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Anticipation of interoceptive threat in highly anxiety sensitive persons

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ABSTRACT

Anticipatory anxiety plays a major role in the etiology of panic disorder. Although anticipatory anxiety elicited by expectation of interoceptive cues is specifically relevant for panic patients, it has rarely been studied. Using a population analogue in high fear of such interoceptive arousal sensations (highly anxiety sensitive persons) we evaluated a new experimental paradigm to assess anticipatory anxiety during anticipation of interoceptive (somatic sensations evoked by hyperventilation) and exteroceptive (electric shock) threat. Symptom reports, autonomic arousal, and defensive response mobilization (startle eyeblink response) were monitored during threat and matched safe conditions in 26 highly anxiety sensitive persons and 22 controls. The anticipation of exteroceptive threat led to a defensive and autonomic mobilization as indexed by a potentiation of the startle response and an increase in skin conductance level in both experimental groups. During interoceptive threat, however, only highly anxiety sensitive persons but not the controls exhibited a startle response potentiation as well as autonomic activation. The anticipation of a hyperventilation procedure thus seems a valid paradigm to investigate anticipatory anxiety elicited by interoceptive cues in the clinical context.

Introduction

Panic disorder is a severe and highly disabling anxiety disorder appearing in about 3–5% of the population (Wittchen & Jacobi, 2005). The core symptoms of panic disorder are repeated panic attacks and a resulting chronic state of anticipatory anxiety targeted at possible new attacks and their consequences (DSM-IV; American Psychiatric Association [APA], 1994). As a result of this anticipatory anxiety, panic disorder patients typically develop avoidance behaviors or safety strategies to prevent exposure to any cues or contexts that signal an increased chance of a new attack. Current etiological models of panic disorder emphasize the important role of this anticipatory anxiety not only for the maintenance of the disorder but also at early stages of its acquisition. Bouton, Mineka, and Barlow (2001) proposed that initial panic attacks are associated with any external (crowds) or interoceptive cues (palpitations) that co-occur during its onset. In consequence of this conditioning process, such cues elicit anticipatory anxiety that a new attack is about to happen.

Two experimental paradigms have been extensively used to study anticipatory anxiety in various non-clinical and clinical populations. In this research, threatening contexts were established to induce anticipatory anxiety by either confronting participants with an inherently insecure environment, such as darkness, or by instructing participants that painful or aversive stimuli (e.g., mild electric shocks or air blasts directed at the larynx) will occur under certain circumstances (for review see Grillon, 2002). These studies have reliably demonstrated that anticipatory anxiety is associated with an increase in subjectively reported anxiety and augmented physiological arousal, such as increased heart rate (Deane, 1961, 1969; Deane & Zeaman, 1958), respiratory rate (Masaoka & Homma, 2000, 2001), and skin conductance level (Chattopadhyay, Cooke, Toone, & Lader, 1980). Moreover, verbal threat of a moderately painful stimulus results in a clear potentiation of the startle reflex (Grillon, Ameli, Merikangas, Woods, & Davis, 1993; Grillon, Ameli, Woods, Merikangas, & Davis, 1991; Melzig, Weike, Zimmermann, & Hamm, 2007). The latter finding is particularly important, because the potentiation of the acoustic startle reflex seems to specifically index the activation of the mammalian defense system (for a review, see Lang, Davis, & Öhman, 2000). It has repeatedly been shown that the startle eyeblink response elicited by a brief acoustic probe stimulus is augmented during viewing of unpleasant pictures and even further potentiated during viewing of phobia-relevant stimuli (Bradley, 2000; Hamm, Cuthbert, Globisch, & Vaitl, 1997). Moreover, this potentiation of the startle reflex by anticipatory anxiety seems to operate on a very fundamental level outside of the subject's awareness and is mediated by the extended amygdala, a subcortical limbic structure located in the anterior temporal lobe (see Davis, 2000).

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While the instructed fear or threat of shock paradigm has been very successfully applied to study anticipatory anxiety in patients with PTSD (see for reviews Grillon, 2002; Grillon & Baas, 2003) the application of verbal threat of shock was less effective in discriminating patients with other anxiety disorders from controls. In a study by Grillon, Ameli, Goddard, Woods, and Davis (1994) patients with panic disorder did not show overall larger potentiation of their startle reflex during anticipation of shock relative to control participants, a finding that was recently replicated by Melzig et al. (2007). These data suggest that patients with panic disorder – in contrast to patients with PTSD – do not show a general hyper-reactivity of their subcortical defense system to the verbal threat of a moderately painful *exteroceptive* stimulus. This does not come as a complete surprise because most etiological models of panic disorder imply that *interoceptive* threats seem to be of specific relevance for these patients.

Cognitive models put forward by Clark (1986, 1988) and Barlow (2004) state that the detection, selective attention to, and misinterpretation of interoceptive symptoms play a key role in the development of panic attacks and panic disorder. In the same vein biological models, such as the false suffocation alarm theory (Klein, 1993), propose that changes in pH homeostasis or carbon dioxide in the blood are detected (perhaps via chemosensitive serotonergic neurons in the midbrain; see Richerson, 2004) and then lead to increased ventilation and intense feelings of anxiety. Implicit evidence that the anticipation of somatic symptoms might serve as an interoceptive threat and thus increases anxiety in panic disorder patients comes from numerous biochemical (e.g., sodium lactate, caffeine, CKK-4, etc.) and respiratory related (hyperventilation, CO_2-inhalation, etc.) provocation studies (see review by Barlow, 2004). In most of these provocation studies patients report increased "baseline anxiety" and show increased "baseline heart rate" in anticipation of the challenge (Coplan et al., 1998; Liebowitz et al., 1985).

