was over.

Prior to each trial, 10 acoustic startle probes were administered to habituate participants to the startle probe. Habituation to the probes was done because startle responses tend to be amplified at the initial

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presentation of the acoustic probes due to their novelty. During habituation the interval between probes varied from 19 to 21 s. After startle probe habituation, a trial started off with a baseline phase of 90 s where subjects breathed through a mouthpiece, fixated their eyes on a computer screen, and received three startle probes at unpredictable times, one during the first, one during the second and one during the last 30 s. Additionally, in the CPT trial, subjects immersed their hand in lukewarm water during baseline. The second phase was the stimulus phase during which either of the three stimuli – cold water, CO_2-enriched air, or loaded breathing – was administered. During this phase, again there were three startle probes at variable times — one during the first 30 s, one during the second, and one during the last. The third phase is referred to as the recovery phase, and was identical to the baseline phase, except in that it followed – instead of preceded – the stimulus phase. After recovery, subjects were free to release the mouthpiece while they filled out the self-report scales, before proceeding to the next trial. In total there were three trials, and only one (continuous) stimulus was presented per trial, during the stimulus phase. Avoiding repeated presentation of stimuli ensured that potential learning behavior and alteration of responses due to recent exposure was minimized. At the end of the experiment, subjects were fully debriefed.

2.4. Data analysis

Eye blink startle EMG responses were calculated by subtracting the mean value from the 0 to 20 ms following probe onset from the peak value found in the 21 to 175 ms time window following probe onset. Excluding startle measured during habituation, there were 27 remaining data points per subject. Data points where there was already blink activity at the onset of startle probe presentation were rejected (<10%) as recommended by Blumenthal et al. (2005). The maximum percentage of missing data points for a single subject was just under 26%, while the mean number of missing data points per person was just over 7%. After removing rejected values, startle amplitudes were transformed to T-scores for each individual, which is a common procedure (see Blumenthal et al., 2005). The reason for this transformation was that we were interested in overall intraindividual differences in response to the different phases and probe delivery times, and not in interindividual differences in response amplitude. Having obtained individual T-scores, missing data were replaced by the mean startle amplitude in response to the same probe of those people who had received all stimuli in the same order. We did this to rule out effects of stimulus presentation order on amplitude. Once missing data were replaced, the data were detrended by using individual regression models with probe order as the predictor (Lüthy et al., 2003). Using this method, the mean of a best fit line was subtracted from the actual T-score. Unless this method is applied, magnitudes in our design are generally higher during baseline, and lower during recovery, simply because startle magnitudes continue to decline linearly, even after initial habituation. By removing this linear reduction in magnitude, the magnitudes at each point in time across the three phases become better comparable, and the differences in amplitude that remain are more likely due to the sensations at that moment of prolonged stimulation.

The detrended data were then entered into repeated measures ANOVA's, with trial type (CPT, CO_2 or load trial), phase (baseline, stimulus, or recovery), and startle probe timing (1st 30 s, 2nd 30 s, or last 30 s) as within subject variables. In order to test our first hypothesis that startle would be reduced during aversive interoceptive stimulation as compared to baseline and recovery phase, we performed a polynomial quadratic contrast for the effect of phase on all data points. In order to test our second hypothesis that startle would progressively decrease during prolonged dyspneic stimulation, we performed a polynomial linear contrast on the effect of startle probe timing on the data points obtained during the stimulus phase of both dyspneic stimuli. And finally, in order to test our third hypothesis that startle in response to the CPT would show a reduction in amplitude overall, except during the 30 to 60 s following the onset, we performed a polynomial quadratic contrast on the data points obtained during the stimulus phase of CPT.

The SAM scores for valence of the three stimuli were compared using a one-way repeated measures ANOVA. The same analysis was done for the SAM scores on arousal as well as for dominance levels and VAS state anxiety scores compared in response to the three stimuli.

An α-level of .05 was set for statistical significance. Analyses were done using the STATISTICA version 10 software package and the means and standard deviations displayed in Table 1 were obtained using the JMP 9 software package.

