

**Defensive mobilization, autonomic arousal, and brain activation
during interoceptive threat
in high and low anxiety sensitive individuals**

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Abstract

The fear of somatic sensations is highly relevant in the etiology and maintenance of various disorders. Nevertheless, little is known about this fear of body symptoms and many questions are yet unanswered. Especially physiological studies on interoceptive threat are rare. Therefore, the present thesis investigated defensive mobilization, autonomic arousal, and brain activation during the anticipation of, exposure to, and recovery from unpleasant body sensations. Symptoms were provoked using a standardized hyperventilation procedure in a sample of high (and as controls: low) anxiety sensitive individuals - a population high at risk for developing a panic disorder and high in fear of internal body symptoms.

In study one, anxious apprehension was investigated during anticipation of interoceptive threat (somatic sensations evoked by hyperventilation) and exteroceptive threat (electric shock). Symptom reports, autonomic arousal, and defensive mobilization assessed by the startle eyeblink response were analyzed. Extending the knowledge on anticipation of interoceptive threat, study two investigated the neural networks activated during anxious apprehension of unpleasant body sensations. Symptom reports and startle response data were collected during a learning session after which participants high and low in fear of somatic symptoms attended a fMRI session anticipating threat (hyperventilation – learned to provoke unpleasant symptoms) or safety (normal breathing). Study three examined the actual exposure to internal body symptoms, investigating symptoms reports, autonomic arousal, and the startle eyeblink response during guided breathing (hyperventilation and, as a non-provocative comparison condition, normoventilation) and during recovery. And finally, study four addressed changes in the defensive mobilization during repeated interoceptive exposure via a hyperventilation procedure. High and low anxiety sensitive persons went through two

guided hyperventilation and normoventilation procedures that were spaced one week apart while symptom reports, breathing parameters, and startle response magnitudes were measured.

In study one it was demonstrated that the anticipation of exteroceptive threat led to a defensive and autonomic mobilization in high and low anxiety sensitive individuals, while during interoceptive threat only high anxiety sensitive participants were characterized by a potentiated startle response and autonomic activation. Imaging data of study two revealed that 1) during anticipation of hyperventilation all participants were characterized by an increased activation of a fear network consisting of anterior insula/ orbitofrontal cortex and rostral parts of the dorsal anterior cingulate cortex/ dorsomedial prefrontal cortex, 2) high fear individuals showed higher anxious apprehension than low fear controls during the entire context (safe and threat conditions), indexed by an overall stronger activation of the described network, and 3) while low fear controls learned that (undisclosed to all participants) in the fMRI scanner the threat cue was not followed by an unpleasant hyperventilation task, high fear participants continued to show stronger fear network activation to this cue. In study three it was demonstrated, that the hyperventilation procedure led to a marked increase in somatic symptoms and to autonomic arousal. While high and low anxiety sensitive groups did not differ during hyperventilation, in the early recovery only high anxiety sensitive individuals showed defensive mobilization, indicated by potentiated startle response magnitudes, and increased autonomic arousal after hyperventilation as compared to after normoventilation. Substantiating these findings, in study four all participants reported more symptoms during hyperventilation than during normoventilation, in both sessions. Nevertheless, only high anxiety sensitive participants displayed a potentiation of startle response magnitudes after the first hyper- vs. normoventilation. One week later, when the

exercise was repeated this potentiation was no longer present and thus both groups no longer differed in their defensive mobilization. Even more, the number of reported baseline symptoms decreased from session one to session two in the high-AS group. While high anxiety sensitive persons reported increased baseline anxiety symptoms in session one, groups did not anymore differ in session two.

These data indicate that the standardized hyperventilation procedure is a valid paradigm to induce somatic symptoms. Moreover, it induces anxious apprehension especially in persons highly fearful of internal body symptoms. The repetition of interoceptive exposure, however, reduces associated fear in highly fearful individuals. Thus, this paradigm might provide an innovative method to study anxious apprehension and also treatment effects in patients with panic disorder. The present findings are integrated and discussed in the light of the current literature.

Zusammenfassung

Die Angst vor körperlichen Empfindungen ist höchst relevant in der Ätiologie und Aufrechterhaltung verschiedener Störungen. Dennoch ist wenig bekannt über Angst vor Körpersymptomen und viele Fragen sind bislang unbeantwortet. Insbesondere physiologische Studien über interozeptive Bedrohung sind rar. Daher untersuchte die vorliegende Arbeit defensive Mobilisierung, autonome Erregung und Hirnaktivierung während der Antizipation, der Exposition und der Erholung von unangenehmen Körperempfindungen. Die Symptome wurden provoziert mittels einer standardisierten Hyperventilationsaufgabe in einer Stichprobe von hoch (und als Kontrollen: niedrig) angstsensitiven Personen - eine Population mit erhöhtem Risiko für die Entwicklung einer Panikstörung und mit starker Angst vor Körpersymptomen.

In Studie eins wurde Erwartungsangst während der Antizipation interozeptiver Bedrohung (körperliche Empfindungen, die durch Hyperventilation hervorgerufen werden) und exterozeptiver Bedrohung (elektrotaktile Stimulation) untersucht. Symptomberichte, autonome Erregung und defensive Mobilisierung, erhoben mittels Messung des Schreckreflexes, wurden analysiert. Studie zwei erweiterte das Wissen über die Antizipation interozeptiver Bedrohung durch die Untersuchung der neuronalen Netzwerke, die während ängstlicher Erwartung unangenehmer Körperempfindungen aktiviert werden. Während einer Trainings-Sitzung zum Kennenlernen der Hyperventilation wurden Symptomberichte und Schreckreflexdaten erhoben. Anschließend nahmen die hoch und niedrig ängstlichen Teilnehmer an einer fMRT-Untersuchung teil, in der sie Bedrohung (Hyperventilation – als Auslöser unangenehmer Symptome) oder Sicherheit (normale Atmung) erwarteten. Studie drei untersuchte die tatsächliche Exposition gegenüber Körpersymptomen. Hier wurden Symptomberichte, autonome Erregung und Schreckreflex während geleiteter Hyperventilation (und als

nicht-symptomauslösende Vergleichsbedingung während geleiteter Normoventilation) sowie während der Erholungsphase untersucht. Studie vier beschäftigte sich mit Veränderungen in der defensiven Mobilisierung bei wiederholter interozeptiver Exposition mittels Hyperventilation. Hoch und niedrig angstsensitive Personen durchliefen zwei geführte Hyperventilations- und Normoventilationsprozeduren im Abstand von einer Woche, während Symptombereiche, Atmungsparameter und Stärke des Schreckreflexes gemessen wurden.

In Studie eins wurde gezeigt, dass die Antizipation exterozeptiver Bedrohung zu einer defensiven und autonomen Mobilisierung in hoch und niedrig angstsensitiven Personen führte, während bei interozeptiver Bedrohung nur hoch angstsensitive Teilnehmer durch eine potenzierte Schreckreaktion und autonome Aktivierung charakterisiert waren. Bildgebungsdaten aus Studie zwei ergaben, 1) dass während der Antizipation der Hyperventilation alle Teilnehmer eine erhöhte Aktivierung eines Angstnetzwerkes bestehend aus anteriorer Insel/ orbitofrontalem Kortex und rostralen Anteilen des dorsalen anterioren cingulären Kortex/ dorsomedialen präfrontalen Kortex zeigten, 2) dass hoch ängstliche Personen während der gesamten Messung (sichere und bedrohliche Bedingungen) stärkere Erwartungsangst zeigten als die niedrig ängstlichen Kontrollen. Dies wurde sichtbar durch eine insgesamt stärkere Aktivierung des beschriebenen Netzwerkes. Und 3) dass während niedrig ängstliche Kontrollpersonen lernten, dass (allen Teilnehmern unbekannt) der Bedrohung anzeigende Hinweisreiz im fMRT-Scanner nicht von einer unangenehmen Hyperventilationsaufgabe gefolgt wurde, hoch ängstliche Teilnehmer auf diesen Reiz weiterhin eine stärkere Aktivierung des Angstnetzwerkes zeigten. In der dritten Studie wurde gezeigt, dass die Hyperventilationsaufgabe zu einem deutlichen Anstieg der körperlichen Symptome und zu autonomer Erregung führte. Während sich hoch und niedrig angstsensitive Gruppen

während der Hyperventilation nicht unterschieden, zeigten in der frühen Erholungsphase nur hoch angst-sensitive Personen eine defensive Mobilisierung - angezeigt durch potenzierte Schreckreaktionen - und eine erhöhte autonome Erregung nach Hyperventilation im Vergleich zu Normoventilation. Studie vier untermauert diese Ergebnisse: Alle Teilnehmer berichteten in beiden Sitzungen mehr Symptome während der Hyperventilation als während der Normoventilation. Jedoch zeigten nur hoch angst-sensitive Teilnehmer eine Potenzierung des Schreckreflexes nach der ersten Hyperventilation vs. Normoventilation. Eine Woche später, als die Übung wiederholt wurde, war diese Potenzierung nicht mehr vorhanden und somit unterschieden sich beide Gruppen nicht mehr in ihrer defensiven Mobilisierung. Außerdem verringerte sich die Anzahl der berichteten Baseline-Symptome von Sitzung eins zu Sitzung zwei in der hoch angst-sensitiven Gruppe. Während hoch angst-sensitive Personen in der ersten Sitzung erhöhte Angstsymptome in der Baselinephase berichteten, unterschieden sich die Gruppen in der zweiten Sitzung nicht mehr.

Diese Daten zeigen, dass das standardisierte Hyperventilationsverfahren ein wirksames Paradigma zur Provokation körperlicher Symptome ist. Außerdem induziert es Erwartungsangst vor allem bei Personen, die sehr ängstlich bezüglich Körpersymptomen sind. Die Wiederholung der interozeptiven Exposition jedoch reduziert entsprechende Angst bei hoch ängstlichen Personen. Daher könnte dieses Paradigma eine innovative Methode zur Untersuchung von Erwartungsangst sowie auch von Behandlungseffekten bei Patienten mit Panikstörung darstellen. Die vorliegenden Erkenntnisse werden in die aktuelle Literatur integriert und in deren Rahmen diskutiert.

1 Panic disorder and anxious apprehension concerning interoceptive symptoms

Panic disorder, often also accompanied by agoraphobic avoidance, is the most prevalent anxiety disorder (Margraf & Schneider, 2000). Patients suffering from panic disorder show exaggerated anxiety concerning internal body sensations and experience recurrent unexpected panic attacks which are brief periods of intense fear during which patients are suffering from somatic symptoms like dizziness, sweating, heart palpitations, and cognitive symptoms like fear of dying or losing control.

For the definition as a panic attack interoceptive symptoms have to appear unexpectedly and they have to increase to a peak within 10 minutes (DSM-V; American Psychiatric Association, 2013). For the diagnosis of a panic disorder, patients additionally have to be worried (for at least one month after an attack) about consequences of the panic attacks or about having further panic attacks, thus displaying anxious apprehension. But also panic attacks without anxious apprehension are quite common (lifetime prevalence of isolated panic attacks about 4%, Pané-Farré et al., 2013, 2014). This indicates, that panic attacks do often not lead to a panic disorder and that anxious apprehension and panic attacks may be regarded as two separate phenomena. An etiological model of panic disorder taking this difference into account is the following neuroscience based learning model of panic disorder.

2 The neuroscience perspective on panic disorder

2.1 Threat imminence model

Fanselow (1994) introduced the threat imminence model based on observations of defensive behaviors in animals. According to this model, defensive behaviors comprise different stages, depending on the proximity of the threat: Threat-nonspecific vigilance if the organism is in a context with former, but not yet detected threat contact, i.e. a predator,

(pre-encounter), increased selective attention and freezing when the threat is detected (post-encounter), and finally active defensive behavior or escape when the threat becomes imminent (circa-strike), see Figure 1.

Stage of defensive behavior	Context	Behavior
Pre-encounter defense	Threat has been encountered previously but has not yet been detected	Threat-nonspecific vigilance/ hypervigilance to potential threat
Post-encounter defense	Threat is detected	Increased selective attention, freezing, potentiation of the startle reflex
Circa-strike defense	Threat is imminent	Active defensive behavior/ avoidance, strong autonomic arousal, escape

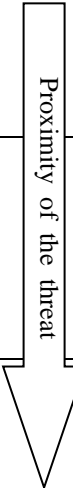


Figure 1. Threat Imminence Model. (adapted from Hamm, Richter, & Pané-Farré, 2014 and Hamm et al., 2016)

2.2 Application of the threat imminence model to panic disorder

Acute threat can also come from inside the body. Hypoxia or hypercapnia result in acute air hunger or dyspnea, which is not only a central symptom during a panic attack but is also a potent interoceptive threat within the respiratory system (see Preter & Klein, 2008; Schimittel et al., 2012). According to the threat imminence model, panic attacks evoked by such interoceptive threats might be defined as circa-strike defense states. This idea was first proposed by Craske (1999) and later elaborated in the “modern learning theory perspective on the etiology of panic disorder” by Bouton et al. (Bouton, Mineka, & Barlow, 2001). The authors presented the idea, that the first panic attack that is accompanied by very strong fear and autonomic arousal could be understood as an

unconditioned circa strike defense, paralleling the circa strike triggered by an unconditioned fear response concerning an external threat like a predator in the threat imminence model. Thus, formerly innocuous mild interoceptive stimuli are now connected to extreme fear and thus, become conditioned stimuli (Hamm et al., 2014).

Applying the threat imminence model to panic disorder, the first stage would be entering a potentially dangerous context in which a panic attack has been encountered previously (i.e. being alone in a shopping mall), followed by anxious apprehension and concerns when mild body symptoms are detected, leading to increased selective attention, freezing and potentiated startle reflex, culminating in the circa-strike of an acute panic attack when interoceptive symptoms increase and active avoidance or escape is initiated, see Figure 2.

Stage of defensive behavior	Context	Behavior
Potentially dangerous context	Threat (panic attack) has been encountered previously in this situation	i.e. Being alone in a shopping mall
Anxious apprehension or concern	Mild interoceptive symptoms are detected	Increased selective attention, freezing, potentiation of the startle reflex
Acute panic	Increasing intensity of interoceptive stimuli	Active defensive behavior/avoidance, strong autonomic arousal, escape

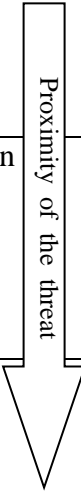


Figure 2. Threat Imminence Model applied to panic disorder. (adapted from Hamm et al., 2014 and Hamm et al., 2016)

3 First empirical support for this model

There is increasing empirical evidence supporting the threat imminence model for panic disorder. While the studies that are part of this work will later examine the match of the anxious apprehension stage with the post-encounter defense of the threat imminence model, first an overview about studies that are concerned with the pre-encounter and circa-strike defense stage will be presented.

3.1 Conditioning process of initial panic attacks

As indicated by the threat imminence model (Figure 2), a conditioning process connecting formerly innocuous mild internal body symptoms to extreme fear is thought to take place with the first pronounced panic attack. As the prevalence of isolated panic attacks (about 4%, Pané-Farré et al., 2013, 2014) is higher than the actual prevalence of panic disorder, not every panic attack results in the development of a panic disorder. The threat-imminence model would predict, that the intensity of the first panic attack (the unconditioned threat) should influence the development of anxious apprehension and panic disorder.

In the Study of Health in Pomerania (SHIP), a large sample of $N = 2259$ adults was interviewed with the Munich Composite International Diagnostic Interview (M-CIDI, Wittchen & Pfister, 1997), resulting in 358 individuals who reported at least one panic attack. These persons were then interviewed in more detail, assessing the first panic attack in order to compare the first panic attacks that marked the beginning of a panic disorder with those that remained isolated. Interestingly, persons who later developed a panic disorder reported more severe cognitive and somatic symptoms during their first panic attack than persons whose panic attacks remained isolated (Hamm et al., 2014). Thus,

supporting the conditioning theory, the intensity of the first panic attack seems to predict the development of a panic disorder.

3.2 Proximity of the threat modulates startle reflex

A more direct test of the threat imminence model in humans was provided by Löw et al. (Löw, Lang, Smith, & Bradley, 2008) who used the startle reflex as a measure for threat during a computer game simulation. Participants could escape from money loss if they quickly responded to pictures that loomed progressively closer during this computer simulation. As to be expected, startle response was potentiated when the anticipated threat (loss of money) was rather distal. But when the distance of the feared stimuli decreased, entering the circa-strike zone, autonomic arousal increased and the startle response was inhibited. This is in line with the threat imminence model, suggesting startle potentiation during action preparation in post-encounter defense and startle inhibition during avoidance or escape behavior when the threat is imminent (Hamm et al., 2014). In a second study, Löw et al. (Löw, Weymar, & Hamm, 2015) further substantiated their findings on the threat imminence model: When participants faced an approaching, uncontrollable threat (electric shock), attentive freezing was augmented. This was indicated by increased skin conductance, fear bradycardia, and potentiated startle reflex. When participants could actively avoid the approaching threat via a button press, response preparation was initialized, indicated by an inhibition of the startle reflex accompanied by a sharp increase in skin conductance prior to the initiation of the motor response and a strong acceleration of the heart rate.

Krause et al. (in press) compared defensive responses to an approaching external threat (electric shock) with an approaching interoceptive threat (feeling of dyspnea evoked by forced breath holding). The threats were either inevitable or avoidable by pressing a

button. During inevitable approaching threats, regardless if external or interoceptive, participants displayed increased skin conductance, potentiation of the startle reflex and bradycardia. Minute ventilation increased during approaching dyspnea. In contrast, when participants were preparing active defensive behavior (of either threat), startle magnitudes were inhibited and heart rate was accelerated. These data further substantiate the assumptions made by the threat imminence model applied to threat processing in humans.

3.3 Proximity of the threat modulates brain activation

Brain activation and its modulation by threat proximity can be investigated using imaging techniques but also a brain stem reflex, the above reported startle response, can provide information about defensive activation of anxiety networks. The startle response is a wave of flexor movements, spreading from cranial to caudal, that is elicited by abrupt sensory stimulation and is potentiated when elicited during a fear conditioning cue or when the organism is set in an unsafe anxiety provoking context (Davis, 2000; Walker & Davis, 2002). As this threat-dependent modulation of the startle response depends on the activation of the central nucleus of the amygdala and the bed nucleus of the stria terminalis, the modulation of the startle reflex can be used as a measure for the activation of the amygdala-dependent defense circuit. Furthermore, the same defense circuit seems to be active in the human brain (Lang & Davis, 2006; LeDoux, 2012) and thus may be used for studying human psychopathology. It has been shown, that the human acoustic startle response, measured via the eyeblink component of the startle reflex, is potentiated during fear conditioning (for review see Hamm & Weike, 2005) and in animal phobic patients during confrontation with pictures of the feared animals (Hamm, Cuthbert, Globisch, & Vaitl, 1997).

Extending the possibilities of startle reflex analyses, imaging techniques provide an opportunity to gain insights about fear processing in a great variety of brain regions. Studying the neural networks of the dynamics of defensive behavior in humans via fMRI, Mobbs et al. (Mobbs et al., 2009; Mobbs, Petrovic, Marchant, Hassabis, & Weiskopf, 2007) used an artificial intelligence predator model to induce threat of varying proximity. As the virtual predator came closer, a shift in brain activation took place, from the ventromedial prefrontal cortex (vmPFC), hippocampus, hypothalamus, and amygdala during anticipation of a possible nociceptive event (post-encounter) to the periaqueductal gray (PAG) and cortical regions like the dorsal anterior cingulate cortex (dACC, known to be involved in analgesia and panic, Petrovic, Kalso, Petersson, & Ingvar, 2002; Tamburin, Cacciatori, Bonato, & Zanette, 2008) during imminent threat or circa-strike. The PAG, especially the dorsal part, is known to control escape behavior in animals (LeDoux, 2012) and thus the found activations are completely in line with the assumptions made by the threat imminence model.

Further support comes from a recent study by Wendt et al. (Wendt, Löw, Weymar, Lotze, & Hamm, 2017), using the approaching threat paradigm established by Löw et al. (2015) in the MRI. When the approaching threat was inevitable, fear bradycardia, potentiated startle reflex, and a dynamic increase in activation of the anterior insula and the periaqueductal grey were present, indicating attentive freezing. Contrary, when participants were preparing for active avoidance a switch in defensive behavior characterized by startle inhibition, heart rate acceleration as well as potentiated activation of the amygdala and the periaqueductal grey was observable. Furthermore, activity in the ventromedial prefrontal cortex was increased only at the beginning of the cascade when anticipated threat was distal, but decreased when threat imminence increased.

3.4 Circa-strike defense in patients with panic disorder

Extending research on the threat imminence model to patient groups, as a part of a large multi-center clinical trial, patients with panic disorder and agoraphobia entered a standardized behavioral avoidance test (being entrapped in a small and dark chamber). Startle reflex modulation and heart rate were measured during anticipation and exposure. Patients were sitting in front of the chamber (10 min, anticipation) and later inside the chamber (max. 10 min., exposure) (see Richter et al., 2012). Patients who refused to enter the chamber were classified as avoiders, patients who entered but did not finish as escapers, patients who remained in the chamber for the whole 10 minutes as completers. The anxious completers displayed strong startle potentiation and an increase in autonomic arousal and reported strong anxious apprehension. Escapers first also showed potentiation of the startle response and augmented heart rate until one minute before the escape heart rate massively increased and the startle reflex was significantly inhibited (Richter et al., 2012), supporting the data by Löw et al. (2008, 2015). During anxious apprehension, with a remote threat encountered (narrow room), patients are concerned and startle response is potentiated. When interoceptive symptoms become more intense (thus, the threat becomes more imminent), autonomic arousal strongly increases, startle reflex is inhibited, and escape behavior is initiated (Hamm et al., 2014).

3.5 Post-encounter defense in high anxiety sensitive persons performing symptom provocation

Investigating the defense stages (pre-encounter, post-encounter and circa-strike defense) as presented in the threat imminence-model (Fanselow, 1994), the reported studies predominantly investigated behavioral and physiological changes when individuals enter the circa-strike defense stage. In order to test if the threat imminence model applied to

panic disorder also holds true for the stage of post-encounter defense, we examined participants high and low in anxiety sensitivity in a symptom provocation paradigm inducing mild internal body sensations. We expected startle response potentiation and beginning sympathetic activation as well as activation of a fear network, when especially high anxiety sensitive persons are in a situation of anxious apprehension, expecting or experiencing mild body symptoms.

3.6 *Anxiety sensitivity*

Anxiety sensitivity represents fear of physiological arousal symptoms (Reiss & McNally, 1985). High anxiety sensitive individuals misinterpret internal body sensations or a change of the body state as a predictor of dramatic consequences (like a change in heart rate being a predictor of a heart attack). As a measure of anxiety sensitivity the Anxiety Sensitivity Index (ASI, Peterson & Reiss, 1992; Reiss, Peterson, Gursky, & McNally, 1986) has been used in numerous studies. High anxiety sensitivity has been considered to be a trait like risk-factor for the development of a panic disorder. In symptom provocation studies using caffeine administration or CO₂ inhalation, high anxiety sensitive participants report similar fear as panic disorder patients (McNally, 2002).

3.7 *Symptom provocation - Inducing internal body sensations*

Symptom provocation studies use a variety of ways to induce internal body sensations. Besides invasive methods like the injection of sodium lactate (Gorman et al., 1984), doxapram (Abelson, Weg, Nesse, & Curtis, 2001), or the tetrapeptide cholecystokinin CCK-4 (Eser et al., 2009) there are non-invasive methods like the inhalation of CO₂ (Blechert, Wilhelm, Meuret, Wilhelm, & Roth, 2010), mechanical ventilation (Banzett et

al., 2000), or the use of inspiratory resistive loads (Alius, Pané-Farré, von Leupoldt, & Hamm, 2013).

Especially in the therapy of panic disorder, the use of interoceptive exposure via symptom provocation is the state of the art treatment. Here, feared internal body symptoms are typically induced by, e.g., breathing through a straw, running, or spinning around (Westphal et al., 2015). These exercises are easy to implement but far less controllable concerning the exact performance and the number and intensity of induced symptoms than the methods used in research environments. A symptom provocation task, however, that combines the controllability of a task used in the laboratory setting with the feasibility of exercises used in the therapeutic context is a guided voluntary hyperventilation exercise.

3.8 Guided hyperventilation procedure

Guided voluntary hyperventilation is one of the most effective symptom provocation tasks concerning intensity of induced symptoms and anxiety. Repetition of the hyperventilation exercise also leads to successful reduction of internal body symptoms and anxiety intensity (Westphal et al., 2015). Guided hyperventilation has been frequently used as a symptom provocation task in laboratory (e.g. Melzig, Holtz, Michalowski, & Hamm, 2011; manuscript 3; Wilhelm, Gerlach, & Roth, 2001) and therapy settings (Antony, Ledley, Liss, & Swinson, 2006). In our current studies we used a guided voluntary hyperventilation procedure to elicit internal body sensations in high and low anxiety sensitive persons. Guided hyperventilation has been frequently used in treatment of panic disorder (Antony et al., 2006; Beck, Shipherd, & Zebb, 1997; Meuret, Ritz, Wilhelm, & Roth, 2005; Schmidt & Trakowski, 2004). During the guided hyperventilation task participants increase their respiratory rate and/ or tidal volume,

exceeding the level of physiological demand, thus inducing a rapid drop of blood partial pressure of carbon dioxide (p_{etCO_2}). Once the partial pressure of p_{etCO_2} falls below approximately 30 mmHg various somatic symptoms like dizziness, heart palpitations, breathlessness, or sweating are elicited (Gardner, 1996).

High anxiety sensitive persons typically report more fear and panic symptoms during such hyperventilation procedures (Asmundson, Norton, Wilson, & Sandler, 1994; Holloway & McNally, 1987; Melzig, Michalowski, Holtz, & Hamm, 2008). Interestingly, physiological measures of anxious apprehension like skin conductance level (Sturges, Goetsch, Ridley, & Whittal, 1998), heart rate (Asmundson et al., 1994; Rapee & Medoro, 1994; Sturges et al., 1998), and blood pressure (Zvolensky et al., 2002) do not differ between high and low anxious participants during hyperventilation. This could be due to the very strenuous task which might make it difficult to identify physiological differences between the groups. Therefore, we decided to focus on the anticipation and the recovery periods surrounding the guided hyperventilation tasks in order to possibly differentiate physiological responding of high and low anxiety sensitive persons.

3.8.1 Anticipation of internal body symptoms (Manuscript 1)

We conducted two studies focusing on the anticipation of interoceptive threat. Up to the first study by Melzig et al. (2008) there was no experimental paradigm available that allowed the explicit investigation of anticipatory anxiety elicited by an interoceptive threat. The current study therefore introduced a completely new paradigm using a well-controlled symptom provocation task to induce fear of internal body symptoms.

High and low anxiety sensitive participants were instructed that one of two colored slides would indicate that a 3 minutes hyperventilation task would follow. During this task individuals were instructed to breath with 20 cycles per minute (cpm) and with a target

p_{etCO_2} of 20 mmHg in order to reliably provoke somatic symptoms. The other slide predicted an upcoming normoventilation task - breathing with 13 cpm and at a comfortable depth. Using acoustic and written instructions, the respiratory rate and breathing depth were adjusted throughout the breathing tasks. The participants were informed that the “fast breathing exercise” could induce internal body symptoms that would disappear when the breathing returned to normal (for the detailed procedure see Melzig et al., 2008; manuscript 1).

3.8.2 Defensive mobilization and autonomic arousal

Startle reflex was potentiated during the anticipation of the symptom-provoking hyperventilation task, but only in high anxiety sensitive participants. In contrast to exteroceptive threat (like an electrotactile stimulus, for more details see Melzig et al., 2008; manuscript 1) that induced anxious apprehension in all participants, this interoceptive threat specifically initiated a defensive response mobilization in those participants who report fear of somatic arousal sensations. Even more interesting is the finding that obviously the pure expectation of such somatic symptoms leads to fear-potentiated startle responses in high anxiety sensitive persons.

Besides this stronger defensive mobilization, also autonomic arousal was present in high anxiety sensitive persons during anticipation of internal body symptoms as indicated by elevated skin conductance and an increase in heart rate.

Taken together, the startle response potentiation and beginning sympathetic activation when high anxiety sensitive persons are in a situation of anxious apprehension (experiencing mild interoceptive symptoms due to anticipatory anxiety or just expecting somatic symptoms to be present soon) clearly support the threat imminence model as

presented above. Maybe the given heart rate acceleration could even be interpreted as a first slight tendency to escape the situation.