Although it seems clear that anticipatory anxiety elicited by an interoceptive threat may be an important phenomenon to study, currently there is no experimental paradigm available that allows its explicit investigation. The current study was therefore designed to evaluate a new experimentally controlled procedure to study anticipatory anxiety elicited by an interoceptive threat in addition to the verbal threat of an exteroceptive aversive stimulus (mild electric shock; Grillon et al., 1991). Interoceptive threat was established by instructing participants that a guided fast and deep breathing challenge would follow the presentation of a colored slide. Participants were informed that this task would produce typical somatic symptoms such as palpitations, sweating, or feeling dizzy. Participants were also instructed that another colored slide would signal a safe context.

Before applying this paradigm in the clinic the current study was designed to test the validity of this experimental manipulation in an analogue sample that parallels panic patients in their fear of somatic arousal sensations due to the belief that these have harmful consequences: Highly anxiety sensitive persons (McNally, 2002). It has repeatedly been shown that persons scoring high on the Anxiety Sensitivity Index (Peterson & Reiss, 1992) exhibit augmented anxiety responses comparable to those of panic disorder patients in biological challenge tasks (McNally, 2002). Also, high anxiety sensitivity constitutes a risk factor for developing panic attacks and panic disorder (Hayward, Killen, Kraemer, & Taylor, 2000; Schmidt, Lerew, & Jackson, 1997, 1999). Finally, persons with high anxiety sensitivity also show increased "baseline" anxiety prior to a hyperventilation challenge (i.e., Donnell & McNally, 1989; Holloway & McNally, 1987; Rapee & Medoro, 1994) although only verbal report data were obtained in these studies and anticipatory anxiety was not compared explicitly with a safe condition. Therefore, in the current study we compared anticipatory anxiety in response to exteroceptive (verbal threat of mild pain induced by an electrical stimulus) and interoceptive (verbal threat of somatic symptoms induced by hyperventilation) threat in participants scoring either high or low on the Anxiety Sensitivity Index (Peterson & Reiss, 1992). Besides the assessment of symptom reports we also recorded heart rate and skin conductance as indices of autonomic arousal. Additionally, we measured the modulation of the startle response, a defensive and protective brain stem reflex that is elicited independently by the same abrupt acoustic probe stimulus that is either presented during the anticipation of threat or during the anticipation of the safe context. If anticipation of the threat condition evokes anticipatory anxiety, a potentiation of the startle reflex should occur as a direct index of defensive mobilization of subcortical networks. While we expected increased anticipatory anxiety during the anticipation of shock in both high and low anxiety sensitive persons, anticipation of somatic symptoms induced by hyperventilation should evoke anticipatory anxiety only in persons with high anxiety sensitivity.

Method

Participants

Two hundred and fifty university students were screened with a German version of the Anxiety Sensitivity Index (ASI; Peterson & Reiss, 1992). Subjects scoring either high or low (at least one standard deviation from the mean [$M \pm SD = 20 \pm 9$]) on the ASI were contacted by telephone and screened for the following inclusion/exclusion criteria: Subjects had to be free of any seizure disorders, cardiovascular or respiratory diseases and should not be in treatment for any psychological disorder. The final sample included 26 participants high in anxiety sensitivity (high-AS, 18 women) and 22 subjects low in anxiety sensitivity (low-AS, 17 women). The mean age of both groups was comparable, *M* (SD) for high vs. low-AS: 22.9 (3.7) vs. 24.2 (3.1), $t(46) = 1.3$, $p = .20$.

For purposes of sample characterization all study participants were assessed using the following questionnaire measures: The trait portion of the State-Trait Anxiety Inventory (STAI; Spielberger, 1983; German version: Laux, Glanzmann, Schaffner, & Spielberger, 1981), the Agoraphobic Cognitions Questionnaire (ACQ; Chambless, Caputo, Bright, & Gallagher, 1984; German version: Ehlers, Margraf, & Chambless, 1993a), and the Body Sensations Questionnaire (BSQ; Chambless et al., 1984; German version: Ehlers, Margraf, & Chambless, 1993b). As expected, the study groups differed significantly on all questionnaires. The high-AS group reported greater trait anxiety, more agoraphobic cognitions, and more severe anxiety symptoms (see Table 1).

Table 1
Means and standard errors of questionnaire measures for participants high and low in anxiety sensitivity

Questionnaire	High-AS	Low-AS	*t*	Significance (*p*)
ASI [0–64]	33.9 (1.1)	8.5 (0.5)	23.4	<.001
STAI-Trait [20–80]	40.9 (1.6)	31.1 (1.2)	6.6	<.001
ACQ [1–5]	1.8 (0.1)	1.3 (0.0)	6.4	<.001
BSQ [1–5]	2.4 (0.1)	1.6 (0.1)	5.1	<.001

Note. ASI: Anxiety Sensitivity Index, STAI: State-Trait Anxiety Inventory, ACQ: Agoraphobic Cognitions Questionnaire, BSQ: Body Sensations Questionnaire. Possible ranges of scores are reported in parentheses behind each questionnaire abbreviation.

Stimulus materials

Warning and safety slides

Four different colored slides were projected onto a screen located in front of the subject to signal the threat/safety conditions. A red slide indicated that a hyperventilation challenge would follow (interoceptive threat), and a green slide indicated a normoventilation task. A yellow slide indicated that a shock would be administered (exteroceptive threat) whereas a blue slide indicated that no shock would be administered during the slide.

Electric shock

The mild electrotactile stimulus, a 500 Hz monopolar DC-pulse with an intensity of 3 mA, was delivered to the participant's left forearm in a 10 ms train of single pulses (1 ms) using an S48 Stimulator, a Constant Current Unit, and a Subject Isolation Unit (all provided by Grass Instruments). Similarly, electrotactile stimuli of such intensity (described by the participant as aversive, but not painful) have successfully been used in previous studies investigating anticipatory anxiety or fear conditioning (Grillon et al., 1991, 1993, 1994; Hamm, Greenwald, Bradley, & Lang, 1993; Hamm & Vaitl, 1996; Melzig et al., 2007).

