Threat from the inside:

Determinants of defensive responses to body sensations and clinical implications

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Content

Abstract

Body sensations play a crucial role in the etiology and maintenance of diverse anxiety and health problems (e.g., in panic disorder or respiratory diseases) as they may be perceived as threatening and consequently elicit anxious responses. The factors that may affect the perception of bodily sensations as a threat and thus modulate the anxious response to body sensations have so far rarely been studied. Therefore, the present thesis targeted at elucidating the effect of contextual (i.e., the predictability, expectation, and proximity of a threat) and dispositional factors (i.e., tendency to fear arousal sensations or trait fear of suffocation) on the defensive response to body sensations.

In study 1, it was investigated how a personality factor, that is, fear of suffocation, affects the acquisition of fear to body sensations (i.e., mild dyspnea induced by inspiratory resistive loads) and contexts when faced with a predictable and unpredictable respiratory threat (i.e., severe dyspnea). Study 2 aimed at examining the main and interactive effects of the tendency to fear arousal sensations, again a personality trait factor, and current arousal expectations as varied by situational variables on anxious responding to arousal sensations. In this study, expected and unexpected arousal sensations were induced by administering caffeine in coffee or bitter lemon soda, respectively. Moreover, in study 3, it was explored how subjective anxiety, bodily symptoms, and defensive respiratory responses change and might culminate into active defense behavior (i.e., escape/active avoidance) during increasing dyspnea that was evoked by inspiratory resistive loads increasing in intensity. For a detailed analysis of the factors that contribute to the initiation and maintenance of avoidance of or escape from increasing dyspnea, in study 4 changes in subjective, autonomic, somatic reflex and brain responses were analyzed during repeated avoidance of increasing dyspnea.

In study 1, it was demonstrated that only individuals who fear suffocation learned to fear mild dyspnea preceding the onset of severe dyspnea and developed anxiety during a context of

unpredictable respiratory threat. Moreover, the data from study 2 indicate that individuals who fear arousal sensations show an increased attention allocation towards unexpected arousal sensations and higher threat appraisal when expecting arousal sensations. Increasing intensity of dyspnea as provoked in study 3 led to increased defensive respiratory responses that were associated with increased symptom reports in individuals with high compared to low fear of suffocation. Moreover, culminating dyspnea elicited repeated avoidance behavior preceded by increases in defensive respiratory mobilization. The analysis of repeated avoidance of increasing dyspnea in study 4 revealed that physiological fear responses might be involved in the initial initiation of this avoidance behavior while no indication of response preparation and physiological arousal was related to persistent avoidance.

Taken together, the present data suggest that the fear of suffocation, as well as the tendency to fear arousal sensations along with the predictability, expectation, or proximity of interoceptive threat, may increase the perceived threat and thus the anxious response to body sensations. Therefore, contextual and dispositional factors may set the stage for the culmination of body sensations into defensive action and might contribute to the development of pathological anxiety and fear of body sensations. The present findings are integrated into the current literature and discussed in relation to the development and maintenance of pathological anxiety and fear of body sensations.

Zusammenfassung

Körperempfindungen spielen eine wesentliche Rolle bei Angststörungen und respiratorischen Erkrankungen, da sie als bedrohlich wahrgenommen werden können und folglich ängstliche Reaktionen hervorrufen. Die Faktoren, die diese wahrgenommene Bedrohung beeinflussen können sowie die ängstliche Reaktion auf Körperempfindungen, wurden bisher kaum untersucht. Aus diesem Grund zielte die vorliegende Arbeit darauf ab, zu untersuchen, wie kontextuelle (sprich, die Vorhersagbarkeit, Erwartung und Nähe von Bedrohung) und dispositionelle Faktoren (Furcht vor Erregungssymptomen bzw. vor Erstickungssensationen) die defensive Reaktion auf Körperempfindungen beeinflussen.

In Studie 1 wurde untersucht, wie die Angst vor Erstickung den Erwerb von Furcht vor Körperempfindungen (sprich vor leichter Atemnot, hervorgerufen durch inspiratorische Atemwiderstände) und Kontexten bei vorhersagbarer und unvorhersagbarer respiratorischer Bedrohung (schwere Atemnot) beeinflusst. Studie 2 zielte darauf ab, die Haupt- und Interaktionseffekte der Furcht vor Erregungssymptomen und der Erregungserwartung auf die ängstliche Reaktion auf Erregungssensationen zu untersuchen. In dieser Studie wurden erwartete und unerwartete Erregungsempfindungen durch die Gabe von Koffein in Kaffee bzw. Bitter Lemon Brause hervorgerufen. Darüber hinaus wurde in Studie 3 untersucht, wie Angst, Körpersymptome und defensive respiratorische Reaktionen sich während der Präsentation ansteigender Atemnot verändern und möglicherweise in aktives Defensivverhalten (d.h. Flucht- und Vermeidungsverhalten) gipfeln. Für eine detaillierte Analyse der Faktoren, die zur Initiierung und Aufrechterhaltung der Vermeidung von oder Flucht vor ansteigender Atemnot beitragen, wurden in Studie 4 Veränderungen in subjektiven, autonomen, somatischen Reflex- und Hirnreaktionen bei wiederholter Vermeidung von ansteigender Atemnot analysiert.

In Studie 1 wurde gezeigt, dass lediglich Personen mit hoher Erstickungsangst Furcht vor leichter Atemnot, die das Einsetzen schwerer Atemnot vorhersagte, und Angst im Kontext einer unvorhersagbaren respiratorischen Bedrohung entwickelten. Darüber hinaus weisen die Daten

aus Studie 2 darauf hin, dass Individuen mit der Furcht vor Erregungssymptomen durch eine erhöhte Aufmerksamkeitszuwendung gegenüber unerwarteten Erregungsempfindungen und eine höhere Einschätzung der Bedrohung bei der Erwartung von Erregungsempfindungen charakterisiert sind. Die in Studie 3 hervorgerufene ansteigende Atemnot führte bei Personen mit hoher im Vergleich zu niedriger Erstickungsangst zu einer gesteigerten defensiven respiratorischen Mobilisierung, die mit stärker berichteten Symptomen assoziiert waren. Zudem wurde wiederholtes Vermeidungsverhalten beobachtet, welches mit starker defensiver respiratorischer Mobilisierung assoziiert war. Die Analyse der wiederholten Vermeidung ansteigender Atemnot in Studie 4 zeigte, dass physiologische Furchtreaktionen bei der ersten Initiierung dieses Vermeidungsverhaltens beteiligt sein könnten, während kein Hinweis auf eine Handlungsvorbereitung oder physiologische Erregung während persistierender, wiederholter Vermeidung beobachtet wurde.

Zusammengenommen deuten die vorliegenden Daten darauf hin, dass die Angst vor Erstickung sowie die Furcht vor Erregungssymptomen, zusammen mit der Vorhersagbarkeit, Erwartung oder Nähe einer interozeptiven Bedrohung die wahrgenommene Bedrohung verstärken und damit die ängstliche Reaktion auf Körpersensationen erhöhen können. Kontextuelle und dispositionelle Faktoren können daher die Kulmination von Körperempfindungen in aktives Defensivverhalten beeinflussen und zur Entstehung von pathologischen Ängsten und Furcht vor Körperempfindungen beitragen. Die vorliegenden Befunde werden in die aktuelle Literatur integriert und in Bezug auf die Entwicklung und Aufrechterhaltung von pathologischen Ängsten und Furcht vor Körperempfindungen diskutiert.

1 Introduction

Excessive and persistent fear and anxiety, that involve defensive responses to threat mediated by defensive brain circuits, are core features of anxiety disorders (American Psychiatric Association, 2013). In the present diagnostic system, the DSM-5, anxiety disorders are grouped according to their critical fear- or anxiety-eliciting situations, e.g., social, circumscribed (i.e., animals, blood/injections), or generalized. In the case of panic disorder (PD) – an anxiety disorder that is characterized by recurrent surges of intense fear (called panic attacks) and persistent anxious apprehension that these attacks may reoccur – the focus of fear is targeted at bodily sensations that may signal the onset of a new panic episode (Barlow, 2002).

Etiological models of pathological anxiety and fear propose that a hyper-reactivity or dysregulation in the brain's defensive survival circuits might mediate this dysfunctional defensive responding (Bouton, Mineka, & Barlow, 2001; Lang, Davis, & Öhman, 2000; Lang, McTeague, & Bradley, 2016; Mineka & Zinbarg, 2006; Rosen & Schulkin, 1998). That is, it is suggested that persons with anxiety disorders may exhibit an exaggerated defensive response to threat or already respond to low levels of threat (e.g., Perusini & Fanselow, 2015). In the case of pathological anxiety and fear of body sensations as observed in patients with PD, it is assumed that actually innocuous body sensations or early indicators of a threat originating from inside the body (e.g., dyspnea indicating suffocation) elicit an increased activation of defensive brain circuits (Bouton et al., 2001; Hamm et al., 2016).

The present thesis manuscript is targeted at elucidating the factors that may contribute to an increased defensive activation and thus dysfunctional anxious responding to body sensations. I will present an overview of the existing evidence revealing the factors that affect to what extent feared body sensations may activate the defensive survival circuit and thus the degree how strongly individuals respond to these body sensations in a specific situation. Before reviewing the literature of modulating factors of defensive responding to feared body

sensations, the present work provides an overview of the defensive responses to threat and its underlying defensive circuits.

2 Defensive responding in the face of interoceptive threat

2.1 Defensive brain networks mediating defensive responses to threat

There is abundant evidence demonstrating that feared body sensations activate the subcortical defensive circuits to initiate and orchestrate the diverse physiological and behavioral defensive responses (Johnson, Federici, & Shekhar, 2014). Studies in animals and humans have identified the amygdala as the key structure within a defensive brain circuit mediating these defensive responses. Animal research has helped to characterize amygdala pathways underpinning defensive responses. Studies have identified the lateral nucleus of the amygdala to directly or indirectly via the basal amygdala convey sensory information about threat-associated cues to the central nucleus of the amygdala (LeDoux, 2000). The central nucleus of the amygdala controls the expression of behavioral, autonomic and endocrine responses as well as somatic reflexes via projections to target regions in the brainstem and hypothalamus (see **Figure 1**). Interestingly, initial evidence indicates that the periaqueductal gray in the brainstem is specialized in detecting internal physiological signals (Schimitel et al., 2012) as well as in integrating these signals with environmental information to detect interoceptive threat and prompting active defensive behavior (Preter & Klein, 2008).

In contrast to defensive reactions as described above, defensive actions, such as escape or avoidance, are mediated by a different amygdala pathway. Threat information is propagated via direct connections from the lateral nucleus of the amygdala to the basal amygdala which projects to the ventral striatum (Amorapanth, LeDoux, & Nader, 2000; Choi, Cain, & LeDoux, 2010; Ramirez, Moscarello, LeDoux, & Sears, 2015). It has been demonstrated that the switch in amygdala pathways is mediated by the prefrontal cortex that suppresses central amygdala

mediated expressions of defensive reactions and thus facilitates aversively motivated actions (Martinez et al., 2013; Moscarello & LeDoux, 2013).

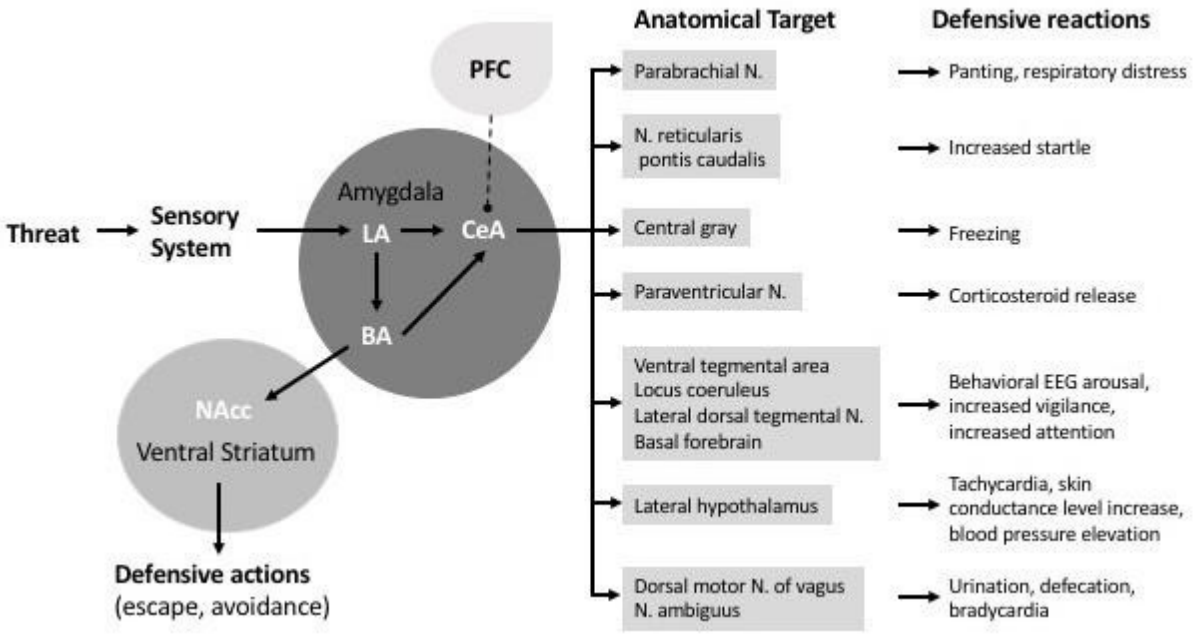

Figure 1. Amygdala pathways underlying defensive reactions and defensive actions (adapted from LeDoux & Pine, 2016 and Davis & Whalen, 2001). Abbreviations: LA, lateral amygdala; CeA, central amygdala; BA, basal amygdala; NAcc, nucleus accumbens; PFC, prefrontal cortex.

2.2 Defensive responses to threat

2.2.1 Autonomic and respiratory responses

The activation of the defensive brain circuit in response to threat is accompanied by an increase in autonomic arousal. It has been demonstrated that skin conductance level and heart rate as indices of autonomic arousal are increased during anticipation or presentation of exteroceptive threat (e.g., electric shocks) or cues that predict its onset (Deane, 1961; Hamm & Vaitl, 1996; Löw, Lang, Smith, & Bradley, 2008; Löw, Weymar, & Hamm, 2015; Wendt, Löw,

Weymar, Lotze, & Hamm, 2017). Increased autonomic arousal has also been shown when persons anticipated interoceptive threat or were exposed to feared body sensations (Lang et al., 2011; Melzig, Holtz, Michalowski, & Hamm, 2011; Melzig, Michalowski, Holtz, & Hamm, 2008; Pappens, Peuter, Vansteenwegen, Van den Bergh, & Van Diest, 2012; Pappens, Van den Bergh, Vansteenwegen, & Van Diest, 2011).

There also is evidence indicating that aversive stimuli elicit a change in breathing pattern that is characterized by a faster and/or deeper breathing (e.g., indicated by increases in breathing frequency, tidal volume or minute ventilation) as well as a decrease in the expired CO_2 (Boiten, Frijda, & Wientjes, 1994). Studies have also demonstrated a respiratory mobilization (e.g., an increase in breathing frequency) during imagination of aversive scenes, anticipation, and provocation of feared bodily sensations (Alius, Pané-Farré, Leupoldt, & Hamm, 2013; Masaoka & Homma, 1997, 2001; Melzig et al., 2011; Pané-Farré et al., 2015; Pappens, Smets, Van den Bergh, & Van Diest, 2012; Van Diest et al., 2001; Van Diest et al., 2005).

2.2.2 Startle eyeblink response

Another physiological measure reflecting the activation of the defensive survival circuit is the startle eyeblink response to an abrupt startle probe (e.g., an acoustic burst of broadband white noise) (Lang et al., 2000). The startle eyeblink response is a rather low-level brain stem measure of defensive response mobilization (Lang, Bradley, & Cuthbert, 1990). The mobilization or priming of the startle eyeblink response to an acoustic startle probe results from direct projections of the amygdala on the primary eyeblink reflex pathway (see Davis, 2006 for a review). Studies in humans and rodents demonstrated that the startle eyeblink response is reliably potentiated when elicited during cues associated with aversive events (e.g., shocks) as well as during anticipation of threat or presentation of unpleasant and feared stimuli (Davis, Walker, Miles, & Grillon, 2010; Grillon, Ameli, Merikangas, Woods, & Davis, 1993; Grillon & Davis, 1997; Hamm, Greenwald, Bradley, & Lang, 1993; Lang et al., 1990; Melzig et al.,

2008). This increase in startle response magnitudes has also been observed during dyspnea predicting respiratory threat (e.g., a suffocation episode) or during anticipation of respiratory threat (Lang et al., 2011; Melzig et al., 2008; Pappens et al., 2013; Pappens, Smets, Vansteenwegen, Van den Bergh, & Van Diest, 2012; Pappens, Vandenbossche, Van den Bergh, & Van Diest, 2015).

2.2.3 Attention

The activation of the defensive survival circuit also engages sensory systems prompting increased attentional and perceptual processing of potential or detected threat, thus facilitating sensory processing of cues or contextual stimuli (Lang & Bradley, 2010; Lang & Davis, 2006; Michalowski, Pané-Farré, Löw, & Hamm, 2015). As the attentional capacity is naturally limited, this increased attention toward threat-relevant stimuli leads to a decrease of processing of threat-irrelevant and distracting stimuli (Lang & Bradley, 2010; Löw et al., 2015). A well described phenomenon related to this attentional shift is the change in the P300 component of the event-related potential to an acoustic startle probe as described in EEG research (Bradley, Codispoti, & Lang, 2006; Cuthbert, Schupp, Bradley, McManis, & Lang, 1998). It has been demonstrated that the amplitudes of the P3 component of the probe-evoked potentials are attenuated when attentional resources are captured by emotional foreground stimuli during emotional picture viewing (Cuthbert et al., 1998; Schupp, Cuthbert, Bradley, Birbaumer, & Lang, 1997), by cues signaling the opportunity to initiate an avoidance response (Löw et al., 2015), interoceptive cues (Alius, Pané-Farré, Löw, & Hamm, 2014) or cues/contexts predicting threat (Nelson & Hajcak, 2017; Nelson, Hajcak, & Shankman, 2015; Nelson, Hodges, Hajcak, & Shankman, 2015). Thus, attention allocation to task or threat-related cues leads to a reduction of the processing of the secondary acoustic probe. It has been demonstrated that this reduced processing of the startle eliciting probe is accompanied by an inhibition of the startle eyeblink response. For example, Alius et al. (2014) showed that a reduction of the amplitudes of the P3 component of the probe-evoked brain potentials during slight dyspnea went along with an

inhibition of the startle blink magnitudes, suggesting that attentional resources are allocated to body sensations in the expense of a reduced capacity to process the acoustic startle probe.