3.8.3 Brain activation during interoceptive threat (Manuscript 2)

In the next step we implemented the new paradigm of anticipation of a hyperventilation task into a fMRI environment to study the neural networks activated during anticipatory anxiety. There were only two prior fMRI studies that assessed neural responses during the anticipation and provocation of panic like symptoms in healthy participants. In these studies body symptoms were evoked by injecting the neuropeptide cholecystokinin in its tetrapeptide form (CCK-4) (Eser et al., 2009; Schunck et al., 2006). This pharmacological symptom provocation resulted in a strong increase of reported panic symptoms compared to placebo injection and stronger activations in the ventral ACC, insula, cerebellum, and the temporal pole including amygdala (Eser et al., 2009). There were no differences between participants who reported a panic attack during the pharmacological challenge and those who did not. Anticipation of the injection resulted in a stronger activation of the dorsal ACC but there was no difference between anticipation of CCK-4 or placebo, suggesting that the injection itself might have been an aversive event, irrespective of the pharmacological challenge (Eser et al., 2009). In the study by Schunck et al. (2006) anticipatory anxiety could not at all be analyzed due to the limited number of high responders.

Instead of using a pharmacological challenge, we used the hyperventilation procedure that was successfully implemented by Melzig et al. (2008; manuscript 1) in the fMRI environment. One methodological challenge is that by the hyperventilation procedure, if carried out correctly, changes in the partial pressure of carbon dioxide in the blood affect the BOLD response. To avoid this, we first introduced the hyperventilation challenge

outside the scanner in the laboratory. After high and low anxiety sensitive participants had run through several hyperventilation anticipation periods and hyperventilation exercises and had practiced that different colored cues predicted either a following hyperventilation challenge or a “safe” phase, the second part of the experiment was implemented in the fMRI scanner (for the detailed procedure see Holtz, Pané-Farré, Wendt, Lotze, & Hamm, 2012; manuscript 2). The participants did the same task in the scanner within a context completely comparable to the practice session but no hyperventilation was conducted during this session. During anticipation of the symptom provoking hyperventilation challenge (compared to anticipation of no hyperventilation) participants reported more intense symptoms and also showed augmented startle responses. High anxiety sensitive participants displayed larger startle response magnitudes (especially during anticipation of interoceptive threat) and symptom reports than low anxiety sensitive participants. During anticipation of the hyperventilation challenges compared with the anticipation of safety, a neural network including the anterior insula/ orbitofrontal cortex (OFC) and rostral parts of the dorsal anterior cingulate cortex (dACC)/ dorsomedial prefrontal cortex (dmPFC) was activated. Interestingly, this activation was more sustained in highly anxious compared to low anxious participants. Furthermore, high anxiety sensitive persons showed stronger activations in the mentioned regions during anticipation of interoceptive symptoms and in insula/ OFC also during the safe period (Holtz et al., 2012; manuscript 2). This suggests, that high anxious individuals showed overall stronger anxious apprehension than low anxious controls. The pattern of activation including insula and dACC/ dmPFC have formerly been found during anticipation of electric shock or aversive pictures (for review see Mechias, Etkin, & Kalisch, 2010). This suggests that anticipation of unpleasant body symptoms activates the same neural network as anticipation of exteroceptive threat. No amygdala activation

was present during anticipation of interoceptive threat supporting recent learning theory perspectives on the etiology of panic disorder suggesting that separate neural networks are involved during panic attacks, an instance of acute fight-flight responses (circa-strike, involving activation of the amygdala), and during anticipatory anxiety. The activations found during anticipation of interoceptive threat perfectly fit into the model as would be expected during a stage of anxious apprehension (Bouton et al., 2001; Mineka & Zinbarg, 2006). Activation of the rostral dmPFC has been suggested to be involved in threat appraisal (see Mechias et al., 2010), which plays a role in cognitive models of panic disorder and seems to be especially important during the stage of anxious apprehension. Additionally, activation of the insula has also been associated with increased awareness of somatic symptoms (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004). Thus, increased activation in the anterior insula during anticipating an unpleasant hyperventilation challenge would support the hypothesis of increased hypervigilance during anxious apprehension of internal body symptoms (Holtz et al., 2012; manuscript 2).

3.8.4 Recovery from internal body symptoms – Defensive mobilization and autonomic arousal (Manuscript 3)

Claiming that mild body symptoms act as conditioned stimuli engaging anxious apprehension that is characterized by increased defensive mobilization like freezing and potentiation of the startle response, Melzig et al. (2011; manuscript 3) used the above described method of a guided voluntary hyperventilation challenge and analyzed the early recovery period after the hyper- and normoventilation exercises. This is a handy possibility to investigate anxious apprehension during mild bodily symptoms that are difficult to explain (individuals do not know that it takes about two minutes before the

$p_{et}CO_2$ level returns back to normal). One advantage is, that with analyzing a time window after the breathing task, the task itself does not interfere with the acoustic startle probes. As a reminder tones of rising and falling pitch signal the subject to inhale and exhale during the task. A second advantage is, that with analyzing the early recovery instead of the phase during the breathing task we can bypass the problem, that differences in physiological responding between high and low anxiety sensitive persons are often hard to detect during a hyperventilation task, which is a highly strenuous exercise that may overlay given differences.

The analysis of the early recovery period is possible, since the $p_{et}CO_2$ level remains under 30 mmHg - the critical threshold for the elicitation of interoceptive symptoms (Gardner, 1996) - for about two minutes after the hyperventilation task. Thus, body symptoms are still present without the interference of the challenge itself. That the end-tidal pCO_2 was indeed under this threshold during the first two minutes, Melzig et al. (2011; manuscript 3) confirmed via measuring the expired $p_{et}CO_2$ using a capnograph (for the detailed procedure see Melzig et al., 2011; manuscript 3). Thus, we can assume that the body symptoms were still present in high and low anxiety sensitive participants during this early recovery phase, when startle reflex and autonomic arousal were measured.

High anxiety sensitive but not low anxiety sensitive controls exhibited a potentiation of the startle response magnitudes during the first two minutes of recovery from the hyperventilation compared to normoventilation exercise. Furthermore, during early recovery high anxiety sensitive persons did not show the compensatory decrease in respiratory rate found in low anxiety sensitive persons and they displayed a delayed recovery of skin conductance level and heart rate. Taken together, this again indicates, that experiencing internal body symptoms engages defensive response mobilization and

that mild body sensations act as conditioned stimuli that elicit post-encounter defense in high anxiety sensitive persons (Hamm et al., 2014).

3.8.5 Defensive mobilization and symptom report after repeated interoceptive exposure (Manuscript 4)

After having tested the applicability of the threat imminence model to panic disorder, focusing on the post-encounter defense stage, in our most recent study we wanted to move on investigating changes in anxious apprehension concerning internal body symptoms over repeated interoceptive exposure. We again used the approved method of guided voluntary hyperventilation. After high and low anxiety sensitive participants had accomplished session one (anticipation, hyperventilation task, and recovery from hyperventilation followed by normoventilation, as described above) they returned for a second session one week later undergoing the same procedure (see Holtz, Hamm, & Pané-Farré, submitted; manuscript 4). While high anxiety sensitive participants, compared to low anxious controls, displayed potentiated startle response magnitudes after the first hyperventilation vs. normoventilation procedure, one week later, when the hyperventilation exercise was repeated, high and low anxiety sensitive groups no longer differed in their defensive mobilization to symptom provocation. Even more, while high anxiety sensitive individuals reported increased baseline anxiety symptoms in session one, groups did not any more differ in session two, as the number of reported baseline symptoms decreased from session one to session two in the high-AS group (Holtz et al., submitted; manuscript 4).

Therapy studies often lack psychophysiological measures or, even if measured, the verbally reported anxiety reduction lacks associated effects in heart rate or skin conductance (Lang & Craske, 2000; Rowe & Craske, 1998). In case of interoceptive

exposure only one study by Forsyth et al. (Forsyth, Lejuez, & Finlay, 2000) found a reduction in subjective distress AND heart rate over several sessions of CO₂ inhalation, in a small sample (N=4). In our recent study, over repetition of symptom provocation, we found a decrease a defensive mobilization (measured via startle response magnitude) and dissolving group differences in symptom report. Thus, startle response magnitude might be a more appropriate measure for activation of and changes in the defensive system. This would also be suggested by the threat imminence model, as we investigate the experience of mild body symptoms (not acute panic) and are therefore in the post-encounter defense (or anxious apprehension) which is characterized by startle potentiation.

4 Summary and future directions

In this work we used anticipation of interoceptive threat in order to investigate anxious apprehension concerning internal body symptoms in high and low anxiety sensitive persons. The results of the studies were associated with the threat imminence model and its application to panic disorder (Hamm et al., 2016, 2014).

Postulating a dimensional construct of defensive reactivity concerning approaching threat, the model claims a pre-encounter, post-encounter, and circa-strike defense stage, applied to panic disorder “potentially dangerous context”, “anxious apprehension”, and “panic attack”. The guided voluntary hyperventilation challenge reliably induced body symptoms in all participants. Especially those participants high in anxiety sensitivity and at high risk for developing a panic disorder (McNally, 2002; Schmidt, Lerew, & Jackson, 1999) reported more symptoms and showed potentiated startle response magnitudes during anticipation and recovery from the interoceptive challenge. This is completely in line with the threat imminence model, claiming that the experience of mild body

symptoms is associated with startle reflex potentiation and reflects a state of anxious apprehension (Hamm et al., 2014). Anticipation of internal body symptoms was associated with an activation in the anterior insula/ orbitofrontal cortex (OFC) and rostral parts of the dorsal anterior cingulate cortex (dACC)/ dorsomedial prefrontal cortex (dmPFC), regions related to processes like appraisal (dmPFC, Mechias et al., 2010) and increased awareness to somatic symptoms (insula, Critchley et al., 2004). These results, again, support the threat imminence model applied to panic disorder as described above. Activation of this network was more sustained in highly anxious compared to low anxious participants and these participants showed an overall higher anxious apprehension than low anxious controls throughout the procedure. With the most recent publication we went a step beyond the model to investigate repeated interoceptive exposure, finding that anxious apprehension in high anxiety sensitive persons (startle potentiation and verbal report of panic symptoms) diminishes with repetition of the interoceptive exposure challenge. This sheds a first light on a possible desensitization of defensive reactivity as a mechanism of action underlying the success of exposure therapy. Nevertheless, the understanding of these mechanisms still remain subject to future research. Our first results on anxious apprehension in high anxiety sensitive individuals have to be substantiated by future investigations on patients with pathological anxiety until final implications for improving therapy of anxiety disorders emerge.

5 References

- Abelson, J. L., Weg, J. G., Nesse, R. M., & Curtis, G. C. (2001). Panic Disorder. *Methods*, 3223(0).
- Alius, M. G., Pané-Farré, C. A., von Leupoldt, A., & Hamm, A. O. (2013). Induction of

- dyspnea evokes increased anxiety and maladaptive breathing in individuals with high anxiety sensitivity and suffocation fear. *Psychophysiology*, 50(5), 488–497. <http://doi.org/10.1111/psyp.12028>
- American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: Author.
- Antony, M. M., Ledley, D. R., Liss, A., & Swinson, R. P. (2006). Responses to symptom induction exercises in panic disorder. *Behaviour Research and Therapy*, 44(1), 85–98. <http://doi.org/10.1016/j.brat.2004.12.005>
- Asmundson, G. J. G., Norton, G. R., Wilson, K. G., & Sandler, L. S. (1994). Subjective symptoms and cardiac reactivity to brief hyperventilation with high anxiety sensitivity. *Behavior Research and Therapy*, 32(2), 237–241.
- Banzett, R. B., Mulnier, H. E., Murphy, K., Rosen, S. D., Wise, R. J., & Adams, L. (2000). Breathlessness in humans activates insular cortex. *Neuroreport*, 11(10), 2117–20. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10923655>
- Beck, J. G., Shipherd, J. C., & Zebb, B. J. (1997). How does interoceptive exposure for panic disorder work? An uncontrolled case study. *Journal of Anxiety Disorders*, 11(5), 541–56. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9407272>
- Blechert, J., Wilhelm, F. H., Meuret, A. E., Wilhelm, E. M., & Roth, W. T. (2010). Respiratory, autonomic, and experiential responses to repeated inhalations of 20% CO₂ enriched air in panic disorder, social phobia, and healthy controls. *Biological Psychology*, 84(1), 104–11. <http://doi.org/10.1016/j.biopsycho.2010.01.002>
- Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A Modern Learning Theory Perspective on the Etiology of Panic Disorder. *Psychological Review*, 108(1), 4–32. <http://doi.org/10.1037//0033-295X.108.1.4>
- Craske, M. G. (1999). *Anxiety disorders: Psychological approaches to theory and*

treatment. New York: Basic Books.

- Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., & Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nature Neuroscience*, 7(2), 189–95. <http://doi.org/10.1038/nn1176>
- Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In J. P. Aggleton (Ed.), *The Amygdala, a Functional Analysis* (pp. 213–287). Oxford.
- Eser, D., Leicht, G., Lutz, J., Wenninger, S., Kirsch, V., Schüle, C., ... Mulert, C. (2009). Functional neuroanatomy of CCK-4-induced panic attacks in healthy volunteers. *Human Brain Mapping*, 30(2), 511–22. <http://doi.org/10.1002/hbm.20522>
- Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review*, 1(4), 429–438. <http://doi.org/10.3758/BF03210947>
- Forsyth, J. P., Lejuez, C. W., & Finlay, C. (2000). Anxiogenic effects of repeated administrations of 20% CO₂-enriched air: Stability within sessions and habituation across time. *Journal of Behavior Therapy and Experimental Psychiatry*, 31(2), 103–121. [http://doi.org/10.1016/S0005-7916\(00\)00014-8](http://doi.org/10.1016/S0005-7916(00)00014-8)
- Gardner, W. N. (1996). The Pathophysiology of Hyperventilation Disorders. *Chest*, 109(2), 516–534. <http://doi.org/https://doi.org/10.1378/chest.109.2.516>
- Gorman, J. M., Askanazi, J., Liebowitz, M. R., Fyer, A. J., Stein, J., Kinney, J. M., & Klein, D. F. (1984). Response to hyperventilation in a group of patients with panic disorder. *American Journal of Psychiatry*, 141(7), 857–861.
- Hamm, A. O., Cuthbert, B. N., Globisch, J., & Vaitl, D. (1997). Fear and the startle reflex: Blink modulation and autonomic response patterns in animal and mutilation

- fearful subjects. *Psychophysiology*. <http://doi.org/10.1111/j.1469-8986.1997.tb02420.x>
- Hamm, A. O., Richter, J., Pané--Farré, C., Westphal, D., Wittchen, H. U., Vossbeck-Elsebusch, A. N., ... Deckert, J. (2016). Panic disorder with agoraphobia from a behavioral neuroscience perspective: Applying the research principles formulated by the Research Domain Criteria (RDoC) initiative. *Psychophysiology*, 53(3), 312–322. <http://doi.org/10.1111/psyp.12553>
- Hamm, A. O., Richter, J., & Pané-Farré, C. A. (2014). When the threat comes from inside the body: A neuroscience based learning perspective of the etiology of panic disorder. *Restorative Neurology and Neuroscience*, 32(1), 79–93.
- Hamm, A. O., & Weike, A. I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology*, 57(1), 5–14. <http://doi.org/10.1016/j.ijpsycho.2005.01.006>
- Holloway, W., & McNally, R. J. (1987). Effects of anxiety sensitivity on the response to hyperventilation. *Journal of Abnormal Psychology*, 96(4), 330–334. <http://doi.org/10.1037/0021-843X.96.4.330>
- Holtz, K., Hamm, A. O., & Pané-Farré, C. A. Repeated interoceptive exposure in high and low anxiety sensitive persons. *Submitted*.
- Holtz, K., Pané-Farré, C. A., Wendt, J., Lotze, M., & Hamm, A. O. (2012). Brain activation during anticipation of interoceptive threat. *NeuroImage*, 61, 857–865. <http://doi.org/10.1016/j.neuroimage.2012.03.019>
- Krause, E., Benke, C., Koenig, J., Thayer, J. F., Hamm, A. O., & Pané-Farré, C. A. (in press). Dynamics of defensive response mobilization to approaching external vs. interoceptive threat. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*.

- Lang, A. J., & Craske, M. G. (2000). Manipulations of exposure-based therapy to reduce return of fear: A replication. *Behaviour Research and Therapy*, 38(1), 1–12. [http://doi.org/10.1016/S0005-7967\(99\)00031-5](http://doi.org/10.1016/S0005-7967(99)00031-5)
- Lang, P. J., & Davis, M. (2006). Emotion, motivation, and the brain: Reflex foundations in animal and human research. *Progress in Brain Research*, 156, 3–29. [http://doi.org/doi.org/10.1016/S0079-6123\(06\)56001-7](http://doi.org/doi.org/10.1016/S0079-6123(06)56001-7)
- LeDoux, J. (2012). Rethinking the emotional brain. *Neuron*, 73(4), 653–676. <http://doi.org/10.1016/j.neuron.2012.02.004>.
- Löw, A., Lang, P. J., Smith, C., & Bradley, M. (2008). Both Predator and Prey: Emotional Arousal in Threat and Reward. *Psychological Science*, 19(9), 865–873. <http://doi.org/10.1111/j.1467-9280.2008.02170.x>
- Löw, A., Weymar, M., & Hamm, A. O. (2015). When Threat Is Near, Get Out of Here. *Psychological Science*, 26(11), 1706–1716. <http://doi.org/10.1177/0956797615597332>
- Margraf, J., & Schneider, S. (2000). Paniksyndrom und Agoraphobie. In J. Margraf (Ed.), *Lehrbuch der Verhaltenstherapie*. Springer Berlin Heidelberg.
- McNally, R. J. (2002). Anxiety sensitivity and panic disorder. *Biological Psychiatry*, 52(10), 938–46. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12437935>
- Mechias, M.-L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *NeuroImage*, 49(2), 1760–1768. <http://doi.org/10.1016/j.neuroimage.2009.09.040>
- Melzig, C. A., Holtz, K., Michalowski, J. M., & Hamm, A. O. (2011). Interoceptive threat leads to defensive mobilization in highly anxiety sensitive persons. *Psychophysiology*, 48(6), 745–754. <http://doi.org/10.1111/j.1469-8986.2010.01150.x>

- Melzig, C. A., Michalowski, J. M., Holtz, K., & Hamm, A. O. (2008). Anticipation of interoceptive threat in highly anxiety sensitive persons. *Behaviour Research and Therapy*, 46(10), 1126–34. <http://doi.org/10.1016/j.brat.2008.07.002>
- Meuret, A. E., Ritz, T., Wilhelm, F. H., & Roth, W. T. (2005). Voluntary hyperventilation in the treatment of panic disorder--functions of hyperventilation, their implications for breathing training, and recommendations for standardization. *Clinical Psychology Review*, 25(3), 285–306. <http://doi.org/10.1016/j.cpr.2005.01.002>
- Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *American Psychologist*, 61(1), 10–26. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/16435973>
- Mobbs, D., Marchant, J. L., Hassabis, D., Seymour, B., Tan, G., Gray, M., ... Frith, C. D. (2009). From Threat to Fear: The Neural Organization of Defensive Fear Systems in Humans. *Journal of Neuroscience*, 29(39), 12236–12243. <http://doi.org/10.1523/JNEUROSCI.2378-09.2009>
- Mobbs, D., Petrovic, P., Marchant, J. L., Hassabis, D., & Weiskopf, N. (2007). When Fear Is Near: Threat Imminence Elicits Prefrontal-Periaqueductal Gray Shifts in Humans. *Science*, 1119(August), 1079–1083. <http://doi.org/10.1126/science.1144298>.
- Pané-Farré, C. A., Fenske, K., Stender, J. P., Meyer, C., John, U., Rumpf, H. J., ... Hamm, A. O. (2013). Sub-threshold panic attacks and agoraphobic avoidance increase comorbidity of mental disorders: Results from an adult general population sample. *Journal of Anxiety Disorders*, 27(5), 485–493. <http://doi.org/10.1016/j.janxdis.2013.06.008>

- Pané-Farré, C. A., Stender, J. P., Fenske, K., Deckert, J., Reif, A., John, U., ... Hamm, A. O. (2014). The phenomenology of the first panic attack in clinical and community-based samples. *Journal of Anxiety Disorders*, 28(6), 522–529. <http://doi.org/10.1016/j.janxdis.2014.05.009>
- Peterson, R. A., & Reiss, S. (1992). *Anxiety sensitivity index manual (2nd ed)*. Worthington, OH: International Diagnostic Systems.
- Petrovic, P., Kalso, E., Petersson, K. M., & Ingvar, M. (2002). Placebo and Opioid Analgesia-- Imaging a Shared Neuronal Network. *Science*, 295(5560), 1737–1740. <http://doi.org/10.1126/science.1067176>
- Preter, M., & Klein, D. F. (2008). Panic, Suffocation False Alarms, Separation Anxiety and Endogenous Opioids. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 32, 603–612. <http://doi.org/10.1038/nature07664>. Anticipatory
- Rapee, R. M., & Medoro, L. (1994). Fear of physical sensations and trait anxiety as mediators of the response to hyperventilation in nonclinical subjects. *Journal of Abnormal Psychology*, 103(4), 693–9. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7822570>
- Reiss, S., & McNally, R. J. (1985). Expectancy model of fear. In S. Reiss & R. R. Bootzin (Eds.), *Theoretical issues in behavior therapy* (pp. 107–121). San Diego, CA: Academic Press.
- Reiss, S., Peterson, R. A., Gursky, D. M., & McNally, R. J. (1986). Anxiety sensitivity, anxiety frequency and the predictions of fearfulness. *Behaviour Research and Therapy*, 24(1), 1–8. [http://doi.org/10.1016/0005-7967\(86\)90143-9](http://doi.org/10.1016/0005-7967(86)90143-9)
- Richter, J., Hamm, A. O., Pané-Farré, C. A., Gerlach, A. L., Gloster, A. T., Wittchen, H. U., ... Arolt, V. (2012). Dynamics of defensive reactivity in patients with panic disorder and agoraphobia: Implications for the etiology of panic disorder.

Biological Psychiatry, 72(6), 512–520.

<http://doi.org/10.1016/j.biopsych.2012.03.035>

- Rowe, M. K., & Craske, M. G. (1998). Effects of an expanding-spaced vs massed exposure schedule on fear reduction and return of fear. *Behaviour Research and Therapy*, 36(7–8), 701–717. [http://doi.org/10.1016/S0005-7967\(97\)10016-X](http://doi.org/10.1016/S0005-7967(97)10016-X)
- Schmitel, F. G., de Almeida, G. M., Pitol, D. N., Armini, R. S., Tufik, S., & Schenberg, L. C. (2012). Evidence of a suffocation alarm system within the periaqueductal gray matter of the rat. *Neuroscience*, 200, 59–73. <http://doi.org/10.1016/j.neuroscience.2011.10.032>
- Schmidt, N. B., Lerew, D. R., & Jackson, R. J. (1999). Prospective Evaluation of Anxiety Sensitivity in the Pathogenesis of Panic : Replication and Extension. *Journal of Abnormal Psychology*, 108(3), 532–537.
- Schmidt, N. B., & Trakowski, J. (2004). Interoceptive Assessment and Exposure in Panic Disorder: A Descriptive Study. *Cognitive and Behavioral Practice*, 11(1), 81–92.
- Schunck, T., Erb, G., Mathis, A., Gilles, C., Namer, I. J., Hode, Y., ... Macher, J.-P. (2006). Functional magnetic resonance imaging characterization of CCK-4-induced panic attack and subsequent anticipatory anxiety. *NeuroImage*, 31(3), 1197–208. <http://doi.org/10.1016/j.neuroimage.2006.01.035>
- Sturges, L. V., Goetsch, V. L., Ridley, J., & Whittal, M. (1998). Anxiety sensitivity and response to hyperventilation challenge: physiologic arousal, interoceptive acuity, and subjective distress. *Journal of Anxiety Disorders*, 12(2), 103–115. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9560174>
- Tamburin, S., Cacciatori, C., Bonato, C., & Zanette, G. (2008). Cingulate Gyrus Tumor Presenting as Panic Attacks. *American Journal of Psychiatry*, 165(5), 651–652.

<http://doi.org/10.1176/appi.ajp.2007.07061005>

Walker, D. L., & Davis, M. (2002). Light-enhanced startle: Further pharmacological and behavioral characterization. *Psychopharmacology*, 159(3), 304–310.

<http://doi.org/10.1007/s002130100913>

Wendt, J., Löw, A., Weymar, M., Lotze, M., & Hamm, A. O. (2017). Active avoidance and attentive freezing in the face of approaching threat. *NeuroImage*,

158(December 2016), 196–204. <http://doi.org/10.1016/j.neuroimage.2017.06.054>

Westphal, D., Gerlach, A. L., Lang, T., Wittchen, H. U., Hamm, A. O., Ströhle, A., ...

Einsle, F. (2015). Die Effekte interozeptiver Expositionsübungen in der Kognitiven

Verhaltenstherapie von Panikstörung mit Agoraphobie. *Verhaltenstherapie*, 25(4),

268–276. <http://doi.org/10.1159/000441508>

Wilhelm, F. H., Gerlach, A. L., & Roth, W. T. (2001). Slow recovery from voluntary hyperventilation in panic disorder. *Psychosomatic Medicine*, 63(4), 638–649.

Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11485118>

Wittchen, H.-U., & Pfister, H. (1997). *DIA-X-Interviews: Manual für Screening-Verfahren und Interview; Interviewheft*. Frankfurt: Swets & Zeitlinger.

Zvolensky, M. J., Goodie, J. L., Ruggiero, K. J., Black, A. L., Larkin, K. T., & Taylor,

B. K. (2002). Perceived stress and anxiety sensitivity in the prediction of anxiety-

related responding: A multichallenge evaluation. *Anxiety, Stress & Coping*, 15(3),

211–229.

Appendix A: Publications

Manuscript 1

Melzig, C. A., Michalowski, J. M., **Holtz, K.**, & Hamm, A. O. (2008). Anticipation of interoceptive threat in highly anxiety sensitive persons. *Behaviour Research and Therapy*, 46(10), 1126–34. <http://doi.org/10.1016/j.brat.2008.07.002>

Manuscript 2

Holtz, K., Pané-Farré, C. A., Wendt, J., Lotze, M., & Hamm, A. O. (2012). Brain activation during anticipation of interoceptive threat. *NeuroImage*, 61, 857–865. <http://doi.org/10.1016/j.neuroimage.2012.03.019>

Manuscript 3

Melzig, C. A., **Holtz, K.**, Michalowski, J. M., & Hamm, A. O. (2011). Interoceptive threat leads to defensive mobilization in highly anxiety sensitive persons. *Psychophysiology*, 48(6), 745–54. <http://doi.org/10.1111/j.1469-8986.2010.01150.x>

Manuscript 4

Holtz, K., Hamm, A. O., & Pané-Farré, C. A. (submitted). Repeated interoceptive exposure in high and low anxiety sensitive persons.

Manuscript 1

Anticipation of interoceptive threat in highly anxiety sensitive persons

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Author contributions:

CM conceived and designed the experiment. CM and KH performed the experiments. CM supervised the data acquisition. CM analyzed the data. All authors contributed to the interpretation of the data and wrote the manuscript (first draft by CM).



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Anticipation of interoceptive threat in highly anxiety sensitive persons

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Hyperventilation

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.

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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, Weihe, 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, CO₂-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	<i>t</i>	Significance (<i>p</i>)
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 electrostatic 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, electrostatic 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 CO₂ 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 hypoxemia 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}CO₂ 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}CO₂ at 20 mmHg. All participants included in this analysis were fully compliant with this procedure.

Normoventilation 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 symptoms¹, 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 electrostatic 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 μ S. 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 magnitudes³ 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 \times 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) μ V, 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 \times 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) μ S, 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 \times 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 \times 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 \times Group interaction, $F(1, 43) = 5.5$, $p < .05$, $\eta_p^2 = .106$, in the between group analysis.