Hyperventilation task

The hyperventilation task was introduced as a "fast breathing exercise" that could induce somatic sensations such as palpitations, sweating, or feeling faint. Participants were informed that the symptoms would disappear once the breathing speed returned to normal. During the hyperventilation task tones of rising and falling pitch were presented via headphones prompting the subjects to breathe in with rising and breathe out with falling pitch of the tone (see Wilhelm, Gerlach, & Roth, 2001 or Wollburg, Meuret, Conrad, Roth, & Kim, 2008 for a similar hyperventilation procedure). Participants were thus led to breathe at a respiratory rate of 20 cpm. During the hyperventilation procedure the respiratory rate as well as the CO_2 of the expired air were monitored continuously by a Nellcor NPB-70 Capnograph to ensure compliance with the hyperventilation procedure. To ensure that the hyperventilation task was executed properly and hypocapnia was obtained in order to provoke physical symptoms in all participants, visual feedback (projected onto a screen) was used instructing the participant to "breathe deeper" until a target $p_{et}CO_2$ of 20 mmHg was reached. Using further written instructions ("breathe more shallow", "deeper", or at a "constant depth") the breathing depth was adjusted throughout the hyperventilation task to keep the $p_{et}CO_2$ at 20 mmHg. All participants included in this analysis were fully compliant with this procedure.

Normoventilaton task

Breathing tones were, again, used to adjust breathing speed to follow a 13 cpm pattern. Participants were instructed to follow the breathing pattern with their own comfortable breathing depth. Normoventilation was chosen as a safe condition to control for the effects of the anticipation of a guided breathing maneuver.

Startle stimulus

A 50 ms burst of white noise with an intensity of 95 dB (A) (rise/fall time < 1 ms) was generated by a Coulbourn S81-02 noise generator and presented binaurally over Sony MDR-CD270 headphones to serve as a startle eliciting stimulus (according to Guidelines for human startle eyeblink electromyographic studies, Blumenthal et al., 2005).

Symptom ratings

To assess reported anxiety symptoms participants were asked to rate the severity of the 14 panic attack symptoms[1], as listed in the DSM-IV (American Psychiatric Association, 1994) on a 4-point Likert-scale ranging from 0 (= not at all) to 3 (= severe). All self-report items and response options were projected onto a 1.50×1.30 m screen in front of the subjects. Ratings were given via a small 4-button parallel port device.

Procedure

All physiological assessments were performed by research assistants blinded to the participants' anxiety sensitivity score. Participants were informed that physiological responding during different kinds of challenges will be assessed, and that each challenge will be explained in detail later. Participants then read and signed the informed consent form before being seated in a reclining chair in a dimly lit sound attenuated room. After attaching all electrodes and checking the signal quality, the assessment started with a 4 min adaptation phase. To habituate startle response magnitudes to a stable baseline, eight startle probes (15 s mean inter-probe interval) were presented during the last 2 min of the adaptation period. At the end of the adaptation phase participants rated the severity of current anxiety symptoms.

After the adaptation phase half of the participants (balanced across groups) started with the exteroceptive threat and the other half with the interoceptive threat condition followed by the other condition, respectively. Before the start of each threat condition participants were informed about the upcoming breathing tasks or shock application, respectively, and again, informed consent was obtained. The *interoceptive threat condition* contained one hyperventilation and one normoventilation block. Each block consisted of 3 min *anticipation*, 3 min *paced breathing* (20 or 13 cpm), and 10 min *recovery*. The order of each paced breathing task was balanced between subjects, i.e., half of the participants within each group started with the hyperventilation task the other half with the normoventilation task. During the 3 min anticipation period nine startle stimuli were presented (20 s mean inter-probe interval), during each recovery period 10 startle stimuli were presented (60 s mean inter-probe interval). No startle probes were presented during the paced breathing to avoid interference with the task. At the beginning of the *exteroceptive threat condition* participants were again instructed about the upcoming procedure. After attaching the shock electrodes, the 3 min *anticipation* period started. Again half of the participants started with the safe, the other half with the threat of shock condition. The order was again balanced across participants. During the anticipation of shock, the electric shock stimulus was delivered 2 s before slide offset. Again, each anticipation phase was followed by a 10 min *recovery* period. Startle stimuli were presented during anticipation and recovery as described above.

After completion of the study procedure all participants were informed that the study was targeted at investigating whether anxiety sensitivity had modulating effects on anticipatory anxiety and psychophysiological responding during hyperventilation as well as electrotactile stimulation.

Apparatus

The eyeblink component of the startle response was measured by recording the electromyographic activity (EMG) over the orbicularis oculi muscle beneath the left eye using two electrolyte-filled

[1] For the present study the item "feeling unsteady, dizzy or faint" from the DSM-IV was split up in 2 separate items "feeling unsteady or dizzy" and "feeling faint".

(Marquette Hellige, Freiburg, Germany) Ag/AgCl miniature surface electrodes (Sensormedics, Yorba Linda, CA, USA). The raw EMG signal was amplified using a Coulbourn S75-01 amplifier with a 30 Hz highpass filter and a Kemo KEM-VBF8-03 400 Hz lowpass filter and digitized at 1000 Hz using a 12 bit A/D converter. Digital sampling started 100 ms before and lasted until 400 ms after the onset of the acoustic startle stimulus. To remove eye movement artifacts, a digital 60 Hz highpass filter was applied to the raw EMG data off-line before the scoring procedure started.

Skin conductance was recorded with Ag/AgCl standard electrodes (8 mm diameter; Marquette Hellige) filled with a 0.05 M sodium chloride electrolyte medium. Electrodes were placed 15 mm apart on the hypothenar eminence of the participant's palmar surface of the non-dominant hand. A Coulbourn S71-22 skin conductance coupler provided a constant voltage of 0.5 V across electrodes and processed the signal with a resolution of 0.01 μS. Digital sampling at 10 Hz was maintained throughout the entire experiment.