2.3 Defensive responses as a function of the imminence of body sensations: Effects of contextual factors and individual differences

The physiological and behavioral responses to a threat in a specific situation fundamentally vary with contexual and dispositional factors (Kozlowska, Walker, McLean, & Carrive, 2015). Evidence from animal and human research with exteroceptive threats (e.g., electric shocks, a predator) demonstrated that the physiological and behavioral responses and its underlying neural circuits differ depending upon the perceived proximity or imminence of the threat (Fanselow & Lester, 1988; Kozlowska et al., 2015; Mobbs et al., 2007; Mobbs et al., 2009; Perusini & Fanselow, 2015). As outlined in the threat imminence model (Blanchard & Blanchard, 1989; Fanselow, 1994; Hamm et al., 2016; Hamm, Richter, & Pane-Farre, 2014), a translational model describing defensive behavior in relation to the proximity of threat, defensive responses may change from enhanced vigilance during potential or more distant threat to flight behavior during proximate and acute threat. The perceived threat is, however, determined by person-specific as well as contextual factors, such as the behavioral repertoire at hand (e.g., availability of escape routes), characteristics of the threat, and the context in which threat occurs (Kozlowska et al., 2015). These factors may interact to affect the perceived imminence determining defensive responses in a specific situation. For example, it has been shown that different levels of imminence in interaction with the behavioral repertoire at hand prompt different patterns of defensive behavior and neural activity (Löw et al., 2015; Wendt et al., 2017).

Clinical observations and experimental studies suggest that defensive response patterns to body sensations may also vary depending on the perceived imminence (resp., the threat intensity) of body sensations (Hamm et al., 2014; Hamm et al., 2016). It has been observed that

low levels of perceived threat of body sensations prompt a defensive response pattern that is characterized by increased attention allocation to body sensations as indexed by reduced amplitudes of the startle eyeblink response and the P3 component of brain potentials to acoustic startle probes (see Alius et al., 2014). In contrast, defensive response mobilization as indicated by increases in autonomic, respiratory and startle eyeblink responses is elicited when the perceived threat of body sensations and the proximity to the interoceptive threat (e.g., suffocation) increases (e.g., Melzig et al., 2011). At the peak of interoceptive threat, acute panic or flight behavior is initiated that is accompanied by a strong surge of autonomic arousal and a relative inhibition of the startle response (e.g., Richter et al., 2012).

Studies on the modulatory factors in anxious responding to body sensation indicate that dispositional and contextual factors may affect the perceived imminence of body sensations, thus determining the defensive response to body sensations (Telch, Harrington, Smits, & Powers, 2011; Zvolensky & Eifert, 2001). From a clinical perspective, these factors may enhance the perceived threat and defensive responding to body sensation spiraling into a vicious circle composed of increased threat perception, defensive responses and body sensations culminating into panic and/or defensive action (Bouton et al., 2001; Clark, 1986; Ehlers & Margraf, 1989; Hamm et al., 2016). Thus, this exaggerated defensive responding to body sensations may set the stage for the development of PD. This is supported by contemporary learning theories of the etiology of PD that propose that the acquisition and expression of defensive responses to body sensations is influenced by several environmental as well as person-specific variables (Bouton et al., 2001; Mineka & Zinbarg, 2006). However, there is only scarce research elucidating the contextual conditions and individual differences that affect the perceived imminence of body sensation and thus determine how persons respond to body sensations. A detailed analysis of these factors will help to refine models of the pathogenesis and maintenance of pathological anxiety related to body sensations. Therefore, the present work summarizes previous evidence and recent studies investigating the effect of contextual (e.g.,

predictability or unexpectedness) and trait-like personality factors (e.g., the tendency to fear arousal sensation) on the modulation of defensive responses to body sensations.

3 Experimental studies of the determinants of defensive responses to body sensations

3.1 Acquisition of defensive responses to dyspnea as a function of predictability and suffocation fear

Body sensations may become threatening and acquire the ability to elicit defensive responses via interoceptive fear conditioning (Barlow, 2002; Bouton et al., 2001; Mineka & Zinbarg, 2006). Previous findings of interoceptive fear conditioning studies have demonstrated that previously innocuous respiratory sensations may become conditioned stimuli (CS) eliciting defensive response mobilization when repeatedly paired with an aversive respiratory unconditioned threat (unconditioned stimuli, US) (Acheson, Forsyth, & Moses, 2012; Acheson, Forsyth, Prenoveau, & Bouton, 2007; Benke, Alius, Hamm, & Pané-Farré, in revision; Ceunen et al., 2016; Pappens et al., 2013; Pappens et al., 2014; Pappens et al., 2015; Pappens, Smets, Vansteenwegen et al., 2012). These studies used episodes of severe dyspnea as interoceptive USs by applying multi-breath inhalations of CO_2, severe inspiratory resistive loads (IRL), or a complete breathing occlusion. In addition to the interoceptive US, in these studies, a brief exposure to IRLs or inhalation of CO_2 was used to induce a mild feeling of dyspnea or breathlessness that served as the CS immediately preceding the interoceptive US (Acheson et al., 2007; Benke et al., in revision; Pappens et al., 2013; Pappens et al., 2015; Pappens, Smets, Vansteenwegen et al., 2012).

However, in the case of PD, body sensations (e.g., dyspnea) preceding a panic attack in one context, e.g., a shopping mall, do not qualitatively differ from the sensations experienced during a different context, e.g., stair-climbing, that may not predict a panic attack. It becomes clear that the very same body sensation may acquire competitive meanings in different contexts and thus become ambiguous (Holland & Bouton, 1999; Maren, Phan, & Liberzon, 2013).

Studies investigating defensive responding to the same CS in different contexts demonstrated that contexts may control the conditioned responses to the same CS (Holland & Bouton, 1999; Kimmel & Gardner, 1981; Kimmel & Ray, 1978; Murrin & Kimmel, 1986).

When faced with an interoceptive threat, not only weak body sensations may acquire the ability to elicit defensive responses (cue conditioning), the context may also be learned as being indicative of the upcoming interoceptive threat and thus may evoke defensive responses (context conditioning) (see Pappens et al., 2012). The latter learning process is facilitated in contexts in which a threat is not signaled by an explicit cue and thus its occurrence remains uncertain and unpredictable (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Grillon, Baas, Cornwell, & Johnson, 2006; Grillon & Davis, 1997; Marschner, Kalisch, Vervliet, Vansteenwegen, & Büchel, 2008; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008).

In a recent study of Benke et al. (in revision), therefore, the acquisition of defensive response mobilization to context as well as to a respiratory CS was investigated using a respiratory US and the same respiratory CS across different contexts. In this study, the startle eyeblink response as an index of defensive response mobilization was measured in three different contexts: mild dyspnea (i.e., the CS) was either paired with strong dyspnea (i.e., the US) in a predictable context, presented unpaired with the US in an unpredictable context or presented without administering the US in a safe context (see Grillon et al., 2006). Moreover, it was examined if associative fear learning processes are moderated by person-specific factors, such as fear of suffocation (SF), as it is assumed that they increase the salience of CS and US stimuli (i.e., the salience of mild and severe dyspnea in the case of suffocation fear) which is discussed to lead to stronger fear conditioning (Barlow, 2002).

The study by Benke et al. (in revision) demonstrated that cued and context-associated defensive response mobilization only developed in those individuals for whom the respiratory

threat was salient, i.e., for individuals who fear suffocation. Only in high SF individuals, mild dyspnea predicting the occurrence of severe dyspnea was enabled to elicit defensive response mobilization indicated by a potentiation of the startle eyeblink response. High SF individuals were also characterized by emerging greater startle response magnitudes during no-cue periods in the unpredictable context as compared to the predictable and safe context, thus indicating successful context conditioning.

The data demonstrate that persons who fear suffocation may show a stronger conditioning of cued and context-associated defensive response mobilization when faced with a respiratory US. Therefore, high SF persons may exhibit exaggerated defensive response mobilization activated by suffocation stimuli and contextual stimuli predicting interoceptive threat that may spiral into PD. Interestingly, a stronger defensive response mobilization has been demonstrated when individuals who reported high SF including patients with PD anticipated threat or were exposed to suffocation sensations (Alius et al., 2013; Benke et al., in revision; Benke, Hamm, & Pané-Farré, 2017; Eifert, Zvolensky, Sorrell, Hopko, & Lejuez, 1999; Eke & McNally, 1996; Grillon et al., 2008; McNally & Eke, 1996; Melzig, Weike, Zimmermann, & Hamm, 2007; Taylor & Rachman, 1994). This observation is of clinical relevance because the observed contextually-induced and cued defensive response mobilization is assumed to further potentiate defensive responses and behaviors, thus increasing the risk for the culmination of symptoms and anxiety into panic or defensive action (Barlow, 2002; Benke et al., in revision; Benke, Hamm et al., 2017; Bouton et al., 2001). Overall, the data suggest that SF might act as a psychological vulnerability factor that may increase associative learning processes, i.e. cue and context conditioning, during respiratory threat and thus pave the way for the development of PD (Barlow, 2002; Bouton et al., 2001; Mineka & Zinbarg, 2006).

The data suggest that individual differences in the level of suffocation fear in combination with contextual factors influence whether body sensations become threatening and

are enabled to prompt defensive responses. Thus, both factors may interact to facilitate the acquisition of anxious responding in the face of body sensations. However, it is assumed that this learning process typically takes place in the context of extreme fear (i.e., during a panic attack) (Barlow, 2002; Bouton et al., 2001). Thus, it is of clinical relevance to examine potential person and context factors that enhance the perceived threat and increase the probability to experience fear or panic attacks (Telch et al., 2011).

3.2 Defensive responses to arousal sensations as a function of arousal expectation and anxiety sensitivity

Recent studies indicate that the interplay of person-specific (e.g., disposition to fear of arousal sensations) and contextual factors (e.g., the unexpectedness or predictability) affect the perception of body sensations as a threat and thus determine defensive responses to body sensations (Pané-Farré et al., 2015; Telch et al., 2010; Telch et al., 2011; Telch, Silverman, & Schmidt, 1996; Zvolensky, Eifert, & Lejuez, 2001). Previous evidence emphasized the role of unexpectedness of emerging bodily sensations as an important contextual factor that is implicated in defensive responses to bodily symptoms as in, for example, unexpected panic attacks (Pané-Farré et al., 2015; Rapee, Mattick, & Murrell, 1986; Telch et al., 2010; Telch et al., 2011; Veltman, van Zijderveld, Van Dyck, & Bakker, 1998). Data from Telch and colleagues (2010; 2011) suggest that the defensive response to unexpected arousal sensations may depend on the level of reported anxiety sensitivity (AS) that refers to the tendency to fear somatic arousal sensations (McNally, 2002). There is ample evidence demonstrating that persons reporting high levels of AS showed an exaggerated anxious responding when confronted with arousal sensations induced by various symptom provocation tasks (e.g., hyperventilation, loaded breathing) (Alius, Pané-Farré, Leupoldt, & Hamm, 2013; Asmundson, Norton, Wilson, & Sandler, 1994; Koszycki, Cox, & Bradwejn, 1993; Melzig, Holtz, Michalowski, & Hamm, 2011; Telch et al., 2010; Telch, Harrington, Smits, & Powers, 2011; Zvolensky, Eifert, & Lejuez, 2001). Moreover, it has been demonstrated that anxiety sensitivity

predicted the occurrence of panic attacks and the onset of PD (Li & Zinbarg, 2007; Schmidt, Zvolensky, & Maner, 2006; Woud, Zhang, Becker, McNally, & Margraf, 2014).

Benke, Blumenthal, Modeß, Hamm, and Pané-Farré (2015) investigated the effect of anxiety sensitivity on the threat appraisal and defensive response to unexpected and unexplained arousal sensations. In this study, caffeine was used to induce arousal sensations in an expectancy-controlled research design (see Rohsenow & Marlatt, 1981). To create a match/mismatch of created arousal expectations and experienced arousal sensations, caffeine at dose of 4 mg/kg body weight vs. no caffeine was mixed either in coffee (context in which arousal sensations are expected) or bitter lemon soda (context in which *no* arousal sensations are expected) (Benke et al., 2015). Therefore, it was possible to assess how anxiety sensitivity affects defensive responses to unexpected arousal sensations induced by administering caffeine in bitter lemon soda.

In line with previous evidence from studies with anxiety-unselected populations, the administration of caffeine led to an increase in respiratory ventilation (i.e., increased minute ventilation, decreased pCO_2) (see Barry, Clarke, Johnstone, & Rushby, 2008; Bell, Kowalchuk, Paterson, Scheuermann, & Cunningham, 1999; Cameron, Modell, & Hariharan, 1990; Sawyer, Julia, & Turin, 1982), autonomic activation (i.e., increased skin conductance level, decreased heart rate) (see Childs, 2006; Flaten & Blumenthal, 1999; Green, Kirby, & Suls, 1996; James, 1994; Lotshaw, Bradley, & Brooks, 1996; Mikalsen, Bertelsen, & Flaten, 2001), and startle response magnitudes (see Andrews, Blumenthal, & Flaten, 1998; Flaten, Aasli, & Blumenthal, 2003; Flaten & Blumenthal, 1999) that peaked 30 minutes after beverage ingestion. No differences in autonomic and respiratory responses were observed between low and high AS individuals (see Asmundson, Norton, Wilson, & Sandler, 1994; Schmidt & Telch, 1994; Sturges & Goetsch, 1996; Sturges, Goetsch, Ridley, & Whittal, 1998; Zvolensky et al., 2001;

Zvolensky, Eifert, Lejuez, & McNeil, 1999), thus indicating a comparable autonomic and respiratory stimulation in both groups.

In contrast, startle responses were inhibited during the unexpected compared to the expected arousal induction by caffeine. This corroborates previous evidence from symptom provocation studies (Alius et al., 2014; Ceunen, Vlaeyen, & Van Diest, 2013; Pappens et al., 2011; Pappens, Peuter et al., 2012) demonstrating a relatively inhibited startle eyeblink response while participants experienced challenge-induced body sensations. This startle inhibition has been interpreted as a shift of attentional resources away from the auditiory channel to the experienced body sensations, thus decreasing available resources for processing of the startle probe (Alius et al., 2014; Ceunen et al., 2013; Pappens et al., 2011). This interpretation is supported by evidence from cross-model experiments demonstrating that startle response magnitudes are inhibited when attention was captured by stimuli that differed from the sensory modality of the startle-eliciting probe (Anthony & Graham, 1985; Filion, Dawson, & Schell, 1998). Moreover, findings from a symptom provocation study demonstrated reduced amplitudes of the startle response and P3 component of the brain potentials to startle probes during dyspnea (see Alius et al., 2014). Thus, the observed pattern might be interpreted as an increased attention allocation to unexpected arousal sensation. This increased attention allocation to unexpectedly arising arousal sensations might limit the capacity available for the processing of the acoustic startle stimulus (Alius et al., 2014; Pappens et al., 2011). Moreover, the data indicate that individuals who fear arousal sensations might engage more attentional resources for processing the unexpected arousal sensations as evidenced by a more accentuated startle response inhibition in persons reporting high anxiety sensitivity. It has been suggested that the mismatch of expected and actual occurring stimuli elicit attentional resource allocation to threat (Gray & McNaughton, 2003) that might be increased in persons who fear arousal sensations as a result of an increased insula activation (Paulus & Stein, 2006) or an increased

threat appraisal (Telch et al., 2011). This increased attentional allocation may facilitate the detection of threat, sensory intake of threat information and the selection of appropriate action.

Extending previous findings from anxiety-unselected populations (Flaten & Blumenthal, 1999; Mikalsen et al., 2001), in high compared to low anxiety-sensitive persons the expectation of arousal induction led to increased alertness ratings following the administration of caffeine and higher reported arousal even if no caffeine was consumed. These data suggest that persons who fear arousal sensations differ from low anxiety-sensitive persons in their threat appraisal of the expected arousal sensations (Telch et al., 2010). Moreover, the results demonstrated that high anxiety sensitivity interacts with contextual factors such as the unexpectedness of arousal sensations by increasing the perceived threat of arousal sensation (see Telch et al., 2010; Telch et al., 2011). The increased threat perception and attention allocation to potential harmful sensations may set the stage for the culmination of body sensations into panic or defensive action.

In the study of Benke et al. (2015), the use of caffeine proved useful to reveal effects of anxiety sensitivity and the expectation of arousal induction on defensive responding to arousal sensations. The caffeine doses used in our study only induced mild arousal sensations as compared to different symptom provocation tasks (e.g., loaded breathing) and led to low defensive activation as evidenced by decreased startle responses indicating increased sensory intake of threat-related information. However, it has not yet been shown how this defensive response pattern might change with increasing intensity of body sensations and thus might contribute to the culmination of body sensation into panic or defensive action as observed in patients with PD.

3.3 Defensive responses as a function of increasing dyspnea and suffocation fear

It is assumed that defensive responses, body sensations and anxiety may amplify each other and thus lead to a culmination into panic or defensive action (Bouton et al., 2001; Clark,

1986; Ehlers & Margraf, 1989; Hamm et al., 2014; Pappens, Smets, Van den Bergh et al., 2012). However, the factors that contribute to this escalating process are not well understood yet. Studies using inspiratory resistive loads inducing dyspnea indicate that individual differences in the way individuals cope with restricted breathing might affect this escalating process (Alius et al., 2013; Pappens, Smets, Van den Bergh et al., 2012). In fact, it was demonstrated that persons who fear suffocation exhibit an increase in respiratory rate that result in an increase in exposure frequency to the loaded inspiration and thus may lead to worsening of dyspnea (Alius et al., 2013; Pappens, Smets, Van den Bergh et al., 2012). Thus, while experiencing an episode of increasing breathing restriction (e.g., during stair climbing, physical activity) defensive respiratory responses in interaction with anxiety and symptoms may possibly culminate into panic or defensive actions.

To study this escalating process, Benke, Hamm et al. (2017) examined defensive respiratory responses as well as symptom and anxiety reports in response to increasing intensity of body sensations (i.e., dyspnea). Increasing intensity and aversiveness of dyspnea were evoked by successively applying IRLs of increasing intensity. Most importantly, in this study the increasing breathing resistance predicted an approaching breathing occlusion that has been demonstrated to be a potent unconditioned respiratory threat as it models a short-lasting suffocation experience (Nardi et al., 2006; Pappens et al., 2014; Pappens, Smets, Vansteenwegen et al., 2012). In this study, it was tested how individual differences in the level of suffocation fear affect the culmination of body sensations into panic or defensive action. To investigate defensive actions all participants had the opportunity to terminate the exposure to increasing dyspnea via a button press.