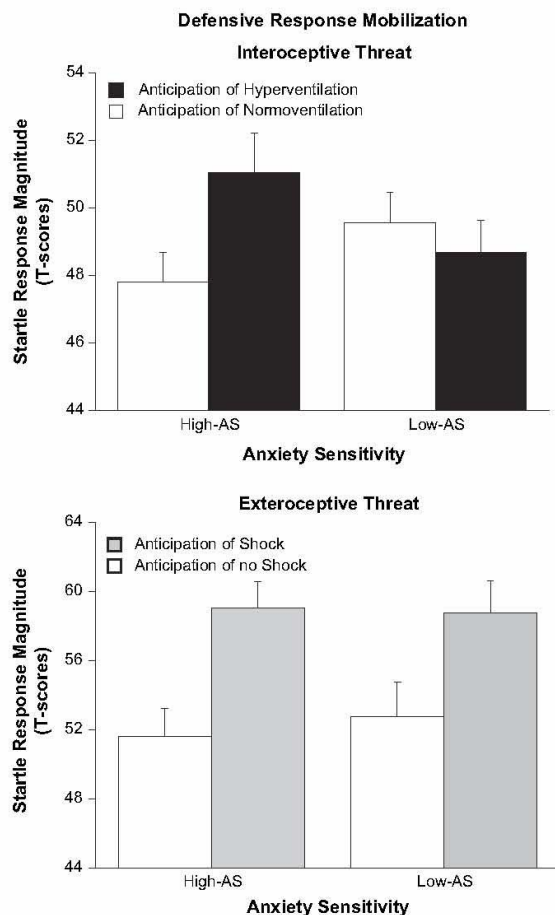


Fig. 1. Mean startle response magnitudes during interoceptive (anticipation of hyperventilation, upper panel) and exteroceptive threat (anticipation of shock, lower panel) in highly anxiety sensitive participants and controls, respectively.

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 \times 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 \times 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 \times Group $F(1, 44) < 1$, $p = .99$, $\eta_p^2 = .000$. However, group specific differences were again detected during anticipation of interoceptive threat, Threat \times 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 \times Group $F(1, 43) < 1$, $p = .36$, $\eta_p^2 = .026$; 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 \times 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 \times 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.

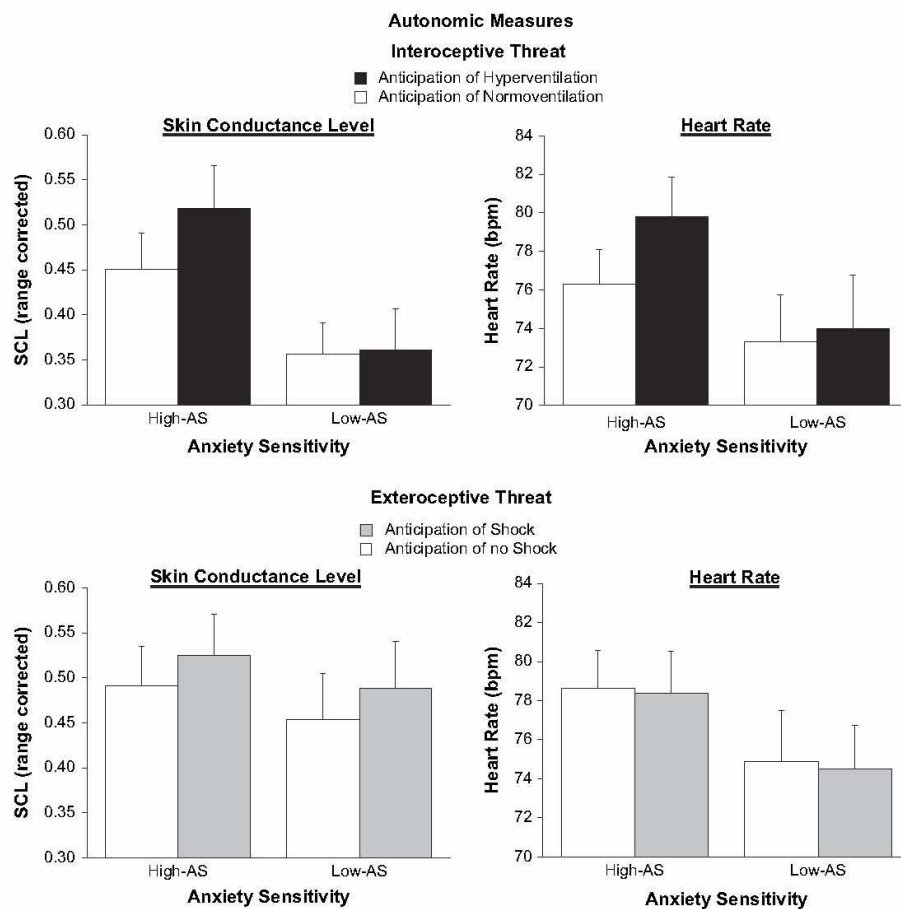


Fig. 2. Mean skin conductance level (left) and heart rates (right) during interoceptive (anticipation of hyperventilation, upper panel) and exteroceptive threat (anticipation of shock, lower panel) in highly anxiety sensitive participants and controls, respectively.

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

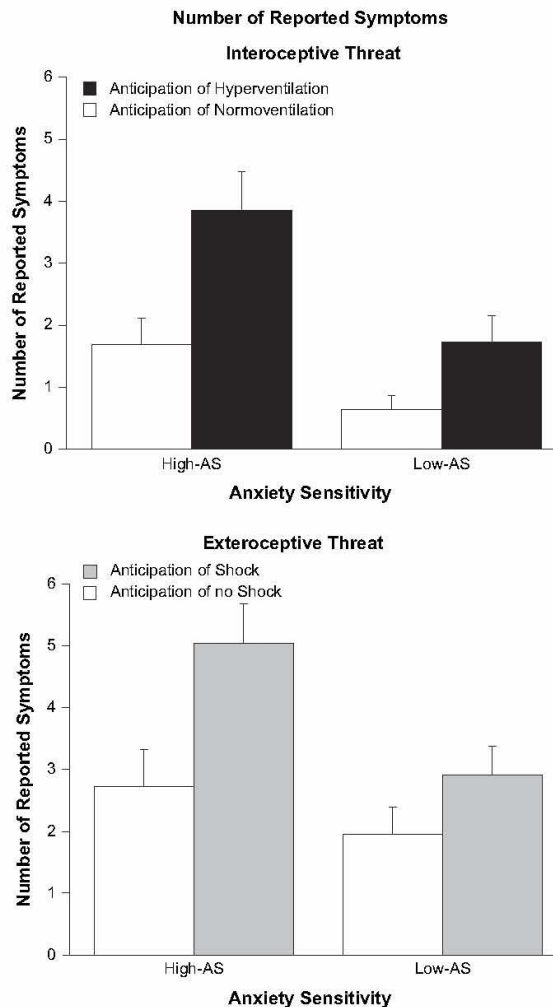


Fig. 3. Mean number of reported symptoms during interoceptive (anticipation of hyperventilation, upper panel) and exteroceptive threat (anticipation of shock, lower panel) in highly anxiety sensitive participants and controls, respectively.

(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, Pelkowski, 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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References

- American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington DC: American Psychiatric Press.
- Barlow, D. H. (2004). *Anxiety and its disorders: the nature and treatment of anxiety and panic* (2nd ed.). New York: Guilford Press.
- Barlow, D. H., & Craske, M. G. (2000). *Mastery of your anxiety and panic (MAP-3): client workbook for anxiety and panic (treatments that work)*. Oxford, England: Oxford University Press.
- Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & Van Bostel, A. (2005). Committee report: guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, 42, 1–15.
- Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychological Review*, 108, 4–32.
- Bradley, M. M. (2000). Emotion and motivation. In J. T. Cacioppo, L. G. Tassinary, & G. G. Bernstein (Eds.), *Handbook of psychophysiology* (pp. 602–642). Cambridge, UK: Cambridge University Press.
- Chambless, D. L., Caputo, G. C., Bright, P., & Gallagher, R. (1984). Assessment of fear of fear in agoraphobics: the body sensations questionnaire and the agoraphobic cognitions questionnaire. *Journal of Consulting and Clinical Psychology*, 52, 1090–1097.
- Chattopadhyay, P., Cooke, E., Toone, B., & Lader, M. (1980). Habituation of physiological responses in anxiety. *Biological Psychiatry*, 15, 711–721.
- Clark, D. M. (1986). A cognitive approach to panic. *Behaviour Research and Therapy*, 24, 461–470.
- Clark, D. M. (1988). A cognitive model of panic attacks. In S. Rachman, & J. D. Maser (Eds.), *Panic: psychological perspectives* (pp. 71–89). Hillsdale, NJ: Erlbaum.
- Cook, E. W., III, Atkinson, L., & Lang, K. G. (1987). Stimulus control and data acquisition for IBM PCs and compatibles. *Psychophysiology*, 24, 726–727.
- Coplan, J. D., Goetz, R., Klein, D. F., Papp, L. A., Fyer, A. J., Liebowitz, M. R., Davies, S. O., & Gorman, J. M. (1998). Plasma cortisol concentrations preceding lactate-induced panic. Psychological, biochemical, and physiological correlates. *Archives of General Psychiatry*, 55, 130–136.
- Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In J. P. Aggleton (Ed.), *The amygdala* (pp. 213–287). Oxford, UK: Oxford University Press.
- Deane, G. E. (1961). Human heart rate responses during experimentally induced anxiety. *Journal of Experimental Psychology*, 61, 489–493.
- Deane, G. E. (1969). Cardiac activity during experimentally induced anxiety. *Psychophysiology*, 6, 17–30.
- Deane, G. E., & Zeaman, D. (1958). Human heart rate during anxiety. *Perceptual and Motor Skills*, 8, 103–106.
- Donnell, C. D., & McNally, R. J. (1989). Anxiety sensitivity and history of panic as predictors of response to hyperventilation. *Behaviour Research and Therapy*, 27, 325–332.
- Ehlers, A., Margraf, J., & Chambless, D. (1993a). *ACQ Fragebogen zu angstbezogenen Kognitionen*. Weinheim: Beltz.
- Ehlers, A., Margraf, J., & Chambless, D. (1993b). *BSQ Fragebogen zur Angst vor körperlichen Symptomen*. Weinheim: Beltz.
- Globisch, J., Hamm, A. O., Schneider, R., & Vaitl, D. (1993). A computer program for scoring reflex eyeblink and electrodermal responses written in PASCAL. *Psychophysiology*, 30, S13.
- Grillon, C. (2002). Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. *Biological Psychiatry*, 52, 958–975.
- Grillon, C., Ameli, R., Goddard, A., Woods, S. W., & Davis, M. (1994). Baseline and fear-potentiated startle in panic disorder patients. *Biological Psychiatry*, 35, 431–439.
- Grillon, C., Ameli, R., Merikangas, K., Woods, S. W., & Davis, M. (1993). Measuring the time course of anticipatory anxiety using the fear-potentiated startle reflex. *Psychophysiology*, 30, 340–346.
- Grillon, C., Ameli, R., Woods, S. W., Merikangas, K., & Davis, M. (1991). Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology*, 28, 588–595.
- Grillon, C., & Baas, J. (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology*, 114, 1557–1579.
- Grillon, C., Pellowski, M., Merikangas, K. R., & Davis, M. (1997). Darkness facilitates the acoustic startle reflex in humans. *Biological Psychiatry*, 42, 453–460.
- Hamm, A. O., Cuthbert, B. N., Globisch, J., & Vaitl, D. (1997). Fear and startle reflex: blink modulation and autonomic response patterns in animal and mutilation fearful subjects. *Psychophysiology*, 34, 97–107.
- Hamm, A. O., Greenwald, M. K., Bradley, M. M., & Lang, P. J. (1993). Emotional learning, hedonic change, and the startle probe. *Journal of Abnormal Psychology*, 102, 453–465.
- Hamm, A. O., & Vaitl, D. (1996). Affective learning: awareness and aversion. *Psychophysiology*, 33, 698–710.
- Hayward, C., Killen, J. D., Kraemer, H. C., & Taylor, C. B. (2000). Predictors of panic attacks in adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, 39, 207–214.
- Hoehn-Saric, R., & McLeod, D. R. (1993). Somatic manifestation of normal and pathological anxiety. In R. Hoehn-Saric, & D. R. McLeod (Eds.), *Biology of anxiety disorders* (pp. 177–222). Washington, DC: American Psychiatric Press.
- Holloway, W., & McNally, R. J. (1987). Effects of anxiety sensitivity on the response to hyperventilation. *Journal of Abnormal Psychology*, 96, 330–334.
- Holtz, K., Melzig, C. A., Hosten, N., & Hamm, A. O. (2006). Watching the fearful brain during anticipation of hyperventilation. *Journal of Psychophysiology*, 43, S47.
- Klein, D. F. (1993). False suffocation alarms, spontaneous panics, and related conditions. An integrative hypothesis. *Archives of General Psychiatry*, 50, 306–317.
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1998). Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. *Biological Psychiatry*, 44, 1248–1263.
- Lang, P. J., Davis, M., & Öhman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of Affective Disorders*, 61, 137–159.
- Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. D. (1981). *STAI State-Trait-Angstinventar*. Weinheim: Beltz.
- Liebowitz, M. R., Gorman, J. M., Fyer, A. J., Levitt, M., Dillon, D., Levy, G., Appleby, I. L., Anderson, S., Palij, M., Davies, S. O., & Klein, D. F. (1985). Lactate provocation of panic attacks. II. Biochemical and physiological findings. *Archives of General Psychiatry*, 42, 709–719.
- Iyken, D. T. (1971). Direct measurement of skin conductance: a proposal for standardization. *Psychophysiology*, 8, 656–672.

- Masaoka, Y., & Homma, I. (2000). The source generator of respiratory-related anxiety potential in the human brain. *Neuroscience Letters*, 283, 21–24.
- Masaoka, Y., & Homma, I. (2001). The effect of anticipatory anxiety on breathing and metabolism in humans. *Respiration Physiology*, 128, 171–177.
- McNally, R. J. (2002). Anxiety sensitivity and panic disorder. *Biological Psychiatry*, 52, 938–946.
- Melzig, C. A., Weike, A. I., Zimmermann, J., & Hamm, A. O. (2007). Startle reflex modulation and autonomic responses during anxious apprehension in panic disorder patients. *Psychophysiology*, 45, 846–854.
- Obrist, P. A. (1976). The cardiovascular-behavioral interaction – as it appears today. *Psychophysiology*, 13, 95–107.
- Peterson, R. A., & Reiss, S. (1992). *Anxiety Sensitivity Index Manual* (2nd ed.). Worthington, OH: International Diagnostic Systems.
- Rapee, R. M., & Medoro, L. (1994). Fear of physical sensations and trait anxiety as mediators of the response to hyperventilation in nonclinical subjects. *Journal of Abnormal Psychology*, 103, 693–699.
- Richerson, G. B. (2004). Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. *Nature Reviews. Neuroscience*, 5, 449–461.
- Schmidt, N. B., Lerew, D. R., & Jackson, R. J. (1997). The role of anxiety sensitivity in the pathogenesis of panic: prospective evaluation of spontaneous panic attacks during acute stress. *Journal of Abnormal Psychology*, 106, 355–364.
- Schmidt, N. B., Lerew, D. R., & Jackson, R. J. (1999). Prospective evaluation of anxiety sensitivity in the pathogenesis of panic: replication and extension. *Journal of Abnormal Psychology*, 108, 532–537.
- Spielberger, C. D. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologist Press.
- Wilhelm, F. H., Gerlach, A. L., & Roth, W. T. (2001). Slow recovery from voluntary hyperventilation in panic disorder. *Psychosomatic Medicine*, 63, 638–649.
- Wittchen, H. U., & Jacobi, F. (2005). Size and burden of mental disorders in Europe – a critical review and appraisal of 27 studies. *European Neuropsychopharmacology*, 15, 357–376.
- Wollburg, E., Meuret, A. E., Conrad, A., Roth, W. T., & Kim, S. (2008). Psychophysiological reactions to two levels of voluntary hyperventilation in panic disorder. *Journal of Anxiety Disorders*, 22, 886–898.

Manuscript 2

Brain activation during anticipation of interoceptive threat

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Brain activation during anticipation of interoceptive threat

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ABSTRACT

The current study investigated the neural networks activated during the anticipation of potentially threatening body symptoms evoked by a guided hyperventilation task in a group of participants reporting either high or low fear of unexplained somatic sensations. 15 subjects reporting high and 14 subjects reporting low fear of somatic symptoms first learned that one of two cues predicted the occurrence of a hyperventilation task reliably producing body symptoms in all participants that were rated as more intense and unpleasant in the high fear group. During anticipation of unpleasant symptoms, high fear participants reported more intense body symptoms and showed potentiation of the startle reflex. After this learning session, participants were taken into the fMRI where the same cues either predicted the occurrence of hyperventilation or normoventilation, although the task was never performed in the scanner. During anticipation of hyperventilation all participants showed an increased activation of anterior insula/orbitofrontal cortex and rostral parts of the dorsal anterior cingulate cortex/dorsomedial prefrontal cortex (dACC/dmPFC). Brain activation of high compared to low fear participants differed in two ways. First, high fear participants showed an overall stronger activation of this network during threat and safe conditions indexing stronger anxious apprehension during the entire context. Second, while low fear participants no longer responded with stronger activation to the threat cue after experiencing that the hyperventilation challenge did not follow this cue, high fear participants continued to show stronger activation of the network to this cue. Activation of the rostral dACC/dmPFC was significantly correlated with reported fear of somatic symptoms. These data demonstrate that anticipation of interoceptive threat activates the same network that has been found to be active during anticipation of exteroceptive threat cues. Thus, the current paradigm might provide an innovative method to study anxious apprehension and treatment effects in patients with panic disorder.

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Introduction

Cues that predict the occurrence of a nociceptive event (e.g., a moderately painful electric shock) reliably elicit a fear response characterized by increased autonomic arousal and defensive response mobilization as indexed by a potentiation of the startle reflex (Davis, 2000; Hamm and Vaitl, 1996; see for review Hamm and Weike, 2005). In humans, the pattern of fear responses elicited by such cues is identical irrespective of whether the individual directly learns that the cue is associated with the aversive event in a fear conditioning procedure or whether participants are just instructed that the nociceptive event will be presented during one cue (threat condition) but not during the other (safe condition) (Grillon et al., 1991; see for review Grillon and Baas, 2003). Findings from neuroimaging studies suggest that the neural networks involved in both fear conditioning and instructed

anticipation of nociceptive events might also be comparable. A conditioned stimulus that has previously been paired with an aversive unconditioned stimulus in a fear conditioning experiment consistently activates a neural network involving the amygdala, the insula, and the anterior cingulate cortex (ACC) (Büchel et al., 1998; LaBar et al., 1998; for a meta-analysis see Sehlmeier et al., 2009). Likewise, if participants are instructed that one of two cues might be followed by an electric shock or by aversive pictures, this warning cue also consistently activates the insular cortex and the ACC (e.g., Chua et al., 1999; Kalisch et al., 2006a; Nitschke et al., 2006; Phelps et al., 2001, 2004). While some studies also found increased activation of the amygdala (particularly in the left amygdala) during instructed fear (Nitschke et al., 2009; Phelps et al., 2001) this finding was not always reported in other studies (Mackiewicz et al., 2006; Nitschke et al., 2006). One reason for these discrepant results might be that amygdala activation is often attenuated during later trials of cued shock anticipation (Phelps et al., 2001).

In a recent meta-analysis of 15 instructed fear studies Mechias et al. (2010) found consistent larger activation to the warning cue in the bilateral anterior insulae but also in a larger cluster including the dorsal anterior cingulate cortex (dACC) and the rostral dorsomedial prefrontal cortex (dmPFC), which the authors interpreted as an

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index of increased threat appraisal of these warning cues. Interestingly, the same neural network is also activated if phobic student volunteers are instructed that a certain cue predicts the occurrence of the picture of a phobic object (Simmons et al., 2006; Straube et al., 2007). Stronger activation in the insular cortex was also found for patients with generalized social phobia during anticipation of public speaking (see Fredrikson and Furmark, 2003). Nitschke et al. (2009) found increased activation of the amygdala and the anterior cingulate cortex in patients with generalized anxiety disorder during anticipation of neutral and aversive pictures, suggesting that such anticipation of threat paradigms could be used as powerful tools to study neural networks of anticipatory anxiety in various anxiety disorder patients.

While these paradigms using anticipation of exteroceptive threat stimuli (such as moderate pain stimuli or symptom-specific aversive pictures or situations) might be useful to study patients with phobias or generalized anxiety disorders these external threat conditions are not suitable to study patients with panic disorder. In fact, if patients with panic disorder are instructed that one of two cues will be followed by a moderately aversive nociceptive stimulus panic disorder patients do show increased autonomic arousal and a clear potentiation of the startle reflex, but this cued fear response is not larger than the defensive mobilization observed in non-anxious control participants (Grillon et al., 1994, 2008; Melzig et al., 2007). Such findings may not come as an absolute surprise, because a second key feature of panic disorder—aside from recurrent panic attacks—is anxious apprehension directed at those body symptoms that are associated with panic attacks (see Bouton et al., 2001). Accordingly, Melzig et al. (2011) recently demonstrated that individuals reporting high fear to unexplained somatic sensations (scoring high on the Anxiety Sensitivity Index, Peterson and Reiss, 1992) showed a significant potentiation of the startle response during recovery from a hyperventilation challenge, i.e., a period during which various body symptoms were still perceived after the challenge. Moreover, even anticipation of such hyperventilation task results in an increase of autonomic arousal and a clear potentiation of the startle reflex in these panic prone individuals but not in those participants who scored low on this dimension (Melzig et al., 2008) providing a good model to study anticipatory anxiety in panic disorder patients.

The current study follows up on this research investigating the neural networks involved during the anticipation of such interoceptive threat cues. Surprisingly, there are only two previous fMRI studies that assessed neural responses during anticipation and provocation of panicogenic symptoms in healthy volunteers injecting the neuropeptide cholecystokinin in its tetrapeptide form (CCK-4) (Eser et al., 2009; Schunck et al., 2006). Pharmacological provocation of somatic symptoms resulted in a strong increase of reported panic symptoms compared to placebo injection and stronger activation in the ventral ACC, the insula, the cerebellum, and the temporal pole including the amygdala (Eser et al., 2009). No differences in brain activation were found in those volunteers who reported a panic attack during the pharmacological challenge and those who did not. Anticipation of CCK-4 injection resulted in a stronger activation of the dorsal ACC but no differences were found in neural responses between anticipation of CCK-4 and placebo, suggesting that the injection procedure might have been an aversive event irrespective of the pharmacological challenge (Eser et al., 2009). In the study by Schunck et al. (2006) anticipatory anxiety to CCK-4 could not be analyzed due to the limited number of high responders in this study. Thus instead of using a pharmacological challenge, the current study investigated anticipation of a hyperventilation challenge (to induce potentially threatening somatic symptoms) and used normoventilation as a non-aversive control condition. Moreover, we introduced the hyperventilation challenge outside of the scanner. So, the context of the scanner was not associated with the provocation of the panic symptoms, which may have produced aversive context conditioning that might have overridden the anticipation of a safe condition in the previous studies (like injection

of a placebo). Finally, to increase the probability of increased anticipatory anxiety to such interoceptive threat conditions we pre-selected our participants so that half of our sample reported high fear to unexplained somatic sensations as assessed by the Anxiety Sensitivity Index (ASI; Peterson and Reiss, 1992) and the Body Sensations Questionnaire (BSQ; Chambless et al., 1984) while the other half of the sample scored low on these dimensions.

Materials and methods

Participants

About 250 students of the University of Greifswald were screened with a German version of the Anxiety Sensitivity Index (ASI; Peterson and Reiss, 1992). Those persons 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 criteria: subjects should not be in treatment for psychological disorders and had to be free of any cardiovascular or respiratory diseases and seizure disorders. After this screening procedure 29 subjects agreed to participate in the study. From these individuals 15 participants (9 females) reported high fear of unexplained somatic sensations and 14 participants (10 females) reported low fear of body symptoms. Both groups were comparable with regard to their age M (Range) 23.6 (18–42) vs. 23.9 (19–37), $t(27) = 0.16$, $p = .88$, and gender distribution, $\chi^2 = 0.42$, $p = .52$.

Participants completed several questionnaires for a more detailed sample characterization: the trait portion of the State-Trait Anxiety Inventory (STAI; Spielberger, 1983; German version: Laux et al., 1981), the Agoraphobic Cognitions Questionnaire (ACQ; Chambless et al., 1984; German version: Ehlers et al., 1993a), and the Body Sensations Questionnaire (BSQ; Chambless et al., 1984; German version: Ehlers et al., 1993b). Participants reporting high fear of unexplained somatic sensations in the ASI and BSQ also reported greater trait anxiety and more agoraphobic cognitions (see Table 1), replicating previous findings (Melzig et al., 2009, 2011) also indicating that this analog sample parallels patients with panic disorder in their pattern of reported concerns (see McNally, 2002).

Stimulus materials

Two different colored slides were projected to a screen (1.50 × 1.30 m) in front of the subjects. A red slide indicated a threat condition (a hyperventilation task might follow—interoceptive threat), a green slide indicated safety (no hyperventilation task will follow—safety). In order to maintain the inherent warning signal of red and to facilitate the association between the warning cue and the possible hyperventilation, colors were not randomized across subjects.

Hyperventilation task

The hyperventilation task was introduced as a “fast breathing exercise” that could induce somatic sensations like palpitations, sweating, dizziness, or feeling faint, but that these symptoms would disappear

Table 1
Means and standard errors of self report measures for participants reporting high and low fear of unexplained somatic symptoms.

Questionnaire	High fear	Low fear	t-score	Significance (p)
ASI (0–64)	36.0 (1.8)	7.8 (0.6)	14.9	<.001
BSQ (1–5)	2.5 (0.1)	1.7 (0.1)	4.1	<.001
STAI-Trait (20–80)	47.2 (3.0)	29.1 (1.9)	5.1	<.001
ACQ (1–5)	1.9 (0.1)	1.3 (0.1)	4.6	<.001

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

when the breathing returned to normal. During the 3 min hyperventilation task, tones of rising and falling pitch were presented via headphones prompting the participants to breathe in with rising and breathe out with falling pitch of the tone (see Melzig et al., 2008, 2011). Participants were thus led to breathe at a respiratory rate of 20 breaths per minute (bpm). 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 (Nellcor Puritan Bennett, Pleasanton, CA) to ensure compliance with the hyperventilation procedure. To ensure that the hyperventilation task was executed properly and hypocapnia was obtained to provoke physical symptoms in all participants, visual feedback (different colored LEDs attached under the screen) was used to instruct the participant to breathe deeper (red LED) until a target $p_{\text{et}}\text{CO}_2$ of 20 mm Hg was reached. Visual feedback (green LED signaled to “breathe more shallow”, no LED signaled to “keep breathing at a constant depth”) was also used to keep the low level of $p_{\text{et}}\text{CO}_2$ during the entire hyperventilation task.

Startle stimulus

A 50 ms burst of white noise (95 dB[A] (rise/fall time < 1 ms) was generated by a Coulbourn S81-02 noise generator (Coulbourn Instruments, Whitehall, PA) and presented binaurally via AKG K-66 headphones.

Symptom ratings

Participants rated the severity of the 14 panic symptoms, as listed in the DSM-IV (American Psychiatric Association, 1994) on a 10-point scale ranging from 1 (not at all) to 10 (very intense) experienced (a) during the hyperventilation task and (b) during the anticipation period. Intensity of anxiety and tension experienced during hyperventilation and anticipation was also rated on a 10-point scale ranging from 1 (not at all) to 10 (very intense). Finally, participants rated how unpleasant they felt during the hyperventilation and during viewing of the red and the green anticipation slide again using the same 10-point scale.