The electrocardiogram (ECG) was obtained using an Einthoven lead II setup with two standard, electrolyte-filled Ag/AgCl electrodes (Marquette Hellige). The raw signal was filtered (0.1–13 Hz bandpass) and amplified using a Coulbourn S75-01 bioamplifier and continuously digitized with a sampling rate of 100 Hz. Additionally, an online Shimuzu R-wave trigger was applied. The digital trigger channel was stored separately with a sampling rate of 1000 Hz.

Data reduction and analysis

The raw orbicularis oculi EMG was integrated off-line (time constant of 10 ms). Reflex eyeblinks were scored using a computer program (Globisch, Hamm, Schneider, & Vaitl, 1993) that identified the latency of blink onset (in milliseconds) and peak amplitude (in microvolts). All blinks occurring within a 20–100 ms time interval after startle probe onset and reaching peak amplitude within 150 ms were scored as valid startle response trials. Trials with clear movement artifacts or excessive baseline activity were rejected (3.8%) and treated as missing trials. Trials in which no response could be detected in the defined time window were scored as zero magnitudes. Digital values were converted to microvolts and group comparisons of overall reactivity were conducted using these raw startle magnitudes. For the analyses of the anticipation data, blink magnitudes were standardized to correct for interindividual variability that was unrelated to the experimental conditions. This transformation was done to ensure that each participant contributes equally to the analysis of the experimental conditions. Responses from each participant were transformed to *z*-scores (raw scores for each participant were subtracted from that person's mean score divided by that person's standard deviation), and converted to *T*-scores (i.e., $50 + [z \times 10]$).

Skin conductance level (SCL) was calculated by averaging across blocks of 10 s excluding those 10 s blocks in which acoustic startle probes were administered. Digital values were converted to microsiemens and group comparisons were conducted using these raw magnitudes. To test the experimental conditions the SCL-scores were range corrected as suggested by Lykken (1971).

Heart rate was derived from the ECG signal using software provided by the VPM data analysis package (Cook, Atkinson, & Lang, 1987). For this purpose, the inter-beat intervals were checked and corrected whenever misplaced R-wave triggers had occurred (due to increased *T*-waves or movement artifacts). Then heart rate was calculated and exported as 10 s mean values excluding those periods in which acoustic startle probes were delivered.

For all statistical analyses, a mixed-model analysis of variance (ANOVA) was applied for each physiological measure. For the adaptation phase, *Group* (low vs. high-AS) was entered as a between-subjects factor and *Block* (third vs. fourth minute) was entered as a within-subjects factor.

The effect of anticipation of threat was – in a first step – analyzed in an overall analysis using *Threat* (threat [hyperventilation, shock] vs. safe [normoventilation, no-shock]) as within-factor and *Group* (low vs. high-AS)[2] as between-factor. In the second step the same analysis was conducted for the interoceptive threat condition (hyperventilation vs. normoventilation) and the exteroceptive threat condition (threat of shock vs. no-threat of shock) separately. All statistical tests used a significance level of $p < .05$. For all *F*-tests effect sizes (partial eta squared) are reported.

Whenever assumptions necessary for conducting ANOVAs were violated, we also report nonparametric tests (Wilcoxon-tests for within-subjects repeated measures or Mann–Whitney-*U*-tests for between-subject comparisons).

Results

Adaptation period

Startle response magnitudes

In both groups startle response magnitudes[3] showed a clear decline within the adaptation period, Block $F(1, 45) = 48.5$, $p < .001$, $\eta_p^2 = .519$; $Z(47) = -5.18$, $p < .001$, Block × Group $F(1, 45) < 1$, $p = .68$, $\eta_p^2 = .002$; group difference in startle eyeblink habituation $U(22, 25) = 252$, $p = .62$. The two experimental groups did not differ significantly in their overall blink magnitudes, *M* (SE) for high vs. low-AS: 73.2 (11.9) vs. 71.3 (14.9) μV, Group $F(1, 45) < 1$, $p = .94$, $\eta_p^2 = .001$; group difference in startle response magnitude $U(22, 25) = 270$, $p = .92$.

Skin conductance level

Due to the activating effect of startle presentation, skin conductance level did not habituate throughout the last 2 min of the adaptation period in both groups, Block $F(1, 46) = 1.7$, $p = .20$, $\eta_p^2 = .036$; $Z(48) = -1.58$, $p = .11$, Block × Group $F(1, 46) < 1$, $p = .64$, $\eta_p^2 = .005$; group difference in skin conductance level habituation $U(22, 26) = 244$, $p = .39$. Overall, persons high and low in anxiety sensitivity did not differ significantly in their baseline skin conductance level (SCL), *M* (SE) for high vs. low-AS: 5.7 (0.9) vs. 4.4 (0.6) μS, Group $F(1, 46) = 1.3$, $p = .26$, $\eta_p^2 = .028$; group difference in skin conductance level $U(22, 26) = 251$, $p = .50$.

Heart rate

Heart rate did not change significantly throughout the adaptation period, Block $F(1, 46) = 2.0$, $p = .16$, $\eta_p^2 = .042$, Block × Group $F(1, 46) < 1$, $p = .42$, $\eta_p^2 = .014$. Overall, baseline heart rate (HR) was slightly enhanced in persons high in anxiety sensitivity, *M* (SE) for high vs. low-AS: 80.9 (2.5) vs. 75.2 (2.2) bpm, however, this group difference did not reach statistical significance, Group $F(1, 46) = 2.7$, $p = .11$, $\eta_p^2 = .056$.

Symptom reports

Highly anxiety sensitive participants reported significantly more symptoms than participants low in anxiety sensitivity, *M* (SE) for high vs. low-AS: 3.7 (0.5) vs. 2.1 (0.4), $t(46) = 2.6$, $p < .05$.