In the study of Benke, Hamm et al. (2017), persons who fear suffocation exhibited a higher respiratory rate that was associated with a higher intensity of panic symptoms during severe dyspnea. Extending previous evidence (Alius et al., 2013; Pappens, Smets, Van den

Bergh et al., 2012), it was demonstrated that the mobilization of defensive respiratory responses depends upon the severity of dyspnea. A higher breathing frequency in high SF participants was only revealed if dyspnea became more severe. These findings suggest that dyspnea predicting an impending suffocation episode initiates the mobilization of defensive respiratory responses in high SF persons (Alius et al., 2013; Benke et al., in revision; Benke, Hamm et al., 2017; Masaoka & Homma, 1997, 2001; Melzig et al., 2011; Pané-Farré et al., 2015; Van Diest et al., 2001; Van Diest et al., 2005). Together with previous evidence (see Benke et al., in revision), the data imply that increased suffocation fear might play a pivotal role for facilitating associative learning processes and might contribute to the development of defensive response mobilization elicited by suffocation stimuli. The observed increased breathing frequency during loaded breathing is assumed to worsen the feelings of dyspnea due to a resulting increase in exposure frequency to the resistive load (Alius et al., 2014; Pappens, Smets, Van den Bergh et al., 2012). In high SF persons, this breathing pattern was additionally accompanied by a higher increase in the intensity of panic symptoms that further increased the intensity of feared symptoms and may culminate into panic attacks or defensive action. Interestingly, a substantial number of persons terminated the exposure to increasing dyspnea.

In high and low SF persons, premature terminations were preceded by an increase in respiratory rate and a decrease in tidal volume. Exploratory analyses revealed that this response pattern was not observed in matched exposure trials that were not terminated. These results are in line with evidence from patients with PD showing a strong surge of autonomic and respiratory responses prior to escapes or panic attacks (Meuret et al., 2011; Richter et al., 2012). The observed respiratory responses prior to terminations suggest an increased activation of the defensive survival circuit that might initiate the mobilization of defensive respiratory responses in preparation for defensive actions (Benke, Hamm et al., 2017). Unfortunately, this increase in respiratory rate may lead to a worsening of dyspnea that may affect the perceived threat, the experienced symptoms and defensive responses which lead to a vicious circle spiraling into

panic or defensive action (Bouton et al., 2001; Clark, 1986; Hamm et al., 2016; Pappens, Smets, Van den Bergh et al., 2012).

Thus, the data suggest that the observed increases in respiration might have triggered repetitive terminations of dyspnea. However, this finding contradicts previous evidence showing a decrease in defensive responses during repeated defensive actions (Boeke, Moscarello, LeDoux, Phelps, & Hartley, 2017; Campese et al., 2016; Delgado, Jou, LeDoux, & Phelps, 2009; LeDoux, Moscarello, Sears, & Campese, 2016; Lovibond, Saunders, Weidemann, & Mitchell, 2008; Vervliet & Indekeu, 2015). Given that avoidance of interoceptive threat is highly prevalent in patients with PD or respiratory diseases (e.g. taking medication to alleviate increasing dyspnea) (Barlow, 2002; Carr, 1999; Lehrer, Feldman, Giardino, Song, & Schmaling, 2002), it is of clinical relevance to reveal the factors mediating the initiation and maintenance of repeated terminations of exposure to interoceptive threat. Thus, a comprehensive characterization of behavioral, subjective and physiological responses prior to repeated terminations of interoceptive threat is essential.

3.4 Defensive responses as a function of repeated terminations of increasing dyspnea

To delineate the factors and motivational basis of repeated avoidance behavior during interoceptive threat, in the study of Benke, Krause, Hamm, and Pané-Farré (2017), a broad array of defensive responses including behavioral, subjective and autonomic responses as well as brain stem reflex measures and startle probe evoked brain potentials were analyzed prior to repeated terminations of the exposure to increasing dyspnea. In this study, these defensive responses preceding defensive actions were compared to subjective and physiological responses during matched control sequences of persons who completed all exposure trials.

It was demonstrated that the first termination of exposure was accompanied by a strong discharge of autonomic arousal and a relatively inhibited startle eyeblink response, supporting evidence from patients with PD who escaped from a situation of entrapment (Richter et al., 2012). The startle inhibition went along with a sharp decrease in the amplitudes of the P3

component of the probe-evoked brain potentials, supporting previous findings from Löw et al. (2015). This attentional and startle reflex pattern suggests that in preparation of the initial defensive action attentional resources are engaged and the elaborated processing of the secondary acoustic probe is reduced (Alius et al., 2014; Löw et al., 2015; Schupp et al., 1997). Thus, prior to defensive action attentional channels for irrelevant and distracting stimuli are dampened to prepare for an effective defensive action.

Following the first termination of the exposure, participants terminated subsequent exposure trials at successively lower threat levels (i.e., already during lower intensities of IRLs) and thus avoided the occurrence of the breathing occlusion. This pattern suggests that increasing dyspnea possibly became a conditioned stimulus signaling the occurrence of the breathing occlusion and thus evoke avoidance behavior to prevent the further increases in dyspnea (Benke et al., in revision; Pappens, Peuter et al., 2012). While the behavioral pattern changed, physiological arousal and the inhibition of the amplitudes of the probe-evoked startle response and P3 component of brain potentials diminished as early as prior to the second termination of increasing intensity of body sensations. This pattern of defensive responses goes in line with evidence in humans showing a decrease in defensive responses during avoidance learning as indexed by diminished autonomic arousal (Boeke et al., 2017; Delgado et al., 2009; Lovibond et al., 2008; Vervliet & Indekeu, 2015). Evidence in animals suggests that this change in defensive reactions during repetitive defensive actions might be the result of a shift in amygdala pathways coordinated by the prefrontal cortex that suppresses central amygdala mediated defensive reactions in favor of proactive coping with the threat (Campese et al., 2016; Martinez et al., 2013; Moscarello & LeDoux, 2013). Together with the present literature, the findings of the study of Benke, Krause et al. (2017) support the view that physiological fear responses might be an important motivator for the initiation of defensive action (see Mowrer, 1939) but might not be involved in the maintenance of repetitive defensive actions (for review see Krypotos, Effting, Kindt, & Beckers, 2015).

In contrast to physiological arousal, selective attention, and defensive reflex pattern, the increase in respiratory rate did not change throughout repeated terminations of increasing dyspnea. This indicates that respiration might not be affected by avoidance learning. It might be that both, the increases in respiratory rate and the omitted avoidance behavior, are defensive behaviors that target at preventing a possible suffocation. However, the increases in respiratory rate prior to terminations might have worsened the experienced dyspnea and symptoms. Since the active respiratory coping with the respiratory threat was ineffective, respiratory coping changed to defensive action that ultimately terminated the threat.

Importantly, the reported response pattern in the study of Benke, Krause et al. (2017) was exclusively observed in persons who prematurely terminated exposure but not in matched control persons, suggesting that the observed effects were not due to the physiological state at the specific time of termination or the repeated exposure to increasing dyspnea.

This study provided a first comprehensive analysis of the processes and motivational basis underlying repeated terminations of interoceptive threat. The data suggest that physiological fear responses might not be the primary motivator for the maintenance of defensive actions. Alternativley, the data may indicate that an exaggerated breathing along with an increased threat perception as indexed by an increased respiratory rate and reports of anxiety might have maintained repetitive defensive actions. Importantly, this dysfunctional defensive behavior possibly prevents extinction of fear and anxiety, maintaining anxiety and irrational fears and leading to an exacerbation of psychopathology and impaired psychosocial functioning (see Krypotos et al., 2015 for review; Vervliet & Indekeu, 2015). Thus, further research should focus on the underlying mechanisms and the factors that possibly set the stage for developing repeated defensive actions to provide effective tools for treatment.

4 Summary and future directions

The present work provided a review of the current evidence elucidating contextual and dispositional factors that may contribute to an increased defensive activation and thus increased defensive responding to body sensations. In our study, we demonstrated that individuals who fear arousal sensations show an increased attention allocation to unexpected arousal sensations and a higher threat appraisal of the arousal induction. The data from our studies also indicate that suffocation fear might be a risk factor that facilitates cue and context conditioning to respiratory threat and affects the defensive responding to body sensations increasing in intensity, thus contributing to the culmination of body sensations into defensive actions. Moreover, we demonstrated that physiological fear responses may initiate avoidance of interoceptive threat but might not contribute to the maintenance of persistent avoidance behavior in the context of an interoceptive threat.

Although our findings shed new light on our understanding of the possible contextual and dispositional factors that contribute to defensive responding to body sensations, several new questions arise. For example, it remains unclear whether the observed effects are specific for interoceptive threats or whether the findings may be generalized to clinical populations. Thus, the current findings should be replicated in clinical populations applying the same research designs used in our studies. Moreover, future research designs ought to include exteroceptive threats (e.g., electric shocks) to study the specificity of the observed effects. Further experimental studies investigating learning or culmination processes in PD and clinical studies using a longitudinal design should more precisely examine the role of suffocation fear and anxiety sensitivity in the development of PD. Moreover, future research using human brain imaging techniques is crucial to reveal the defensive brain circuits underlying the observed findings. Further studies should also focus on the underlying mechanisms and the factors that possibly set the stage for developing persistent avoidance of interoceptive threat.

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Appendix A: Publications

Manuscript 1

Benke, C., Alius, M. G., Hamm, A. O., & Pané-Farré, C. A. (in revision). Cue and context conditioning to respiratory threat: Effects of suffocation fear and implications for the etiology of panic disorder.

Manuscript 2

Benke, C., Blumenthal, T., Modeß, C., Hamm, A., & Pané-Farré, C. (2015). Effects of anxiety sensitivity and expectations on the modulation of the startle eyeblink response during a caffeine challenge. *Psychopharmacology*, *232*(18), 3403–3416. doi: 10.1007/s00213-015-3996-9

Manuscript 3

Benke, C., Hamm, A. O., & Pané-Farré, C. A. (2017). When dyspnea gets worse: Suffocation fear and the dynamics of defensive respiratory responses to increasing interoceptive threat. *Psychophysiology*, *43*(1), 174. doi: 10.1111/psyp.12881

Manuscript 4

Benke, C., Krause, E., Hamm, A. O., & Pané-Farré, C. A. (2017). Dynamics of defensive response mobilization during repeated terminations of exposure to increasing interoceptive threat. *Int J Psychophysiol.* doi: 10.1016/j.ijpsycho.2017.09.013

Manuscript 1

Cue and context conditioning to respiratory threat: Effects of suffocation fear and implications for the etiology of panic disorder

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In revision

Author contributions:

CPF, MGA and AOH designed the experiment. CPF supervised the data acquisition. CB analyzed the data and provided the first draft of the manuscript. All authors contributed to the interpretation of the data and wrote the manuscript.

Cue and context conditioning to respiratory threat: Effects of suffocation fear and implications for the etiology of panic disorder

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Abstract

Interoceptive threats play a crucial role in the etiology of panic disorder (PD). While body sensations may become conditioned stimuli (CS) when paired with such interoceptive threats (cue conditioning), the environment in which such interoceptive threats occur may also be learned as a predictor of threat (context conditioning). Suffocation fear (SF) might facilitate these associative learning processes if threats of suffocation (e.g. feelings of dyspnea) become relevant as unconditioned stimuli (US). To investigate whether SF affects associative learning during such respiratory threat, we used mild dyspnea as CS that was either paired with strong dyspnea serving as the US in a predictable context, presented unpaired with the US (unpredictable context) or presented without administering the US (safe context). Startle eyeblink responses as an index of defensive network activation and subjective reports were assessed in 34 participants during learning. Individuals reporting high SF showed a clear potentiation of the startle response during the interoceptive CS predicting the occurrence of interoceptive threat (US). Such startle potentiation was not observed when the CS remained unpaired (safe or unpredictable context). Moreover, high SF persons also showed a significant startle potentiation to the context, when there was no temporal contiguity between CS and US. No such learning effects were observed for low SF individuals. The data support the view that defensive response mobilization can be triggered by cues but also by contexts predicting the occurrence of interoceptive threats if these threats are relevant for the individuals, supporting learning accounts for the development of PD.

1 Introduction

According to the DSM-5 (American Psychiatric Association, 2013a) excessive and persistent fear and anxiety as well as related behavioral disturbances, e.g., avoidance or escape, are the core features of the anxiety disorders. Etiological models propose that pathological fear and anxiety are acquired through associative learning processes (Duits et al., 2015; Hamm & Weike, 2005; Lissek et al., 2005; Mineka & Oehlberg, 2008; Mineka & Zinbarg, 2006). Thus, animal, as well as translational human research, has used fear conditioning studies to elucidate the pathogenesis and maintenance of pathological anxiety as well as its underlying neural networks (Davis, 2006; Grillon, 2002; Hamm & Weike, 2005; LeDoux, 2000; Tovote, Fadok, & Lüthi, 2015). The acquisition of fear to a specific stimulus is typically investigated by repeatedly pairing an affectively neutral stimulus (conditioned stimulus, CS, typically a light, a picture, or a tone) with an emotionally aversive event (unconditioned stimulus, US, typically a mildly painful stimulus or a loud noise) (see Lonsdorf et al., 2017 for a review). As a result of this association, the previously neutral stimulus is enabled to elicit a fear response. While fear conditioning paradigms using external threat as unconditioned stimuli might provide reliable animal and human analogue models to better understand the development of pathological anxiety (see Duits et al., 2015; Lissek et al., 2005; Michael et al., 2007), there are some mental disorders where fear and anxiety are centered around potential threat coming from inside the body.

Fear of potentially dangerous inexplicable body symptoms (like chest pain; dizziness; dyspnea etc.) is a core symptom in panic disorder (Barlow, 2002; Bouton et al., 2001) but also in somatic symptom and illness anxiety disorders. Associative learning processes have been discussed as central mechanisms in etiological models of such disorders as well (Barlow, 2002; Bouton et al., 2001; Peuter, Van Diest, Vansteenwegen, Van den Bergh, & Vlaeyen, 2011; Zaman, Vlaeyen, van Oudenhove, Wiech, & Van Diest, 2015). Particularly in patients with panic disorder, body sensations or physiological signals linked to risk of suffocation – e.g.,

dyspnea, breathlessness, or air hunger – elicit anxious apprehension, fear, or even panic (Barlow, 2002; Bouton et al., 2001; Johnson et al., 2014). Respiratory restriction or obstruction which ultimately poses the risk of suffocation is known as a stimulus activating the defensive survival circuits in the brain. A neural network located in the periaqueductal gray of the brainstem is specialized in integrating physiological (brain O_2, CO_2, and lactate) as well as environmental information (Preter & Klein, 2008) in detecting such respiratory threat and initiating a defensive alarm reaction to initiate effective coping (Schimitel et al., 2012). In line with this, evidence from experimental studies demonstrated that early interoceptive signals of respiratory threat, e.g., dyspnea or breathlessness -- either induced pharmacologically or by respiratory challenges -- are potent elicitors of defensive mobilization (Johnson et al., 2014; Schimitel et al., 2012).

A number of studies have investigated associative fear learning processes to respiratory threat in humans in more detail. In these studies, severe dyspnea served as a US evoked either by multi-breath CO_2 inhalations, severe inspiratory resistive loads or complete breathing occlusions (e.g., Acheson et al., 2007; Pappens et al., 2013; Pappens, Smets, Vansteenwegen et al., 2012). Focusing on intero-interoceptive conditioning, these studies used mild inspiratory resistive loading or brief CO_2 inhalations as CSs preceding the unconditioned interoceptive stimulus. Consequently, a fear response to the previously innocuous body sensations (e.g., mild dyspnea) was acquired when repeatedly paired with the unconditioned strong respiratory threat (e.g., strong dyspnea indicating possible suffocation) (Acheson et al., 2007; Acheson et al., 2012; Ceunen et al., 2016; Pappens et al., 2013; Pappens et al., 2014; Pappens et al., 2015; Pappens, Smets, Vansteenwegen et al., 2012).

Cue conditioning, as described above, occurs when cue (CS) and US are paired in close temporal contiguity. However, the associative learning, that is, the association of CS and US, always takes place in an environmental context, that is, a greater set of stable, complex, and multisensory features including diverse internal and external stimuli (Holland & Bouton, 1999;

Maren et al., 2013; Urcelay & Miller, 2014). Thus, the US is not only associated with the preceding cue but also to the context. The salience of such context information is enhanced when the US is not preceded by a specific cue and thus is presented unexpectedly or unpredictably at least according to its temporal contiguity (Alvarez et al., 2008; Grillon et al., 2006; Grillon & Davis, 1997; Marschner et al., 2008; Vansteenwegen et al., 2008). On a functional level, the context may directly be associated with the CS or the US. Also, the context may become a signal to whether the CS predicts or does not predict the occurrence of the US in a specific context (Holland & Bouton, 1999; Rescorla & Wagner, 1972).

Clinically this latter effect becomes relevant in case of PD where the CS, e.g., a feeling of dyspnea as explained above, may predict a panic attack in one context, e.g., in a shopping mall, but may not predict a panic attack when encountered in another context, e.g., during stair-climbing. Of note, the critical CSs in these situations do not differ and defensive responding to same CS in different contexts is only controlled by the context itself (Holland & Bouton, 1999; Kimmel & Gardner, 1981; Kimmel & Ray, 1978; Maren et al., 2013; Murrin & Kimmel, 1986). Despite its clinical relevance for the understanding of the development of PD, there are no experimental studies characterizing the acquisition of defensive response mobilization to an interoceptive CS in different contexts that signal whether the CS is followed by an aversive US or not. Moreover, CS and US (dyspnea and panic) may occur independently of each other in a given context and thus the CS may not provide predictive value while the context would be the best predictor. The latter was implicated to lead to context conditioning (see Pappens et al., 2012 for preliminary evidence) but this hypothesis has not yet been tested in a within-group design incorporating the above features. Therefore, in the present study, we applied an experimentally-controlled within-subject design using a respiratory threat and the same respiratory CS across different contexts. Thus, we assessed conditioning of defensive response mobilization in three different contexts: (1) a distinct signal cue (respiratory CS) predicted the occurrence of the respiratory threat (US) in the *predictable context*, (2) the CS did not predict

the occurrence of the US in the *unpredictable context*, or (3) no US was delivered at all, thus this *safe context* and the cue predicted the absence of the US (cf. Grillon et al., 2006).

Feelings of dyspnea might not activate the defensive survival circuits in all individuals in the same way. Thus, fear of respiratory sensations in a conditioning paradigm employing such stimuli might be established only in a subset of participants, that is, in persons who specifically fear respiratory or suffocation sensations (fear of suffocation, SF) (Alius et al., 2013; Benke, Hamm et al., 2017; Eifert et al., 1999; Eke & McNally, 1996; McNally & Eke, 1996; Taylor & Rachman, 1994). In the present study, we, therefore, explored the influence of suffocation fear on associative fear learning in the face of a respiratory threat. The unconditioned respiratory threat (i.e., the US) was a severe IRL producing maximally tolerable dyspnea, while a mild IRL producing mild dyspnea served as the CS. In the predictable context, the presentation of the CS (i.e., the mild IRL) was paired with the administration of the US (i.e., the severe IRL), while the CS and US were presented in an unpaired manner during the unpredictable context. In the safe context, the CS was presented without administering the US. The three different contexts were signaled via different colored ambient lighting.