3. Results

3.1. Eye blink EMG

To test our first hypothesis that startle is reduced during interoceptive stimulation (all three stimuli), a univariate test of significance for planned comparisons of least square means for the effect of PHASE confirmed the existence of a quadratic contrast, $F(1, 35) = 6.19$, $p < .05$, meaning that startle dropped from baseline phase to stimulus phase, and rose again from stimulus phase to recovery phase (see Fig. 1a). To test our second hypothesis that dyspneic stimuli lead to a progressive decrease in startle responding, another test for planned comparisons was performed for the effect of startle probe timing during stimulus phase of both dyspneic stimuli, and found a linear decrease in startle magnitude, $F(1, 35) = 4.20$, $p < .05$ (see Fig. 1b). In contrast, the effect of startle probe timing during the stimulus phase of the CPT displayed a quadratic pattern, $F(1, 35) = 4.68$, $p < .05$. That is, during the stimulus phase where the CPT was administered, there was an initial reduction in startle amplitude, followed by an increase in amplitude, which was in its turn followed by a decrease in amplitude again (see Fig. 1c).

3.2. Self-report

As evident from Table 1, the three stimuli evoked similar levels of arousal, dominance, and fear. The only significant difference between the stimuli was in perceived valence, $F(2, 70) = 5.52$, $p < .01$, with Tukey–Kramer post-hoc tests indicating that CP was rated as more unpleasant than both CO_2 inhalation ($p = .01$) and loaded breathing ($p < .05$).

4. Discussion

The current study aimed to elucidate the startle response pattern during aversive interoceptive stimulation, and used a sample of 36 young adult females to do so. To date affective modulation of startle has almost exclusively been studied using visual and auditory stimuli,

Table 1
Means and standard deviations for valence, arousal, dominance, and fear experienced during stimulation.

	Cold pain		CO_2 inhalation		Loaded breathing	
	Mean	SD	Mean	SD	Mean	SD
Valence	3_b	1.6	3.8_a	1.5	3.8_a	1.6
Arousal	6_a	1.9	5.6_a	1.7	5.4_a	1.8
Dominance	3.9_a	1.9	4.4_a	1.9	4.5_a	2.2
Fear	43_a	24	47_a	25	45_a	30

Note. Valence, arousal, and dominance all ranged from 1 to 9, respectively unpleasant versus pleasant, calm versus excited, and a lack of control versus a sense of control. Fear ranged from 0 to 100, respectively from not at all scared to extremely scared. Means in the same row which share a subscript are not significantly different from one another according to Tukey–Kramer post-hoc tests.

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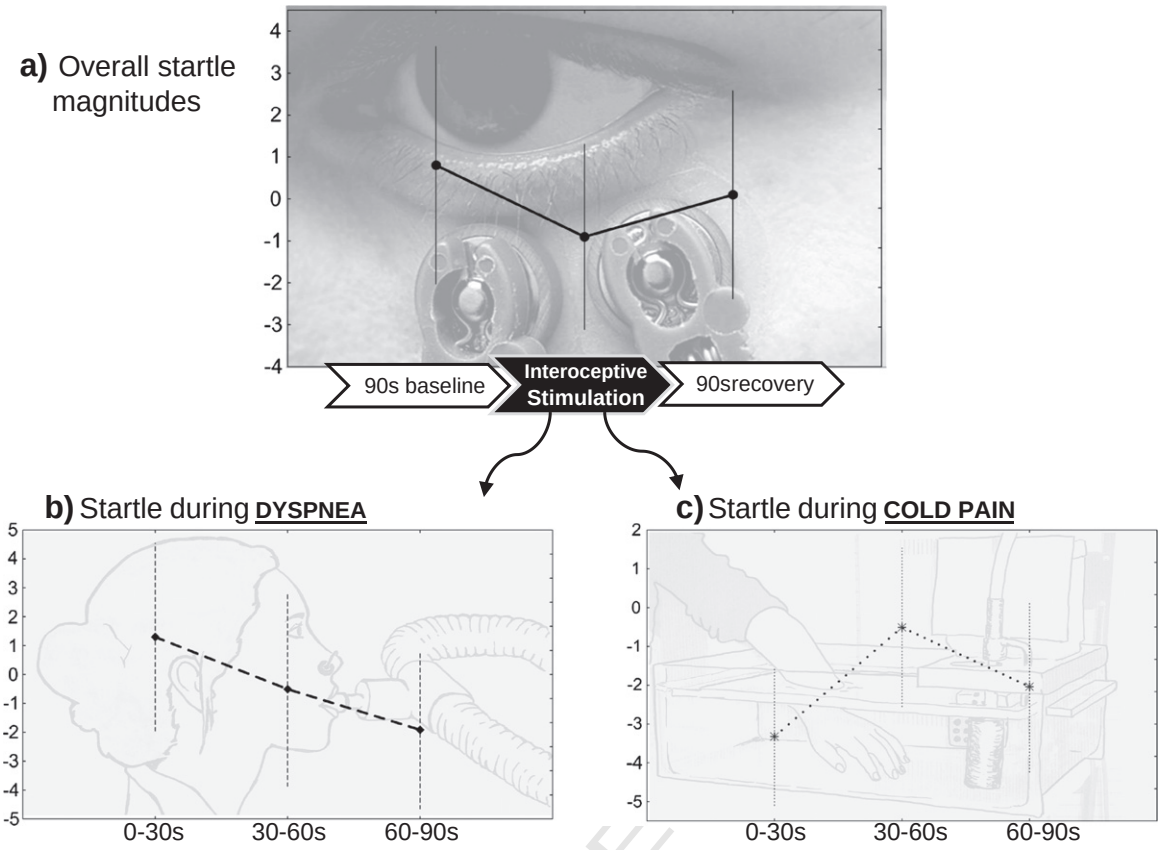