[2] Irrespective of kind of threat (exteroceptive vs. interoceptive) we observed a significant effect of the order of presentation of safe and threat conditions that was due to habituation of responses over time. Although the orders were carefully balanced within and between groups, we included *Order* as a factor in all analyses to evaluate whether the order effect would modulate the main findings. Throughout all parameters, no significant interactions of the order with other effects of interest, especially group-interactions, were discovered.

[3] For all analyses of startle response magnitudes one person had to be removed from the dataset due to a large amount of missing trials (> 30%).

Anticipation of threat

Startle response magnitudes

Fig. 1 shows *T*-standardized scores of the mean blink magnitudes for the safe vs. threat conditions in the interoceptive and exteroceptive threat condition, respectively. Overall, anticipation of threat resulted in a substantial potentiation of startle response magnitudes, Threat $F(1, 43) = 92.2$, $p < .001$, $\eta_p^2 = .500$; $Z(48) = -2.70$, $p < .01$. This threat induced startle potentiation, however, differed for the two groups and the type of anticipated threat: When anticipating an aversive electric shock, all participants showed a significant potentiation of startle response magnitudes, Threat $F(1, 43) = 42.9$, $p < .001$, $\eta_p^2 = .499$; $Z(48) = -3.15$, $p < .01$, with no differences between both groups Threat × Group $F(1, 43) < 1$, $p = .73$, $\eta_p^2 = .003$; group difference in startle eyeblink potentiation $U(22, 26) = 253$, $p = .64$ (see lower panel of Fig. 1). In contrast, when anticipating the hyperventilation task only participants high in anxiety sensitivity exhibited a significant potentiation of startle eyeblink responses, Threat $F(1, 23) = 5.7$, $p < .05$, $\eta_p^2 = .196$, but not controls, Threat $F(1, 20) < 1$, $p = .46$, $\eta_p^2 = .021$ (see upper panel of Fig. 1). This effect was substantiated by a significant Threat × Group interaction, $F(1, 43) = 5.5$, $p < .05$, $\eta_p^2 = .106$, in the between group analysis.

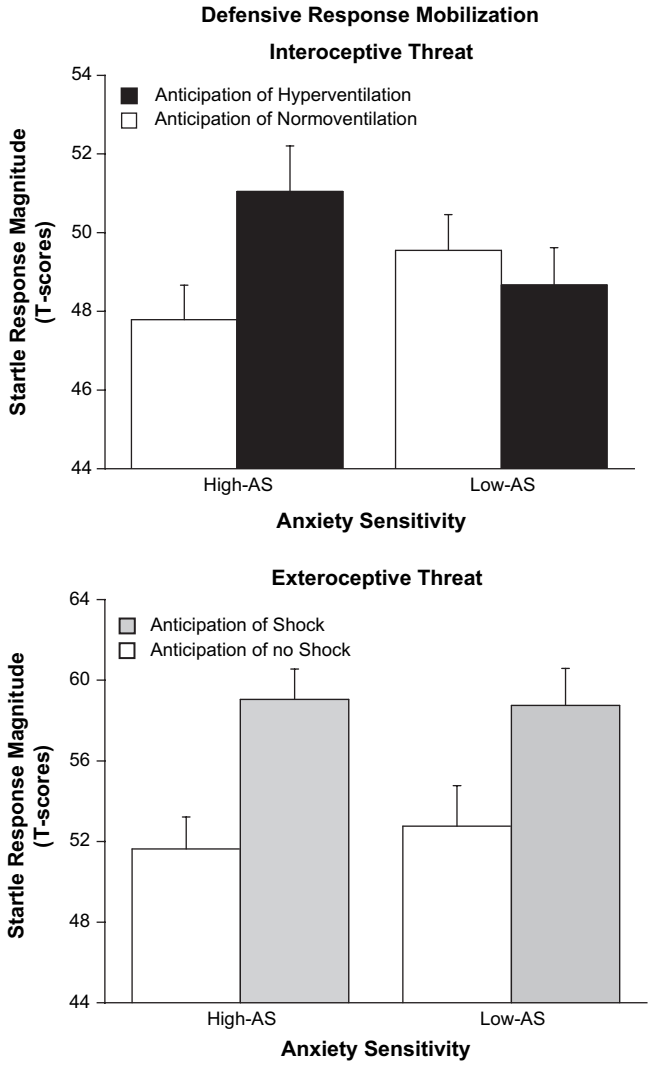

Fig. 1. Mean startle response magnitudes during interoceptive (anticipation of hyperventilation, upper panel) and exteroceptive threat (anticipation of shock, lower panel) in highly anxiety sensitive participants and controls, respectively.

Skin conductance level

The left panel of Fig. 2 shows the range corrected skin conductance level (SCL) for the safe vs. threat conditions in the interoceptive and exteroceptive threat condition, respectively.

Overall, anticipation of threat resulted in a significant increase in skin conductance level, *Threat* $F(1, 44) = 8.2$, $p < .01$, $\eta_p^2 = .126$. This SCL increase, again, differed for the two groups and the type of anticipated threat: When anticipating an aversive electric shock, all participants showed an increase in SCL, Threat $F(1, 44) = 3.7$, $p = .06$, $\eta_p^2 = .077$, again equally pronounced in both groups, Threat × Group $F(1, 44) < 1$, $p = .58$, $\eta_p^2 = .007$ (see lower left panel of Fig. 2). In contrast, as depicted in the upper left panel of Fig. 2, only participants high in anxiety sensitivity exhibited increased SCL during the anticipation of the hyperventilation task, Threat $F(1, 24) = 3.7$, $p = .07$, $\eta_p^2 = .135$ [4]. Again, controls did not differentially respond to the anticipation of normo- or hyperventilation, Threat $F(1, 20) < 1$, $p = .69$, $\eta_p^2 = .008$, Threat × Group $F(1, 44) = 1.3$, $p = .25$, $\eta_p^2 = .030$[5].