Procedure

During the first part of the study a learning session was conducted in which participants went through three guided hyperventilation tasks. The learning session started with the presentation of the red warning slide for 3 min followed by the first hyperventilation task as described above followed by a 3 min recovery period. After recovery, participants were instructed to rate the intensity of anxiety, tension, unpleasantness, and severity of symptoms during the hyperventilation task. Then, the green slide was presented for 3 min signaling the safe condition followed by a 3 min recovery period. Afterwards, participants rated symptom severity and feeling states during anticipation of threat (red slide) and safety (green slide). Following this learning phase participants were instructed that the colored slides were now presented for a shorter duration and that a hyperventilation task might follow after the red slide (instructed interoceptive threat condition) and that the green slide will definitely not be followed by a hyperventilation task. The red (R) and green (G) slides were presented for 18 s six times each, alternating with a 15 s presentation of a fixation cross in two different pseudorandomized orders, (order 1: GRRGRGRRGRR; order 2: RGGRRGRRGRR), randomly assigned to the subjects and balanced out within and between the groups. Startle probes were presented either at 3, 8, or 11 s after the onset of the red and green slide (overall 12 startle probes were delivered). The 3 min hyperventilation task was introduced after the third and the sixth presentation of the red slide resulting in two additional hyperventilation tasks in this session. Again, each hyperventilation was followed by a 3 min recovery period after which participants rated the severity of the panic symptoms and anxiety intensity experienced during the preceding hyperventilation task. Ratings of symptom severity and anxiety intensity during anticipation of threat and safety were obtained at the end of the entire procedure. The upper panel of Fig. 1

shows the $p_{\text{et}}\text{CO}_2$ levels during the three hyperventilation tasks, demonstrating that all participants included in the analysis were compliant with this procedure (see Fig. 1A) and even slightly improved with the repetition of the task. The lower panel of Fig. 1 depicts the rated intensity of the experienced panic symptoms during the hyperventilation procedure (see Fig. 1B), indicating that participants with high fear of unexplained somatic symptoms reported more severe panic symptoms than low fear participants. These group differences remained stable across all three hyperventilation tasks.

After this procedure subjects participated in the fMRI experiment at the Functional Imaging Unit of the Center for Diagnostic Radiology at the University of Greifswald as soon as the scanner was available (on average within the same month). After receiving a detailed introduction to the method of the magnetic resonance imaging (MRI), participants were again instructed that, paralleling the previous session outside the scanner, the red slide will signal that the hyperventilation task might follow while the green slide indicated safety (definitely no hyperventilation task). Participants were provided with the nasal cannula that was used to measure expired CO_2 in the previous session and were instructed that the rising and falling tone would be presented via loudspeakers to maximize the credibility that the red cue would indeed be followed by the hyperventilation task. In fact, the hyperventilation task was never performed in the scanner.

After these instructions, and signing informed consent, subjects rated their current tension and anxiety prior to the experiment on an 11-point scale ranging from 0 (no anxiety/tension at all) to 10 (extremely strong anxiety/tension).

Participants were then placed in the fMRI scanner. The experiment started with anatomical scans that took about 6 min. Each section of

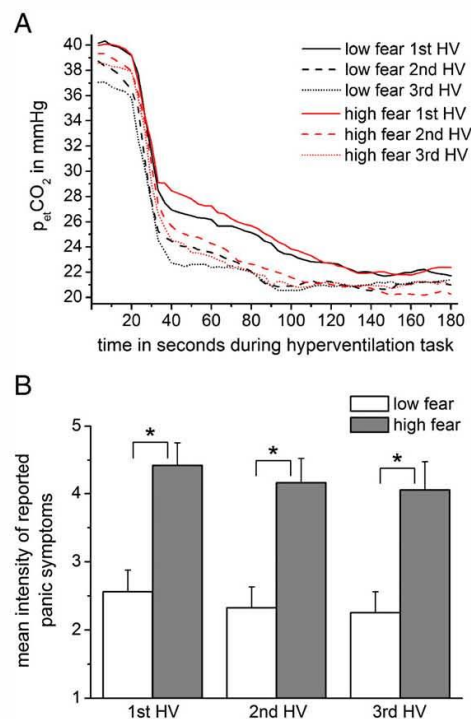


Fig. 1. A. $p_{\text{et}}\text{CO}_2$ during the three hyperventilation tasks. Course of $p_{\text{et}}\text{CO}_2$ during 3 min of hyperventilation (HV). Hyperventilation task 1, 2, and 3 in the low fear and high fear group. B. Reported panic symptoms during the three hyperventilation tasks. Mean intensities and standard errors of reported panic symptoms during hyperventilation task 1, 2, and 3 in the low fear and high fear group, * indicates a significant high vs. low fear group difference, all $ps < .01$.

the experiment was introduced via intercom that allowed the experimenter to talk to the participant while he or she was lying in the scanner. The anatomical scans were followed by the experiment starting with the presentation of a fixation cross followed by the red (R) and green (G) slides for 18 s each, alternating with a 15 s presentation of a fixation cross exactly in the same order as during the learning session (except that the hyperventilation task did not follow the third and the last red slide). The slides were presented via a projector onto a screen outside the scanner that was placed about 2 m in front of the participants' eyes. Subjects looked at the screen by help of a mirror system in the scanner. The session ended with recording of 3D anatomical scans that took about 10 min. After leaving the scanner, participants again rated their anxiety and tension during the scanning procedure as well as after the scanning.

Before leaving the laboratory all participants were informed that the study was aimed at investigating whether fear of unexpected somatic sensations would be associated with the activation of specific neural networks.

Apparatus and data acquisition

To record the eyeblink component of the startle response, two electrolyte filled Ag/AgCl miniature surface electrodes (Marquette Hellige, Freiburg, Germany; Sensormedics, Yorba Linda, CA) measured the electromyographic activity (EMG) over the orbicularis oculi muscle beneath the left eye. The raw EMG signal was amplified by a Coulbourn S75-01 amplifier using a 30 Hz high-pass filter and a Kemo KEM-VBF8-03 400 Hz low-pass filter (Kemo Limited, Beckenham, UK) and further digitized at 1000 Hz using a 12-bit A/D converter. Digital sampling lasted from 100 ms before until 400 ms after the onset of the acoustic startle stimulus. Before the scoring procedure started, a digital 60 Hz high-pass filter was applied to the raw EMG data off-line to remove eye movement artifacts.

MRI data were acquired using a 1.5 Tesla Magnetom Symphony system (Siemens, Erlangen, Germany) that was additionally equipped with an 8-channel headcoil. Prior to each session, field homogeneity was optimized by using a shimming sequence. After aligning the images in a transversal direction parallel to the AC–PC line on the basis of a localizer scan a T1-weighted anatomical volume (TE 4.88 ms, flip angle 40°, FoV 192 mm, matrix 256 × 256, voxel size 1 × 1 × 1 mm) was recorded. Then, the stimulus presentation was started, during which 137 volumes with 33 slices each (3 mm thick, 0.75 mm gap) were acquired using echo-planar images (EPIs; TR 3000 ms, TE 50 ms, flip angle 90°, FoV 192 mm, matrix 64 × 64, voxel size 3 × 3 × 3 mm).

Data processing and analysis

The raw orbicularis oculi EMG was integrated off-line with a time constant of 10 ms and reflex eyeblinks were then scored using a computer program (Globisch et al., 1993) that identified the latency of blink onset in ms and peak amplitude in μ V. All blinks occurring between 20 and 100 ms after startle probe onset and reaching their peak amplitude within 150 ms were scored as valid startle response trials. Trials showing clear movement artifacts or excessive baseline activity were rejected and defined as missing trials. If no response could be detected in the defined time window, the trial was scored as zero magnitude. Digital values were converted into μ V. Baseline group comparisons of overall reactivity were conducted using the raw startle magnitudes. Blink magnitudes were standardized to correct for interindividual variability that was unrelated to the experimental conditions before statistical analyses of the effects of interoceptive threat were performed (as suggested by the guidelines for human startle eyeblink electromyographic studies, Blumenthal et al., 2005). This transformation could ensure an equal contribution of each participant to the analysis of the experimental conditions. Responses of 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 then converted into t-scores, i.e. $50 + [z \times 10]$.

For the statistical analyses, a mixed-model analysis of variance (ANOVA) was applied for verbal report and startle eyeblink measures. For all analyses, Condition (anticipation of threat vs. safety) was entered as a within-subjects factor and Group (low vs. high fear participants) as a between-subjects factor. For the analysis of startle eyeblink, Block (first vs. second half of the session) was entered as an additional within-subjects factor. Post-hoc t-tests were applied whenever a significant main effect was present. All statistical tests used a significance level of $p < .05$ and were accomplished using SPSS 19.0.

The fMRI data were preprocessed and analyzed using the statistical parametric mapping software (SPM5, Wellcome Department of Imaging Neuroscience, London, UK). The preprocessing included spatial realignment, co-registration, normalization into the MNI space, and spatial smoothing (FWHM 12 mm). A high-pass filter with a cut off of 128 s was used to correct for low-frequency components. Statistical analyses were performed using the general linear model (as implemented in SPM5). On the first level, for each participant a design matrix was created using a canonical hemodynamic response function for the following conditions: threat and safety, each divided into two blocks (first half of the experiment: mean of the red/green slides 1 to 3—and second half: mean of the red/green slides 4 to 6), as well as the fixation cross period which all together constituted the regressors. The six movement parameters estimated during the realignment were introduced into the model as covariates to control for variance caused by head displacements. This resulted in the output of 12 beta-estimates (4 threat conditions (threat and safe periods each divided into first and second half), 1 fixation cross period, 6 movement parameters, 1 constant). Beta estimates of the 4 threat conditions, separately for low and high fear groups, were then taken to the second level full factorial model.

Contrast maps were created on the basis of simple t-contrasts (threat > safe; safe > threat) that were then analyzed for the entire sample. After the whole brain analysis, small volume corrected analyses were conducted for the regions of interest (ROI) (anterior insula, OFC, ACC, dmPFC) using masks provided by the "Automated Anatomical Labeling" software (AAL; Tzourio-Mazoyer et al., 2002). The statistical threshold was set to $p < 0.05$ (family wise error (FWE) corrected; Brown and Russell, 1997). MNI-coordinates of the highest activated voxels are reported in the Results section. For the between group analyses (high > low fear) and analyses regarding differences between the blocks (threat > safe first half; threat > safe second half of the experiment), parameter estimates (betas of the full factorial model, 10 mm sphere) were extracted to SPSS 19.0 (SPSS for Windows, SPSS Inc.).¹ Possible relations of personality traits (questionnaire scores) and brain activation were investigated using bivariate Pearson correlations (.05 level of significance). ANOVAs and t-tests to check for group differences and effects in subjective report data were also accomplished using SPSS 19.0. In addition to the factors introduced in the analysis of startle response and symptom reports, the factor Time was entered as a within-subjects factor in the analysis of verbal anxiety and tension reports concerning the scanning procedure.

Results

First session

Verbal report measures

High fear participants reported more severe symptoms, higher anxiety, tension, and unpleasantness during hyperventilation

¹ fMRI results concerning the factors group and block as well as correlation analyses are based on the parameter estimates extracted to SPSS (10 mm sphere). Thus, these results always refer to the corresponding sub-region within the specific ROI.

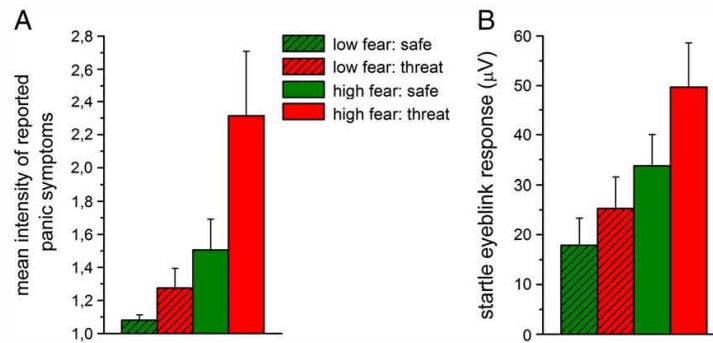


Fig. 2. A/B. Reported panic symptoms and startle eyeblink responses during the anticipation periods. Means and standard errors of the intensity of reported panic symptoms (A) and of the startle eyeblink responses (B) during the anticipation of safety vs. interoceptive threat in the low fear and high fear groups.

compared to low fear participants, Group, $F(4, 24) = 5.87, p < .01$. During anticipation of body symptoms, all participants reported more severe panic symptoms, higher anxiety, tension, and unpleasantness than during the anticipation of safety, Condition, $F(4, 24) = 29.15, p < .001$. Again, high fear participants reported overall more anxiety than participants with low fear, Group $F(4, 24) = 5.63, p < .01$. All means and statistical analyses of the verbal report data can be obtained from Supplementary Table 1.

Startle response magnitudes

Startle response magnitudes were significantly potentiated when elicited during viewing of threat compared to safety signals, Condition, $F(1, 27) = 17.04, p < .001$. Moreover, startle response magnitudes were significantly larger for high compared to low fear persons, Group, $F(1, 27) = 4.64, p < .05$. These group differences were more pronounced during the anticipation of interoceptive threat, $t(27) = 2.23, p < .05$, and only marginally significant during safety periods, $t(27) = 1.90, p = .07$ (see Fig. 2B).

fMRI session

Verbal report measures

High fear participants rated the scanner environment as more aversive than low fear subjects, Group, $F(1, 26) = 31.33, p < .001$. Tension ratings, as well as anxiety ratings were significantly higher for high fear compared to low fear participants prior to the fMRI experiment² (tension: $M (SD) = 4.1 (2.2)$ vs. $1.1 (1.3)$; anxiety: $M (SD) = 3.1 (2.2)$ vs. $0.7 (1.2)$ for high and low fear participants, respectively, $t(26) = 4.54, p < .001$ and $t(26) = 3.60, p < .01$, for tension and anxiety ratings respectively) and during the scanning procedure (tension: $M (SD) = 5.7 (2.3)$ vs. $1.5 (1.5)$; anxiety: $M (SD) = 3.9 (2.5)$ vs. $0.8 (1.5)$ for high and low fear subjects, respectively, $t(26) = 5.76, p < .001$ and $t(26) = 3.94, p = .001$, for tension and anxiety ratings respectively). Tension and anxiety ratings significantly decreased after the scanning procedure, particularly in the high fear group (tension: $M (SD) = 1.1 (1.0)$ vs. $0.2 (0.6)$; anxiety: $M (SD) = 0.6 (1.1)$ vs. $0.1 (0.3)$ for high and low fear participants, respectively, Time, $F(2, 52) = 41.69, p < .001$.

fMRI data

Whole brain analysis

Fig. 3 depicts activated voxels for the contrast threat > safe in the whole brain analysis for the entire sample. As expected, increased activation during processing of cues predicting interoceptive threat was

observed in clusters that comprised those areas that were defined as regions of interest (see Table 2). The only significant activation going beyond the regions of interest in the whole brain analysis was a cluster in the right supramarginal/angular gyrus.

ROI analyses

ROI analyses supported the whole brain analysis. Processing of the cue that predicted the occurrence of the hyperventilation task was associated with significantly increased activations in the anterior insula/orbitofrontal region (OFC) and rostral parts of the dorsal anterior cingulate cortex/dorsomedial prefrontal cortex (dACC/dmPFC) when compared to the activation elicited during processing of the safety cue (see Table 3).

Between group comparisons

Participants with high fear of somatic symptoms showed overall stronger activation in the right anterior insula/OFC during processing of the threat cue than did persons with low fear of somatic symptoms (all MNI coordinates as presented in Table 3; anterior insula/OFC R: Group, $t(27) = 2.38, p < .05$. Activation was also stronger in the anterior insula/OFC L: Group, $t(27) = 1.95, p = .06$ and rostral dACC/dmPFC: Group, $t(27) = 1.71, p = .10$, marginally significant). High fear participants also showed stronger activation in this network during processing of the safety cue compared to low fear participants (anterior insula/OFC R: Group, $t(27) = 2.18, p < .05$, n.s. in anterior insula/OFC L and rostral dACC/dmPFC). Due to this increased activation during processing of the safety cue in the high fear participants the overall between group comparison of the activation differences between threat and safety cues was not significant. Fig. 4A illustrates these overall between group effects in the different regions of interest.

During the learning session the hyperventilation task was delivered after the third threat cue. During the session in the scanner the third cue was not followed by the hyperventilation task. Therefore the activation during the first three trials of threat and safety cues (block 1) was compared with the last three trials (block 2). Increased activation during processing of the threat cues compared to the safety cues was overall significantly larger during the first block compared to the second block, Condition \times Block, $F_s(1, 27) = 8.37, 14.84$, all $p_s < .05$, for the anterior insula/OFC L and rostral dACC/dmPFC, respectively. This pattern of activation, however, differed between groups. While low fear participants showed significant conditioning effects in these ROIs only consistently during block 1 of the experiment, but not during block 2, high fear participants continued to show increased activation of this network during processing of the threat cue throughout the entire experiment (see statistics in Table 4). Fig. 4B illustrates these group differences in the activation pattern for the rostral dACC/dmPFC.

² One low fear person had to be excluded from the analyses due to missing data.

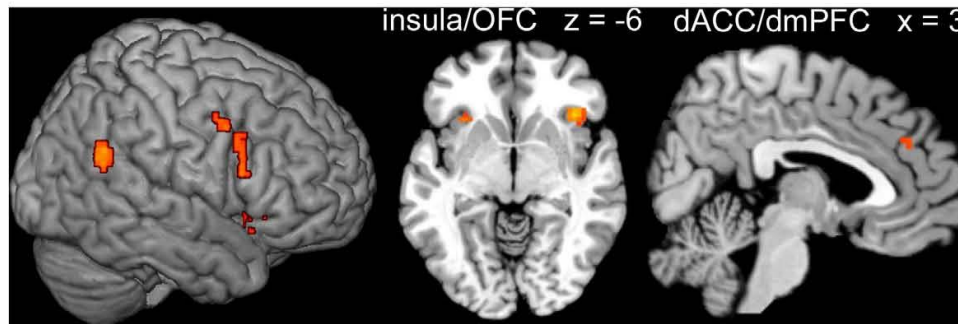


Fig. 3. Whole brain analysis of the contrast threat > safe. Whole brain analysis activations for the contrast threat > safe in the whole sample ($p_{FWE} = .05$). Activation foci are overlaid on a T1 reference brain (position of the slice indicated above the image).

Correlation analyses

Correlation analyses between reports of fear of somatic symptoms as assessed by the BSQ and potentiation of activation during processing of the threat compared to the safety cues showed strongest associations between reported fear of somatic symptoms and increase in activation in the rostral dACC/dmPFC. As depicted in Fig. 5, the increase in activation (threat–safe) in the rostral dACC/dmPFC was significantly correlated with the participants' score in the Body Sensations Questionnaire, $r_{\text{Pearson}} = .492$, $p < .01$. Moreover, a significant correlation between the BSQ scores and the increase in activation (threat–safe) in the anterior insula was observed during the second block of the experiment, $r_{\text{Pearson}} = .387$, $p < .05$.

Discussion

The present study investigated the neural network activated during anticipation of a hyperventilation task. We found increased activations of the anterior insula/OFC and the rostral dACC/dmPFC during processing of cues that predicted the occurrence of a hyperventilation task that reliably evoked somatic symptoms in all participants. Increased activation of this neural network, however, was more sustained in high fear compared to low fear persons. High fear participants also showed clear fear potentiated startle and reported more severe panic symptoms during anticipation of body symptoms. Moreover, these individuals reported higher tension prior to and during the fMRI session. Correlation analyses supported the association between the fear of somatic symptoms—as assessed

by questionnaires—and the increased activation of the neural network, comprising the rostral dACC/dmPFC and the anterior insula.

Neural network activated during the anticipation of interoceptive threat

Cues that predicted the occurrence of a hyperventilation task activated a neural network involving the anterior insula/orbitofrontal cortex and rostral parts of the dorsal anterior cingulate cortex/dorsomedial prefrontal cortex. Increased activations of the dACC/dmPFC and the insula have formerly consistently been found during anticipation of electric shock or aversive pictures (for review see Mechias et al., 2010), suggesting that anticipation of unpleasant somatic symptoms activates the same neural network that is activated during the anticipation of exteroceptive threat. Interestingly, no increased activation of the amygdala was found during the anticipation of interoceptive threat. In the current experiment, participants were instructed that a hyperventilation challenge would follow one but not the other cue, and increased activation of the amygdala have not consistently been found under these instructed fear conditions. Accordingly, while activation of the amygdala has been reported during the pharmacological challenge (Eser et al., 2009), no amygdala activation was found during the anticipation of injection of CCK-4 in this study. Together with the data of the current experiment this pattern of results supports recent learning theory perspectives on the etiology of panic disorder that clearly separates between panic attacks as an acute fight–flight response (presumably involving the activation of the amygdala) and the emotional state anticipatory anxiety characterized by hypervigilance of somatic symptoms and anxious apprehension (Bouton et al., 2001; Mineka and Zinbarg, 2006). Moreover, increased activation of the insular cortex has been associated with increased awareness of somatic symptoms (Critchley et al., 2004). Accordingly, increased activation in the anterior insula during anticipation of hyperventilation would also support the hypothesis of increased hypervigilance during anxious apprehension of unpleasant somatic symptoms.

In the context of instructed fear paradigms Mechias et al. (2010) reported activation in the dmPFC, as a region involved in the

Table 2

Significant activations during threat vs. safe condition in the whole sample (whole brain analysis) with MNI coordinates and cluster size (k_E).

Region	Side	MNI coordinates			t-score	k_E	p_{FWE}
		x	y	z			
<i>Inferior frontal gyrus</i>							
OFC/	R	39	24	−9	8.55	70	<.001
Insula ^a		45	21	3	7.57		=.001
Operculum	R	57	18	18	6.78	18	<.01
		54	18	27	6.43		<.05
dIPFC	R	48	9	39	6.77	10	<.01
Insula	L	−30	21	−6	6.69	6	.01
<i>Medial frontal gyrus</i>							
ACC/mPFC		3	42	30	6.21	5	<.05
<i>Supramarginal gyrus</i>							
Angular gyrus	R	54	−51	27	7.49	29	=.001

OFC, orbitofrontal cortex; dIPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex; mPFC, medial prefrontal cortex; L, left; R, right; (x, y, z), MNI coordinates of maximally activated voxel (whole brain analysis p_{FWE}).

^a Maximally activated voxel lies close to ROI.

Table 3

Significant activations during threat vs. safe condition in the whole sample (ROI analyses).

Region	Side	MNI coordinates			t-score	p_{FWE}
		x	y	z		
Anterior insula/OFC	L	−33	21	−6	6.56	<.001
	R	39	21	0	7.02	<.001
Rostral dACC/dmPFC		3	42	27	6.09	=.001

OFC, orbitofrontal cortex; dACC, dorsal anterior cingulate cortex; dmPFC, dorsomedial prefrontal cortex; L, left; R, right; (x, y, z), MNI coordinates of maximally activated voxel (small volume corrected p_{FWE}).

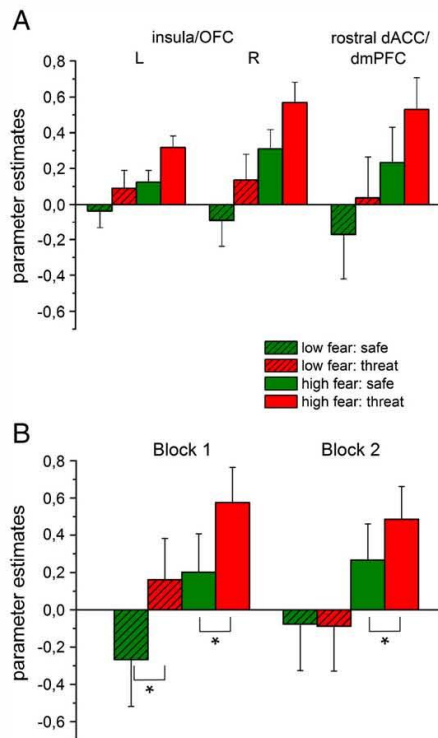


Fig. 4. A. Activation in ROIs during safe and threat conditions. Hemodynamic responses in the regions of interest (ROIs) during threat and safe conditions in high and low fear participants. Mean parameter estimates for both groups were derived from the clusters (10 mm sphere) of maximum activation within the ROIs for the contrast threat > safe in the complete sample. B. Activation in rostral dACC/dmPFC during safe and threat conditions—first vs. second block of the experiment. Hemodynamic responses in the rostral dACC/dmPFC during threat and safe conditions in high and low fear participants during block 1 vs. block 2 of the experiment. Mean parameter estimates derived as for Fig. 4A. * indicates a significant threat vs. safe difference, all $p < .05$.

conscious appraisal of threat and the cognitive regulation of own emotional behavior (Ochsner et al., 2004). Especially the region around rostral dACC/dmPFC has been found to be activated during anticipatory anxiety in several studies (Kalisch et al., 2005, 2006b). The peak activation at 3/42/27 very closely corresponds to the coordinates reported by Kalisch et al. (2005, 2006a) that were derived from different imaging studies on anticipatory anxiety (Kalisch et al., 2006b; Mechias et al., 2010; Raczka et al., 2010). In line with this research, high fear participants displayed increased activation in the rostral dACC/dmPFC during a task in which unpleasant body symptoms were anticipated.

The increased activation of the right supramarginal gyrus has previously been reported by Schienle et al. (2005) who presented fear-relevant stimuli to patients with obsessive-compulsive disorder. In

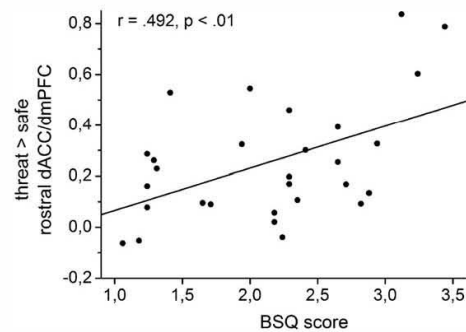


Fig. 5. Correlation between BSQ and rostral dACC/dmPFC. Correlation of BSQ score and rostral dACC/dmPFC activation (mean parameter estimates from the cluster (10 mm sphere) of maximum activation during threat > safe).

the current study, the stronger activation in the supramarginal gyrus during threat but not during safety might be related to the activation of a motor program that is involved in the action tendency to escape. The activation of a neural network that is typically found during anticipation of aversive events (Chua et al., 1999; Nitschke et al., 2006; Straube et al., 2007) is in line with this interpretation.

High vs. low fear of somatic symptoms

Brain activation of participants reporting high fear of somatic symptoms differed from the activation of participants with low fear in two ways. First participants reporting high fear of somatic symptoms showed overall stronger startle potentiation and stronger activations in the anterior insula/OFC and the rostral dACC/dmPFC both during the threat and (in insula/OFC) the safe conditions compared to the controls. These data suggest that these participants showed overall higher anxious apprehension in the experimental context compared to the low fear group. The verbal report data are in line with such an interpretation. High fear participants reported more tension and anxiety prior to and during the experimental procedures. Thus, although the activation of the anterior insula/OFC and the rostral dACC/dmPFC was stronger during anticipation of hyperventilation as compared to the safe condition in both groups, high fear participants showed an overall sensitization effect. These data are in line with a recent study from Nitschke et al. (2009) who found that patients with generalized anxiety disorder showed generally increased ACC activation during the anticipation of both aversive and neutral pictures, suggesting the same sensitizing effect of the context in this group of patients. Furthermore, those patients who showed the strongest pretreatment activation in this area also showed the strongest reduction in reported anxiety and worries after treatment.

Second, low fear participants showed modest startle potentiation and stronger activation of the network described above during processing of the threat compared to the safety cue in the first block (trials 1–3) suggesting that the hyperventilation task might also be slightly aversive for low fear participants. Indeed the quite strenuous hyperventilation

Table 4
Analyses of threat vs. safe conditions during first and second half of the experiment (Block 1 vs. Block 2).

Region	Condition × Block				Block 1: threat > safe				Block 2: threat > safe			
	Low fear		High fear		Low fear		High fear		Low fear		High fear	
	F	p	F	p	t	p	t	p	t	p	t	p
Ant insula/OFC L	13.69	<.01	0.90	=.36	4.89	<.001	3.75	<.01	0.42	=.68	2.64	<.05
R	5.30	<.05	0.00	=.96	4.45	=.001	3.14	<.01	3.81	<.01	3.06	<.01
Rostral dACC/dmPFC	16.75	=.001	1.98	=.18	5.92	<.001	3.82	<.01	−0.20	=.84	2.42	<.05

Ant insula, anterior insula; OFC, orbitofrontal cortex; dACC, dorsal anterior cingulate cortex; dmPFC, dorsomedial prefrontal cortex; L, left; R, right. MNI coordinates as reported in Table 3.

challenge was designed to induce somatic symptoms in all participants, and low fear participants also experienced unpleasant somatic symptoms. Moreover, previous data from our laboratory clearly showed that low fearful participants also report experiencing unpleasant somatic symptoms—albeit less intense than high fear participants—during the anticipation of the hyperventilation challenge (Melzig et al., 2008). After the third presentation of the threat cue, activation during threat no longer differed from that evoked by the safe cue suggesting that anticipatory anxiety waned in low fearful participants. In contrast, the high fear group continued to show fear potentiated startle and stronger activation during processing of the threat cue in the anterior insula/OFC and the rostral dACC/dmPFC. Anxious apprehension of somatic symptoms during the threat cue was maintained even though participants did not experience any further hyperventilation challenge. These data again support clinical data from Grillon and colleagues who found that patients with panic disorders show an overgeneralization of their defensive response mobilization and a reduction in safety learning (Grillon et al., 2008; Lissek et al., 2010).