In the present study, we predicted that when mild dyspnea was paired with severe dyspnea stronger cued fear learning would evolve in persons who fear suffocation. In a predictable context, we expected that at the end of the acquisition phase startle responses, as a low-level reflex indicating defensive mobilization, to the CS would be potentiated as compared no-cue intervals (see Pappens et al., 2013; Pappens et al., 2015; Pappens, Smets, Vansteenwegen et al., 2012) in persons reporting high suffocation fear but not in those persons reporting low SF. Moreover, evidence from context conditioning studies in humans demonstrated that persons showed increased context conditioning during unpredictable threat as indexed by increased context associated defensive response mobilization to an unpredictable context as compared to a safe context (Alvarez et al., 2008; Andreatta et al., 2015; Grillon, 2002; Grillon et al., 2006; Marschner et al., 2008; Vansteenwegen et al., 2008). In the present study, we assumed that only

persons who report high SF would show increased startle response magnitudes in the unpredictable context as compared to the safe context (see Davis, Walker, Miles, and Grillon (2010) for a review of studies demonstrating increased context associated defensive responding in patients with anxiety disorders). In accordance with previous evidence (e.g., Pappens et al., 2013; Pappens et al., 2015), we predicted that this conditioning process might be evident in low-level startle reflex modulation but may not be observed in reported valence and arousal.

2 Methods

2.1 Participants

Thirty-four students (19 females, age: M = 23.06, SD = 2.85) participated in the study. Before study inclusion participants were screened for the following exclusion criteria via telephone interview: cardiovascular, respiratory (e.g., asthma, COPD), or neurological (e.g., epileptic or apoplectic seizures, multiple sclerosis) diseases, mental health problems, significant hearing impairment, or pregnancy. All participants provided written informed consent prior to the study and either received course credit or financial compensation for their participation. The study protocol was approved by the ethics committee of the German Psychological Society. All participants rated their suffocation fear (SF) on the Suffocation Fear subscale of the Claustrophobia Questionnaire (Radomsky, Rachman, Thordarson, McIsaac, & Teachman, 2001). Participants´ scores ranged between 0 and 27, with a mean of 8.97 (SD = 5.46).

2.2 Materials

2.2.1 Breathing circuit

Participants breathed through a tightly fitting face mask (7400 series; Hans Rudolph, Inc., Kansas City, MO) connected to a flow sensor that was mounted to the mouth port of the two-way y-shaped non-rebreathing valve (no. 2630; Hans Rudolph, Inc.) enabling unrestricted

expiration through the expiratory port of the valve. A plastic tube (length: 2.75 m; diameter: 35 mm) was connected to the inspiratory port of the valve and mounted to the common port of a Five-Way Gatlin-Shape™ Inflatable-Balloon-Type™ valve (2440 series, Hans Rudolph, Inc.), placed in the adjacent control room. Closing and opening of the valves were controlled via VPM software that triggered a pneumatic controller (2430 series, Hans Rudolph, Inc.). This system allowed a prompt and easy switching between two different inspiratory resistive loads, i.e., the CS and the US, and unrestricted breathing.

2.2.2 Unconditioned and conditioned stimuli

Nylon flow resistors of linear type (7100 series, Hans Rudolph, Inc., range: 0.05-23.19 kPa/l/s) were used to induce dyspnea. These inspiratory resistive loads (IRLs) were either attached separately or combined in groups of two or more resistors to two different ports of the valve. The combination of separate resistors followed a fixed scheme resulting in the total resistance (range: 0.05-23.19 kPa/l/s). IRLs producing slight dyspnea, as individually determined preceding the experiment (see section 2.3), served as the CS, while IRLs producing maximally tolerable dyspnea were used as the US. The CS was presented for 15 s and the US lasted 20 s.

2.2.3 Contexts

In the predictable threat context, the US was presented twice and always immediately preceded by the CS (see Fig. 1). In the unpredictable threat context, one to three USs (two on average) and two CSs were presented in an unpaired fashion. In the safe context, no US was presented. The CS was presented twice. To define the different experimental contexts (safe, predictable, unpredictable) the room was illuminated either in blue, green, or yellow color by indirect lighting. The colors were counterbalanced across contexts. Each context was presented for 210 s.

2.2.4 Startle stimulus

The acoustic startle probe, a 50 ms burst of broadband white noise (rise/fall time < 1 ms), was presented binaurally with an intensity of 95 dB(A) through AKG K-66 headphones.

2.2.5 Subjective ratings

Participants rated the intensity and unpleasantness of dyspnea experienced during loaded breathing on the following scale: 1 (*not at all*), 2 (*slight*), 3 (*moderate*), 4 (*strong*), 5 (*very strong*), and 6 (*maximally tolerable*). Valence (*pleasant – unpleasant*) and arousal (*calm – aroused*) ratings were obtained for each context using the Self-Assessment Manikin (SAM; Bradley & Lang, 1994). Moreover, participants were asked to rate how certain a slight breathing load predicted the presentation of a strong breathing load on a 9-point scale ranging from 1 (*not at all*) to 9 (*absolutely certain*). Ratings were obtained using a computer keyboard. Rating items and response options were projected onto a 1.50 x 1.30 m screen in front of the subjects.

2.3 Procedure

First, all sensors and the face mask were attached and the individual CS and US IRLs were determined. For this, the individual detection threshold of IRLs was determined (see Benke, Hamm et al., 2017, for detailed information) and then, participants were familiarized with the possible range of IRLs by presenting a mild (0.49 kPa/l/s above threshold) and a more severe load (4.90 kPa/l/s above threshold). Afterward, IRLs were gradually increased following an exponential curve. Each load was presented 30 s, followed by a recovery phase lasting 30 s. The participants rated the intensity and unpleasantness of dyspnea experienced during IRL presentation. Once an unpleasantness rating of 6 (=maximally tolerable) was reached, loads were not further increased.

The experiment started with a rating of the valence and arousal for each context. Then, to habituate startle response magnitudes to a stable baseline 6 startle probes were presented during a 90-s resting interval. During the acquisition phase, each context was presented four times (see Fig. 1). The order of presentation of the three contexts was pseudo-randomized (3

orders; restriction that each context was not to be presented multiple times in a row) and balanced out between participants. Between contexts, a black screen was presented for 10 s. Startle probes were delivered randomly between eleven and thirteen seconds after CS onset as well as between 16 and 18 s following the onset of the US. Per context, five startle probes were delivered in non-CS/US intervals[1]. At the end of the acquisition phase, valence and arousal ratings were repeated for each context.

Finally, participants rated the valence and arousal for each context and completed the Claustrophobia Questionnaire (CLQ, Radomsky et al., 2001).

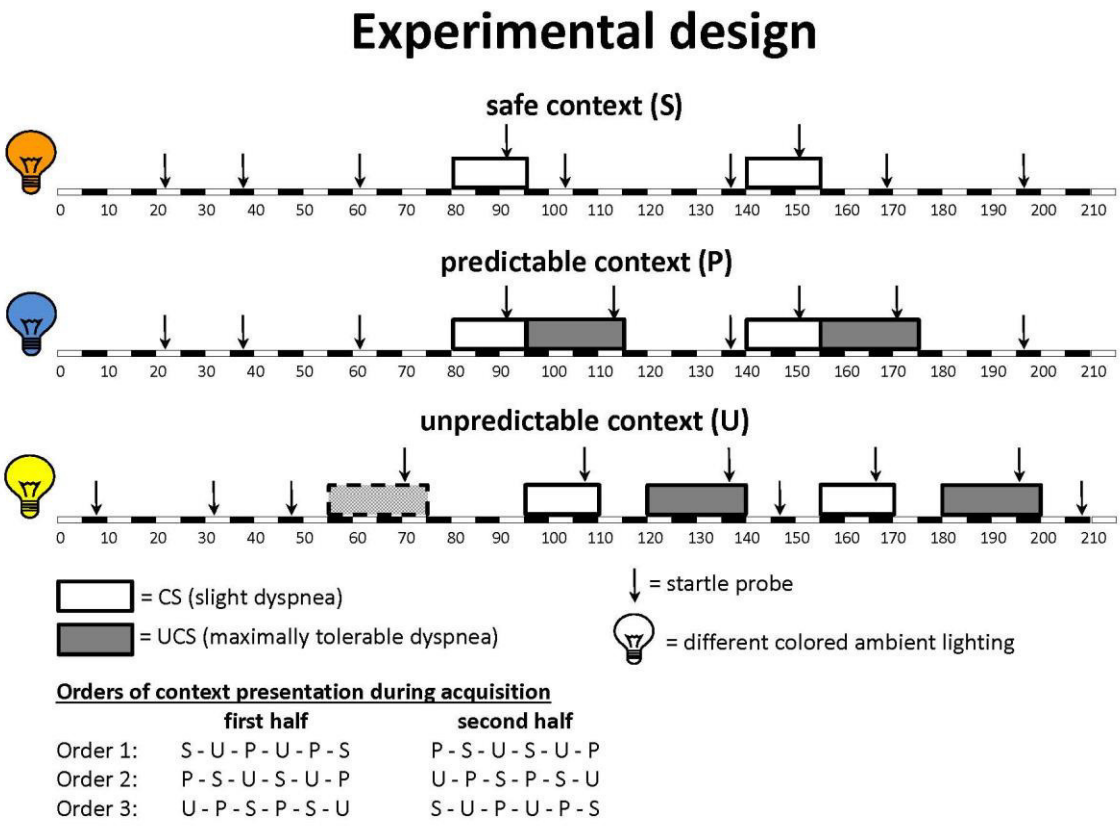

Fig. 1 Experimental design of the present study. During the acquisition, slight dyspnea (CS) was either paired with maximally tolerable dyspnea (US) in the predictable context, administered unpaired with the US (one to three US presentations, two on average) in the

[1] Seven startle probes were presented during the safe context to keep the total number of delivered startle probes constant across contexts.

unpredictable context or presented without administering the US in the safe context (upper panel). Different contexts were signaled via different colored indirect lighting (blue, green, or yellow). Each context was presented four times during acquisition. The order of the safe, predictable and unpredictable context was counterbalanced across participants (lower panel).

2.4 Physiological recordings and response definitions

2.4.1 Startle response

To measure the eyeblink component of the startle response the EMG activity was recorded with two electrolyte-filled (Marquette Hellige, Freiburg, Germany) Ag/AgCl miniature surface electrodes (Sensormedic, Yorba Linda, CA) attached over the orbicularis oculi muscle beneath the lower left eyelid. The raw EMG signal was amplified by a Coulbourn S75-01 amplifier and filtered with a 30 Hz high-pass and a Kemo KEM-VBF8-03 400 Hz low-pass filter. Digital sampling was carried out at a rate of 1000 Hz via a 12-bit A/D converter starting 100 ms before the onset of the startle stimulus and lasting 400 ms after probe onset.

The raw EMG signal was filtered off-line with a 60 Hz high-pass filter to remove eye movement artifacts and then rectified and smoothed using a first-order low-pass filter with a time constant of 10 ms. Then, the startle eyeblinks were scored using a computer program (Globisch, Hamm, Schneider, & Vaitl, 1993) that identified blink onset and peak amplitude. Only trials in which blinks started during 20-100 ms after delivery of the startle probe and reached their peak amplitude within 150 ms were scored as valid startle responses. If no blink was detected in the defined time window the trials were scored as zero responses. Trials were rejected and treated as missing values if there was excessive baseline activity or movement artifacts. Digital values were converted to μV and then exported. To remove inter-individual variability not related to the experimental manipulation, all values were transformed to T-scores (M = 50, SD = 10) as recommended by the guidelines for human startle eyeblink studies

(Blumenthal et al., 2005). For statistical analyses, startle response magnitudes were averaged for CSs vs. no-cue interval and context (no-cue intervals per safe, predictable, and unpredictable context) for the first and second half of the acquisition phase.

2.4.2 Respiratory parameters

A pneumotachography system (ZAN 600, nSpire Health, Inc., Oberthulba, Germany) continuously measured mouth pressure. The mouth pressure signal was visually inspected. Artifacts, such as coughing, were deleted in BrainVision Analyzer (version 2.0, Brain Products GmbH, Gilching, Germany) and treated as missing values. Then, maximal mouth pressure during inspiration (P_I) was automatically detected and exported in 5s bins. The maximal mouth pressure was averaged separately for load-free periods, CSs, and USs.

2.4 Data analysis

To check whether the experimental manipulation was successful in the present study, mouth pressure and physical intensity of IRLs were analyzed applying an analysis of variance (ANOVA) with the repeated-measures factor *inspiratory load* (CS vs. US, resp. load free periods vs. CS. vs. US for mouth pressure) and *SF* (as a continuous variable).

The startle data were analyzed for the acquisition phase using an analysis of variance (ANOVA) with the repeated-measures factors *half* (first vs. second half), *cue* (CS vs. no-cue) and *context* (safe vs. predictable vs. unpredictable) as well as suffocation fear (*SF*) as a continuous predictor (see Cohen, 2003; Miller & Chapman, 2001). The moderating effect of SF was clarified by computing analyses separately for persons reporting low (1 *SD* below the mean of the sample; SF < 4), medium (between −1 *SD* and +1 *SD*; 4 ≤ SF ≤ 14) and high SF (1 *SD* above the mean of the sample; SF > 14). Our hypothesis concerning cued fear conditioning was tested by conducting planned comparisons to analyze response differences between no-cue and CS intervals (fear-potentiated startle). To further validate SF-dependent effects and test for positive associations SF scores were correlated with fear-potentiated (CS vs. no-cue) startle

magnitudes. We also conducted planned comparisons to test whether startle potentiation would differ depending upon contexts. To evaluate context conditioning planned contrasts were calculated for no-cue intervals comparing different contexts, i.e., unpredictable (resp., predictable) vs. safe context, or unpredictable vs. predictable context. To further validate SF-dependent effects and test for positive associations, SF scores were correlated with context-potentiated (no-cue of the unpredictable [resp., predictable] vs. safe context) startle magnitudes.

To test whether valence and arousal ratings after acquisition differ from ratings obtained before the acquisition started, an ANOVA was calculated using the within-subjects factors *time* (pre- vs. post-acquisition) and *context* as well as the continuous factor *SF*. Contingency ratings were evaluated using the within-subjects factor *context* and the continuous factor *SF*.

All statistical tests used a significance level of $p < .05$. Whenever necessary, a Greenhouse-Geisser correction was applied. Effect sizes (partial eta squared) are reported for all *F* tests. According to our directional hypotheses, correlations were tested one-tailed. All data were processed using SPSS 22.0 (SPSS for windows, IBM).

3 Results

3.1 Manipulation check

The physical intensity of IRLs was significantly higher during the US as compared to during the CS, $F(1, 32) = 13.80$, $p = .001$, $\eta_p^2 = .301$, but did not differ as a function of SF, $F(1, 32) < 1$, $p = .841$, $\eta_p^2 = .001$, SF $F(1, 32) < 1$, $p = .674$, $\eta_p^2 = .006$. According to the applied physical resistance, mouth pressure, as an index of respiratory muscle work load, rose from load-free periods to the CS to the US, $F(2, 64) = 30.89$, $p < .001$, $\eta_p^2 = .491$, and did not vary with SF, $F(2, 64) < 1$, $p = .491$, $\eta_p^2 = .016$, SF $F(1, 32) < 1$, $p = .608$, $\eta_p^2 = .008$.

3.2 Startle responses

Figure 2 illustrates blink magnitudes to probes elicited during the CS and the non-cue periods (ITIs) in the predictable and unpredictable context – as well as in the safe context. Overall, there was a significant Cue x Context x Half x SF interaction, $F(2, 64) = 4.17$, $p = .020$, $\eta_p^2 = .115$. Follow up comparisons revealed that high SF persons, showed a significant potentiation of the startle response during the CS relative to the ITI when the CS predicted the US. This fear potentiated startle developed during the second half of the acquisition, Cue x Context x Half $F(2, 8) = 17.78$, $p = .001$, $\eta_p^2 = .816$; cue $F(1, 4) = 13.30$, $p = .022$, $\eta_p^2 = .769$ for the second half. This cue specific startle potentiation did not occur in the unpredictable or safe condition, Cue x Context $F(2, 8) = 17.67$, $p = .001$, $\eta_p^2 = .815$. Moreover, in the second half, participants reporting low or medium suffocation fear did not show any significant potentiation but rather a slight inhibition of the startle response during the cue predicting strong IRLs, cue Fs > 6.00, ps < .038, $\eta_p^2 > .206$.

Correlational analyses supported these significant group effects. Startle potentiation during the CS correlated significantly with the reported suffocation fear, $r = .397$, $p = .010$, if the cue predicted the US during the second half of acquisition. No significant correlations were found in the safe, $p = .127$, or unpredictable contexts, $p = .267$.

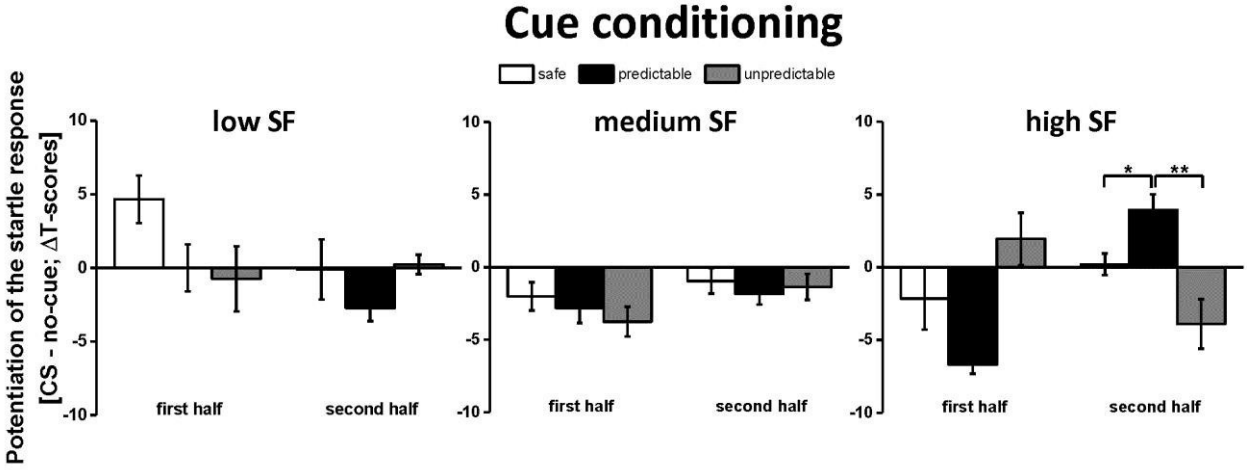

Fig. 2 Cue conditioning during the first and second half of the acquisition. Means and standard errors of the potentiation of the startle response to the CS vs. no-cue intervals during the safe,

predictable and unpredictable context in low, medium and high SF persons. In high SF persons, asterisks indicate significant differences between potentiation of the startle response to CS vs. no-cue periods during the predictable context and startle responding during the safe (* $p < .05$) and unpredictable context (** $p < 0.01$) within the second half of the acquisition.