Heart rate

The right panel of Fig. 2 shows the mean heart rate for the safe vs. threat conditions in the interoceptive and the exteroceptive threat condition, respectively.

As reported previously (Melzig et al., 2007), heart rate did not differentiate between the safe and threat phases of the shock anticipation task (see lower right panel of Fig. 2), in neither group, Threat $F(1, 44) < 1$, $p = .66$, $\eta_p^2 = .005$, Threat × Group $F(1, 44) < 1$, $p = .99$, $\eta_p^2 = .000$. However, group specific differences were again detected during anticipation of interoceptive threat, Threat - × Group $F(1, 44) = 4.1$, $p < .05$, $\eta_p^2 = .085$; group difference in heart rate increase $U(22, 26) = 182$, $p < .05$. As depicted in the upper right panel of Fig. 2, only participants high in anxiety sensitivity showed an increase in heart rate when anticipating the hyperventilation procedure, Threat $F(1, 24) = 14.3$, $p < .001$, $\eta_p^2 = .373$; $Z(26) = -3.01$, $p < .01$, but not controls, Threat $F(1, 20) = 1.0$, $p = .32$, $\eta_p^2 = .049$; $Z(22) = -0.34$, $p = .73$.

Symptom report

Fig. 3 shows the mean number of reported symptoms during the safe vs. threat conditions in the interoceptive and the exteroceptive threat condition, respectively.

Overall, anticipation of threat was associated with an increase in the number of anxiety symptoms reported, Threat $F(1, 44) = 56.5$, $p < .001$, $\eta_p^2 = .429$; $Z(48) = -4.72$, $p < .001$. Both groups equally responded with an increase in the number of reported symptoms during threat of shock, Threat $F(1, 44) = 51.7$, $p < .001$; $Z(48) = -4.17$, $p < .001$, $\eta_p^2 = .355$, Threat × Group $F(1, 43$[6]$) < 1$, $p = .36$, $\eta_p^2 = .026$; group difference in symptom report increase $U(22, 26) = 180$, $p < .05$, as well as during anticipation of hyperventilation, Threat $F(1, 44) = 23.5$, $p < .001$, $\eta_p^2 = .332$; $Z(48) = -4.16$, $p < .001$, Threat - × Group $F(1, 43) < 1$, $p = .43$, $\eta_p^2 = .004$; group difference in symptom report increase $U(22, 26) = 214$, $p = .12$. Importantly, high-AS participants continued to report a larger number of symptoms in both threat conditions, Group $F(1, 44) = 6.5$, $p < .05$, $\eta_p^2 = .122$; group difference in symptom report $U(22, 26) = 150$, $p < .01$, thus showing a dissociation to the threat-specific physiological response pattern.

[4] Threat $F(1, 23) = 8.0$, $p < .05$ after exclusion of one outlier person who had strong sensitization during anticipation of normoventilation, which was the very first phase after baseline for this person.

[5] Again, after exclusion of the outlier mentioned earlier, Threat × Group $F(1, 43) = 2.8$, $p = .10$.

[6] Number of reported symptoms at baseline was entered as a covariate, due to significant baseline group differences.