Although the breathing patterns of high and low fear participants were identical during the hyperventilation challenge high fear participants reported more intense panic symptoms during this task, suggesting that high fear participants evaluated these somatic symptoms as much more threatening. The increased co-activation of the anterior insula/OFC and the rostral dACC/dmPFC during the anticipation of such symptoms in high fear participants might index the neural network involved in such appraisal process. Correlation analyses support such interpretation.

Correlations between brain activation and self report measures

Scores in the BSQ—a questionnaire that measures the person's anxiety concerning somatic symptoms and how much these bodily symptoms are interpreted as threatening—correlated significantly with an increase in activation during threat compared to safe in the rostral dACC/dmPFC. The rostral dmPFC is consistently activated during instructed fear paradigms suggesting that this area might be involved in the conscious appraisal of threat (see Mechias et al., 2010). Participants who evaluated somatic symptoms as more threatening showed stronger activation of this region during processing of cues that predicted the occurrence of such symptoms. Moreover, positive correlations between the BSQ scores and the anterior insula during the second half the experiment also support previous data from Melzig et al. (2008), demonstrating that participants with high fear of somatic symptoms also show increased autonomic arousal as indexed by elevated skin conductance responses during the anticipation of such symptoms.

Conclusions

In the current experiment, participants learned that one of two cues was associated with a hyperventilation challenge that successfully evoked unpleasant somatic symptoms in all participants. Presenting these cues later, in a scanner environment, to the same individuals reliably activated a neural network in the brain including the anterior insula/OFC and the rostral dACC/dmPFC, a network that has been reported frequently in other instructed fear studies using exteroceptive aversive stimuli. Although the anticipation of unpleasant somatic symptoms activated this network in all participants, high fear persons showed an overall stronger activation in this network, indicating an increased sensitization probably evoked by the unpleasant experimental environment. The increased anxiety and tension ratings obtained from these participants support such interpretation. Moreover, high fear participants maintained the increased activation during anticipation of the somatic symptoms throughout the entire experiment despite that the hyperventilation challenge was no longer required, suggesting that increased anxious apprehension of somatic symptoms is associated with

the activation of this network. The significant correlation of rostral dACC/dmPFC activation, that plays a major role in conscious threat appraisal and the explicit evaluation of threat (Etkin et al., 2011; Mechias et al., 2010; Raczka et al., 2010), with a trait measure of fear of somatic symptoms substantiates the assumption that the current paradigm might be an innovative method to study anxious apprehension of unpleasant somatic sensations in clinical samples like patients with panic disorder or somatoform disorders.

Supplementary materials related to this article can be found online at doi:10.1016/j.neuroimage.2012.03.019.

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References

- American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Press, Washington, DC.
- Blumenthal, T.D., Cuthbert, B.N., Filion, D.L., Hackley, S., Lipp, O.V., van Boxtel, A., 2005. Committee report: guidelines for human startle eyeblink electromyographic studies. *Psychophysiol.* 42 (1), 1–15.
- Bouton, M.E., Mineka, S., Barlow, D.H., 2001. A modern learning theory perspective on the etiology of panic disorder. *Psychol. Rev.* 108 (1), 4–32.
- Brown, B.W., Russell, K., 1997. Methods of correcting for multiple testing: operating characteristics. *Stat. Med.* 16 (22), 2511–2528.
- Büchel, C., Morris, J., Dolan, R.J., Friston, K.J., 1998. Brain systems mediating aversive conditioning: an event-related fMRI study. *Neuron* 20 (5), 947–957.
- Chambless, D.L., Caputo, G.C., Bright, P., Gallagher, R., 1984. Assessment of fear in agoraphobia: the body sensations questionnaire and the agoraphobic cognitions questionnaire. *J. Consult. Clin. Psychol.* 52 (6), 1090–1097.
- Chua, P., Krams, M., Toni, I., Passingham, R., Dolan, R., 1999. A functional anatomy of anticipatory anxiety. *Neuroimage* 9 (6 Pt 1), 563–571.
- Critchley, H.D., Wiens, S., Rotshtein, P., Ohman, A., Dolan, R., Raymond, J., 2004. Neural systems supporting interoceptive awareness. *Nat. Neurosci.* 7 (2), 189–195.
- Davis, M., 2000. The role of the amygdala in conditioned and unconditioned fear and anxiety. In: Aggleton, J.P. (Ed.), *The Amygdala*. Oxford University Press, Oxford, UK, pp. 213–287.
- Ehlers, A., Margraf, J., Chambless, D., 1993a. ACQ Fragebogen zu angstbezogenen Kognitionen. Beltz, Weinheim.
- Ehlers, A., Margraf, J., Chambless, D., 1993b. BSQ Fragebogen zur Angst vor körperlichen Symptomen. Beltz, Weinheim.
- Eser, D., Leicht, G., Lutz, J., Wenninger, S., Kirsch, V., Schüle, C., Karch, S., et al., 2009. Functional neuroanatomy of CCK-4-induced panic attacks in healthy volunteers. *Hum. Brain Mapp.* 30 (2), 511–522.
- Etkin, A., Egner, T., Kalisch, R., 2011. Emotional processing in anterior cingulate and medial prefrontal cortex. *TICS* 15 (2), 85–93.
- Fredrikson, M., Furmark, T., 2003. Amygdaloid regional cerebral blood flow and subjective fear during symptom provocation in anxiety disorders. *Ann. N. Y. Acad. Sci.* 985, 341–347.
- Globisch, J., Hamm, A.O., Schneider, R., Vaitl, D., 1993. A computer program for scoring reflex eyeblink and electrodermal responses written in PASCAL. *Psychophysiology* 30 (S1), S13.
- Grillon, Christian, Baas, J., 2003. A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clin. Neurophysiol.* 114 (9), 1557–1579.
- Grillon, C., Ameli, R., Woods, S.W., Merikangas, K., Davis, M., 1991. Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology* 28 (5), 588–595.
- Grillon, C., Ameli, R., Goddard, A., Woods, S.W., Davis, M., 1994. Baseline and fear-potentiated startle in panic disorder patients. *Biol. Psychiatry* 35 (7), 431–439.
- Grillon, Christian, Lissek, S., Rabin, S., McDowell, D., Dvir, S., Pine, D.S., 2008. Increased anxiety during anticipation of unpredictable but not predictable aversive stimuli as a psychophysiological marker of panic disorder. *Am. J. Psychiatry* 165 (7), 898–904.
- Hamm, A.O., Vaitl, D., 1996. Affective learning: awareness and aversion. *Psychophysiology* 33 (6), 698–710.
- Hamm, A.O., Weike, A.I., 2005. The neuropsychology of fear learning and fear regulation. *Int. J. Psychophysiol.* 57 (1), 5–14.
- Kalisch, R., Wiech, K., Critchley, H.D., Seymour, B., O'Doherty, J.P., Oakley, D.A., Allen, P., Dolan, R.J., 2005. Anxiety reduction through detachment: subjective, physiological, and neural effects. *J. Cogn. Neurosci.* 17 (6), 874–883.
- Kalisch, R., Wiech, K., Critchley, H.D., Dolan, R.J., 2006a. Levels of appraisal: a medial prefrontal role in high-level appraisal of emotional material. *Neuroimage* 30 (4), 1458–1466.
- Kalisch, R., Wiech, K., Herrmann, K., Dolan, R.J., 2006b. Neural correlates of self-distraction from anxiety and a process model of cognitive emotion regulation. *J. Cogn. Neurosci.* 18 (8), 1266–1276.
- LaBar, K.S., Gatenby, J.C., Gore, J.C., LeDoux, J.E., Phelps, E.A., 1998. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron* 20, 937–945.

- Laux, L., Glanzmann, P., Schaffner, P., Spielberger, C.D., 1981. STAI State-Trait-Angstinventar. Beltz, Weinheim.
- Lissek, S., Rabin, S., Heller, R.E., Lukenbaugh, D., Geraci, M., Pine, D.S., Grillon, C., 2010. Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *Am. J. Psychiatry* 167 (1), 47–55.
- Mackiewicz, K.L., Sarinopoulos, I., Clevon, K.L., Nitschke, J.B., 2006. The effect of anticipation and the specificity of sex differences for amygdala and hippocampus function in emotional memory. *Proc. Natl. Acad. Sci. U. S. A.* 103 (38), 14200–14205.
- McNally, R.J., 2002. Anxiety sensitivity and panic disorder. *Biol. Psychiatry* 52 (10), 938–946.
- Mechias, M.-L., Elkin, A., Kalisch, R., 2010. A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *NeuroImage* 49 (2), 1760–1768.
- Melzig, C.A., Weike, A.I., Zimmermann, J., Hamm, A.O., 2007. Startle reflex modulation and autonomic responding during anxious apprehension in panic disorder patients. *Psychophysiology* 44 (6), 846–854.
- Melzig, C.A., Michalowski, J.M., Holtz, K., Hamm, A.O., 2008. Anticipation of interoceptive threat in highly anxiety sensitive persons. *Behav. Res. Ther.* 46 (10), 1126–1134.
- Melzig, C.A., Weike, A.I., Hamm, A.O., Thayer, J.F., 2009. Individual differences in fear-potentiated startle as a function of resting heart rate variability: implications for panic disorder. *Int. J. Psychophysiol.* 71 (2), 109–117.
- Melzig, C.A., Holtz, K., Michalowski, J.M., Hamm, A.O., 2011. Interoceptive threat leads to defensive mobilization in highly anxiety sensitive persons. *Psychophysiology* 48 (6), 745–754.
- Mineka, S., Zinbarg, R., 2006. A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *Am. Psychol.* 61 (1), 10–26.
- Nitschke, J.B., Sarinopoulos, I., Mackiewicz, K.L., Schaefer, H.S., Davidson, R.J., 2006. Functional neuroanatomy of aversion and its anticipation. *NeuroImage* 29 (1), 106–116.
- Nitschke, J.B., Sarinopoulos, I., Oathes, D.J., Johnstone, T., Whalen, P.J., Davidson, R.J., Kalin, N.H., 2009. Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. *Am. J. Psychiatry* 166 (3), 302–310.
- Ochsner, K.N., Ray, R.D., Cooper, J.C., Robertson, E.R., Chopra, S., Gabrieli, J.D.E., et al., 2004. For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. *NeuroImage* 23 (2), 483–499.
- Peterson, R.A., Reiss, S., 1992. Anxiety Sensitivity Index Manual, 2nd ed. International Diagnostic Systems, Worthington, OH.
- Phelps, E.A., O'Connor, K.J., Gatenby, J.C., Gore, J.C., Grillon, C., Davis, M., 2001. Activation of the left amygdala to a cognitive representation of fear. *Nat. Neurosci.* 4 (4), 437–441.
- Phelps, E.A., Delgado, M.R., Nearing, K.I., LeDoux, J.E., 2004. Extinction learning in humans: role of the amygdala and vmPFC. *Neuron* 43 (6), 897–905.
- Raczka, K.A., Gartmann, N., Mechias, M.-L., Reif, A., Büchel, C., Deckert, J., Kalisch, R., 2010. A neuropeptide S receptor variant associated with overinterpretation of fear reactions: a potential neurogenetic basis for catastrophizing. *Mol. Psychiatry* 15 (11), 1–8.
- Schienze, A., Schäfer, A., Stark, R., Walter, B., Vaitl, D., 2005. Neural responses of OCD patients towards disorder-relevant, generally disgust-inducing and fear-inducing pictures. *Int. J. Psychophysiol.* 57, 69–77.
- Schunck, T., Erb, G., Mathis, A., Gilles, C., Namer, I.J., Hode, Y., Demaziere, A., et al., 2006. Functional magnetic resonance imaging characterization of CCK-4-induced panic attack and subsequent anticipatory anxiety. *NeuroImage* 31 (3), 1197–1208.
- Sehlmeyer, C., Schöning, S., Zwitserlood, P., Pfleiderer, B., Kircher, T., Arolt, V., Konrad, C., 2009. Human fear conditioning and extinction in neuroimaging: a systematic review. *PLoS One* 4 (6), e5865.
- Simmons, A., Strigo, I., Matthews, S.C., Paulus, M.P., Stein, M.B., 2006. Anticipation of aversive visual stimuli is associated with increased insula activation in anxiety-prone subjects. *Biol. Psychiatry* 60, 402–409.
- Spielberger, C.D., 1983. Manual for the State-Trait Anxiety Inventory. Consulting Psychologist Press, Palo Alto, CA.
- Straube, T., Mentzel, H.-J., Miltner, W.H.R., 2007. Waiting for spiders: brain activation during anticipatory anxiety in spider phobics. *NeuroImage* 37 (4), 1427–1436.
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage* 15 (1), 273–289.

Manuscript 3

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Interoceptive threat leads to defensive mobilization in highly anxiety sensitive persons

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Abstract

To study defensive mobilization elicited by the exposure to interoceptive arousal sensations, we exposed highly anxiety sensitive students to a symptom provocation task. Symptom reports, autonomic arousal, and the startle eyeblink response were monitored during guided hyperventilation and a recovery period in 26 highly anxiety sensitive persons and 22 controls. Normoventilation was used as a non-provocative comparison condition. Hyperventilation led to autonomic arousal and a marked increase in somatic symptoms. While high and low anxiety sensitive persons did not differ in their defensive activation during hyperventilation, group differences were detected during early recovery. Highly anxiety sensitive students exhibited a potentiation of startle response magnitudes and increased autonomic arousal after hyper- as compared to after normoventilation, indicating defensive mobilization evoked by the prolonged presence of feared somatic sensations.

Descriptors: Interoceptive threat, Fear-potentiated startle, Hyperventilation, Symptom provocation

Contemporary learning accounts of panic disorder (Bouton, Mineka, & Barlow, 2001; Forsyth & Eifert, 1996) propose that a variety of interoceptive cues may act as elicitors of increasing anxious apprehension because they are identified as signals or predecessors of upcoming danger, that is, an evolving panic attack or possible suffocation. This conditioned fear to interoceptive cues might then serve to augment future panic reactions (see Bouton et al., 2001). It is further suggested that these learning experiences might be modulated by non-specific as well as specific vulnerability factors that predispose individuals for developing a panic disorder. There is evidence that previous learning experiences regarding potential dangers of bodily sensations might serve as a specific psychological vulnerability factor that leads to beliefs that somatic sensations might signal danger (Watt, Stewart, & Cox, 1998). A measure that was developed to assess this set of beliefs is the Anxiety Sensitivity Index (ASI; Peterson & Reiss, 1992).

Accordingly, numerous studies (see McNally, 2002, for a review) have measured fear responses to unexplained somatic sensations in participants that either scored high or low on this dimension. One of the most frequently used challenges in this field of research is the voluntary hyperventilation procedure. In

this task, participants are guided to increase their respiratory rate and/or tidal volume, exceeding the level of physiological demand. This leads to a rapid drop in blood partial pressure of carbon dioxide ($p\text{CO}_2$) level, thus evoking typical sensations such as dizziness, paresthesias, heart racing, or breathlessness, which start as soon as the partial pressure of end-tidal CO_2 ($p_{\text{et}}\text{CO}_2$) falls below an approximate value of 30 mmHg (Gardner, 1996). Using this paradigm, it has repeatedly been shown that highly anxiety sensitive participants report greater subjective distress, fear, or more panic symptoms during hyperventilation (Asmundson, Norton, Wilson, & Sandler, 1994; Donnell & McNally, 1989; Holloway & McNally, 1987; Liebman & Allen, 1995; Rapee & Medoro, 1994; Zvolensky et al., 2002). In contrast, physiological indices of anxious apprehension, such as heart rate (Asmundson et al., 1994; Rapee & Medoro, 1994; Sturges, Goetsch, Ridley, & Whittal, 1998; Zvolensky et al., 2002), skin conductance level (Sturges et al., 1998), and blood pressure (Sturges et al., 1998; Zvolensky et al., 2002) were found to be equally high in highly anxiety sensitive participants as well as controls, when symptoms were provoked by hyperventilation. These dissociations between self-report measures and physiological responses seem to support cognitive models emphasizing that symptom reports are primarily determined by specific appraisal processes (such as catastrophic misinterpretations of the somatic symptoms, see Clark, 1986) rather than by increased conditioned fear responses to the interoceptive threat cues.

A number of methodological concerns, however, have to be considered before such conclusions can be drawn. In none of the studies mentioned above was $p\text{CO}_2$ assessed during hyperventilation, thus, no control over the actual adherence to the hyper-

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ventilation procedure and the intended changes in $p\text{CO}_2$ was possible. Furthermore, several studies (e.g., Sturges et al., 1998) used very short hyperventilation periods resulting in only small changes in $p\text{CO}_2$ that may not be sufficient to reliably provoke somatic symptoms (Hornsveld, Garssen, & van Spiegel, 1995). Finally, the hyperventilation procedure is a highly strenuous task, which renders it very difficult to detect differences in physiological responding between high and low anxiety sensitive individuals during the hyperventilation task (for a review, see Meuret, Ritz, Wilhelm, & Roth, 2005). Therefore, in the current study participants completed the hyperventilation task during which we continuously controlled for adherence to the given task by assessing the expired $p_{\text{et}}\text{CO}_2$ and having the participants follow a given breathing pattern that was continuously adjusted to reach a target $p\text{CO}_2$ level of 20 mmHg. In contrast to the previous studies, we not only measured the subjective and physiological indices of fear during the hyperventilation period itself, but additionally analyzed physiological responses during *early recovery*, when hyperventilation symptoms and thus the interoceptive threat were still expected to be present. Moreover, to get a more direct measure of the subcortical fear networks (see Öhman & Mineka, 2001), modulation of the acoustic startle reflex during the early recovery period was assessed.

The acoustic startle response is a low-level non-cognitive automatic protective brain stem reflex that operates outside of the subjects' awareness. Any abrupt sensory event will prompt a startle response (see Lang, Bradley, & Cuthbert, 1990). Substantial evidence from animal research shows that the magnitude of the startle response is potentiated when elicited during a fear conditioned cue and that this fear potentiated startle is mediated by the amygdala, a subcortical limbic structure located in the anterior temporal lobe (see Davis, 2000). In the same vein, human subjects show an elevated startle amplitude elicited by a brief acoustic probe stimulus in the presence of a cue that has previously been paired with shock (Hamm & Vaitl, 1996), and this fear-potentiated startle is reduced in patients with unilateral lesions of the amygdala (Weike et al., 2005). Moreover, the probe startle reflex is also potentiated when individuals view unpleasant or threatening phobia-relevant pictures (Bradley, 2000; Hamm, Cuthbert, Globisch, & Vaitl, 1997). Thus, if somatic symptoms serve as interoceptive threat cues for individuals who are vulnerable to develop a panic disorder, persons who score high on the ASI should respond with a clear potentiation of the startle reflex when they experience somatic symptoms during early recovery from hyperventilation. Participants who are not vulnerable should not exhibit such defensive response mobilization. Moreover, when defensive activation is associated with increased autonomic arousal, we would also expect a delayed autonomic recovery from hyperventilation in the high anxiety sensitive group. Based on the findings reported previously, *during* the hyperventilation procedure we expected to find increased subjective symptom report in high anxiety sensitive individuals but no clear-cut group differences in physiological responding.

Method

Participants

Two hundred and fifty university students were screened with a German version of the ASI (Peterson & Reiss, 1992). Subjects scoring either high or low (at least one standard deviation from

Table 1. Means and Standard Errors (in brackets) of Questionnaire Measures for Participants High and Low in Anxiety Sensitivity

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

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 square brackets following each questionnaire abbreviation.

the mean [$M = 20 \pm SD = 9$]) 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 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), $t(46) = 1.3$, $n.s.$

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).

Stimulus Materials

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; Wollburg, Meuret, Conrad, Roth, & Kim, 2008). Participants were thus led to breathe at a respiratory rate of 20 breaths per minute (bpm). During the hyperventilation procedure, the respiratory rate as well as the CO_2 of the expired air was monitored continuously by a Nellcor NPB-70 Capnograph (Nellcor Puritan Bennett, Pleasanton, CA) 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 to instruct the participant to “breathe deeper” until a target $p_{\text{et}}\text{CO}_2$ 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 this low level of p_{etCO_2} . All participants included in this analysis were fully compliant with this procedure.

Normoventilation task. Breathing tones were, again, used to adjust breathing speed to follow a 13 bpm 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 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 (Coulbourn Instruments, Whitehall, PA) 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 symptoms¹, 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 recordings were performed by research assistants blind to the participants' anxiety sensitivity score. After reading and signing the informed consent form, participants were 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.

To induce somatic symptoms, one hyperventilation task was applied as described above. As a control condition, all participants additionally completed one normoventilation task. The hyperventilation as well as the normoventilation task consisted of 3 min anticipation, 3 min paced breathing (20 or 13 bpm), and 10 min recovery. The order of the breathing tasks 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, 9 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.

In addition to the described breathing tasks, all participants went through an exteroceptive threat (threat of shock) condition. The exact procedure of this task is described elsewhere (Melzig, Michalowski, Holtz, & Hamm, 2008).

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 electrocutaneous 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 (Marquette Hellige, Freiburg, Germany) Ag/AgCl miniature surface electrodes (Sensormedics, Yorba Linda, CA). The raw EMG signal was amplified using a Coulbourn S75-01 amplifier with a 30 Hz high-pass filter and a Kemo KEM-VBF8-03 400 Hz low-pass filter (Kemo Limited, Beckenham, UK) 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 high-pass 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 Eindhoven lead II setup with two standard, electrolyte filled Ag/AgCl electrodes (Marquette Hellige). The raw signal was filtered (0.1–13 Hz band pass) and amplified using a Coulbourn S75-01 bio-amplifier and continuously digitized with a sampling rate of 100 Hz. Additionally, an online Shimizu R-wave trigger (Shimizu, 1978) was applied. The digital trigger channel was stored separately with a sampling rate of 1000 Hz.

Respiratory rate and end-tidal $p\text{CO}_2$ (p_{etCO_2}) were registered by a capnograph NPB-70 by Nellcor. Air was drawn from both nostrils through a 1.2-mm diameter nasal cannula (Adult Nasal CO_2 filterline, Salter Labs, Arvin, CA). Using infrared spectroscopy, the Nellcor NPB-70 monitor continuously measures the amount of $p\text{CO}_2$ during every breath, the amounts of $p\text{CO}_2$ present at the end of exhalation (p_{etCO_2}), as well as during inhalation (FiCO_2). The time difference between p_{etCO_2} peaks is automatically registered by the monitor, making a calculation of respiratory rate possible. The monitor continuously creates an output step function for p_{etCO_2} as well as respiratory rate that is refreshed for every breath. This output was continuously digitized with a sampling rate of 10 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 ms) and peak amplitude (in μV). 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 μV , and baseline group comparisons of overall reactivity were conducted using these raw startle magnitudes. As suggested by the guidelines for

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

human startle eyeblink electromyographic studies (Blumenthal et al., 2005), blink magnitudes were standardized to correct for interindividual variability that was unrelated to the experimental conditions before statistical analyses of the effects of hyperventilation and normoventilation were performed. 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 then 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 μ S, and group comparisons were conducted using these raw magnitudes. To test the experimental conditions, the SCL-scores were range corrected as suggested by Lykken and Venables (1971).

Heart rate (HR) 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, HR was calculated and exported as 10-s mean values excluding those periods in which acoustic startle probes were delivered.

Respiratory rate and $p_{et}CO_2$ were averaged in 10-s intervals and exported to SPSS software.

For all statistical analyses, a mixed-model analysis of variance (ANOVA) was applied for each physiological and self-report measure. For all analyses, *Group* (low vs. high-AS) was entered as a between-subjects factor. For the adaptation phase, *Block* (3rd vs. 4th minute) was entered as an additional within-subjects factor. To test the initial effects of the onset of the different breathing tasks on autonomic arousal, *Onset* (last minute of adaptation vs. first minute of paced breathing) was entered as a within-subjects factor. The effects of the paced breathing tasks itself were evaluated entering *Task* (normoventilation vs. hyperventilation) as well as *Block* (1st vs. 2nd vs. 3rd minute) as within-subjects factors. The effects of symptom provocation during recovery from the paced breathing tasks were evaluated using *Task* (normoventilation vs. hyperventilation) as well as *Block* (minutes 1 through 10) as within-subjects factors. All statistical tests used a significance level of $p < .05$. Greenhouse-Geisser corrections of degrees of freedom were applied whenever necessary. For all *F*-tests, effect sizes (partial eta squared) are reported.

Results

Adaptation Period

Persons high and low in anxiety sensitivity did not differ significantly in their baseline end-tidal pCO_2 , respiratory rate, startle response magnitude, skin conductance level, or HR, all $F(1,46) < 2.7$, *n.s.* However, 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), $t(46) = 2.6$, $p < .05$.

Symptom Provocation (Guided Hyperventilation) vs. Control Condition (Guided Normoventilation)

Respiratory rate. The shaded areas of the upper panels of Figure 1 show the respiratory rate of persons low (left) and high

(right) in anxiety sensitivity during the guided normo- and hyperventilation task. Participants high and low in anxiety sensitivity adjusted their breathing to the required pattern equally well, *Group* $F < 1$, both during hyper- as well as during normoventilation, *Task* \times *Group* $F(1,46) = 1.9$, *n.s.* In both tasks, all participants constantly followed the breathing tones throughout the whole 3-min period, *Block* $F < 1$, *Block* \times *Group* $F(2,92) = 1.8$, *n.s.*

End-tidal pCO_2 . As depicted in the shaded areas of the lower panels of Figure 1, the hyperventilation procedure led to the desired rapid decrease in $p_{et}CO_2$ over the 3-min hyperventilation period, *Block* $F(2,92) = 521.1$, $p < .001$, $\epsilon = .59$, $\eta_p^2 = .989$, that did not differ between groups, *Block* \times *Group* $F < 1$. The target $p_{et}CO_2$ of 20 mmHg was reached equally well in both experimental groups, *Group* $F < 1$. Due to the strain of having to follow a fixed breathing pattern, $p_{et}CO_2$ also slightly dropped during normoventilation, *Block* $F(2,92) = 48.1$, $p < .001$, $\epsilon = .58$, $\eta_p^2 = .511$. This drop in $p_{et}CO_2$ was equally pronounced in both groups, *Block* \times *Group* $F < 1$. Also, the average $p_{et}CO_2$ during normoventilation was equal for both groups and above the critical level of 30 mmHg, *Group* $F < 1$.

Skin conductance level. As depicted in the shaded areas of the upper panels of Figure 2, the hyperventilation task led to a pronounced initial increase in SCL that was greater for high vs. low-AS participants, *Onset* $F(1,46) = 53.3$, $p < .001$, $\eta_p^2 = .550$, *Onset* \times *Group* $F(1,46) = 4.4$, $p < .05$, $\eta_p^2 = .087$. Throughout the hyperventilation exercise, SCL showed a minor decline in both groups, *Block* $F(2,92) = 16.8$, $p < .001$, $\epsilon = .63$, $\eta_p^2 = .267$, *Block* \times *Group* $F < 1$. During normoventilation, only a marginal initial increase of SCL was observed, *Onset* $F(1,46) = 3.7$, $p = .06$, $\eta_p^2 = .075$, *Onset* \times *Group* $F < 1$, which was, again, followed by a steady decline throughout the paced breathing task, *Block* $F(2,92) = 57.3$, $p < .001$, $\epsilon = .62$, $\eta_p^2 = .555$, *Block* \times *Group* $F < 1$. Overall, the SCL was higher during hyper- as compared to normoventilation, *Task* $F(1,46) = 71.0$, $p < .001$, $\eta_p^2 = .61$, and did not differ significantly between the two groups, neither during the normo- nor during the hyperventilation task, *Group* $F(1,46) = 2.5$, *n.s.*, *Group* \times *Task* $F(1,46) = 2.5$, *n.s.*

Heart rate. As depicted in the shaded areas of the lower panels of Figure 2, in both groups the hyperventilation task led to a pronounced initial increase in HR at the start of the exercise, *Onset* $F(1,46) = 73.5$, $p < .001$, $\eta_p^2 = .615$, *Onset* \times *Group* $F < 1$, which proceeded throughout the first and second minute and was then followed by a minor decline towards minute 3, *Block* $F(2,92) = 13.0$, $p < .001$, $\epsilon = .78$, $\eta_p^2 = .220$, *Block* \times *Group* $F < 1$. The onset of normoventilation had, as intended, no effect on HR in low-AS participants, *Onset* $F(1,21) = 1.1$, *n.s.* In high-AS persons, HR was actually slightly lower at the beginning of normoventilation than during adaptation, *Onset* $F(1,25) = 6.2$, $p < .05$, $\eta_p^2 = .198$. Throughout the normoventilation task in both groups, HR was characterized by a steady but small increase, *Block* $F(2,92) = 18.6$, $p < .001$, $\epsilon = .69$, $\eta_p^2 = .288$, *Block* \times *Group* $F(2,92) = 2.9$, *n.s.* Overall, HR was higher during hyper- as compared to during normoventilation, *Task* $F(1,46) = 177.3$, $p < .001$, $\eta_p^2 = .79$, and did not differ significantly between the two groups, neither during the normo- nor during the hyperventilation task, *Group* $F(1,46) = 2.0$, *n.s.*, *Group* \times *Task* $F < 1$ (Figure 2).