Figure 3 shows the blink magnitudes elicited during the context in which the unconditioned stimulus was unpredictable relative to the predictable, or safe context. Again, overall analyses revealed a significant Context x Half x SF interaction, $F(2, 64) = 3.95$, $p = .024$, $\eta_p^2 = .110$. Follow up analyses showed that individuals reporting high-SF exhibited a significant potentiation of the startle response magnitudes elicited in the non-cue (ITI) condition in the unpredictable compared to the safe, $F(1, 4) = 24.30$, $p = .008$, $\eta_p^2 = .859$, and to the predictable contexts, $F(1, 4) = 14.21$, $p = .020$, $\eta_p^2 = .780$ (see right panel of Fig. 3) during the second half of acquisition. No context dependent modulation of the startle responses was observed for participants who reported either low, Context x Half $F(2, 8) < 1$, $p = .193$, $\eta_p^2 = .337$, or medium SF, Context x Half $F(2, 46) < 1$, $p = .466$, $\eta_p^2 = .033$.

Again, correlational analyses supported the findings of the group analysis. Potentiation of the blink magnitudes evoked by probes presented in the no-cue condition of the unpredictable context during the second half of the acquisition (relative to the safe condition) was significantly correlated with the reported levels of SF, $r = .325$, $p = .030$. This correlation was not significant in the predictable context ($r = -.062$, $p = .363$) context.

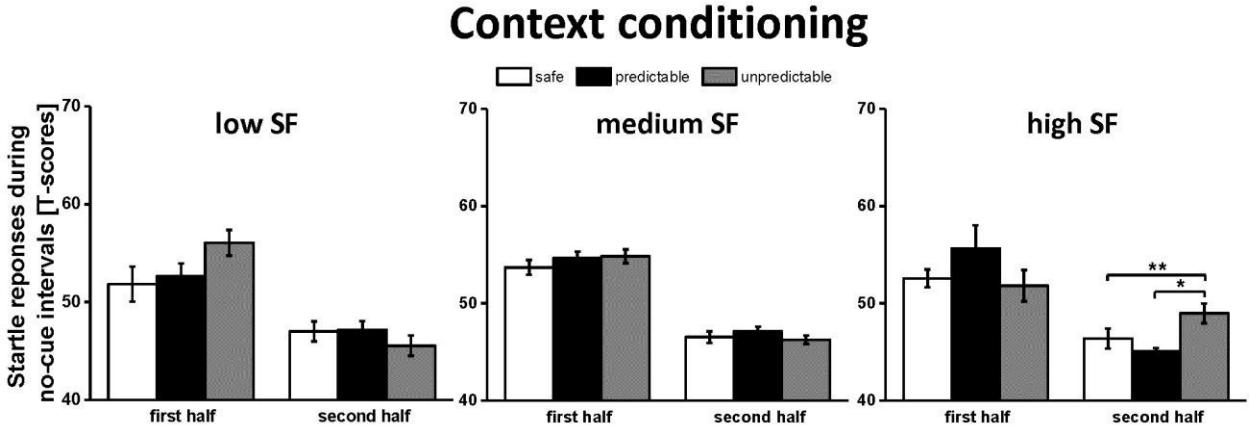

Fig. 3 Context conditioning during the first and second half of the acquisition in low, medium and high SF persons. Means and standard errors of startle eyeblink magnitudes during no-cue intervals in the different contexts. In high SF persons, asterisks indicate a significant difference in startle eyeblink magnitudes between the unpredictable context and the safe (** $p < 0.01$) and predictable (* $p < .05$) context.

3.3 Subjective ratings

Overall, the contexts were rated as more arousing after the acquisition, $F(1, 32) = 5.31$, $p = .028$, $\eta_p^2 = .142$. However, valence and arousal ratings did not differ significantly between the three contexts, Context $F(2, 64) < 1.06$, $p > .354$, $\eta_p^2 < .033$, Context x Time $F(2, 64) < 1$, $p > .850$, $\eta_p^2 < .006$, Context x Time x SF $F(2, 64) < 1$, $p > .552$, $\eta_p^2 < .019$, suggesting that the participants did not explicitly process the context information. Arousal and valence ratings, however, were affected by SF, Fs$(1, 32) > 1.53$, ps $< .035$, $\eta_p^2 > .132$, that is, high SF persons rated all contexts as more arousing und unpleasant. This finding was supported by a positive correlation of SF scores with arousal ($r = .373$) and valence ($r = .365$) ratings.

Participants were more confident that slight dyspnea was followed by strong dyspnea in the predictable as compared to the safe context, $F(1, 33) = 5.46$, $p = .026$, $\eta_p^2 = .142$, but not in comparison to the unpredictable context, $F(1, 33) = 2.93$, $p = .096$, $\eta_p^2 = .082$, context $F(2, 64)$

= 2.93, p = .061, η_p^2 = .061, suggesting that participants had no explicit declarative memory of the contingencies in the current experiment. Contingency ratings did not differ as a function of SF, Context x SF $F(2, 64) < 1$, p = .481, η_p^2 = .023.

4 Discussion

In the present study, we aimed at investigating cue and context conditioning using the same interoceptive CS in different contexts. A benign respiratory stimulus, i.e., mild dyspnea (CS), either immediately preceded a respiratory threat, i.e., severe dyspnea (US) induced by IRLs (predictable context), was presented without predicting the respiratory threat that occurred at any time during the context (unpredictable context), or was presented in the absence of a respiratory threat (safe context). Also, we sought to examine how the level of SF, a trait-like personality factor that increases the salience of suffocation stimuli in those participants characterized by high levels of fear, affects cue and context conditioning in the face of predictable and unpredictable respiratory threat.

In the present study, associative fear learning only occurred in high SF individuals, i.e., in those individuals for whom the respiratory threat was salient. Only persons reporting high levels of SF showed defensive response mobilization during mild dyspnea that predicted the immediate onset of severe dyspnea. Moreover, in the unpredictable context, context conditioning was established as indexed by greater startle response magnitudes during no-cue intervals in the unpredictable as compared to the safe and the predictable context, respectively. Again, fear of suffocation was positively correlated with anxiety-potentiated startle responses.

4.1 Effects of SF on cue and context conditioning during interoceptive threat

The present data replicate previous findings of interoceptive fear conditioning studies showing that mild respiratory sensations may become CSs eliciting defensive response mobilization when repeatedly paired with an aversive interoceptive US such as severe dyspnea or a

suffocation experience (Acheson et al., 2007; Pappens et al., 2013; Pappens et al., 2014; Pappens et al., 2015; Pappens, Smets, Vansteenwegen et al., 2012). However, present results also extend these findings in demonstrating that the contexts acquired the ability to control defensive responses to the same CS (see Maren et al., 2013). Moreover, the current results also substantiate and add to existing data in that context conditioning – a model of the acquisition of anxious apprehension - was established during unpredictable interoceptive threat in comparison to an adequate control condition (see Pappens, Smets, Vansteenwegen et al., 2012 for preliminary evidence).

The findings of the current study extend the current literature in demonstrating that trait SF plays a role in these associative learning processes. In line with assumptions derived from the modern learning theory, our data indicate that the disposition to fear suffocation sensations (i.e., fear of suffocation) might act as a psychological vulnerability factor that may increase cue and context conditioning during respiratory threat (Barlow, 2002; Bouton et al., 2001; Mineka & Zinbarg, 2006). On a functional level, the current data suggest that SF may predispose to acquiring cued fear and contextual anxiety when faced with respiratory threat. The finding that significantly elevated fear of suffocation was found in a large group of patients with PD/AG (Hamm et al., 2016) may be interpreted as corroborating this assumption. Unfortunately, so far there are longitudinal epidemiological data available that clarifies the exact functional role of SF in the etiology of PD.

Nevertheless, the present results provide first evidence that persons with high trait SF show exaggerated defensive response mobilization during a context in which interoceptive threats occur at unpredictable when they occur. This is in line with evidence demonstrating that persons reporting high SF including persons with PD showed stronger defensive response mobilization during anticipation of (unpredictable) threat as well as during provocation of strong suffocation sensations (Alius et al., 2013; Benke, Hamm et al., 2017; Eifert et al., 1999; Eke & McNally, 1996; Grillon et al., 2008; McNally & Eke, 1996; Melzig et al., 2007; Taylor

& Rachman, 1994). Of clinical relevance is that contextual-induced anxious apprehension and cued fear may further potentiate defensive responses and behaviors, thus increasing the risk for the culmination of symptoms into panic (Barlow, 2002; Bouton et al., 2001). Experimental data indicate that such culminating symptoms may trigger escape or persistent avoidance behavior in response to early indicators of the increasing interoceptive threat (Benke, Hamm et al., 2017; Benke, Krause, Hamm, & Pané-Farré, 2017b).

It is to note that context conditioning was reflected in the modulation of an amygdala-dependent brainstem reflex but not in verbal report measures. This is in line with evidence indicating that mobilization of defensive responses is mediated by a neural defensive network that differs from the neural circuitry involved in the report of subjective feelings of fear and anxiety (Hamm et al., 2003; Hamm & Vaitl, 1996; Hamm & Weike, 2005; Lang et al., 2000; LeDoux & Pine, 2016). Thus, it has repeatedly been shown that fear learning is evidenced in the potentiation of the startle response but not necessarily in subjective reports and declarative knowledge of the CS-US contingencies (Clark, 1998; Hamm & Vaitl, 1996; Pappens et al., 2013; Pappens et al., 2015; Sevenster, Beckers, & Kindt, 2014). This pattern was also replicated in interoceptive fear conditioning studies (Pappens et al., 2013; Pappens et al., 2015; Pappens, Smets, Vansteenwegen et al., 2012). In line with this, only one person with high SF had a declarative memory of the contingency between the CS and US in the present study. In sum, this would suggest that behavioral adjustments to contextual cues occurred automatically which goes in line with the conditioning model of PD and might explain why panic is perceived as occurring unexpectedly in several contexts (Bouton et al., 2001).

4.2 Strengths and limitations

The present study demonstrated that individuals with high SF acquire fear responses to mild dyspnea when these body symptoms predict more severe symptoms. Moreover, these individuals also show stronger defensive response mobilization in a context where strong dyspnea occurs unpredictably. Although our data indicate that conditioned fear and anxiety

continuously increased with increasing levels of SF, the relatively small sample size limits the generalizability of results. Thus, further studies with larger samples ideally covering the full range of possible SF scores are warranted to replicate these findings. Moreover, in the present study, the assessment of verbal reports was limited and for a comprehensive analysis, future research should include US expectancy ratings as well as the ratings of fear and anxiety.

4.3 Conclusion and future directions

The current study aimed at investigating associative learning processes involved in the development of fear and anxiety in the context of interoceptive sensations and interoceptive threat. The present data suggest that persons who fear suffocation are prone to develop fear to respiratory sensations and anxiety when exposed to a context signaling unpredictable respiratory threat. Fear and anxiety in high SF persons were assessed by the potentiation of the startle eyeblink response that indicated a stronger mobilization of defensive responses during mild dyspnea paired with strong dyspnea and to contexts during which strong dyspnea could occur unpredictably. Importantly, in high SF persons, the very same body sensation presented across different contexts elicited stronger defensive response mobilization in the predictable context. Thus, SF might support the differentiation of the same CS in different contexts signaling different meanings of the CS. These data are in line with the interoceptive learning account of understanding the etiology of PD. The current data suggest that fear of suffocation might be an important risk factor that plays a pivotal role in facilitating associative fear and anxiety learning and thus set the stage for the development of PD. Of course, further experimental studies investigating learning processes in PD and clinical studies using a longitudinal design should more precisely examine the role of suffocation fear in the development of PD.

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Manuscript 2

Effects of anxiety sensitivity and expectations on the modulation of the startle eyeblink response during a caffeine challenge

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All authors designed the experiment. CPF and CB supervised the data acquisition. CB analyzed the data and provided the first draft of the manuscript. All authors contributed to the interpretation of the data and wrote the manuscript.

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ORIGINAL INVESTIGATION

Effects of anxiety sensitivity and expectations on the modulation of the startle eyeblink response during a caffeine challenge

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Abstract
Rationale The way in which the tendency to fear somatic arousal sensations (anxiety sensitivity), in interaction with the created expectations regarding arousal induction, might affect defensive responding to a symptom provocation challenge is not yet understood.
Objectives The present study investigated the effect of anxiety sensitivity on autonomic arousal, startle eyeblink responses, and reported arousal and alertness to expected vs. unexpected caffeine consumption.
Methods To create a match/mismatch of expected and experienced arousal, high and low anxiety sensitive participants received caffeine vs. no drug either mixed in coffee (expectation of arousal induction) or in bitter lemon soda (no expectation of arousal induction) on four separate occasions. Autonomic arousal (heart rate, skin conductance level), respiration (end-tidal CO_2, minute ventilation), defensive reflex responses (startle eyeblink), and reported arousal and alertness were recorded prior to, immediately and 30 min after beverage ingestion.
Results Caffeine increased ventilation, autonomic arousal, and startle response magnitudes. Both groups showed comparable levels of autonomic and respiratory responses. The startle eyeblink responses were decreased when caffeine-induced arousal occurred unexpectedly, e.g., after administering caffeine in bitter lemon. This effect was more accentuated in high anxiety sensitive persons. Moreover, in high anxiety sensitive persons, the expectation of arousal (coffee consumption) led to higher subjective alertness when administering caffeine and increased arousal even if no drug was consumed.
Conclusions Unexpected symptom provocation leads to increased attention allocation toward feared arousal sensations in high anxiety sensitive persons. This finding broadens our understanding of modulatory mechanisms in defensive responding to bodily symptoms.

Keywords Interoceptive threat · Attention · Unexpectedness · Arousal · Alertness

Introduction

It has been demonstrated that the degree of anxious responding to the provocation of somatic arousal sensations is determined not only by biological factors but also by context variables as well as psychological factors, such as anxiety sensitivity. Anxiety sensitivity (AS) is a personality trait that defines the tendency to fear somatic arousal sensations which are assumed to signify potentially dangerous consequences (McNally 2002). A number of experimental studies have demonstrated that high anxiety sensitive persons report greater fear as well as more anxiety symptoms and panic attacks when exposed to somatic sensations provoked by challenge procedures such as hyperventilation, CO_2 inhalation, loaded breathing, and CKK-4 administration (Alius et al. 2013; Asmundson

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et al. 1994; Koszycki et al. 1993; Melzig et al. 2011; Telch et al. 2010; Telch et al. 2011; Zvolensky et al. 2001).

As mentioned earlier, contextual factors such as availability of explanations or predictability may also play a modulatory role in anxious responding to symptom provocation procedures (Clark 1993; Zvolensky and Eifert 2001). In an exemplary study by Rapee et al. (1986), either only minimal explanations vs. very detailed descriptions of a variety of possible symptoms and causes of these symptoms were provided before a CO_2 inhalation procedure. It was demonstrated that those persons who received only minimal information and were thus confronted with a variety of unexpected and unexplained sensations reported a greater intensity of panic during the actual challenge than those who were extensively informed.

Interestingly, recent studies have indicated that person-specific and contextual factors may interact (Pané-Farré et al. 2014; Telch et al. 2010; Telch et al. 2011; Telch et al. 1996; Zvolensky et al. 2001). An experimental study by Telch et al. (2011) revealed a more frequent report of panic attacks to CO_2-induced arousal in high anxiety sensitive persons but not controls when the participants did not expect such sensations but were given information that the inhalation would have a relaxant effect. Similarly, Pané-Farré et al. (2014) showed an exaggerated respiratory response in high-AS low-caffeine users after unexpected (as compared to expected) arousal provocation using caffeine mixed into a caffeine-free soft drink. This effect was reversed in low-AS controls. The mechanism of this observed interaction of personality trait and context has not been completely understood. It has been proposed that the mismatch of a safety or non-arousal interoceptive state expectation and the actual experience of feared arousal sensation either directly leads to an initiation of a defensive response via the insula (Paulus and Stein 2006) or that the fear response is mediated via reappraisal processes (Telch et al. 2011). While both explanations seem generally plausible, further studies are needed to characterize the specific processes that are associated with unexpected and unexplained symptom provocation in high-AS individuals.

The present study aimed at following up on the presented research aiming for a more comprehensive characterization of the defensive response to unexpected and unexplained arousal symptoms. For this purpose, the assessment of autonomic activation and respiration was complemented with measurement of the startle eyeblink response. The startle eyeblink response to an acoustic startle probe is a brain stem reflex which is typically potentiated when elicited during anticipation or presentation of unpleasant or feared stimuli (Hamm et al. 1997; Lang et al. 1990; Melzig et al. 2008), thus indicating activation of the defense system (for a review, see Lang et al. 2000). Moreover, it has been demonstrated that the startle reflex magnitude is also modulated by other processes such as arousal or attention. The observed startle response magnitude in response to a probe stimulus reflects the net effect of those multiple processes that may concurrently or sequentially occur (Bradley et al. 2006).

In the present study, caffeine was used to induce somatic arousal sensations. Applying an expectancy-controlled research design as introduced by Rohsenow and Marlatt (1981), the active drug (caffeine at a dose of 4 mg/kg body weight) vs. no drug was delivered either in coffee (creation of a context where arousal symptoms are expected) or bitter lemon soda (no arousal expectation). This research design enables delineation of expectancy and drug effects and, most importantly, allows investigation of defensive responding to an unexpected arousal experience that is evoked when bitter lemon soda containing caffeine is administered. To maximize expectancy effects and therefore discrimination between experimental conditions, we recruited regular caffeine users who were, due to their daily coffee consumption, highly familiar with the arousing effects of caffeine.

It has been demonstrated that the consumption of caffeine can reliably be detected at the dose administered in the current study (Silverman and Griffiths 1992). In accordance with the literature on the physiological and psychological effects of caffeine, we expected caffeine to result in physiological arousal associated with elevated skin conductance level (Barry et al. 2005; Barry et al. 2008; Flaten and Blumenthal 1999; Mikalsen et al. 2001), a blood pressure increase which is typically associated with a heart rate decrease (Benowitz 1990), increased minute ventilation (Barry et al. 2008; Sawyer et al. 1982), and lowered expiratory CO_2 (Bell et al. 1999; Cameron et al. 1990; Sawyer et al. 1982). Moreover, we expected to observe an increase of startle response magnitudes after caffeine consumption, which has been attributed to its arousal-inducing effects (Andrews et al. 1998; Flaten et al. 2003; Flaten and Blumenthal 1999). On a subjective level, the ingestion of caffeine was expected to lead to an increase in arousal and alertness (Flaten and Blumenthal 1999; Mikalsen et al. 2001; Rogers et al. 2013).