Fig. 1. Graphs showing detrended startle magnitudes per phase (a), and of individual startles during stimulus phase only for respiratory stimuli (b) and for peripheral hypothermia (c). Values are presented as magnitude means ± 0.95 confidence intervals.

mental imagery, and sometimes using anticipation of aversive interoceptive sensations (e.g., Lang et al., 2011; Melzig et al., 2008). However, reports on startle during aversive interoceptive stimulation are still very scarce. In this initial study on within-subject responses to different types of interoceptive stimulation, two respiratory stimuli commonly used in studies on dyspnea and fear, and one cold stimulus commonly used in research on pain were presented to participants. In order to effectively document startle not as a conditioned response, but as an unconditioned response, all stimuli were administered only once, which necessitated that they be administered for a relatively prolonged duration in order to allow for the administration of multiple startle probes.

The findings from our current study are in accordance with the scarce amount of previously published data, in that startle responding to these three interoceptive stimuli is reduced overall. A new insight from the current study is the presence of a linear decrease of startle responding in face of dyspneic stimulation, and a non-linear, quadratic startle response pattern during the CPT.

The overall reduction of startle for all three stimuli is evident despite that all three stimuli are rated as fearful, unpleasant, arousing, and associated with sub-maximal levels of dominance. Although the ratings of unpleasantness are not at the extreme end of the valence-scale, an earlier study of Pappens (2010) found that the intensity of respiratory loaded breathing which we also used in the current study, was more aversive than aversive pictures from the International Affective Picture System (IAPS). Moreover, the CO_2 inhalation in the current study was equally aversive as loaded breathing, and CP was even more aversive. That participants in the current study refrained from filling in the extremes of the valence and fear scales does not indicate the stimuli were ineffective in inducing unpleasantness or fear. Rather, we argue this underreport to be due to the lack of milder unpleasant stimuli (e.g., unpleasant pictures), and the anticipation that a potentially more aversive stimulus might be presented in a subsequent trial (requiring that the extremes of the scale need to remain unused until then). Though we did not let subjects rate the baseline and recovery phases, right after the experiment was over subjects informally informed the experimenter that those phases were dull (they had to stare at a fixation cross and knew no stimuli would be presented during those phases), thus ruling out that the affective tone was constantly negative.

From the perspective of the emotional priming model, which posits that startle magnitude should be greater when the aversive motivational system is active (Lang et al., 1998), the overall reduction in startle is puzzling. Although the few previous studies that found similar results forwarded a number of explanations, currently there is no satisfactory answer to the mechanisms underlying this unusual response pattern. Nevertheless, based on prior explanatory speculations, some suggestions for future research can be made.