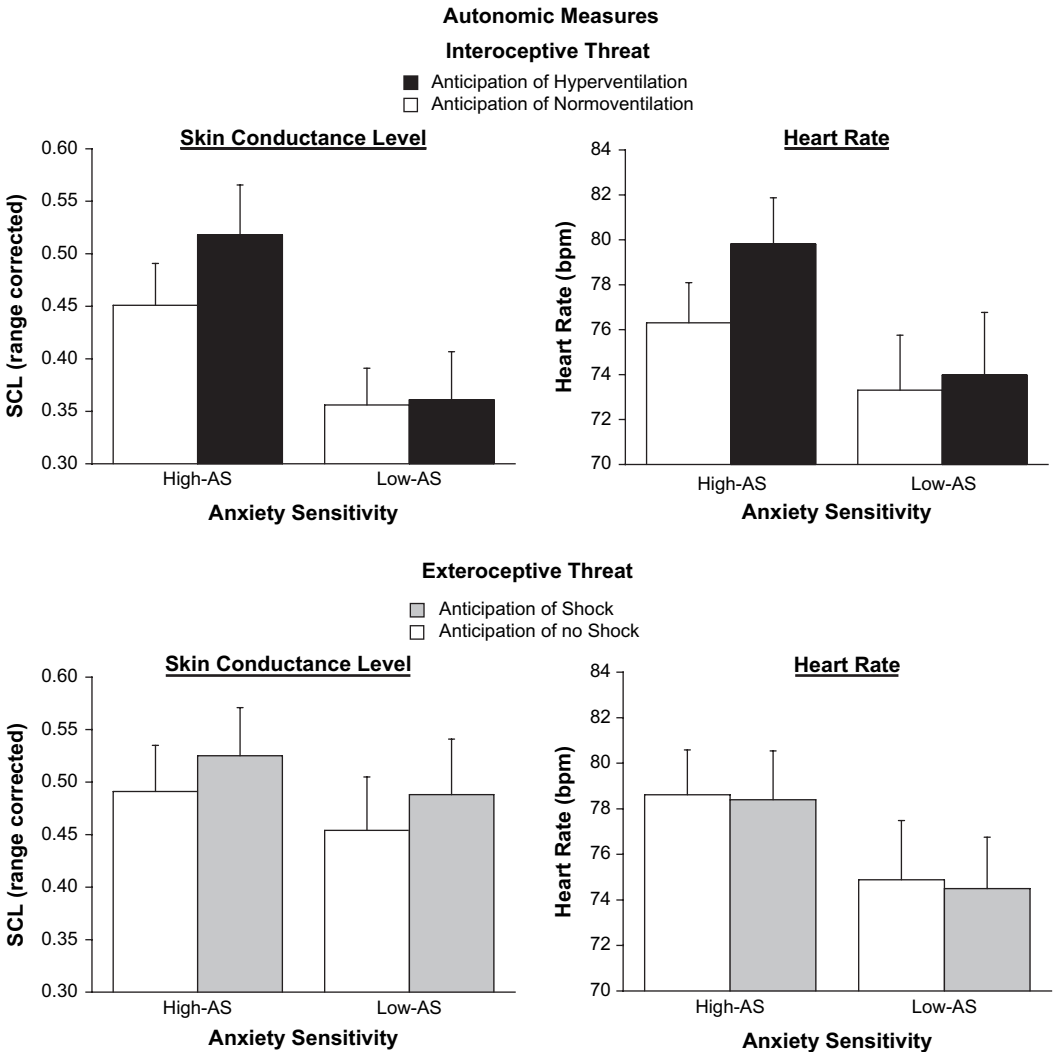

Fig. 2. Mean skin conductance level (left) and heart rates (right) during interoceptive (anticipation of hyperventilation, upper panel) and exteroceptive threat (anticipation of shock, lower panel) in highly anxiety sensitive participants and controls, respectively.

Discussion

The current study compared two experimental procedures to investigate anticipatory anxiety in persons who either reported high or low fear of somatic symptoms. The basic finding was that verbal threat of a mildly painful stimulus evoked comparable anticipatory anxiety in both groups, while the anticipation of somatic symptoms induced by hyperventilation evoked anticipatory anxiety only in those persons scoring high on the Anxiety Sensitivity Index, thus rendering this procedure a valid paradigm to investigate anticipatory anxiety to interoceptive cues in the clinical context. Moreover, the current study revealed an interesting dissociation between the verbal report of anxiety symptoms and the physiological response pattern evoked during anticipation of threat.

Startle potentiation and autonomic arousal during exteroceptive threat

Replicating previous findings, verbal threat of an aversive electrical stimulation to the forearm resulted in a clear potentiation of the acoustic startle reflex supporting the view that those subcortical networks that are involved in the anxiety induced potentiation of this obligatory defensive reflex are activated by this experimental condition (for extensive reviews see Davis, 2000; Grillon, 2002). Moreover, threat of shock also resulted in an augmentation of autonomic arousal as indexed by an increase in skin conductance level in the threat relative to the safe condition. On the other hand, heart rate was not affected by the threat of shock. Such an autonomic response pattern, however, is typically observed in so called passive coping conditions (Obrist, 1976) in which the organism is passively waiting for the aversive event to happen. Under these circumstances, the organism is in the state of defensive immobility that is characterized by increased orienting and hypervigilance to the environment (as indexed by increased skin conductance), and by the potentiation of protective reflexes (see Lang, Bradley, & Cuthbert, 1998). Importantly, this physiological response pattern to the anticipation of shock did not vary between participants with high or low concerns about their somatic symptoms. These data are in line with clinical observations showing that startle potentiation as well as skin conductance increase did not overall differ between panic patients and controls during threat of shock (Grillon et al., 1994; Melzig et al., 2007). These data indicate that patients with panic disorder and also persons who fear arousal sensations and are described to be at risk to develop such disorder (Hayward et al., 2000; Schmidt et al., 1997, 1999) are not characterized by a generally increased sensitivity of the anxiety network as can be observed for patients with PTSD

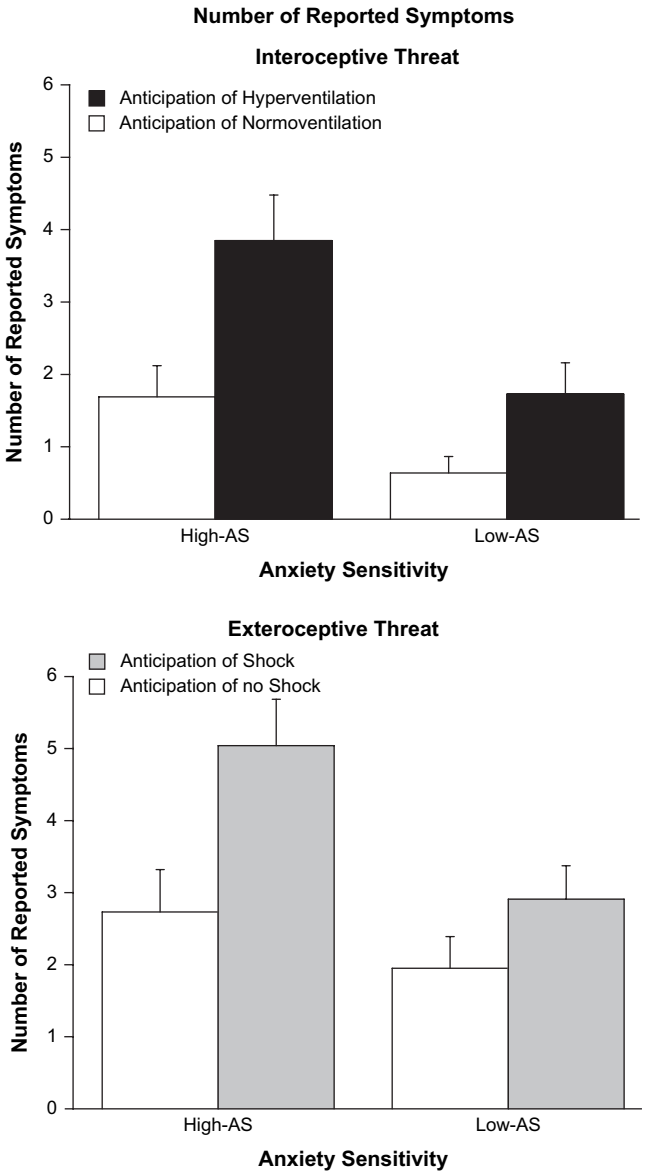

Fig. 3. Mean number of reported symptoms during interoceptive (anticipation of hyperventilation, upper panel) and exteroceptive threat (anticipation of shock, lower panel) in highly anxiety sensitive participants and controls, respectively.