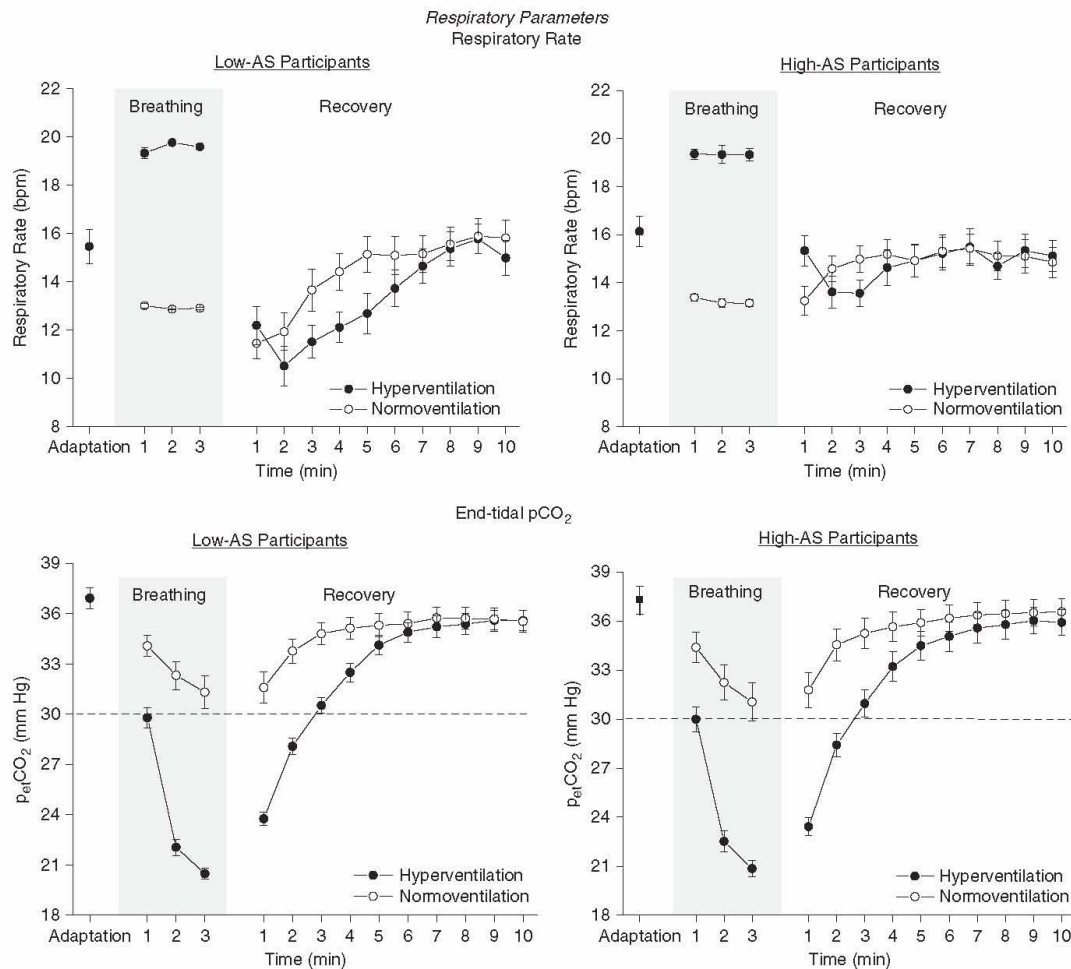


Figure 1. Respiratory rate and end-tidal pCO₂ at the end of the adaptation period and during paced breathing tasks (shaded in gray) and the recovery period in highly anxiety sensitive participants and controls, respectively.

Symptom reports. As depicted in Figure 3, the hyperventilation procedure induced several arousal sensations that are usually associated with fear and panic. All participants reported significantly more symptoms during hyperventilation as compared to during adaptation, *Onset* $F(1,46) = 59.8$, $p < .001$, $\eta_p^2 = .565$, *Onset* \times *Group* $F < 1$, or normoventilation, *Task* $F(1,46) = 68.7$, $p < .001$, $\eta_p^2 = .599$. The number of symptoms reported during normoventilation was in both groups even lower than the symptom number reported during adaptation, *Onset* $F(1,46) = 6.5$, $p < .05$, $\eta_p^2 = .124$, *Onset* \times *Group* $F < 1$, thus supporting the view that normoventilation can serve as a non-symptom-inducing control task to hyperventilation. Overall, high anxiety sensitive participants reported more symptoms than controls during both experimental conditions, that is, during hyperventilation and during normoventilation, *Group* $F(1,46) = 10.0$, $p < .01$, $\eta_p^2 = .178$.

Recovery After Symptom Provocation (Guided Hyperventilation) vs. Control Condition (Guided Normoventilation)

Respiratory rate. The upper panels of Figure 1 depict the respiratory rate of persons low (left) and high (right) in anxiety sensitivity during the recovery from guided normo- and hyperventilation. While low-AS participants showed a significant compensatory decrease in respiration rate during early recovery (minutes 1–5) after hyperventilation compared to normoventilation *Task* $F(1,21) = 7.2$, $p < .05$, $\eta_p^2 = .256$, high AS-participants did not show such compensatory response pattern *Task* $F < 1$. These group differences were also supported by a significant *Group* effect $F(1,46) = 10.5$, $p < .01$, $\eta_p^2 = .185$ during the first 5 min of recovery after hyperventilation. No such group differences occurred during the late (minutes 6–10) recovery phase (see Figure 1).

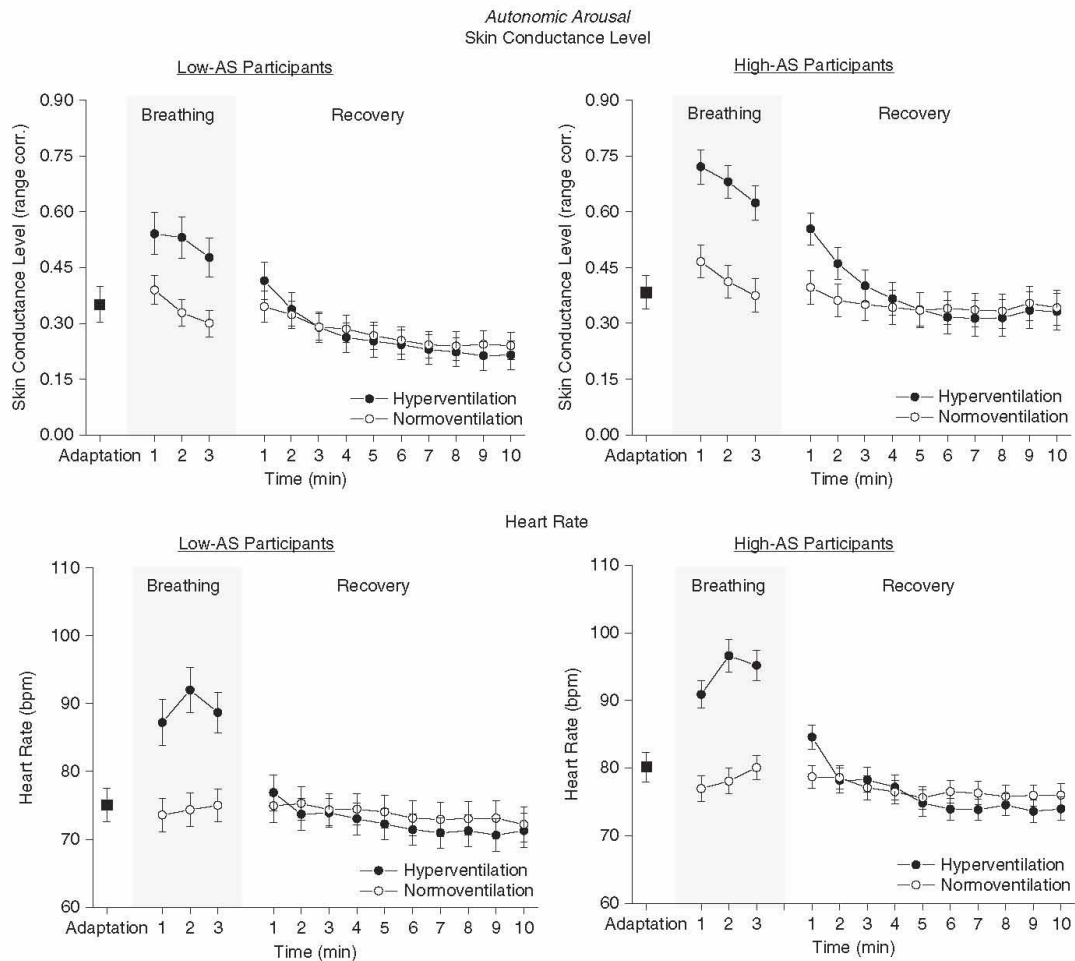


Figure 2. Skin conductance level and heart rate at the end of the adaptation period and during paced breathing tasks (shaded in gray) and the recovery period in highly anxiety sensitive participants and controls, respectively.

End-tidal pCO_2 . Despite obvious group differences in respiratory rate during recovery from hyperventilation, no differences in $p_{et}CO_2$ recovery were detected, *Group* $F < 1$, *Block* \times *Group* $F < 1$, see lower panels of Figure 1. During recovery, i.e., minutes 1 through 8, all $ts(47) > 2.0$, all $ps < .05$, $p_{et}CO_2$ was significantly lowered after hyper- as compared to normoventilation in both groups, *Task* \times *Block* $F(9,414) = 95.0$, $p < .001$, $\varepsilon = .24$, $\eta_p^2 = .674$, *Task* \times *Block* \times *Group* $F < 1$. During the first 2 min, in both groups the $p_{et}CO_2$ level was below the critical threshold of 30 mmHg, $ts(47) > 3.9$, $ps < .001$. Thus, one can assume that hyperventilation symptoms were still present in both groups during this early recovery period.

Skin conductance level. The upper panels of Figure 2 depict the SCL of participants low (left) and high (right) in anxiety sensitivity during the recovery from guided normo- and hyperventilation. Both groups quickly recovered after hyperventilation, *Task* \times *Block* $F(9,414) = 19.0$, $p < .001$, $\varepsilon = .30$, $\eta_p^2 = .292$.

This recovery was delayed in high-AS participants, *Task* \times *Block* \times *Group* $F(9,414) = 3.2$, $p < .05$, $\varepsilon = .30$, $\eta_p^2 = .066$. *Post hoc* analyses indicated that SCL was significantly increased during the first minute after hyperventilation $t(21) = 2.1$, $p < .05$ in low-AS participants, while high-AS participants showed increased SCL for minutes 1 through 3, all $ts(25) > 2.1$, $p < .05$ after hyperventilation.

Heart rate. Similarly to SCL, HR (see lower panels of Figure 2) decreased during early recovery from hyperventilation rapidly reaching a level comparable to after normoventilation or even dropping below this level during late recovery, *Task* \times *Block* $F(9,414) = 13.5$, $p < .001$, $\varepsilon = .59$, $\eta_p^2 = .227$. The early recovery was, again, delayed in the high-AS group, *Task* \times *Block* \times *Group* $F(9,414) = 2.3$, $p < .05$, $\varepsilon = .59$, $\eta_p^2 = .047$. The group difference in the course of early HR recovery after hyperventilation was substantiated by a significant between group comparison of the course of recovery, *Block* \times *Group* $F(9,414) = 3.3$, $p < .01$,

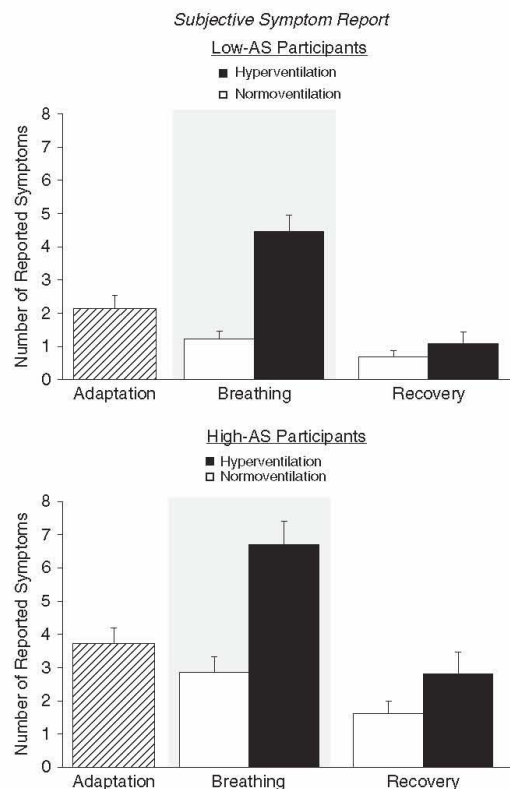


Figure 3. Number of reported symptoms at the end of the adaptation period, during the paced breathing tasks (shaded in gray), and at the end of the recovery period in highly anxiety sensitive participants and controls, respectively.

$\epsilon = .47$, $\eta_p^2 = .068$. Moreover, HR was significantly increased in highly anxiety sensitive participants as compared to controls during the first minute of recovery, $t(46) = 2.5$, $p < .05$.

Startle response magnitudes. As expected, high-AS participants² showed a significant potentiation of the startle response during early recovery after hyperventilation as compared to after normoventilation, $Task \times Block$ $F(9,216) = 2.2$, $p < .05$, $\epsilon = .70$, $\eta_p^2 = .085$, i.e., $ts(24) > 2.2$, $ps < .05$ during the first 2 min. In contrast, no such potentiation was observed for low-AS participants, $Task \times Block$ $F < 1$, see Figure 4. The within-group comparisons were supported by a marginally significant $Task \times Block \times Group$ interaction $F(9,405) = 2.0$, $p = .08$, $\epsilon = .60$, $\eta_p^2 = .042$. The difference in early defensive activation was also substantiated by a significant between-group comparison of raw startle response magnitudes immediately after hyperventilation, $Block \times Group$ $F(9,405) = 2.3$, $p < .05$, $\epsilon = .51$, $\eta_p^2 = .050$.

Symptom reports. During the recovery period, the number of reported symptoms decreased from either paced breathing task,

$F(1,46) = 38.4$, $p < .001$, $\eta_p^2 = .455$. All participants reported slightly more symptoms during recovery from hyperventilation as compared to during recovery from normoventilation, $Task$ $F(1,46) = 5.5$, $p < .05$, $\eta_p^2 = .106$, see Figure 3. Participants high in anxiety sensitivity again generally reported more symptoms, $Group$ $F(1,46) = 6.1$, $p < .05$, $\eta_p^2 = .116$, after both experimental conditions, $Task \times Group$ $F(1,46) = 1.3$, $n.s.$

Discussion

The current study investigated the defensive response mobilization during exposure to somatic symptoms in individuals who either reported high or low fear of interoceptive arousal sensations. Somatic symptoms were induced by a voluntary hyperventilation exercise. Replicating previous findings, the guided hyperventilation task successfully increased physiological arousal and also induced several somatic symptoms that are typically reported during a panic attack. While physiological arousal responses did not differ between low and high anxiety sensitive individuals during the hyperventilation task, the latter group reported overall more panic symptoms. In contrast, during early recovery, when the hyperventilation-induced somatic symptoms were still present, highly anxiety sensitive individuals showed stronger defensive response mobilization, as indexed by a potentiation of the startle reflex, as well as an increase in respiratory rate and autonomic arousal.

Physiological Arousal Induced by Hyperventilation

During the guided hyperventilation task, all participants reduced their $p_{et}CO_2$ level below the critical level of 30 mmHg within the first minute and also successfully held this low $p_{et}CO_2$ level during the entire period of 3 min, resulting in strong increases in skin conductance level and HR. Thus, the implementation of the continuous visual feedback of the expired pCO_2 during the tone-cued paced breathing enabled all participants to adhere to the task, which is often a methodological concern when hyperventilation challenges are applied. Replicating previous findings (Asmundson et al., 1994; Donnell & McNally, 1989; Holloway & McNally, 1987; Liebman & Allen, 1995; Rapee & Medoro, 1994; Zvolensky et al., 2002), highly anxiety sensitive individuals reported more symptoms during the hyperventilation task than low anxiety sensitive individuals. In contrast, but again replicating previous studies (Asmundson et al., 1994; Rapee & Medoro, 1994; Sturges et al., 1998; Zvolensky et al., 2002), no differences between high and low anxiety sensitive individuals were observed in physiological arousal measures. A possible reason for the dissociation of subjective and physiological measures might be that the hyperventilation procedure is an equally strenuous task for both groups that induces the same shifts in blood biochemistry in both groups with strong impact on physiological measures.

Increased Defensive Activation in Highly Anxiety Sensitive Participants During Early Recovery from Hyperventilation

The complete recovery of $p_{et}CO_2$ after hyperventilation back to about baseline level takes 6 to 7 min. After about 3 min, the rise of $p_{et}CO_2$ typically crosses the critical level of 30 mmHg, and hyperventilation symptoms start to disappear. However, during the first 3 min of recovery, $p_{et}CO_2$ is still sufficiently lowered that the somatic symptoms induced by the hyperventilation task persist. Thus, the somatic sensations are still present during this period and are not influenced by the task itself. Accordingly, clear group differences between high and low anxiety sensitive

²For the analyses of startle response magnitudes, one person had to be removed from the dataset due to a large amount of missing trials (>30%).

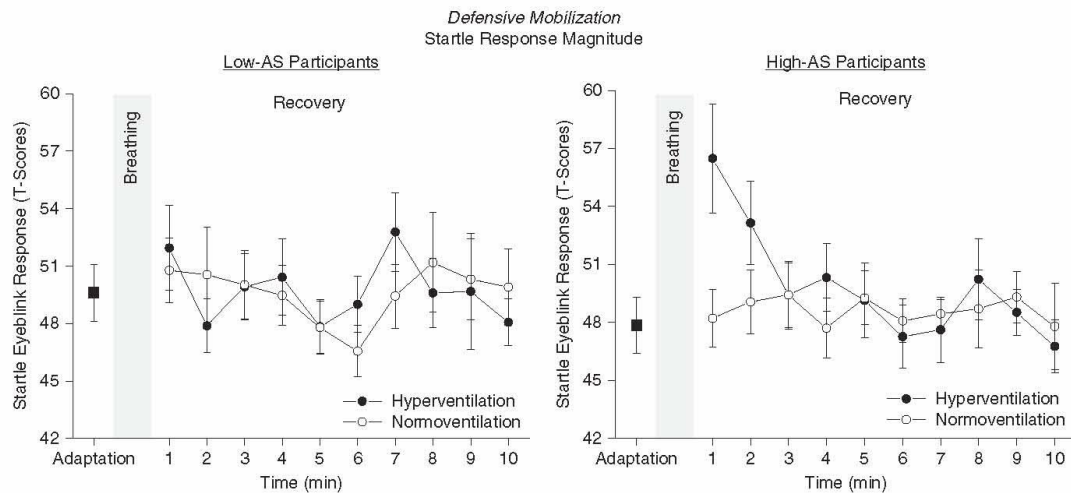


Figure 4. Startle response magnitudes at the end of the adaptation period and during recovery period in highly anxiety sensitive participants and controls, respectively.

individuals were discovered during this early recovery period, although the course of $p_{et}CO_2$ recovery was absolutely congruent for both groups.

During early recovery from hyperventilation, highly anxiety sensitive participants showed a clear potentiation of the startle response during early recovery after hyperventilation compared to the recovery after normoventilation—an effect that did not occur in low anxiety sensitive persons. This finding clearly indicates that the perception of the somatic symptoms during early recovery activates a defensive response mobilization in highly anxiety sensitive individuals. The reflex potentiation is limited to the threat situation, i.e., the time period where the somatic symptoms are present. It only occurs during very early recovery from hyperventilation, while there are no group differences during baseline or when no somatic sensations are present, e.g., during recovery after normoventilation. As soon as the symptoms disappeared, startle potentiation was no longer observed, supporting the view that the perception of somatic symptoms activated the subcortical defense networks in the brain.

This is the first study demonstrating startle potentiation in highly anxiety sensitive participants when exposed to feared interoceptive somatic sensations. One might argue that, instead of fear of somatic symptoms, increased *attention* to somatic symptoms might be responsible for startle potentiation, because it is a well known finding that startle reflex is also modulated by selective attention (see Filion, Dawson, & Schell, 1998). The startle response magnitude is augmented if attention is focused on the sensory modality in which the probe stimulus is delivered (Anthony & Graham, 1985). In the current paradigm, the probe stimuli were presented in the auditory modality. Thus, assuming that highly anxiety sensitive individuals would show increased selective attention to somatic symptoms, we would predict *decreased* instead of potentiated startle responses evoked by acoustic probe stimuli during early recovery after hyperventilation.

The current data are more in line with the emotional priming model (see Lang et al., 1990) suggesting that protective reflexes—like the startle response—are potentiated if the organism

is in an ongoing aversive emotion. Likewise, animal data as well as human research show that the startle reflex is also potentiated during presentation of visual cues that have previously been paired with aversive shock stimuli (Davis, 1998; Hamm & Weike, 2005). Thus, the current finding strongly supports the view that somatic symptoms induce anxious apprehension in highly anxiety sensitive individuals, which then primes protective reflexes like the startle response.

One major advantage of the study presented is the multi-measure approach that made it possible to not only describe defensive mobilization on the reflex level, but also to acquire an extensive characterization of autonomic and, specifically, respiratory correlates of the anxious activation that is triggered by the interoceptive threat. As expected, highly anxiety sensitive individuals showed increased autonomic arousal during early recovery from hyperventilation that was indexed by—in comparison to the control group—increased HR and skin conductance level. A similar finding was previously reported by Wilhelm et al. (2001) who studied panic disorder patients during recovery after a 3-min hyperventilation phase. In this study, patients were characterized by a slowed recovery of SCL and HR but also by a delayed pCO_2 recovery that was accompanied by increased tidal volume instability. Although in our study no differences were found in pCO_2 recovery, between-group differences in respiration pattern during early recovery after hyperventilation were detected: Highly anxiety sensitive persons showed a reduced compensatory decrease in respiratory rate relative to controls. It remains to be investigated whether this different compensatory respiratory pattern might be responsible for the delayed SCL and HR recovery or whether these systems covary due to a common path activation of the autonomic system.

Taken together, these data would support a symptom progression model of panic based on a learning theory perspective (Bouton et al., 2001) proposing that somatic symptoms associated with the initial panic attack turn into threat cues and become conditioned elicitors of anxious apprehension. Accordingly, individuals who believe that somatic symptoms are potentially

dangerous show a clear defensive response mobilization and autonomic activation once such somatic symptoms are present during a recovery period, where no further task is involved.

Responses to Interoceptive Threat: Implications for Clinical Research

The findings of the current study suggest that the guided hyperventilation procedure, as presented in the present paper, is a valid experimental paradigm to investigate defensive activation induced by the presence of an interoceptive threat. During early recovery from hyperventilation, those persons who are characterized by high fear of somatic arousal sensations showed a clear potentiation of startle responses, which was accompanied by heightened autonomic arousal delaying the course of recovery. Thus, we propose that this paradigm may prove useful in further investigations regarding clinical populations that also suffer from increased fear of somatic arousal sensations, such as panic disorder patients. In fact, data from an ongoing study investigating the fearful response to hyperventilation in panic disorder patients demonstrated similarly potentiated startle responses as described for highly anxiety sensitive participants (Melzig, Holtz, & Hamm, 2009). Interestingly, in this patient sample the degree of startle potentiation was positively associated with panic disorder severity, as assessed with the Panic and Agoraphobia Scale (Bandelow, 1999). The high degree of standardization of the

hyperventilation procedure and adherence to this procedure could be of specific interest for research paradigms that need repeated and equal levels of symptom provocation. For instance, this paradigm could be useful to study therapeutic effects of panic disorder therapy and, particularly, the effects of interoceptive exposure.

Limitations of the Study

In the context of etiological models of panic disorder, the present study investigated defensive mobilization in response to a confrontation with feared interoceptive arousal sensations provoked by a guided hyperventilation task. Highly anxiety sensitive participants are often studied as an analogue sample for panic disorder patients, since they equal those patients in their high fear of somatic arousal sensations. We were able to show that defensive mobilization in response to interoceptive threat is found in this group of fearful participants, but, of course, these results need to be replicated in patients currently suffering from panic disorder before interpreting the results in the context of the disorder. Furthermore, future studies investigating defensive mobilization to interoceptive threat in highly anxiety sensitive persons should take into account that this studied population may be prone to show heightened reactivity to affective procedures in general and, therefore, introduce non-interoceptive comparison conditions.

REFERENCES

- American Psychiatric Association (1994). *Diagnostic and statistical manual of mental disorders* (4th ed). Washington, DC: American Psychiatric Press.
- Anthony, B. J., & Graham, F. K. (1985). Blink reflex modification by selective attention: Evidence for the modulation of 'automatic' processing. *Biological Psychology*, 21, 43–59.
- Asmundson, G. J., Norton, G. R., Wilson, K. G., & Sandler, L. S. (1994). Subjective symptoms and cardiac reactivity to brief hyperventilation in individuals with high anxiety sensitivity. *Behaviour Research and Therapy*, 32, 237–241.
- Bandelow, B. (1999). *Panic and agoraphobia scale (PAS)*. Göttingen, Germany: Hogrefe & Huber Publishers.
- Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, 42, 1–15.
- Bouton, M. E., Mineka, S., & Barlow, D. H. S. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychological Review*, 108, 4–32.
- Bradley, M. M. (2000). Emotion and motivation. In J. T. Cacioppo, L. G. Tassinary, & G. G. Bernstein (Eds.), *Handbook of Psychophysiology* (pp. 602–642). Cambridge UK: Cambridge University Press.
- Chambless, D. L., Caputo, G. C., Bright, P., & Gallagher, R. (1984). Assessment of fear of fear in agoraphobics: The body sensations questionnaire and the agoraphobic cognitions questionnaire. *Journal of Consulting and Clinical Psychology*, 52, 1090–1097.
- Clark, D. M. (1986). A cognitive approach to panic. *Behaviour Research and Therapy*, 24, 461–470.
- Cook III, E. W., Atkinson, L., & Lang, K. G. (1987). Stimulus control and data acquisition for IBM PCs and compatibles. *Psychophysiology*, 24, 726–727.
- Davis, M. (1998). Are different parts of the extended amygdala involved in fear versus anxiety? *Biological Psychiatry*, 44, 1239–1247.
- Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In J. P. Aggleton (Ed.), *The amygdala* (pp. 213–287). Oxford, UK: Oxford University Press.
- Donnell, C. D., & McNally, R. J. (1989). Anxiety sensitivity and history of panic as predictors of response to hyperventilation. *Behaviour Research and Therapy*, 27, 325–332.
- Ehlers, A., Margraf, J., & Chambless, D. (1993a). *ACQ Fragebogen zu angstbezogenen Kognitionen*. Weinheim: Beltz.
- Ehlers, A., Margraf, J., & Chambless, D. (1993b). *BSQ Fragebogen zur Angst vor körperlichen Symptomen*. Weinheim: Beltz.
- Filion, D. L., Dawson, M. E., & Schell, A. M. (1998). The psychological significance of human startle eyeblink modification: A review. *Biological Psychology*, 4, 1–43.
- Forsyth, J. P., & Eifert, G. H. (1996). Systemic alarms in fear conditioning—I: A reappraisal of what is being conditioned. *Behavior Therapy*, 27, 441–462.
- Gardner, W. D. (1996). The pathophysiology of hyperventilation disorders. *Chest*, 109, 516–534.
- Globisch, J., Hamm, A. O., Schneider, R., & Vaitl, D. (1993). A computer program for scoring reflex eyeblink and electrodermal responses written in PASCAL. *Psychophysiology*, 30(S1), S13.
- Hamm, A. O., Cuthbert, B. N., Globisch, J., & Vaitl, D. (1997). Fear and the startle reflex: Blink modulation and autonomic response patterns in animal and mutilation fearful subjects. *Psychophysiology*, 34, 97–107.
- Hamm, A. O., & Vaitl, D. (1996). Affective learning: Awareness and aversion. *Psychophysiology*, 33, 698–710.
- Hamm, A. O., & Weike, A. I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology*, 57, 5–14.
- Holloway, W., & McNally, R. J. (1987). Effects of anxiety sensitivity on the response to hyperventilation. *Journal of Abnormal Psychology*, 96, 330–334.
- Hornsveld, H., Garssen, B., & van Spiegel, P. (1995). Voluntary hyperventilation: The influence of duration and depth on the development of symptoms. *Biological Psychology*, 40, 299–312.
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review*, 97, 377–395.
- Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. D. (1981). *STAI State-Trait-Angstinventar*. Weinheim: Beltz.
- Lieberman, S. E., & Allen, G. J. (1995). Anxiety sensitivity, state anxiety and perceptions of facial emotions. *Journal of Anxiety Disorders*, 9, 257–267.
- Lykken, D. T., & Venables, P. H. (1971). Direct measurement of skin conductance: A proposal for standardization. *Psychophysiology*, 8, 656–672.
- McNally, R. J. (2002). Anxiety sensitivity and panic disorder. *Biological Psychiatry*, 52, 938–946.