One speculation that has been made earlier, is that the reduction in startle responding is due to the interoceptive nature of the stimuli (Pappens et al., 2010), implying that aversive interoceptive stimulation of any kind would fail to evoke potentiation. Although the current study did not find any counterevidence for this claim, further research with other types of interoceptive stimulation is necessary in order to truly falsify this claim. Moreover, resorting to the interoceptive nature of the stimuli as an explanation for the unusual startle response, requires a predefined and well-outlined working definition of interoception, given that consensus on its definition is lacking, in particular with regard to the 'outer boundaries' of the concept (Dworkin, 2007).

Another explanation forwarded by Pappens et al. (2010) is that according to the defense cascade model (Lang et al., 1997), startle potentiation should no longer be evident during the circastrike (fight/

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flight) phase of defensive responding, despite aversiveness of stimulation (e.g., Low et al., 2008; Richter et al., 2012). This circastrike phase of defensive responding is distinct from earlier defensive phases, not only in startle responding and threat imminence, but also in autonomous responses such as heart rate, skin conductance, and presumably in respiration as well (Van Diest et al., 2009a). In order to test whether the startle response pattern in face of interoceptive stimuli might be due to activation of circastrike responding, inclusion of additional autonomous measures could theoretically provide further conclusive evidence. In practice however, many interoceptive stimuli, including the stimuli used in this experiment, elicit regulatory homeostatic responses, which may complicate interpretation of autonomous measures, making this hypothesis hard to test for at least a number of interoceptive stimuli.

Finally, orientation of attention to bodily processes has been speculated to be responsible for a reduction in responsiveness to auditory stimuli such as the startle probe (Pappens et al., 2012). This speculation could be tested by manipulating orientation of attention to bodily processes or to surrounding stimuli such as acoustic probes. To date, only one such study has been done and suggests orientation of attention inward may be responsible for a reduction in startle responding to respiratory loads (Pappens et al., 2011), but it remains unclear whether this could also explain startle in response to CO_2 or the CPT. An alternative method to corroborate this explanation, is to include a measure of attention requiring subjects to indicate whether their attention was oriented predominantly at bodily sensations, predominantly at surrounding stimuli, or divided between both.

In the current study, these explanatory hypotheses were not extensively put to the test, as the primary aim was to describe, not explain the response pattern to the interoceptive stimuli we selected. Nevertheless, the present findings provide sufficient reason for taking these hypotheses and the methods to test them into account in future studies. Outlining the definition of interoception, testing startle in response to other forms of aversive interoceptive stimulation, inclusion of other psychophysiological measures in some instances, and manipulation and/or measures of orientation of attention are all potential avenues for future research, which may elucidate the mechanism responsible for the atypical startle patterns observed in the current and previous studies. Additionally, possible sex differences in the subjective experience and/or in the psychophysiological response pattern may require more attention in future studies, given that psychosomatic complaints are predominantly present in women (Kroenke and Spitzer, 1998; Şar, 2010). Until these issues are addressed in further studies, any explanatory hypotheses remain speculative at best. For now, we are left with only a descriptive model of startle to aversive interoceptive stimulation.

In this respect, it needs to be mention that the startle-by-startle analysis, a method usually rejected in favor of averaging magnitudes of startles delivered at different times, may actually provide additional insight into the pattern of responding over the course of time. The startle-by-startle analysis accounts for discrepancies between the study of Tavernor (2000) and an earlier study of ourselves (De Peuter et al., 2009); our current findings illustrate that although startle responding may be generally reduced following CPT, it is not necessarily reduced at all points in time following the onset of this stimulus. The magnitude increase during the 30 to 60 second interval that we have found a second time now, warrants a startle-by-startle analysis in addition to the more common averaging method, especially when startles are administered during prolonged aversive stimulation. Moreover, further research on the CPT and its concomitant fluctuations of sensory discomfort over the course of time are necessary, as these sensory fluctuations may underlie the fluctuations in startle responding. Currently, such research is very limited (e.g., Davis and Pope, 2002).

In conclusion, the evidence for an unusual startle response pattern during interoceptive stimulation is becoming more substantial. Although it is commonly assumed that startle is potentiated during aversive emotional states including fear, an opposite pattern has been found for a number of fearful interoceptive stimuli. A startle-by-startle analysis suggests this to be dependent on subjective fearfulness which generally increases following the onset of respiratory stimulation, but presumably fluctuates for CP induced by the CPT. Further research is needed to test this hypothesis more thoroughly, and to find out if the results are specific to women, or whether they also apply to men.

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