Most importantly, based on the studies presented above, we proposed that the unexpected arousal provocation in the bitter lemon soda with caffeine condition should lead to defensive mobilization in high anxiety sensitive persons but not controls, which in accordance with Pané-Farré et al. (2014), we expected to be reflected in greater respiratory activation. Moreover, we expected that the usually observed arousal-related increase of the startle reflex under caffeine would be modulated by expectation leading to a different startle response magnitude in the expected arousal provocation condition (coffee with caffeine) in contrast to the unexpected arousal provocation condition (bitter lemon soda with caffeine). As mentioned earlier, it has been demonstrated that defensive mobilization evoked in a threat context leads to startle potentiation (Grillon and Baas 2003). However, recent studies have suggested that this response pattern may deviate in the

presence of an interoceptive threat originating from within a person's body. This specific situation leads to a decrease of startle response magnitudes, possibly reflecting an inward orientation of attention toward the feared somatic sensations (Alius et al. 2014; Pappens et al. 2011). We therefore expected that in high-AS individuals, startle response potentiation by caffeine would be further modulated, with an additional second process (to the drug effect) being present in the unexpected arousal condition: Here, the attention allocation toward the unexpected somatic arousal sensations should lead to a relative startle magnitude decrease in the unexpected as compared to the expected caffeine consumption condition.

Methods

Participants

About 300 university students were screened and selected according to their scores on the German version of the Anxiety Sensitivity Index (ASI; Peterson and Reiss 1992) and their caffeine consumption habits as assessed with the Caffeine Consumption Questionnaire (CCQ; Schicatano and Blumenthal 1995). Subjects who consumed at least 200 mg caffeine per day, including at least one cup of caffeinated coffee, were defined as habitual caffeine users. Participants classified as habitual caffeine users were contacted by telephone if they scored either low (≤11) or high (≥29) on the ASI (one standard deviation [*SD*=9] above and below the mean [*M*=20]). The following exclusion criteria were assessed during the telephone interview: presence of cardiovascular, respiratory (asthma, COPD), neurological (epileptic or apoplectic seizures, multiple sclerosis), gastrointestinal, liver, or skin diseases; being in a psychotherapeutic treatment due to an anxiety disorder; pregnancy; hyperthyroidism/hypothyroidism; and long-term medication use (except contraceptive agents and dietary supplements). Moreover, all subjects had to be infrequent or non-smokers (<4 cigarettes/week) and Caucasians aged between 18 and 35 years with a body mass index (BMI) ranging from 19 to 27. Prior to inclusion in the study, all participants additionally underwent a medical examination involving blood pressure and electrocardiogram (ECG) assessment and a structured medical anamnestic interview.

The final sample consisted of 19 low and 19 high anxiety sensitive (AS) habitual caffeine users (14 females per group). A further description of the group characteristics is presented in Table 1, indicating that the groups neither differed regarding the mean age nor the average amount of daily consumed caffeine. However, high anxiety sensitive persons reported increased distress as indicated by significantly increased scores in all self-report measures.

Stimulus materials

Startle stimulus The startle-eliciting stimulus, a 50 ms burst of broadband white noise (fall/rise time <1 ms), was generated by a Coulbourn S81-02 noise generator (Coulbourn Instruments, Whitehall, PA) and presented binaurally with an intensity of 85, 95, or 105 dB(A) through AKG K-66 headphones.

Subjective ratings Subsequent to each physiological recording phase, participants rated the extent of alertness, contentedness, and arousal using a bipolar rating scale with numerical anchors (from −5 to +5, excluding zero) and verbally labeled scale endpoints consisting of 16 pairs of antonymous adjectives according to Bond and Lader (1974). All items and response options were projected onto a 1.50×1.30 m screen in front of the participants, while ratings were given via a keyboard.

Beverages Participants received four different beverages: 125 ml of brewed decaffeinated coffee (Dallmayr Prodomo) or bitter lemon soda (Glashaeger) mixed with either 4 mg caffeine per kg body weight or no caffeine. For the caffeine solution, 100 % anhydrous caffeine was dissolved in distilled water at the ratio of 8 mg caffeine to 1 ml distilled water. Even though the bitter lemon soda and the coffee were partially substituted by the caffeine solution to result in a 125 ml beverage, due to the inherently bitter taste of the caffeine solution, the typical taste and the strength of both beverages were not affected by this slight dilution. Also, no taste difference between caffeinated and not caffeinated beverages was detectable. As it was assumed that caffeine-associated stimuli such as the taste, smell, and warm temperature of coffee contribute to elicit expectancy effects, coffee was served warm, with an approximate temperature of 50 °C (130 °F). Bitter lemon soda was served at room temperature.

Apparatus and materials To measure the eyeblink component of the startle response, the electromyographic (EMG) activity was recorded by means of two electrolyte-filled (Marquette Hellige, Freiburg, Germany) Ag/AgCl miniature surface electrons (Sensormedic, Yorba Linda, CA) attached over the left orbicularis oculi muscle beneath the lower eyelid. The amplification of the raw EMG signal was realized using a Coulbourn S75-01 amplifier modified with a 30-Hz high-pass filter and a Kemo KEM-VBF8-03 400-Hz low-pass filter. Digital sampling at a rate of 1000 Hz was carried out via a 12-bit A/D converter starting 100 ms before the onset of the startle stimulus and lasting 400 ms following the startle probe.

Skin conductance was recorded from the hypothenar eminence on the palm of the participant's non-dominant hand using two silver-silver chloride standard electrodes (8 diameter, Marquette Hellige) filled with a 0.05 M sodium chloride

Table 1 Means and standard deviations of questionnaire measures, age, and daily caffeine intake for high and low anxiety sensitive participants

	Anxiety sensitivity		*t*	Significance
	Low	High		
ASI [0–64]	8.68 (2.11)	36.21 (5.08)	21.80	$p<.001$
STAI—Trait [20–80]	32.32 (5.10)	45.11 (9.44)	6.10	$p<.01$
BVS [0–40]	11.68 (5.11)	18.61 (4.75)	4.33	$p<.001$
ACQ [1–5]	1.33 (0.17)	1.90 (0.55)	4.29	$p<.001$
BSQ [1–5]	1.47 (0.31)	2.38 (0.46)	7.08	$p<.001$
Daily caffeine intake [mg]	567.05 (259.50)	742.80 (418.34)	1.56	$p=.13$
Age	23.84 (2.91)	22.68 (2.71)	1.27	$p=.21$

ASI Anxiety Sensitivity Index, *STAI* State-Trait Anxiety Inventory, *BVS* Body Vigilance Scale, *ACQ* Agoraphobic Cognitions Questionnaire, *BSQ* Body Sensations Questionnaire; possible questionnaire score ranges are listed in parentheses

electrolyte medium. A constant DC voltage of 0.5 V was applied across electrodes (attached 15 mm apart) by a Coulbourn S71-22 skin conductance coupler that processed the signal with a resolution of 0.01 μS. The DC voltage amplified signal was continuously sampled at 10 Hz by a 12-bit A/D converter.

Electrocardiogram was measured with electrolyte filled Ag/AgCl standard electrons (Marquette Hellige) placed in a Einthoven II setup. The raw ECG signal was amplified and filtered through a 0.1–13 Hz band-pass filter using a Coulbourn S75-01 bioamplifier. Digital sampling rate was set to 100 Hz.

Respiratory parameters were registered by an inductive plethysmography system (Respitrace, Q.D.C., SensorMedics, NewMedics GmbH, Öhringen, Germany) applying two stretch respiration belts with insulated coils which were placed over the chest and the abdomen. In order to measure volume-related parameters (minute ventilation, tidal volume), a spirometry system (SpiroJet, Ganshorn Medicine Electronics, Niederlauer, Germany) simultaneously recorded respiratory volumes during a calibration procedure. End-tidal carbon dioxide partial pressures ($p_{et}CO_2$) were registered by a Nellcor NPB-70 capnograph analyzing the amount of pCO_2 present at the end of exhalation ($p_{et}CO_2$) via infrared spectroscopy. For this purpose, nasal prongs were placed in both nostrils from which the expired air was continuously drawn and delivered through a 1.2-mm diameter tube to the monitor. All outputs were continuously digitalized with a sampling rate of 10 Hz.

Procedure

Participants attended five laboratory assessment sessions (one desensitization/taste test session and four beverage administration sessions) in the morning (8.00–12.00 a.m.) spaced 2 to 4 days apart. Participants were informed about the procedure of the study and provided written informed consent. The study protocol and the informed consent form were approved by the local Ethic Committee of the Medical Faculty of the University Greifswald. Subjects were told that the study was targeted at examining the effects of the ingestion of different beverages on subjective and physiological reactions and their replicability. During the first laboratory session, a caffeine taste test adapted from DeMet et al. (1989) was conducted, and participants got acquainted with the laboratory and the experimental procedures to be completed in the following four sessions. Participants were asked to eat breakfast before each lab session and to refrain from caffeine-containing foods or beverages for at least 12 h (overnight). During the following four sessions, participants ingested decaffeinated coffee with 4 mg/kg body weight caffeine, decaffeinated coffee, bitter lemon soda with 4 mg/kg body weight caffeine, and bitter lemon soda. The solutions were administered in a random order across subjects.

Each laboratory recording session (sessions 2 to 5) followed a fixed procedure: First, all sensors were attached, while participants were seated in a reclining chair in a sound attenuated and dimly lit room. The experimenters were blind to participants' anxiety sensitivity score and whether the beverage contained caffeine or no caffeine. After the signal quality was checked, heart rate variability was measured during a 5-min rest period followed by an 8-min *adaptation* phase. At the end of the adaptation phase, the current extent of arousal, contentedness, and alertness were rated. Next, during the 8-min *anticipation* phase, a slide projected onto a screen signaled whether the participant was going to receive coffee or bitter lemon. Then, the participants received the announced beverage and were instructed to drink it immediately. Following complete ingestion of the beverage, physiological parameters were recorded in blocks of 8 min immediately and after 15 and 30 min, respectively. During the first 6 min of each recording phase, 18 startle stimuli (one startle stimulus of each probe intensity per minute) were presented with a mean interprobe interval of 20 s (range 17–23 s). The order of presentation of the three startle probe intensities were randomized within and among participants with the restriction that startle

probes of the same intensity were not delivered consecutively. Again, subjective ratings were obtained subsequent to each physiological recording phase. Finally, in a volume calibration procedure, participants breathed through a spirometer for 3 min. Following the last session, subjects completed the questionnaires mentioned above and were debriefed afterwards.

Data reduction and statistical analysis

The raw EMG signal was filtered off-line with a 60-Hz high-pass filter, rectified, and integrated with a time constant of 10 ms. Then, the startle eyeblinks were scored using a computer program (Globisch et al. 1993) that identified latency of blink onset (in milliseconds) and peak amplitude (in microvolts). Trials in which blinks started 20–100 ms after stimulus onset and reached their peak amplitude within 150 ms were scored as a valid startle response; however, if no blinks were detected in the defined time windows, the trials were scored as zero responses. If, due to excessive baseline activity or movement artifacts, trials were rejected, resulting missing values were replaced with their predicted values of a calculated linear regression (linear trend for that point). Digital values were converted to microvolts and exported. To remove interindividual variability not related to the experimental manipulation, for further analyses, all values were transformed to *T*-scores (*M= 50*, *SD= 10*) as recommended by the guidelines for human startle eyeblink studies (Blumenthal et al. 2005).

Digital values of skin conductance were converted to microSiemens, exported in 5-s blocks, and then, range corrected as suggested by Lykken and Venables (1971). To analyze the level of skin conductance (SCL), means of blocks were computed.

The ECG signals were visually inspected, and artifacts as well as misplaced R-wave triggers were corrected using ANSLAB version 2.4 (Autonomic Nervous System Laboratory, University of Basel, Switzerland). Interbeat intervals were then calculated, converted to heart rate (HR in bpm), and averaged across 5-s bins.

Values of end-tidal pCO_2 were exported in 5-s means. To enable a calculation of volume data for the entire session, a regression coefficient was calculated which expresses the association between the digital outputs of the spirometry (volume) and Respitrace system (band stretch). For this purpose, the Respitrace sum channel and the spirometry outputs were checked using ANSLAB version 2.4. Technical (resets of the system) and body movement artifacts were removed and treated as missing values. Finally, respiratory rate (in bpm), tidal volume (in ml), and minute ventilation (in l) were calculated for each breath and exported in 30-s means.

For all physiological parameters, change scores were calculated with reference to each adaptation phase by subtracting the mean of the entire adaptation phase (last 2 min for startle, respectively) from each minute mean after the ingestion of the beverages, resulting in eight intervals per phase. For subjective reports, the baseline scores were subtracted from scores after the ingestion of beverages. Each parameter was analyzed applying a mixed-model analysis of variance (ANOVA). In all analyses, *group* (low AS vs. high AS) was included as a between-subject factor. For analyzing adaptation across the four *adaptation* phases (baseline levels), *session* (sessions 1 through 4) was entered as a within-subject factor. The overall effects of expectancy and caffeine across time were evaluated including the within-subject factors *drug (caffeine vs. no drug)*, *solution (bitter lemon vs. coffee)*, *phase* (*immediate assessment* vs. *30-min delayed assessment*), and *time (minute 1 through 8, resp. block* [first vs. second vs. third] for startle). Additionally, the modulation of the startle responses was analyzed by adding *probe intensity* (85 vs. 95. vs. 105 dB) as a within-subject factor. To test for expectancy effects in the presence or absence of an arousal provoking substance (caffeine vs. no drug), planned comparisons were conducted within the caffeine vs. no drug condition including the factors *solution*, *time*, and *group.* All statistical tests used a significance level of $p<.05$. Whenever necessary, a Greenhouse-Geisser correction was applied. All data were processed using SPSS 21.0 (SPSS for windows, IBM).

Results

Adaptation phases

Across all adaptation phases, high-AS as compared to low-AS participants had lower $p_{et}CO_2$ level and reported increased arousal as well as decreased alertness, group all Fs(1, 36)> 4.20, ps<0.05, η_p^2 >0.11. Baseline levels of startle response magnitude (for 85 and 95 dB but not for 105 dB), skin conductance level, and reported symptoms adapted across the four laboratory sessions, session all Fs>3.15, ps<0.05, η_p^2 > 0.08, linear trends across sessions ps<0.01. Session did not interact with group for any variable. Although the order of beverage administration was balanced across sessions, random baseline differences between sessions were present for startle reflex magnitudes (for 85 and 95 dB but not for 105 dB) and reported alertness, all ps<0.01. We therefore conducted the main analyses using difference scores between baseline and immediate/late assessment phases.

Effects observed immediately after beverage ingestion

Skin conductance level As depicted in the upper panel of Fig. 1, SCL was increased immediately after ingestion of the beverages and adapted throughout the first 4 min, time $F(3, 81)=34.72$, $p<.001$, $\eta_p^2 =0.56$, $\varepsilon=0.22$. This adaptation proceeded when no drug was administered, time $F(3,81)=$

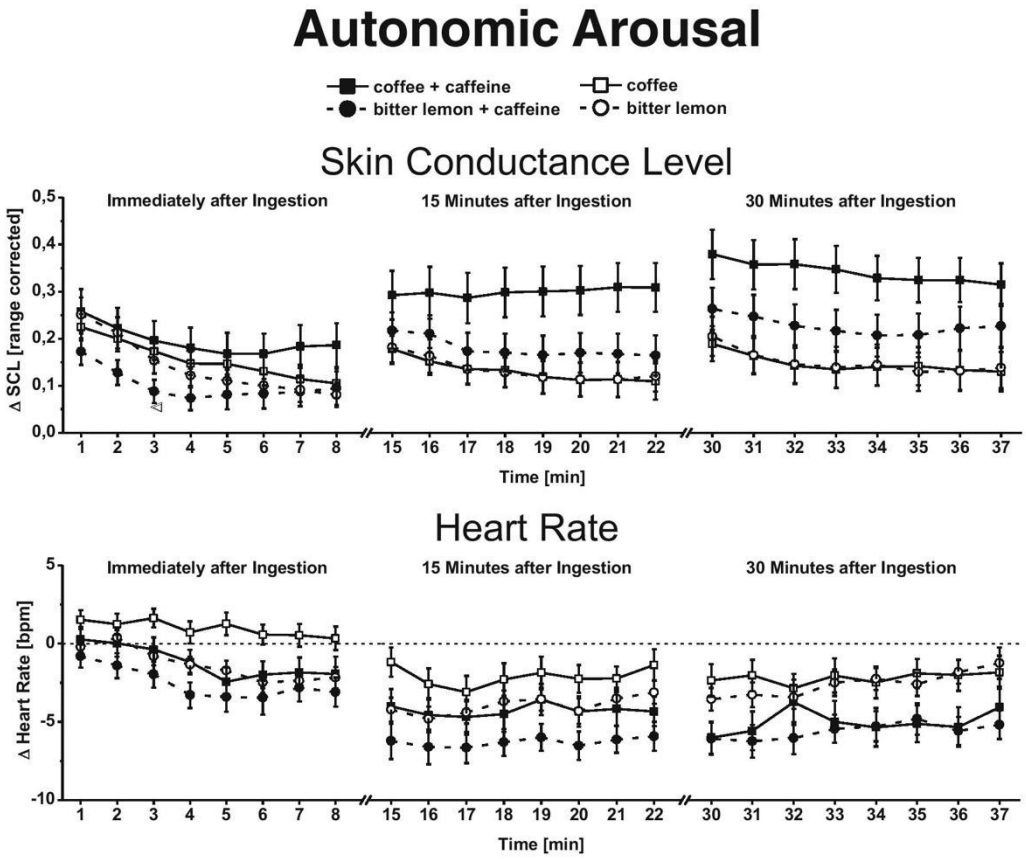

Fig. 1 Means and standard errors of change scores (each minute minus baseline) for skin conductance level (*upper panel*) and heart rate (*lower panel*) in blocks of 8 min immediately, 15, and 30 min after the ingestion of a caffeinated or decaffeinated beverage (coffee or bitter lemon)

7.27, p=.005, η_p^2 =0.21, ε=0.47, and stagnated when caffeine was added to the administered solution, time $F(3,81)$=1.81, p=.175, η_p^2 =0.06, ε=0.62, drug × time $F(7,189)$=7.83, p<.001, η_p^2 =0.23, ε=0.36. SCL was neither modulated by the created expectation nor anxiety sensitivity; solution, group, and by-group interactions all Fs<3.68, ps>0.065, η_p^2 <0.13.