(Grillon & Baas, 2003). Instead, the current data strongly suggest that the anxiety network in persons who dread somatic arousal sensations is specifically prone to respond to interoceptive cues and their anticipation.

Startle potentiation and autonomic arousal during interoceptive threat

In contrast to the exteroceptive threat, potentiation of the startle reflex during anticipation of the somatic symptoms provoking hyperventilation task was only observed in highly anxiety sensitive participants. Those persons scoring low on the ASI questionnaire did not show any augmentation of their startle responses elicited during anticipation of the hyperventilation challenge compared to those evoked in the safe condition. These data clearly support the view that anticipation of somatic symptoms specifically initiates a defensive response mobilization only in those participants who report to fear somatic sensations associated with anxious arousal. The current findings make an important contribution to the existing data base in showing that a defensive brain stem reflex is potentiated during anticipatory anxiety elicited by expectation of such somatic symptoms. As outlined above, potentiation of the startle reflex is regulated by subcortical networks, with the amygdala being the core structure within this circuit. The findings of this study suggest that anticipation of interoceptive cues might specifically activate those networks in highly anxiety sensitive persons priming defensive behavior. Recent imaging data from our laboratory support the view that anticipation of somatic symptoms evokes a stronger activation relative to the safe condition of the anxiety network including the amygdala, the insula, and the anterior cingulate cortex (Holtz, Melzig, Hosten, & Hamm, 2006).

The autonomic response patterns corresponded to the group specific startle potentiation. Only highly anxiety sensitive participants exhibited increased autonomic arousal, indexed by both, elevation of skin conductance and an increase in heart rate during the anticipation of somatic symptoms evoked by hyperventilation. No such autonomic arousal response was detected in participants with low anxiety sensitivity. These data support the view that the anticipation of somatic symptoms not only evoked a stronger defensive mobilization but also a stronger sympathetic activation in participants afraid of arousal sensations. The increase in heart rate in this group during anticipation of hyperventilation is specifically interesting because such increase was not observed during anticipation of shock. These data replicate and extend previous findings of the study by Melzig et al. (2007) in which threat of shock alters heart rate neither in panic patients nor in controls. In contrast, when panic patients were confronted with darkness (an insecure context for diurnal organisms; Grillon, Pellowski, Merikangas, & Davis, 1997) these patients showed a clear increase in heart rate that additionally correlated with the amount of agoraphobic avoidance and the tendency to escape. Interestingly, the same cardiac acceleration is evoked when animal phobic volunteers (who tend to run away from the feared animal) view symbolic representations of their phobic objects (Hamm et al., 1997), while blood injection phobic participants (who freeze or faint when they view blood) show a heart rate deceleration when confronted with pictures of mutilated bodies. In the current experiment anticipation of somatic symptoms evoked a significant heart rate acceleration suggesting that anticipatory anxiety elicited by expectation of interoceptive cues might also activate a tendency to escape.

Number of reported symptoms

In contrast to the physiological responses which did not differ between groups in the adaptation phase, highly anxiety sensitive participants already reported more anxiety symptoms before any of the threat conditions were introduced. This pattern of increased numbers of reported complaints was maintained during the entire experiment. Moreover, although symptom reports increased during both threat conditions, highly anxiety sensitive persons also reported more symptoms than controls during the *safe* conditions. Thus, the reported symptoms deviated from the physiological data indicating that both measures may assess different aspects of the anxiety response: In contrast to the physiological data that specifically indicate anxious network activation by the anticipated threat conditions, the generally increased symptom reports may indicate hypervigilance towards somatic sensations either triggered by the experimental context or generally present in this population. It may thus be a result of more pronounced negative affectivity in highly anxiety sensitive persons. The questionnaire data, namely the heightened trait anxiety scores, would support such a view. At least the current data suggest a clear dissociation between the physiological pattern of anticipatory anxiety and the verbal report of

perceived symptoms. These data support findings from ambulatory measures of anxiety and panic which often show a clear dissociation between physiological responses and symptom reports (see for review Hoehn-Saric & McLeod, 1993).

General conclusions and implications for future panic disorder research

The findings of the current study suggest that the anticipation of hyperventilation is a valid experimental paradigm to investigate anticipatory anxiety elicited by expectation of somatic symptoms. Anticipation of such interoceptive threat results in clear increase in the number of reported symptoms, autonomic arousal, and potentiation of the startle response but only in participants high in anxiety sensitivity. Thus, the paradigm seems useful in studying those populations that are characterized by fear of somatic arousal sensations, including panic disorder patients.

It needs to be noted that the sample size of the current study is relatively small and replications with larger and more diverse samples (e.g., regarding age or educational background) are needed before the presented findings can be generalized to a larger population. In this context it should also be tested, whether panic disorder patients in fact show increased anticipatory anxiety when expecting interoceptive threat.

If, as suggested by etiological models of panic, a sensitization of panic disorder patients towards interoceptive threat can be experimentally validated, a number of interesting research questions arise: It would, for instance, be interesting to see whether in these patients anticipatory anxiety elicited by expectation of somatic symptoms would be reduced as a result of systematic exposure to interoceptive cues as it has been proposed in the panic control treatment by Barlow & Craske (2000). Given the dissociation between the physiological response pattern and the symptom reports it would be important to include these physiological measures as an additional outcome to the verbal report data. Different treatment ingredients might differentially influence changes in physiological responses and verbal report. One could speculate that changes in physiological responding might depend primarily on the direct exposure to interoceptive cues and that the amount of exposure might predict the extent of change. However, cognitive interventions might be critical for changes in symptom reports. Thus, it would be interesting to see how a repeated hyperventilation challenge would influence physiological responses and symptom reports. Finally, the current paradigm can be used in fMRI experiments to elucidate the therapy induced changes in the anxiety networks of the brain.

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