- Melzig, C. A., Michalowski, J. M., Holtz, K., & Hamm, A. O. (2008). Anticipation of interoceptive threat in highly anxiety sensitive persons. *Behaviour Research and Therapy*, 46, 1126–1134.
- Melzig, C. A., Holtz, K., & Hamm, A. O. (2009). Activation of subcortical fear networks in panic disorder patients during anticipation and provocation of interoceptive threat. *Psychophysiology*, 46(S1), S8.
- Meuret, A. E., Ritz, T., Wilhelm, F. H., & Roth, W. T. (2005). Voluntary hyperventilation in the treatment of panic disorder—Functions of hyperventilation, their implications for breathing training, and recommendations for standardization. *Clinical Psychology Review*, 25, 285–306.
- Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review*, 108, 483–522.
- Peterson, R. A., & Reiss, S. (1992). *Anxiety sensitivity index manual* (2nd ed). Worthington, OH: International Diagnostic Systems.
- Rapee, R. M., & Medoro, L. (1994). Fear of physical sensations and trait anxiety as mediators of the response to hyperventilation in nonclinical subjects. *Journal of Abnormal Psychology*, 103, 693–699.
- Shimizu, H. (1978). Reliable and precise identification of R-waves in the EKG with a simple peak detector. *Psychophysiology*, 15, 499–501.
- Spielberger, C. D. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologist Press.
- Sturges, L. V., Goetsch, V. L., Ridley, J., & Whittal, M. (1998). Anxiety sensitivity and response to hyperventilation challenge: Physiologic arousal, interoceptive acuity, and subjective distress. *Journal of Anxiety Disorders*, 12, 103–115.
- Watt, M. C., Stewart, S. H., & Cox, B. J. (1998). A retrospective study of the learning history origins of anxiety sensitivity. *Behaviour Research and Therapy*, 36, 505–525.
- Weike, A. I., Hamm, A. O., Schupp, H. T., Runge, U., Schroeder, H. W., & Kessler, C. (2005). Fear conditioning following unilateral temporal lobectomy: Dissociation of conditioned startle potentiation and autonomic learning. *Journal of Neuroscience*, 25, 11117–11124.
- Wilhelm, F. H., Gerlach, A. L., & Roth, W. T. (2001). Slow recovery from voluntary hyperventilation in panic disorder. *Psychosomatic Medicine*, 63, 638–649.
- Wollburg, E., Meuret, A. E., Conrad, A., Roth, W. T., & Kim, S. (2008). Psychophysiological reactions to two levels of voluntary hyperventilation in panic disorder. *Journal of Anxiety Disorders*, 22, 886–898.
- Zvolensky, M. J., Goodie, J. L., Ruggiero, K. J., Black, A. L., Larkin, K. T., & Taylor, B. K. (2002). Perceived stress and anxiety sensitivity in the prediction of anxiety-related responding: A multichallenge evaluation. *Anxiety, Stress & Coping: An International Journal*, 15, 211–229.

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Manuscript 4

Repeated interoceptive exposure in high and low anxiety sensitive persons

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Submitted

Author contributions:

CPF conceived and designed the experiment. CPF and KH performed the experiments.

CPF supervised the data acquisition. KH analyzed the data. All authors contributed to the interpretation of the data and wrote the manuscript (first draft by KH).

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Abstract

Interoceptive exposure is one component in cognitive behavioral therapy of panic disorder. The present investigation addressed changes in defensive mobilization during repeated interoceptive exposure using a standardized hyperventilation procedure.

26 high and 22 low anxiety sensitive persons (ASI, Peterson & Reiss, 1992) went through two guided hyperventilation and normoventilation procedures, spaced one week apart. Breathing parameters, startle response magnitudes and symptom reports were measured. All participants successfully adhered to the guided breathing procedures. Both groups comparably reported more symptoms during hyperventilation than normoventilation in both sessions. Only high-AS participants displayed potentiated startle magnitudes after the first hyperventilation vs. normoventilation. One week later, when the hyperventilation exercise was repeated, this potentiation was no longer present. Thus, high and low-AS groups no longer differed in their defensive mobilization to symptom provocation. Furthermore, the number of reported baseline symptoms also decreased from session one to session two in the high-AS group. While high-AS reported increased baseline anxiety symptoms in session 1, groups did not differ in session 2.

Results indicate a reduction of defensive mobilization during repeated interoceptive exposure.

Introduction

Interoceptive exposure is one component of cognitive behavioral therapy in the treatment of panic disorder (Gloster et al., 2011; White et al., 2013). The specific aim of this intervention is to reduce the fear of somatic symptoms by repeatedly engaging patients in a series of exercises that provoke physical sensations resembling those experienced during a panic attack or anxious apprehension. It is assumed that the fear networks activated by these triggers change as a result of inhibitory learning processes (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Hamm, Richter, & Pané-Farré, 2014).

The activation of fear networks, i.e., defensive mobilization, can be readily assessed using multiple measures of fear expression including the potentiation of the startle eyeblink response – a low-level brain stem protective reflex modulated by outputs from the central nucleus of the amygdala - increases in heart rate and skin conductance level reflecting enhanced autonomic arousal and changes in respiration. Using these measures it has been demonstrated, that persons suffering from fear of somatic sensations, e.g., panic disorder patients (Blechert, Wilhelm, Meuret, Wilhelm, & Roth, 2010; Wilhelm, Gerlach, & Roth, 2001), patients suffering from somatic symptom or pain related disorders (Elsenbruch & Orr, 2001; Glombiewski et al., 2015) but also individuals reporting high trait anxiety sensitivity or suffocation fear (Alius, Pané-Farré, Löw, & Hamm, 2015; Melzig, Holtz, Michalowski, & Hamm, 2011), show augmented defensive mobilization when exposed to feared somatic sensations.

In multiple experimental studies, systematic interoceptive exposure, i.e., exposure to somatic symptoms by physical exercise or respiratory challenges (e.g., breathing through a straw, hyperventilation, inhalation of CO₂ enriched air) was demonstrated to be effective in reducing reported fear of somatic sensations in the described populations and – in panic disorder patients –decreasing the frequency of panic attacks (Arntz, 2002;

Beck, Shipherd, & Zebb, 1997; for an overview see Boettcher, Brake, & Barlow, 2015; Deacon et al., 2013; van den Hout, van der Molen, Griez, Lousberg, & Nansen, 1987). Most importantly, fear reduction was observed to increase with repetitions of interoceptive exposure exercises. The current study was designed to follow up on this research assessing not only subjective report of fear but also physiological indicators of defensive mobilization, e.g., the startle response magnitude as well as respiratory parameters.

In the present study, a guided hyperventilation task was used to induce somatic symptoms. During this guided hyperventilation task participants increase their respiratory rate and/ or tidal volume, exceeding the level of physiological demand, thus inducing a rapid drop of blood partial pressure of carbon dioxide (p_{etCO_2}). Once the partial pressure of p_{etCO_2} falls below approximately 30 mmHg (Gardner, 1996) various somatic symptoms like dizziness, heart palpitations, breathlessness, or sweating are elicited. Using this hyperventilation task it has been demonstrated, that - in comparison to individuals reporting low levels of fear of somatic symptoms - persons scoring high in anxiety sensitivity report more distress and panic symptoms, show increased autonomic arousal and a potentiation of the startle response during early recovery from hyperventilation when the somatic symptoms of hyperventilation are still present (Asmundson, Norton, Wilson, & Sandler, 1994; Donnell & McNally, 1989; Holloway & McNally, 1987; Liebman & Allen, 1995; Melzig et al., 2011; Rapee & Medoro, 1994; Zvolensky et al., 2002).

In the present study we wanted to assess defensive response mobilization using multiple levels of fear expression during repetitive exposure to the described guided hyperventilation task that took place in two separate sessions spaced one week apart. Using the Anxiety Sensitivity Index (Peterson & Reiss, 1992) as a screening instrument,

we selected individuals reporting high fear of somatic symptoms and compared defensive responses to individuals reporting low fear of somatic symptoms. According to previous studies (Melzig, Holtz, Michalowski, & Hamm, 2011; Melzig, Michalowski, Holtz, & Hamm, 2008) we expected high anxiety sensitive (high-AS) and low anxiety sensitive (low-AS) persons to differ during the very first hyperventilation task and recovery. We expected greater startle response magnitudes showing that this interoceptive exposure prompted stronger fear responses in high but not in low-AS participants. According to the rationale of interoceptive exposure (Gerlach & Neudeck, 2012; Ito et al., 2001; Lee et al., 2006) we expected a decrease of reported fear but also a reduction in the physiological indices of fear with repetition of the symptom provocation. There is initial evidence that high anxiety sensitive individuals show a reduction of heart rate acceleration and tidal volume to repeated CO₂ inhalations along with a decrease of reported fear (Beck, Shipherd, & Read, 1999; Beck et al., 1997; Beck & Wolf, 2001; Forsyth, Lejuez, & Finlay, 2000). In contrast, there are also data by Li et al. (2006, 2008) showing that regardless of reported trait anxiety individuals show a decrease of reported air hunger and a later onset of a compensatory respiratory response to cumulating CO₂ across three rebreathing tasks. Thus, it currently remains an open question, whether the reduction of fear responses is specific for high fear individuals or a result of physiological adaptive processes to the challenge.

Methods

Sample

250 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 not be in treatment for any mental disorder. The final sample included 48 participants, 26 with high fear of somatic symptoms (high-AS, 18 women) and 22 participants low in anxiety sensitivity (low-AS, 17 women). The mean age of both groups was comparable, $M (SD)$ for high vs. low-AS: 22.88 (3.70) vs. 24.18 (3.14), $t(46) = 1.30$, $p = .202$. For purposes of further sample characterization trait anxiety was assessed using the State-Trait Anxiety Inventory (Laux, Glanzmann, Schaffner, & Spielberger, 1981) and fear of body sensations and agoraphobic cognitions were assessed using the Agoraphobic Cognitions Questionnaire (Ehlers, Margraf, & Chambless, 1993a) and the Body Sensations Questionnaire (Ehlers, Margraf, & Chambless, 1993b). As expected, the high-AS group reported greater trait-anxiety, more agoraphobic cognitions, and more severe anxiety symptoms (see **Table 1**).

Stimulus Materials

Hyperventilation. The hyperventilation (HV) task was introduced as a “fast breathing exercise” that could induce somatic sensations such as palpitations, sweating or to feel faint. Participants were informed that the symptoms would disappear once breathing would return 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 for a similar hyperventilation procedure). Participants were thus led to breathe at a respiratory rate of 20 cpm. To ensure compliance with 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 during the hyperventilation procedure. Visual feedback (instruction slides) was provided to lead the participant to “breathe deeper” until a target

$p_{et}CO_2$ of 20 mmHg was reached. Using further visual feedback participants were then led to “breathe more shallow”, or “keep breathing at a constant depth”, respectively, so that the target $p_{et}CO_2$ level was maintained. All participants included in the present analyses were fully compliant with the hyperventilation procedure.

Normoventilation. In the normoventilation (NV) control condition participants paced their breathing to a tone at 13 cpm which corresponds to a normal breathing frequency. During this guided normoventilation procedure the depth of breathing was to be freely adjusted to a comfortable level by the participant.

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.

Symptom ratings. Participants were asked to rate the severity of 14 symptoms¹, that are listed as symptoms in the panic attack specifier in the DSM-5 (American Psychiatric Association, 2013) on a 4-point Likert-scale ranging from 0 (not at all) to 3 (severe) via a small 4 button parallel port device. All symptoms and response options were projected onto a 1.50 x 1.30 m screen in front of the subjects.

Procedure²

All physiological recordings were performed by research assistants blind to the participants' anxiety sensitivity score. After reading and signing the informed consent form participants were seated in a reclining chair in a dimly lit, sound attenuated room.

All electrodes were attached and signal quality was checked.

¹For the present study, the item „feeling dizzy, unsteady, light headed, or faint“ was split up in two separate items: „feeling unsteady or dizzy“ and „feeling faint“, thus providing 14 symptom severity ratings.

² As described in Melzig, Michalowski, Holtz, & Hamm, 2008, in addition to the described paced breathing tasks, a threat of shock condition (shock/ no shock) was also established in a separate part of the experiment.

Adaptation. 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 two minutes of the adaptation period.

Anticipation. Prior to each guided breathing task, a 3 min anticipation period was implemented during which 9 startle stimuli were presented (20 s mean inter-probe interval).

Guided breathing task. Participants went through one 3 min hyperventilation (HV) and one 3 min normoventilation (NV) task. The order of paced breathing tasks was balanced out, i.e., half of the participants within each group started with the hyperventilation task and the other half with the normoventilation task. No startle probes were presented during the paced breathing to avoid interference with the task.

Recovery. Every breathing task was followed by a 10 min recovery period during which 10 startle stimuli were presented (60 s mean inter-probe interval). Retrospective symptom ratings for paced breathing and recovery periods were obtained at the end of each recovery period.

Participants returned for a second assessment session exactly one week after the first laboratory assessment. Session 2 was structured in parallel to session 1, with the only difference that those participants who received the HV-NV order in session 1 now received the NV-HV order and vice versa. After completion of the study procedure all participants were informed about study goals and received course credit for participation.

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 (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.

Respiratory rate and end-tidal $p\text{CO}_2$ ($p_{\text{et}}\text{CO}_2$) were registered by a capnograph NPB-70 by Nellcor (Nellcor Puritan Bennett, Pleasanton, CA, USA). Air was drawn from both nostrils through a 1.2 mm diameter nasal cannula (Adult Nasal CO_2 filterline, Salter Labs, Arvin, CA, USA). Using infrared spectroscopy the Nellcor NPB-70 monitor continuously measures the amount of $p\text{CO}_2$ during every breath, the amounts of $p\text{CO}_2$ present at the end of exhalation ($p_{\text{et}}\text{CO}_2$) as well as during inhalation (FiCO_2). The time difference between $p_{\text{et}}\text{CO}_2$ peaks is automatically registered by the monitor, making a calculation of respiratory rate possible. The monitor continuously creates an output step function for $p_{\text{et}}\text{CO}_2$ as well as respiratory rate that is refreshed for every breath. This output was continuously digitized with a sampling rate of 10 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 μV). 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 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 μV . Blink magnitudes were then 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., $50 + [z \times 10]$) to ensure that each participant contributes equally to the analysis of the experimental conditions.

Respiratory rate and $p_{\text{et}}\text{CO}_2$ were averaged in 10 s intervals and exported to SPSS software.

All statistical analyses were performed using separate mixed-model analyses of variance for each physiological and self-report measure and each experimental phase.

Adaptation. The adaptation period was analyzed using session (1 vs. 2) as within-subjects factor and group (high-AS vs. low-AS) as between-subjects factor.

Guided breathing task. The effects of repeated performance of breathing tasks were evaluated using session (1 vs. 2), task (HV vs. NV), and minute (1 through 3) as within-subjects factors and group (high-AS vs. low-AS) as a between-subjects factor.

Recovery. As reported by Melzig et al. (2011) group differences in high vs. low anxiety sensitive persons occurred most prominently during early recovery. Thus, we narrowed our analysis down to the first three minutes of the recovery periods. The immediate effects of repeated symptom provocation were evaluated for the early recovery window using session (1 vs. 2), task (HV vs. NV), and minute (recovery minutes 1 and 2 for startle response, minutes 1 through 3 for respiratory measures) as within-subjects factors and group (high-AS vs. low-AS) as a between-subjects factor.

For the analysis of session (1 vs. 2) and group (high- vs. low-AS) effects of symptom reports concerning the breathing tasks, the significant group difference during adaptation period of session 1 was entered as a covariate. All statistical tests used a

significance level of $p < .05$ and were performed using SPSS 19.0. Greenhouse-Geisser corrections of degrees of freedom were applied whenever necessary. For all F-tests effect sizes (partial eta squared) are reported.

Results

Adaptation

During the adaptation phase of session 1 high-AS individuals reported more symptoms than low-AS individuals. No baseline differences were detected for startle response magnitude, respiratory rate, and end-tidal $p\text{CO}_2$, see **Table 2**. With repetition of the experimental session one week later startle response magnitudes equally decreased from session 1 to session 2 for both groups, while respiratory rate and $p_{\text{et}}\text{CO}_2$ did not differ between sessions. The number of reported symptoms during the adaptation period showed a greater decrease from session 1 to session 2 for high- as compared to low-AS persons. In consequence, the groups did not differ in symptom report during the adaptation phase of session 2.

Guided breathing tasks: Manipulation check

Respiratory parameters

Participants were able to adhere to the paced breathing procedures in both sessions, see **Figure 1** for end-tidal $p\text{CO}_2$ during all hyperventilation and normoventilation procedures. During hyperventilation, participants increased their respiratory rate to the target rate of 20 cpm and kept it constant throughout the task, minute $F(2, 92) = 1.20$, $p = .304$, $\eta^2_p = .03$, $\varepsilon = .93$, Minute x Session $F(2, 92) = 1.31$, $p = .269$, $\eta^2_p = .03$, $\varepsilon = .69$. High- and low-AS participants were comparable in their respiratory pattern throughout the hyperventilation task, group and Minute x Group $F <$

1. During normoventilation in both sessions, both groups were comparably able to successfully adjust their respiratory rate to the target rate of 13 cpm, group $F(1, 46) = 1.05, p = .312, \eta^2_p = .02$, Minute x Group $F < 1$.

As intended by the application of the guided hyperventilation procedure both groups were characterized by an almost identical decrease of $p_{et}CO_2$ in both sessions, Minute x Session $F < 1$, Minute x Group $F(2, 92) = 1.38, p = .255, \eta^2_p = .03, \varepsilon = .70$. $P_{et}CO_2$ equally decreased towards the target level of 20 mmHg in high- and low-AS participants, minute $F(2, 92) = 722.36, p = .000, \eta^2_p = .94, \varepsilon = .70$, group $F < 1$. Also during normoventilation both groups were characterized by a comparable course of $p_{et}CO_2$ in both sessions, Minute x Session $F < 1$, Minute x Group $F(2, 92) = 1.62, p = .211, \eta^2_p = .03, \varepsilon = .55$, minute $F(2, 92) = 82.21, p = .000, \eta^2_p = .64, \varepsilon = .55$, group $F < 1$. Although $p_{et}CO_2$ slightly decreased it never dropped below the threshold of 30 mmHg which is critical for symptom elicitation.

Symptom reports

As depicted in **Figure 2**, in both sessions both groups reported more panic symptoms during hyperventilation than during normoventilation, task $F(1, 46) = 96.36, p = .000, \eta^2_p = .68$, Task x Group $F < 1$, Task x Session $F(1, 46) = 2.00, p = .164, \eta^2_p = .04$. Independent of the breathing task, i.e., similarly for hyperventilation and normoventilation, the overall number of symptoms equally decreased from session 1 to session 2 for both groups, session $F(1, 45) = 6.10, p = .017, \eta^2_p = .12$, Session x Group $F < 1$, Task x Group x Session $F(1, 46) = 1.64, p = .206, \eta^2_p = .04$.

Recovery

Respiratory parameters

P_{et}CO₂: After termination of the breathing procedures (hyper- as well as normoventilation), high- and low-AS participants showed a steady p_{et}CO₂ recovery, minute $F(2, 92) = 303.67, p = .000, \eta^2_p = .87, \varepsilon = .71$, Minute x Group $F < 1$, group $F < 1$. After the hyperventilation challenges, participants crossed the critical threshold for hyperventilation-related symptom elicitation between minutes 2 and 3 of the recovery, see **Figure 1**. Most importantly, at no point of time p_{et}CO₂ after normoventilation dropped below the critical symptom-eliciting threshold of 30 mmHg, see Figure 1. Across all conditions no differences in the course of recovery were detected between groups and sessions, Minute x Session $F(2, 92) = 1.33, p = .269, \eta^2_p = .03, \varepsilon = .94$, Minute x Session x Group $F(2, 92) = 1.17, p = .314, \eta^2_p = .03, \varepsilon = .94$.

Respiratory rate: In session 1, as expected, respiratory rate was relatively decreased after hyperventilation as compared to after normoventilation, in low-AS, task $F(1, 21) = 5.43, p = .030, \eta^2_p = .21$, but not in high-AS participants, task $F(1, 25) = 3.12, p = .090, \eta^2_p = .11$, Task x Group $F(1, 46) = 8.72, p = .005, \eta^2_p = .16$, see Figure 3.

After the repetition of the paced breathing tasks one week later, both groups showed a comparable respiratory response characterized by a relative decrease of the respiratory rate after hyperventilation as compared to after normoventilation, task $F(1, 46) = 5.50, p = .023, \eta^2_p = .11$, Task x Group $F(1, 46) = 1.02, p = .318, \eta^2_p = .02$.

Differences in group responding between sessions were only supported in form of a main group effect, i.e., high AS had generally higher respiratory rates in session 1, group $F(1, 46) = 11.09, p = .002, \eta^2_p = .19$, but groups did not differ in session 2, group $F(1, 46) = 2.08, p = .156, \eta^2_p = .04$, Group x Session $F(1, 46) = 5.98, p = .018, \eta^2_p = .12$. More complex by task interactions did not turn out significant, $F < 1$.

Startle response magnitudes

As depicted in **Figure 4**, only high-AS but not low-AS participants were characterized by a potentiated startle response during early recovery from the first hyperventilation exercise compared to the normoventilation, Group x Task $F(1, 45) = 4.94, p = .031, \eta^2_p = .10$; task: high-AS $F(1, 24) = 7.63, p = .011, \eta^2_p = .24$, low-AS $F < 1$.

This startle potentiation in the high-AS group was not anymore present one week later, when the hyperventilation exercise was repeated, task $F < 1$, Task x Session $F(1, 24) = 8.38, p = .008, \eta^2_p = .26$. No effects of repetition were observed in low-AS participants, task $F < 1$, Task x Session $F < 1$. Consequently, no group difference in task-dependent startle modulation was present in session 2, Group x Task $F < 1$.

Discussion

The present investigation addressed changes in defensive mobilization in individuals with high vs. low fear of somatic sensations during repeated interoceptive exposure using a standardized hyperventilation procedure. All participants repeatedly successfully adhered to the guided hyperventilation and normoventilation procedures. Consequently, both groups comparably reported more symptoms during hyperventilation compared to normoventilation in both sessions. Only high-AS participants displayed potentiated startle response magnitudes after the first hyperventilation vs. normoventilation, while low-AS participants did not. One week later, when the hyperventilation exercise was repeated, this potentiation was no longer present and thus high and low-AS groups no longer differed in their defensive mobilization to symptom provocation. Concurrently, the number of reported baseline symptoms also decreased from session one to session two in

the high-AS group. Thus, while high-AS reported increased baseline anxiety symptoms in session 1, groups did not differ in session 2.

Symptom provocation through guided hyperventilation

In the present study, we applied a well-controlled repeated interoceptive exposure procedure, i.e., a paced breathing task accompanied by visual feedback regarding breathing depth. All participants were able to successfully adhere to the hyperventilation and normoventilation procedures in both sessions. In consequence, due to the induced over-breathing above and beyond physiological demands high- and low-AS study participants showed a reduction of $p_{et}CO_2$ that was absolutely comparable between groups and sessions due to the high level of procedural standardization. The course of $p_{et}CO_2$ recovery was also comparable between groups. In concordance with previous studies (Holtz, Pané-Farré, Wendt, Lotze, & Hamm, 2012; Wilhelm et al., 2001; Wollburg, Meuret, Conrad, Roth, & Kim, 2008) both groups reported a variety of somatic symptoms in response to hyperventilation and more symptoms during hyperventilation compared to normoventilation clearly demonstrating the success of the symptom induction procedure. Applying this standardized procedure we were able to investigate effects of repeated HV on defensive mobilization to the feared interoceptive threat, i.e., the provoked sensations of hyperventilation.

Changes in defensive mobilization with repetition of symptom provocation

Previous studies that were targeted at elucidating the effects of repeated interoceptive exposure have demonstrated a reduction of fear (Deacon et al., 2013; Sabourin, Stewart, Watt, & Krigolson, 2015) and greater toleration of somatic symptoms (Deacon et al., 2013) in high anxiety sensitive participants when these were repeatedly

confronted with feared somatic sensations. Further extending these findings Li et al. (2008) demonstrated that the tolerance for aversive somatic sensations (i.e., air hunger) increased for high *and* low trait anxious persons over the course of repeated confrontations with hypercapnia. In the present study we found a decrease of provoked symptoms in both, high and low anxiety sensitive persons, when they were confronted with repeated hyperventilation as compared to normoventilation. Further supplementing and extending these findings we demonstrated that startle response magnitudes during recovery from the first hyperventilation vs. normoventilation were potentiated only in high-AS participants and that this potentiation was no longer present when the hyperventilation exercise was repeated one week later. Together with the reported changes in breathing parameters in high anxiety sensitive participants this reduction in fear potentiated startle in response to the elicitation of feared somatic sensations indicates a reduction of mobilization of central defense networks in the context of repeated hyperventilation, i.e., repeated interoceptive exposure.

A similar decrease of defensive network activation has been demonstrated to occur in spider phobics with successful psychotherapy (Straube, Glauer, Dilger, Mentzel, & Miltner, 2005). While phobics, as compared to controls, showed greater responses to spider vs. control videos in the insula and anterior cingulate cortex (ACC), cognitive-behavioral therapy (CBT) strongly reduced phobic symptoms as well as insula and ACC hyperactivity in the treatment but not in the waiting-list group. Further evidence for the reduction of defensive network activation by repeated exposure therapy comes from Kircher et al. (2013). After CBT treatment, patients with panic disorder and agoraphobia, compared to healthy controls, showed reduced activation in the left inferior frontal gyrus (IFG) during a conditioning paradigm, reduced agoraphobic symptoms, and increased

connectivity between the IFG and fear network regions (amygdalae, insulae, ACC) (Kircher et al., 2013).

As central mechanisms of action for the decrease in defensive mobilization recent research suggests a form of fear extinction, namely inhibitory learning, as a key process responsible for the treatment outcome of repeated (interoceptive) exposure. It is assumed that the conditioned stimuli (somatic symptoms - formerly associated with aversive outcomes) are now - via experiences from repeated interoceptive exposure - associated with competing associations like tolerability or the non-occurrence of aversive outcomes, that now inhibit previous associations (for a review see Boettcher et al., 2015; Craske et al., 2008, 2014). Further, elucidating principles behind the particular effect of anxiety reduction across *repeated* exposure sessions, Berry et al. (Berry, Rosenfield, & Smits, 2009) point out the importance of consolidation of extinction learning into long-term memory between several sessions of exposure. In a similar vein, Pace-Schott and co-workers (Pace-Schott et al., 2014) underline the relevance of sleep between two sessions. In their study sleep augmented the between session reduction of physiological measures recorded during a loud-tone habituation paradigm, which can also be explained by consolidation processes.