Heart rate As can be seen in the lower panel of Fig. 1, the administration of caffeine led to a decreased HR, drug $F(1, 33)$=9.52, p=.004, η_p^2 =0.22, drug × solution $F(1,33)$<1, p=.420. The HR response to caffeine was not significantly modulated by the created expectation, solution $F(1,33)$= 2.27, p=.141, η_p^2 =0.06. When no drug was added to the beverages, heart rate exhibited a decrease after bitter lemon which was not observed for coffee, solution $F(1,33)$=18.39, p<.001, η_p^2 =0.36. The described effects were not differentially modulated by anxiety sensitivity, all by-group interactions $F(1,33)$<2.87, p>.099, η_p^2 <0.09. However, HR levels were generally higher (i.e., all decreases less pronounced, all increases more pronounced) in high as compared to low-AS persons, group $F(1,33)$=9.19, p=.005, η_p^2 =0.22.

Minute ventilation As depicted in the upper panel of Fig. 2, minute ventilation did not differ from baseline levels after the ingestion of a decaffeinated beverage but continuously increased when caffeine was administered, drug × time $F(7, 210)$=2.61, p=.044, η_p^2 =0.08, ε=0.53, drug $F(1,30)$=5.67, p=.024, η_p^2 =0.16. Planned comparisons within the caffeine vs. no drug condition revealed that the observed respiration patterns were neither influenced by the created expectation nor anxiety sensitivity; solution, group, and all by-group interactions Fs(1,30)<1, ps>0.54, η_p^2 <0.02.

End-tidal pCO_2 As depicted in the lower panel of Fig. 2 and in accordance with the observed minute ventilation patterns, there was a steady decline in $p_{et}CO_2$ after the administration of caffeine compared to no drug, drug × time $F(7231)$=15.21, p<.001, η_p^2 =0.32, ε=0.58, that led to an overall lower $p_{et}CO_2$ after the ingestion of caffeinated beverages, drug $F(1,33)$=

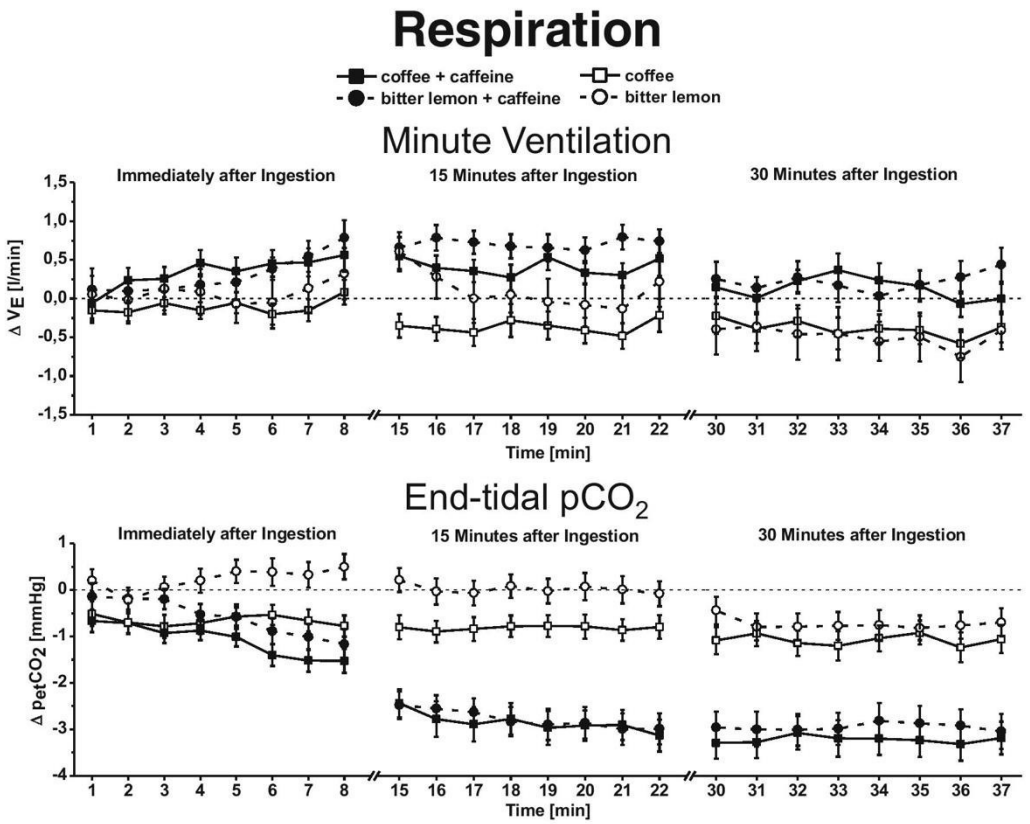

Fig. 2 Means and standard errors of change scores (each minute minus baseline) in blocks of 8 min immediately, 15, and 30 min after ingestion of beverages for minute ventilation (V_E) and end-tidal pCO_2 ($p_{et}CO_2$)

7.98, p=.008, η_p^2 =0.20. This effect of caffeine was not modulated by the created expectation or anxiety sensitivity; solution, group, and all by-group interactions Fs(1,33)<2.47, ps> 0.125, η_p^2 <0.07. When no caffeine was administered, the consumption of coffee resulted in a lower $p_{et}CO_2$ than the ingestion of bitter lemon, solution F(1,33)=14.07, p=.001, η_p^2 =0.30, which did not differ by group, solution × group F(1,33)=1.57, p=.219, η_p^2 =0.05.

Startle response magnitudes The ingestion of caffeinated as compared to non-caffeinated beverages led to higher startle response magnitudes in both groups, for 95 and 105 dB startle probes: drug F(1,36)>7.14, p<.008, η_p^2 >0.184; for 85 dB probes: drug F(1,36)=2.39, p=.131, η_p^2 =0.06, all drug × group F(1,36)<2.35, p<.136, η_p^2 <0.07. As depicted in the left panel of Fig. 3, startle response magnitudes were decreased in both experimental groups when caffeine was consumed unexpectedly vs. when consumed in coffee, for 95 and 105 dB probes: solution F(1,36)>4.25, p<.05, η_p^2 >0.11, solution × group F<1, p>.703. For the less intense 85 dB startle probes, this effect was only observed in high-AS participants, solution F(1,18)=7.24, p=.015, η_p^2 =0.287, but not low-AS controls, F <1, p=.959, solution × group F(1,36)=4.47, p=.041, η_p^2 = 0.11. Planned comparisons in the no drug condition revealed no significant effects of expectation or anxiety sensitivity; solution, solution × group F<1.72, p>.199, η_p^2 <0.05.

Subjective reports As depicted in the upper panel of Fig. 4, the immediate ratings of alertness tended to be slightly increased by caffeine in low- and high-AS participants, drug F(1,36)=3.19, p=.083, η_p^2 =0.08, drug × group F<1, p=.641. When caffeinated beverages were ingested, high-AS as compared to low-AS persons reported a marginally greater increase of alertness when caffeine was consumed expectedly, solution × group F(1,36)=3.36, p=.075, η_p^2 =0.085, and p=.047 in a group comparison limited to the upper quartile of high-AS participants, group for coffee + caffeine F(1, 36)=8.10, p=.007, η_p^2 =0.18. When no drug was added to the beverages, a similarly greater increase in alertness for coffee as compared to bitter lemon was observed in both experimental groups, solution F(1,36)=5.66, p=.023, η_p^2 =0.14, solution × group F<1 p=.419.

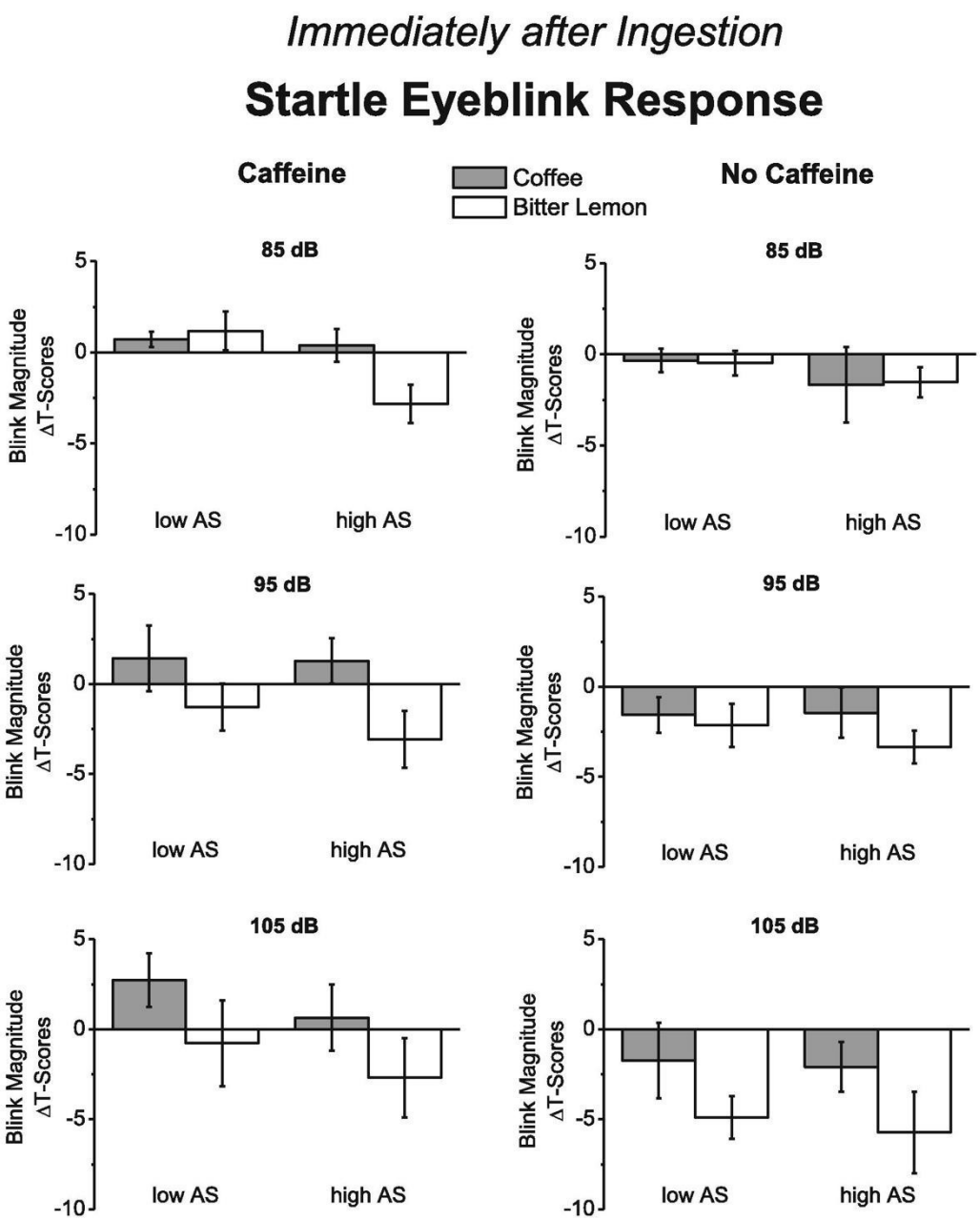

Fig. 3 Means and standard errors of change scores (early minus baseline) for startle response magnitudes (elicited by a 85, 95, and 105 dB startle probe, resp.) after an expected or unexpected administration of caffeine (*left panel*) vs. no drug (*right panel*)

There were no differences in ratings of arousal shortly after consumption of caffeinated or not caffeinated beverages, drug $F<1$, $p=.658$, drug × group $F<1$, $p=.570$. Independent of drug or no drug administration, high-AS participants reported a greater increase of arousal when consuming coffee, solution $F(1,18)=6.14$, $p=.023$, $\eta_p^2=0.25$, an effect that was not observed in low-AS controls, solution $F(1,18)<1$, $p=1.00$, solution × group $F(1,36)=4.25$, $p=.046$, $\eta_p^2=0.11$.

Effects observed 30 min after beverage ingestion

Skin conductance level SCL continued to rise from the immediate assessment after beverage consumption to 30 min later, phase $F(1,27)=11.85$, $p=.002$, $\eta_p^2=0.305$, and was, again, higher when caffeinated beverages were ingested, drug $F(1,27)=9.02$, $p=.006$, $\eta_p^2=0.250$, see Fig. 1. Neither in the drug nor in the no drug condition was SCL modulated by the

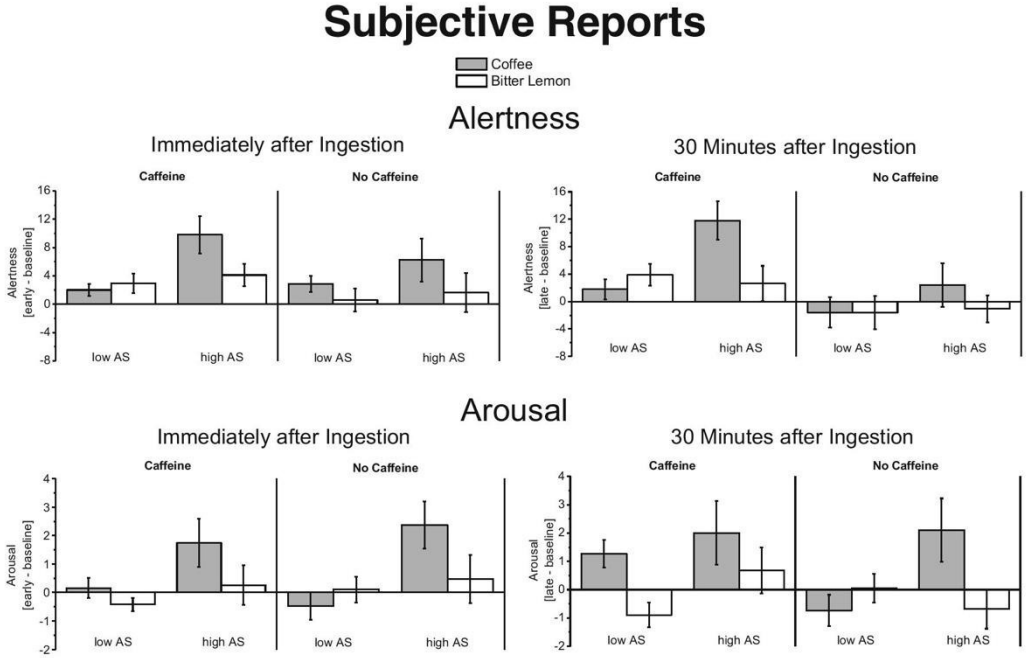

Fig. 4 Means and standard errors of change scores for the reported alertness (*upper panel*) and arousal (*lower panel*) comparing low- and high-AS participants immediately and 30 min after consumption of coffee or bitter lemon mixed with caffeine or no drug

created expectation or anxiety sensitivity; solution, group, and all by-group interactions all Fs<3.76, ps>0.062.

Heart rate HR decelerated from the immediate to the 30-min delayed assessment, phase $F(1,33)=59.24$, $p<.001$, $\eta_p^2 = 0.642$, see lower panel of Fig. 1. Beverages containing caffeine led to a more pronounced deceleration of HR in both experimental groups, drug $F(1,33)=15.54$, $p<.001$, $\eta_p^2 = 0.320$, drug × group $F(1,33)<1$, $p=.329$. In the caffeine condition, high-AS participants showed a less accentuated decrease in HR as compared to low-AS persons, group $F(1,33)=5.70$, $p=.023$, $\eta_p^2 =0.147$. This effect was not significant when no drug was administered, group $F(1,33)=2.86$, $p=.100$, $\eta_p^2 =0.080$. In both groups, the created expectation did not affect HR, neither in the caffeine condition, solution $F(1,33)<1$, $p=.629$, solution × group $F(1,33)<1$, $p=.820$, nor in the no drug condition, solution $F(1,33)<1$, $p=.584$, solution × group $F(1,33)<1$, $p=.648$.

Minute ventilation Minute ventilation slightly decreased from the immediate to the 30-min delayed assessment, phase $F(1,30)=8.34$, $p=.007$, $\eta_p^2 =0.218$. As depicted in the upper panel of Fig. 2, compared to the baseline assessment, caffeine consumption led to an elevated minute ventilation and minute ventilation was reduced after no caffeine, drug $F(1,30)=10.26$, $p=.003$, $\eta_p^2 =0.255$. When tested separately for caffeine vs. no drug, neither the created expectation nor anxiety sensitivity had any further effects on minute ventilation; solution, group, and all by-group interactions all Fs(1,30)<2.73, ps>0.108.

End-tidal pCO_2 $p_{et}CO_2$ kept decreasing from the immediate to the 30-min delayed assessment, phase $F(1,35)=157.86$, $p<.001$, $\eta_p^2 =0.827$, with a much more pronounced decline after ingestion of caffeine, phase × drug $F(1,35)=72.56$, $p<.001$, $\eta_p^2 =0.687$. Consequently, $p_{et}CO_2$ was again lower after administration of caffeine than after no drug, drug $F(1,33)=54.00$, $p<.001$, $\eta_p^2 =0.621$, see lower panel of Fig. 2. $P_{et}CO_2$ was not affected by the created expectation or anxiety sensitivity, neither in the caffeine nor in the no drug condition; solution, group, and all by-group interactions Fs(1,33)<1.25, ps>0.272.

Startle response magnitudes Overall, startle reflex magnitudes (for 85, 95, and 105 dB, resp.) were higher after ingestion of caffeinated compared to decaffeinated beverages in both groups, drug Fs>7.06, ps<0.013, η_p^2 >0.167, drug × group Fs<3.02, ps>0.091. When caffeine was administered, in both groups startle reflex magnitudes elicited by 105 dB startle probes were inhibited after administration of bitter lemon vs. coffee, solution $F(1,34)=7.85$, $p=.008$, $\eta_p^2 =0.188$, solution × group $F(1,34)<1$, $p=.536$; for 95 dB probe: solution $F(1,34)=2.10$, $p=.157$, $\eta_p^2 =0.058$, solution × group $F(1,34)<1$, $p=.438$. As observed for 85 dB startle probes

immediately after ingestion of caffeine, high-AS participants exhibited a trend of inhibition after bitter lemon as compared to after coffee, solution $F(1,16)=3.89$, $p=.065$, $\eta_p^2=0.186$, see upper left panel of Fig. 5. This effect was not observed in low-AS participants, solution $F(1,18)<1$, $p=.575$, $\eta_p^2=0.018$, solution × group $F(1,34)=2.89$, $p=.098$, $\eta_p^2=0.076$. When no drug was administered, no significant effects of the created expectation or anxiety sensitivity on startle reflex magnitudes were observed, solution, solution × group $Fs<1.71$, $p>.199$.