Anxious apprehension during adaptation

Already during adaptation phase, that means even before the first hyperventilation exercise was introduced, high anxiety sensitive persons reported more anxiety symptoms than low-AS persons, indicating greater anxious apprehension. At the same time, no baseline differences were present for startle response magnitude, respiratory rate, and end-tidal pCO₂. This finding is in agreement with a diverse range of studies demonstrating increased subjective anxious apprehension or increased report of anxiety symptoms when

high fearful participants were at baseline but expected to be confronted with a symptom provocation task (Alius, Pané-Farré, von Leupoldt, & Hamm, 2013; Holtz et al., 2012; Stegen, Van Diest, Van de Woestijne, & Van den Bergh, 2000).

Interestingly, during adaptation in session two, i.e., after one performance of the hyperventilation task one week earlier, the groups no longer differed in their symptom reports as the number of reported symptoms showed a greater decrease from session 1 to session 2 for high- as compared to low-AS persons. Additionally, startle response magnitudes equally decreased from adaptation in session 1 to session 2 for both groups, while respiratory rate and $p_{et}CO_2$ did not differ between sessions. The reduction of symptoms, especially in high-AS participants, and the decrease in startle magnitudes are in line with experiences made in psychotherapy of anxiety disorders that is found to reduce anxious apprehension.

Limitations of the study

As the current study investigated anxiety reduction in an analogue sample to patients, which must be kept in mind as a limiting factor when drawing clinical conclusions, future investigations need to be transferred to the clinical context before definite statements about therapeutic processes in the treatment of actual patients can be made.

Summary and clinical implications

In the present study we were able to establish a highly standardized hyperventilation challenge that was successfully accomplished by all participants and that induced anxiety symptoms in high and low anxiety sensitive persons. High-AS participants were characterized by potentiated startle magnitude during recovery from

hyper- vs. normoventilation in session one. With repetition of the challenge these group differences were no longer present.

The results from the present study give first indication that defensive activation in anxiety networks can be changed with repetition of a symptom provocation task. Thus, established anxiety networks may be desensitized by repeated exposure to the feared somatic sensations.

References

- Alius, M. G., Pané-Farré, C. A., Löw, A., & Hamm, A. O. (2015). Modulation of the blink reflex and P3 component of the startle response during an interoceptive challenge. *Psychophysiology*, 52(1), 140–148. <http://doi.org/10.1111/psyp.12295>
- Alius, M. G., Pané-Farré, C. A., von Leupoldt, A., & Hamm, A. O. (2013). Induction of dyspnea evokes increased anxiety and maladaptive breathing in individuals with high anxiety sensitivity and suffocation fear. *Psychophysiology*, 50(5), 488–497. <http://doi.org/10.1111/psyp.12028>
- American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: Author.
- Arntz, A. (2002). Cognitive therapy versus interoceptive exposure as treatment of panic disorder without agoraphobia. *Behaviour Research and Therapy*, 40(3), 325–41. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11863242>
- Asmundson, G. J. G., Norton, G. R., Wilson, K. G., & Sandler, L. S. (1994). Subjective symptoms and cardiac reactivity to brief hyperventilation with high anxiety sensitivity. *Behavior Research and Therapy*, 32(2), 237–241.
- Beck, J. G., Shipherd, J. C., & Read, J. (1999). Response patterns to repeated CO₂ inhalation in individuals with high anxiety sensitivity. *Behaviour Research and*

- Therapy*, 37(11), 1073–89. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10500321>
- Beck, J. G., Shipherd, J. C., & Zebb, B. J. (1997). How does interoceptive exposure for panic disorder work? An uncontrolled case study. *Journal of Anxiety Disorders*, 11(5), 541–56. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9407272>
- Beck, J. G., & Wolf, M. S. (2001). Response to repeated CO₂ in individuals with elevated anxiety sensitivity: replication with 20% CO₂. *Journal of Behavior Therapy and Experimental Psychiatry*, 32(1), 1–16. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11729942>
- Berry, A. C., Rosenfield, D., & Smits, J. A. J. (2009). Extinction retention predicts improvement in social anxiety symptoms following exposure therapy. *Depression and Anxiety*, 26(1), 22–7. <http://doi.org/10.1002/da.20511>
- Blechert, J., Wilhelm, F. H., Meuret, A. E., Wilhelm, E. M., & Roth, W. T. (2010). Respiratory, autonomic, and experiential responses to repeated inhalations of 20% CO₂ enriched air in panic disorder, social phobia, and healthy controls. *Biological Psychology*, 84(1), 104–11. <http://doi.org/10.1016/j.biopsycho.2010.01.002>
- Boettcher, H., Brake, C. A., & Barlow, D. H. (2015). Origins and outlook of interoceptive exposure. *Journal of Behavior Therapy and Experimental Psychiatry*, 1–11. <http://doi.org/10.1016/j.jbtep.2015.10.009>
- Craske, M. G., Kircanski, K., Zelikowsky, M., Mystkowski, J., Chowdhury, N., & Baker, A. (2008). Optimizing inhibitory learning during exposure therapy. *Behaviour Research and Therapy*, 46(1), 5–27. <http://doi.org/10.1016/j.brat.2007.10.003>
- Craske, M. G., Treanor, M., Conway, C. C., Zbozinek, T., & Vervliet, B. (2014). Maximizing exposure therapy: an inhibitory learning approach. *Behaviour*

- Research and Therapy*, 58, 10–23. <http://doi.org/10.1016/j.brat.2014.04.006>
- Deacon, B., Kemp, J. J., Dixon, L. J., Sy, J. T., Farrell, N. R., & Zhang, A. R. (2013). Maximizing the efficacy of interoceptive exposure by optimizing inhibitory learning: a randomized controlled trial. *Behaviour Research and Therapy*, 51(9), 588–96. <http://doi.org/10.1016/j.brat.2013.06.006>
- Donnell, C. D., & McNally, R. J. (1989). Anxiety sensitivity and history of panic as predictors of response to hyperventilation. *Behaviour Research and Therapy*, 27(4), 325–332. [http://doi.org/10.1016/0005-7967\(89\)90002-8](http://doi.org/10.1016/0005-7967(89)90002-8)
- Ehlers, A., Margraf, J., & Chambless, D. (1993a). *ACQ Fragebogen zu angstbezogenen Kognitionen*. Weinheim: Beltz.
- Ehlers, A., Margraf, J., & Chambless, D. (1993b). *BSQ Fragebogen zur Angst vor körperlichen Symptomen*. Weinheim: Beltz.
- Elsenbruch, S., & Orr, W. C. (2001). Diarrhea- and constipation-predominant IBS patients differ in postprandial autonomic and cortisol responses. *Am J Gastroenterol*, 96(2), 460–466. Retrieved from <http://dx.doi.org/10.1111/j.1572-0241.2001.03526.x>
- Forsyth, J. P., Lejuez, C. W., & Finlay, C. (2000). Anxiogenic effects of repeated administrations of 20% CO₂-enriched air: Stability within sessions and habituation across time. *Journal of Behavior Therapy and Experimental Psychiatry*, 31(2), 103–121. [http://doi.org/10.1016/S0005-7916\(00\)00014-8](http://doi.org/10.1016/S0005-7916(00)00014-8)
- Gardner, W. N. (1996). The Pathophysiology of Hyperventilation Disorders. *Chest*, 109(2), 516–534. <http://doi.org/https://doi.org/10.1378/chest.109.2.516>
- Gerlach, A. L., & Neudeck, P. (2012). Interoceptive exposure. In P. Neudeck & H.-U. Wittchen (Eds.), *Exposure Therapy. Rethinking the Model - Refining the Method*. New York: Springer-Verlag. <http://doi.org/10.1007/978-1-4614-3342-2>

- Globisch, J., Hamm, A. O., Schneider, R., & Vaitl, D. (1993). A computer program for scoring reflex eyeblink and electrodermal responses written in Pascal. *Psychophysiology*, (30), S13.
- Glombiewski, J. A., Riecke, J., Holzapfel, S., Rief, W., König, S., Lachnit, H., & Seifart, U. (2015). Do patients with chronic pain show autonomic arousal when confronted with feared movements? An experimental investigation of the fear–avoidance model. *Pain*, 156(3), 547–554.
<http://doi.org/10.1097/01.j.pain.0000460329.48633.ce>
- Gloster, A. T., Wittchen, H.-U., Einsle, F., Lang, T., Helbig-Lang, S., Fydrich, T., ... Arolt, V. (2011). Psychological treatment for panic disorder with agoraphobia: A randomized controlled trial to examine the role of therapist-guided exposure in situ in CBT. *Journal of Consulting and Clinical Psychology*.
<http://doi.org/10.1037/a0023584>
- Hamm, A. O., Richter, J., & Pané-Farré, C. A. (2014). When the threat comes from inside the body: A neuroscience based learning perspective of the etiology of panic disorder. *Restorative Neurology and Neuroscience*, 32(1), 79–93.
- Holloway, W., & McNally, R. J. (1987). Effects of anxiety sensitivity on the response to hyperventilation. *Journal of Abnormal Psychology*, 96(4), 330–334.
<http://doi.org/10.1037/0021-843X.96.4.330>
- Holtz, K., Pané-Farré, C. a, Wendt, J., Lotze, M., & Hamm, A. O. (2012). Brain activation during anticipation of interoceptive threat. *NeuroImage*, 61, 857–865.
<http://doi.org/10.1016/j.neuroimage.2012.03.019>
- Ito, L. M., De Araujo, L. A., Tess, V. L. C., De Barros-Neto, T. P., Asbahr, F. R., & Marks, I. (2001). Self-exposure therapy for panic disorder with agoraphobia: Randomised controlled study of external v. interoceptive self-exposure. *The British*

- Journal of Psychiatry*, 178(4), 331–336. <http://doi.org/10.1192/bjp.178.4.331>
- Kircher, T., Arolt, V., Jansen, A., Pyka, M., Reinhardt, I., Kellermann, T., ... Straube, B. (2013). Effect of cognitive-behavioral therapy on neural correlates of fear conditioning in panic disorder. *Biological Psychiatry*, 73(1), 93–101. <http://doi.org/10.1016/j.biopsych.2012.07.026>
- Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. D. (1981). *STAI State-Trait-Angstinventar*. Weinheim: Beltz.
- Lee, K., Noda, Y., Nakano, Y., Ogawa, S., Kinoshita, Y., Funayama, T., & Furukawa, T. A. (2006). Interoceptive hypersensitivity and interoceptive exposure in patients with panic disorder: specificity and effectiveness. *BMC Psychiatry*, 6, 32. <http://doi.org/10.1186/1471-244X-6-32>
- Li, W., Daems, E., Van de Woestijne, K. P., Van Diest, I., Gallego, J., De Peuter, S., ... Van den Bergh, O. (2006). Air hunger and ventilation in response to hypercapnia: effects of repetition and anxiety. *Physiology & Behavior*, 88(1–2), 47–54. <http://doi.org/10.1016/j.physbeh.2006.03.001>
- Li, W., Van Diest, I., De Peuter, S., Bogaerts, K., Oyen, N., Hombroux, N., ... Van den Bergh, O. (2008). Repeated experiences of air hunger and ventilatory behavior in response to hypercapnia in the standardized rebreathing test: effects of anxiety. *Biological Psychology*, 77(2), 223–32. <http://doi.org/10.1016/j.biopsycho.2007.10.013>
- Liebman, S. E., & Allen, G. J. (1995). Anxiety sensitivity, state anxiety and perceptions of facial emotions. *Journal of Anxiety Disorders*, 9(4), 257–267. [http://doi.org/10.1016/0887-6185\(95\)00007-B](http://doi.org/10.1016/0887-6185(95)00007-B)
- Melzig, C. A., Holtz, K., Michalowski, J. M., & Hamm, A. O. (2011). Interoceptive threat leads to defensive mobilization in highly anxiety sensitive persons.

- Psychophysiology*, 48(6), 745–54. <http://doi.org/10.1111/j.1469-8986.2010.01150.x>
- Melzig, C. A., Michalowski, J. M., Holtz, K., & Hamm, A. O. (2008). Anticipation of interoceptive threat in highly anxiety sensitive persons. *Behaviour Research and Therapy*, 46(10), 1126–34. <http://doi.org/10.1016/j.brat.2008.07.002>
- Pace-Schott, E. F., Tracy, L. E., Rubin, Z., Mollica, A. G., Ellenbogen, J. M., Bianchi, M. T., ... Orr, S. P. (2014). Interactions of time of day and sleep with between-session habituation and extinction memory in young adult males. *Experimental Brain Research*, 232(5), 1443–58. <http://doi.org/10.1007/s00221-014-3829-9>
- Peterson, R. A., & Reiss, S. (1992). *Anxiety sensitivity index manual (2nd ed)*. Worthington, OH: International Diagnostic Systems.
- Rapee, R. M., & Medoro, L. (1994). Fear of physical sensations and trait anxiety as mediators of the response to hyperventilation in nonclinical subjects. *Journal of Abnormal Psychology*, 103(4), 693–9. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7822570>
- Sabourin, B. C., Stewart, S. H., Watt, M. C., & Krigolson, O. E. (2015). Running as Interoceptive Exposure for Decreasing Anxiety Sensitivity: Replication and Extension. *Cognitive Behaviour Therapy*, 44(4), 264–74. <http://doi.org/10.1080/16506073.2015.1015163>
- Stegen, K., Van Diest, I., Van de Woestijne, K. P., & Van den Bergh, O. (2000). Negative affectivity and bodily sensations induced by 5.5% CO₂ enriched air inhalation: Is there a bias to interpret bodily sensations negatively in persons with negative affect? *Psychology & Health*, 15(4), 513–525. <http://doi.org/10.1080/08870440008402010>
- Straube, T., Glauer, M., Dilger, S., Mentzel, H.-J., & Miltner, W. H. R. (2005). Effects

of cognitive-behavioral therapy on brain activation in specific phobia.

NeuroImage, 29(1), 125–35. <http://doi.org/10.1016/j.neuroimage.2005.07.007>

Van den Hout, M. A., Van der Molen, G. M., Griez, E., Lousberg, H., & Nansen, A.

(1987). Reduction of CO₂ -induced anxiety in patients with panic attacks after repeated CO₂ exposure. *American Journal of Psychiatry*, 144(6), 788–791.

White, K. S., Payne, L. A., Gorman, J. M., Shear, M. K., Woods, S. W., Saksa, J. R., &

Barlow, D. H. (2013). Does maintenance CBT contribute to long-term treatment response of panic disorder with or without agoraphobia? A randomized controlled clinical trial. *Journal of Consulting and Clinical Psychology*, 81(1), 47–57.

<http://doi.org/10.1037/a0030666>

Wilhelm, F. H., Gerlach, A. L., & Roth, W. T. (2001). Slow recovery from voluntary hyperventilation in panic disorder. *Psychosomatic Medicine*, 63(4), 638–49.

Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11485118>

Wollburg, E., Meuret, A. E., Conrad, A., Roth, W. T., & Kim, S. (2008).

Psychophysiological reactions to two levels of voluntary hyperventilation in panic disorder. *Journal of Anxiety Disorders*, 22(5), 886–98.

<http://doi.org/10.1016/j.janxdis.2007.09.004>

Zvolensky, M. J., Goodie, J. L., Ruggiero, K. J., Black, A. L., Larkin, K. T., & Taylor,

B. K. (2002). Perceived stress and anxiety sensitivity in the prediction of anxiety-related responding: A multichallenge evaluation. *Anxiety, Stress & Coping*, 15(3), 211–229.

Table 1. Means (standard errors) for questionnaire measures.

Measure	high-AS	low-AS	<i>t</i>	<i>p</i> (high vs. low-AS)
ASI [0-64]	33.92 (1.10)	8.52 (.51)	19.39	.000
STAI-Trait [20-80]	40.89 (1.56)	31.14 (1.20)	4.82	.000
ACQ [1-5]	1.79 (.09)	1.26 (.04)	5.22	.000
BSQ [1-5]	2.36 (.10)	1.59 (.11)	5.08	.000

Table 2. Means (standard errors) for baseline values of physiological and subjective measures for session 1 and session 2.

Measure	Session 1				Session 2							
	High-AS	Low-AS	High- vs. Low-AS		High-AS	Low-AS	High- vs. Low-AS		Session effect		Session x Group effect	
			<i>t</i>	<i>p</i>			<i>t</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Startle response magnitude (μ V)	62.67 (11.72)	47.37 (8.04)	1.08	.288	57.17 (12.04)	39.96 (8.39)	1.13	.265	4.66	.036*	.10	.752
RR (cpm)	16.14 (0.60)	15.22 (0.67)	1.03	.307	16.66 (0.54)	14.99 (0.74)	1.86	.069	.19	.668	1.33	.255
p _{et} CO ₂ (mmHg)	37.43 (0.88)	37.07 (0.65)	.32	.747	37.40 (0.77)	37.68 (0.63)	.27	.786	.76	.387	.91	.346
Number of symptoms	3.73 (0.46)	2.14 (0.40)	2.57	.014*	1.08 (0.26)	0.55 (0.21)	1.56	.125	68.02	.000*	4.27	.045*

Paced breathing tasks: End-tidal pCO₂

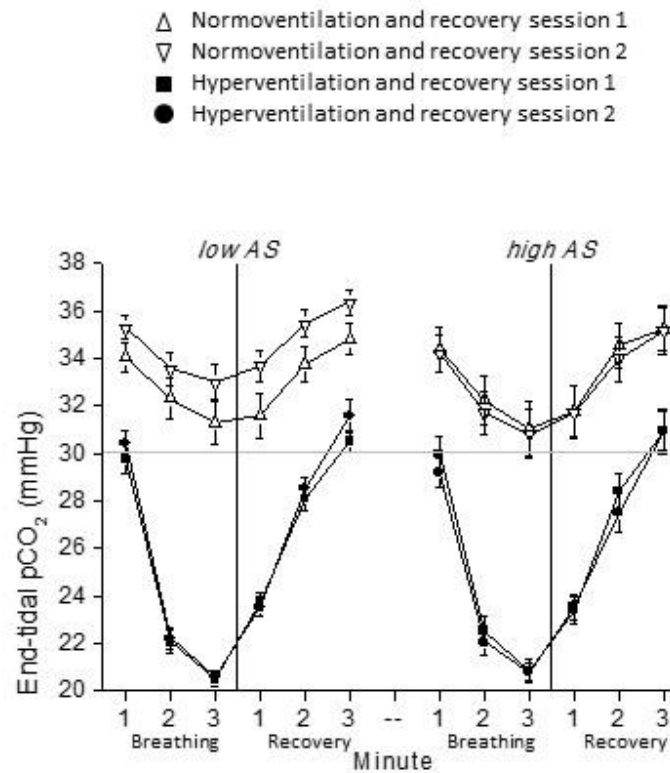


Figure 1. End-tidal pCO₂ during normo- and hyperventilation and early recovery after normo- and hyperventilation in low and high anxiety sensitive participants during session 1 and 2, respectively.

Paced breathing tasks: Symptom report

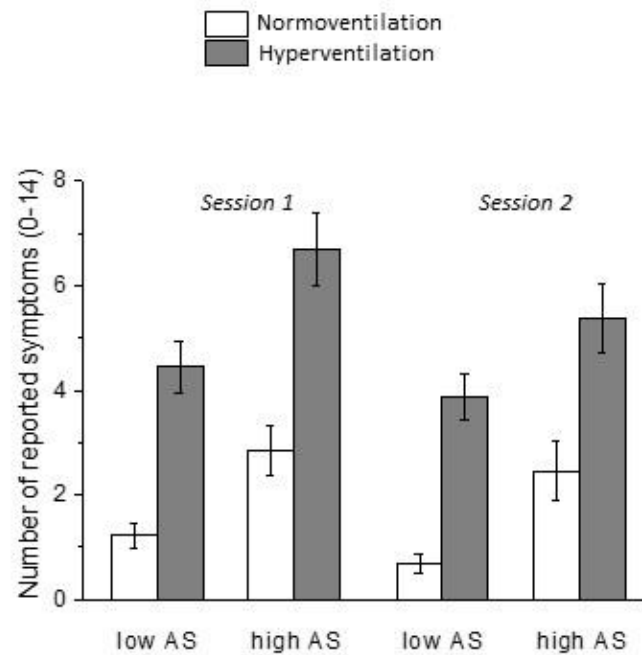


Figure 2. Number of reported symptoms during normo- and hyperventilation in low and high anxiety sensitive participants during session 1 and 2, respectively.

Early recovery: Respiratory rate

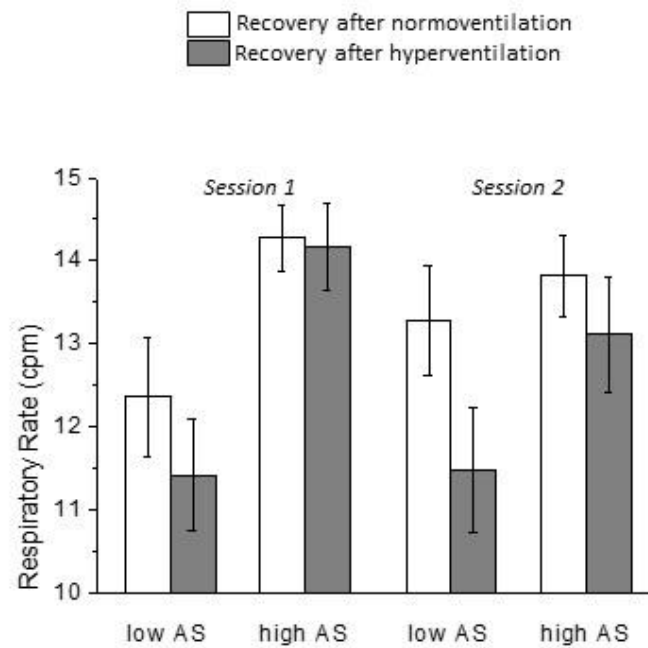


Figure 3. Respiratory rate during early recovery after normo- and hyperventilation in low and high anxiety sensitive participants during session 1 and 2, respectively.

Early recovery: Defensive mobilization

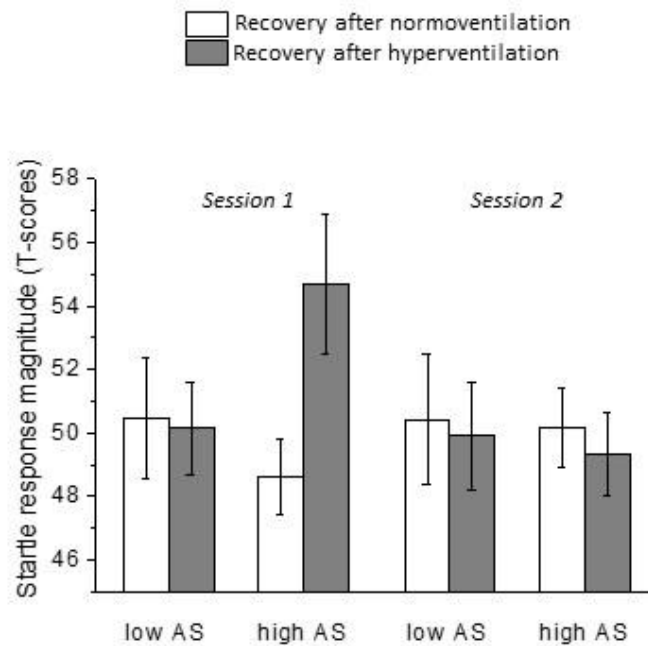


Figure 4. Startle response magnitudes during early recovery after normo- and hyperventilation in low and high anxiety sensitive participants during session 1 and 2, respectively.

Biographical statements

Katharina Holtz is currently finishing her PhD thesis at the Department of Biological and Clinical Psychology at the University of Greifswald and works as a psychologist at a psychiatric outpatient clinic.

Alfons O. Hamm is full professor at the Department of Biological and Clinical Psychology at the University of Greifswald and chief scientist of the associated psychotherapeutic outpatient clinic.

Christiane A. Pané-Farré is a postdoc at the Department of Biological and Clinical Psychology at the University of Greifswald and is an approbated psychotherapist.

Appendix B: Erklärungen

Anteil aller Autoren bei kumulativen Dissertationen

Manuskript 1

Melzig, C. A., Michalowski, J. M., **Holtz, K.**, & Hamm, A. O. (2008). Anticipation of interoceptive threat in highly anxiety sensitive persons. *Behaviour Research and Therapy*, 46(10), 1126–34. <http://doi.org/10.1016/j.brat.2008.07.002>

CM konzipierte und designte das Experiment. CM und KH führten die Experimente durch. CM supervidierte die Datenakquisition. CM analysierte die Daten. Alle Autoren interpretierten die Daten und wirkten an der Manuskripterstellung mit (erster Entwurf durch CM).

Manuskript 2

Holtz, K., Pané-Farré, C. A., Wendt, J., Lotze, M., & Hamm, A. O. (2012). Brain activation during anticipation of interoceptive threat. *NeuroImage*, 61, 857–865. <http://doi.org/10.1016/j.neuroimage.2012.03.019>

Alle Autoren konzipierten und designten das Experiment. KH führte die Experimente durch. CPF und ML supervidierten die Datenakquisition. KH und ML analysierten die Daten. Alle Autoren interpretierten die Daten und wirkten an der Manuskripterstellung mit (erster Entwurf durch KH).

Manuskript 3

Melzig, C. A., **Holtz, K.**, Michalowski, J. M., & Hamm, A. O. (2011). Interoceptive threat leads to defensive mobilization in highly anxiety sensitive persons. *Psychophysiology*, 48(6), 745–54. <http://doi.org/10.1111/j.1469-8986.2010.01150.x>

CM konzipierte und designte das Experiment. CM und KH führten die Experimente durch. CM supervidierte die Datenakquisition. CM analysierte die Daten. Alle Autoren interpretierten die Daten und wirkten an der Manuskripterstellung mit (erster Entwurf durch CM).

Manuskript 4

Holtz, K., Hamm, A. O., & Pané-Farré, C. A. (submitted). Repeated interoceptive exposure in high and low anxiety sensitive persons.

CPF konzipierte und designte das Experiment. CPF und KH führten die Experimente durch. CPF supervidierte die Datenakquisition. KH analysierte die Daten. Alle Autoren interpretierten die Daten und wirkten an der Manuskripterstellung mit (erster Entwurf durch KH).

Datum

Prof. Dr. A. O. Hamm

Katharina Holtz

Appendix D: List of Publications

Melzig, C. A., Michalowski, J. M., **Holtz, K.**, & Hamm, A. O. (2008). Anticipation of interoceptive threat in highly anxiety sensitive persons. *Behaviour Research and Therapy*, 46(10), 1126–1134. <http://doi.org/10.1016/j.brat.2008.07.002>

Melzig, C. A., **Holtz, K.**, Michalowski, J. M., & Hamm, A. O. (2011). Interoceptive threat leads to defensive mobilization in highly anxiety sensitive persons. *Psychophysiology*, 48(6), 745–754. <http://doi.org/10.1111/j.1469-8986.2010.01150.x>

Holtz, K., Pané-Farré, C. A., Wendt, J., Lotze, M., & Hamm, A. O. (2012). Brain activation during anticipation of interoceptive threat. *NeuroImage*, 61, 857–865. <http://doi.org/10.1016/j.neuroimage.2012.03.019>

Yang, Y., Lueken, U., Wittmann, A., **Holtz, K.**, Kleint, N. I., Herrmann, M. J., Sass, K., Jansen, A., Konrad, C., Ströhle, A., Pfleiderer, B., Lotze, M., Hamm, A., Deckert, J., Arolt, V., Wittchen, H-U., Kircher, T., & Straube, B. (2016). Neural correlates of individual differences in anxiety sensitivity: An fMRI study using semantic priming. *Social Cognitive and Affective Neuroscience*, 11(8), 1245–1254. <http://doi.org/10.1093/scan/nsw024>

Grunkina, V., **Holtz, K.**, Klepzig, K., Neubert, J., Horn, U., Domin, M., Hamm, A. O., & Lotze, M. (2017). The Role of Left Hemispheric Structures for Emotional Processing as a Monitor of Bodily Reaction and Felt Chill – a Case-Control Functional Imaging Study. *Frontiers in Human Neuroscience*, 10(January), Article 670. <http://doi.org/10.3389/fnhum.2016.00670>

Holtz, K., Hamm, A. O., & Pané-Farré, C. A. (submitted). Repeated interoceptive exposure in high and low anxiety sensitive persons.

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