Subjective reports Alertness was increased 30 min after the administration of caffeine as compared to no drug, drug $F(1,36)=15.16$, $p<.001$, $\eta_p^2=0.30$ (see upper right panel of Fig. 4). Coffee compared to bitter lemon potentiated the effect of caffeine on the reported alertness exclusively in high-AS persons, solution $F(1,19)=9.94$, $p=.005$, $\eta_p^2=0.36$, but not low-AS controls, solution $F(1,19)=1.24$, $p=.281$, $\eta_p^2=0.06$, solution × group $F(1,36)=10.56$, $p=.003$, $\eta_p^2=0.23$. No such effect was observed when administering no drug, solution $F(1,36)<1$, $p=.437$, $\eta_p^2=0.02$, solution × group $F<1$, $p=.451$.

Overall, the presence or absence of caffeine in the ingested beverages did not influence ratings of arousal in either group, drug $F(1,36)=2.19$, $p=.148$, $\eta_p^2=0.06$, drug × group $F(1,36)<1$, $p=.894$. However, planned analyses of the drug vs. no drug condition revealed that the created expectations influenced the subjective level of arousal. Both groups reported an increased arousal after ingestion of caffeinated coffee as compared to caffeinated bitter lemon, solution $F(1,36)=8.62$, $p=.006$, $\eta_p^2=0.19$; solution × group $F<1$, $p=.481$. Even when no caffeine was added to the beverages, high-AS participants reported an elevated arousal when an arousal expectation was created by administration of coffee, solution $F(1,19)=6.41$, $p=.021$, $\eta_p^2=.26$. This was not observed in low-AS persons, solution $F(1,19)=1.26$, $p=.276$, $\eta_p^2=0.07$, solution × group $F(1,36)=7.50$, $p=.01$, $\eta_p^2=0.17$.

Discussion

The current study aimed at investigating physiological arousal, respiratory and defensive reflex responses, and subjective reports to expected and unexpected caffeine-induced arousal in low and high anxiety sensitive persons who regularly consume caffeine.

As expected, caffeine started to increase ventilation, autonomic activation, and startle response magnitudes within the first minutes after ingestion of the beverages and peaked 30 min after administration. Both AS groups were characterized by a comparable level of autonomic arousal and respiratory responses. Immediately after the ingestion of decaffeinated coffee, both groups showed a lower level of expired pCO_2 and no decrease in heart rate compared to after the consumption of caffeine-free bitter lemon soda.

Overall, administration of caffeine led to an increase in startle response magnitude. Moreover, when caffeine was administered, startle response magnitudes were modulated by expectation. In both AS groups, the unexpected as compared to the expected administration of caffeine led to decreased startle response magnitudes. However, for low intensity 85 dB startle probes, this effect was only observed for high-AS persons, possibly indicating a greater sensitivity in modulation of startle response magnitudes by expectancy in high-AS participants. In the absence of caffeine, startle response magnitudes were not influenced by anxiety sensitivity or expectancy.

The administration of caffeine resulted in increased alertness ratings immediately and 30 min after drug ingestion. Moreover, immediately after beverage ingestion, the expectation of arousal led to a greater increase of alertness ratings in both groups, but only when no caffeine was administered. When caffeine was given, only high-AS individuals showed a greater increase of alertness when caffeine was expected. This pattern was also found in the high-AS group 30 min after beverage ingestion. No main effect of caffeine was found for subjective arousal. However, high-AS individuals reported an increase in arousal whenever they expected it, i.e., consumed coffee. This was observed immediately as well as 30 min after beverage consumption. In contrast, low-AS controls showed this expectancy-congruent increase in arousal only 30 min after beverage ingestion and only when receiving caffeine.

Autonomic and respiratory responses

The observed autonomic and respiratory response patterns were in accordance with previous evidence from studies with anxiety-unselected populations (Flaten and Blumenthal 1999; Green et al. 1996; Mikalsen et al. 2001; Sawyer et al. 1982). As reported earlier, Pané-Farré et al. (2014) revealed an exaggerated respiratory response to unexpected caffeine administration in high compared to low anxiety sensitive persons who were classified as non-habitual caffeine users. This finding was not replicated in the present study for a population of habitual caffeine users. This effect may be explained by a possible development of tolerance to caffeine's physiological effect reducing the physiological responsivity to caffeine in this group (Gupta and Gupta 1999) and thus changing response patterns.

Expectancy effects on startle response modulation during a caffeine challenge

Caffeine has been shown to increase startle response magnitudes (Andrews et al. 1998; Flaten and Blumenthal 1999;

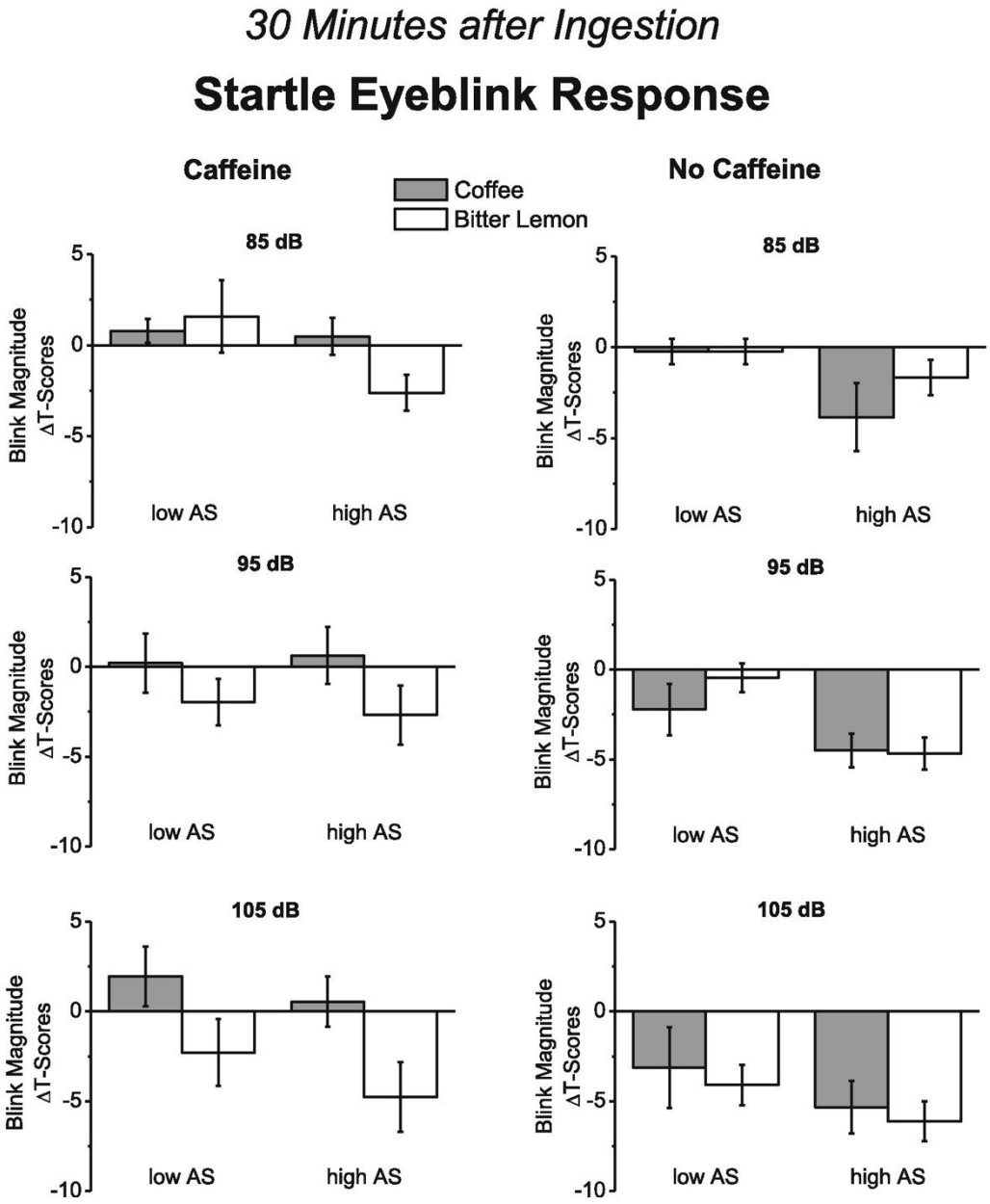

Fig. 5 Means and standard errors of change scores (late minus baseline) for startle response magnitudes (elicited by a 85, 95, and 105 dB startle probe, resp.) comparing low- and high-AS individuals 30 min after an expected or unexpected administration of caffeine (*left panel*) vs. no drug (*right panel*)

Schicatano and Blumenthal 1994; Schicatano and Blumenthal 1995; Schicatano and Blumenthal 1998). The present study demonstrated that this effect was modulated by expectation. When caffeine was unexpectedly administered in bitter lemon and thus unexpectedly led to autonomic arousal and respiratory stimulation, startle response magnitudes were reduced in comparison to the expected administration in coffee. This finding of reduced startle response magnitude during stimulation of body sensations is in line with studies that reported attenuated startle eyeblink responses during a variety of interoceptive challenge tests including CO_2 inhalation and loaded breathing (Ceunen et al. 2013; Pappens et al. 2012; Pappens et al. 2011). It has been suggested that this reduction of startle reflex magnitudes may be attributed to increased attention allocation to interoceptive sensations (Alius et al. 2014; Pappens et al. 2011). In accordance with this

interpretation, Alius et al. (2014) demonstrated that decreased startle reflex magnitudes during a dyspneic challenge were accompanied by reduced amplitudes of the P300 component of the probe-evoked potential as an index of reduced processing of auditory startle probes (Cuthbert et al. 1998; Keil et al. 2007; Schupp et al. 1997; Schupp et al. 1997). It has been proposed that the attentional shift toward the interoceptive stimulation leads to a reduced capacity of resources for processing the auditory startle-eliciting probe, leading to a reduction of startle response magnitudes. In accordance with this hypothesis, decreased startle response magnitude has been demonstrated when attention was captured by stimuli that differed from the sensory modality of the startle-eliciting probe (Anthony and Graham 1985; Filion et al. 1998, for review). Finally, Schicatano and Blumenthal (1998) showed that auditory startle response amplitudes were decreased when attention was captured by a visual search task after the administration of caffeine. Thus, it has been suggested that sufficient attentional resources are necessary for the processing of the startle probe to reveal caffeine's typical effect of increasing startle response magnitudes (Schicatano and Blumenthal 1998).

In the current study, we substantiate and further extend previous findings by demonstrating reduced startle eyeblink magnitudes in response to an unexpected arousal induction by caffeine. Moreover, our data provides the first evidence that this effect may be more accentuated in high anxiety sensitive persons. That is, persons who fear bodily perturbations may engage more attentional resources for processing unexpected somatic arousal cues.

Gray and McNaughton (2003) stated that a mismatch of expected and actual stimuli engages the organism's "control mode," leading to increased attention allocation toward the threat. Or, put it in terms of Paulus and Stein (2006), a mismatch of predicted and observed physiological states generates an error signal in the anterior insula cortex, prompting attentional resource allocation for facilitating information gathering and selecting appropriate action. Interestingly, this insula-mediated error signal is suggested to be increased in high anxiety sensitive persons. If altered body state expectations were implicated in the generation of higher error signal, one would also assume a more pronounced attenuation of the startle reflex when arousal induction occurs unexpectedly after participants experienced no arousal in the same context. In fact, this pattern was observed when bitter lemon was administered without caffeine in a first session but with caffeine in a subsequent session (data not shown). In contrast, when bodily changes in habitual caffeine users correspond to the expected arousal induction by caffeine, upcoming arousal may be attributed to the familiar caffeine effect such that somatic arousal sensations became threat-irrelevant even for high-AS persons (Telch et al. 2011). Importantly, differences in startle response modulation cannot be explained by higher interoceptive stimulation in high-AS persons as respiration and autonomic arousal did not differ between both AS groups.

Effects of anxiety sensitivity and arousal expectation on subjective reports

During the entire assessment, participants who fear arousal sensations reported an elevated arousal after ingestion of coffee, even when no arousal provoking agent was administered. Importantly, low and high anxiety sensitive persons showed a comparable level of physiological arousal, as indicated by measures of autonomic arousal and respiration, that goes in line with previous findings (Asmundson et al. 1994; Schmidt and Telch 1994; Sturges and Goetsch 1996; Sturges et al. 1998; Zvolensky et al. 2001; Zvolensky et al. 1999). However, high-AS participants differed from low-AS participants in their appraisal of the evoked sensations. The observed overestimation or misinterpretation of interoceptive stimuli has inherently been linked to the concept of anxiety sensitivity (McNally 1990). These findings broaden previous evidence in regular caffeine consumers (Flaten and Blumenthal 1999; Mikalsen et al. 2001), in demonstrating that anxiety sensitivity affects this arousal response.

This finding is additionally substantiated by the context-sensitivity vulnerability model that emphasizes the interplay of person and contextual factors such as the dispositional sensitivity to fear arousal sensations and an expected arousal induction (Telch et al. 2011). Thus, high AS potentiated the effect of the instructional set (arousal induction) by increasing the perceived threat of the expected arousal induction based on beliefs that arousal sensations signify potential dangerous consequences (McNally 2002; Telch et al. 2010). This notion is similarly supported by higher alertness rating in high-AS persons when an arousal expectation was created. Following Flaten and Blumenthal (1999), these findings in habitual caffeine users can also be interpreted in the light of conditioned responses to caffeine-associated stimuli; that is, high-AS individuals may have acquired a stronger conditioned arousal and alertness response.

Limitation and future directions

The present study discerned lowered startle reflex magnitudes as an index of increased attentional resource allocation toward unexpected arousal in individuals who fear associated sensations. Further interoceptive challenge studies are required that replicate and strengthen the current evidence by simultaneously measuring both the startle eyeblink response and event-related potentials to startle probes. Additionally, as habitual caffeine consumers might have acquired tolerance to the effect of caffeine, firstly, reversal of withdrawal symptoms including aversive bodily sensations possibly masked effects; secondly, compared to various challenge tests (hyperventilation, CO_2

inhalation) caffeine may provoke only mild interoceptive stimulation which rendered it difficult to reveal pronounced anxiety induction (Rogers et al. 2010; Rogers et al. 2013). In conclusion, subsequent studies investigating the effect of caffeine administration should take into account the effects of expectancy and anxiety sensitivity.

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Conflict of interest The authors declare no conflict of interest.

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Manuscript 3

When dyspnea gets worse: Suffocation fear and the dynamics of defensive respiratory responses to increasing interoceptive threat

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When dyspnea gets worse: Suffocation fear and the dynamics of defensive respiratory responses to increasing interoceptive threat

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Abstract

In patients with anxiety and/or respiratory diseases, body sensations, particularly from the respiratory system, may increase in intensity and aversiveness and thus lead into defensive action (e.g., escape) or panic. The processes, however, that might contribute to the culmination of symptoms and the switch into defensive action have not been well understood yet. The current study aimed at evaluating an experimental paradigm to characterize the dynamics of defensive mobilization to body sensations increasing in intensity and aversiveness. Persons reporting low and high suffocation fear (SF; $N = 69$) were exposed to increasingly unpleasant feelings of dyspnea induced by inspiratory resistive loads and a breathing occlusion requiring voluntary breath holding. Respiratory responses were assessed along with subjective reports of anxiety and panic symptoms. Presentation of respiratory loads with increasing physical resistance led to increasingly unpleasant feelings of dyspnea. Twenty-eight participants terminated the exposure prematurely at least once. When dyspnea was severe, high compared to low SF persons exhibited an increased respiratory rate that was accompanied by reports of more intense panic symptoms. Premature terminations of exposure were preceded by a surge in anxiety, breathing frequency, and mouth pressure, and a decrease in tidal volume. We successfully established an experimental paradigm to assess changes in defensive responding with increasing intensity of an interoceptive threat. The current data foster our understanding of behavioral expression patterns observed in patients with anxiety and/or respiratory diseases and the processes involved in the culmination of bodily sensations and anxiety into panic.

KEYWORDS

asthma, COPD, defense cascade, inspiratory load, panic disorder, respiration

1 | INTRODUCTION

Internal body sensations (e.g., those arising from the respiratory system) bear high relevance for a number of anxiety and health problems such as panic disorder (PD) or respiratory diseases including asthma and chronic obstructive pulmonary disease (COPD). For example, these patients often perceive even slight feelings of dyspnea as threatening as they may signal an impending catastrophe (e.g., a critical somatic state or even possible suffocation), or a panic or an asthma attack (Bouton, Mineka, & Barlow, 2001). As a consequence, the experience of dyspnea may trigger anxious arousal (Pappens et al., 2013; Pappens, Smets, Vansteenwegen, Van den Bergh, & Van Diest, 2012), thus further amplifying body symptoms and potentially culminating into defensive action (e.g., escape or active avoidance behavior) or a panic attack (e.g., Barlow, 2002; Bouton et al., 2001; Clark, 1986; Ehlers & Margraf, 1989; Hamm, Richter, & Pané-Farré, 2014; Lehrer, Feldman, Giardino, Song, & Schmaling, 2002; Pappens et al., 2013; Pappens, Smets, Vansteenwegen et al., 2012). In

this patient group, defense behaviors (e.g., frequent flight from critical situations) are associated with an exacerbation of anxiety psychopathology, increased psychosocial impairment, thus causing increased societal burden and costs (Barlow, 2002; Carr, 1999; Lehrer et al., 2002; Wittchen & Jacobi, 2005). Therefore, it is desirable to prevent the culmination of symptoms into defensive action. However, to provide effective tools for treatment to stop this escalating process and alleviate anxiety, it is important to reveal the mechanisms that contribute to the culmination of body sensations.

The culmination of symptoms as well as a behavioral switch into defensive action can be explored from the perspective of defensive response mobilization. As explained above, respiratory sensations, such as feelings of dyspnea or shortness of breath, may be perceived as indicators of an upcoming aversive event (e.g., a panic or asthma attack). Thus, in addition to various other defensive responses, the mobilization of defensive respiratory responses is initiated, that is, an increase in respiratory rate, which sometimes even goes above and beyond actual physiological demand (Alius, Pané-Farré, von Leupoldt, & Hamm, 2013; Homma & Masaoka, 2008; Masaoka & Homma, 1997, 2001; Melzig, Holtz, Michalowski, & Hamm, 2011; Van Diest et al., 2001, 2005). This defensive mobilization of respiratory responses is mediated by a defensive neural network that has been described extensively (Davis & Whalen, 2001; Lang, Davis, & Öhman, 2000; von Leupoldt et al., 2008). As breathing changes, various internal body sensations are elicited that may escalate the perception of threat predicting an imminent catastrophe (e.g., a critical somatic state or even possible suffocation), or a panic or an asthma attack (Hamm et al., 2014, 2016). Thus, internal body sensations may be assumed to culminate resulting from an interaction of symptom perception, affective response, and thus at peak levels of defensive mobilization leading to defensive action. Supporting this view, it has been demonstrated that the switch into defensive action is preceded by a surge of sympathetic arousal and inhibition of the startle reflex (Löw, Weymar, & Hamm, 2015; Richter et al., 2012).

An experimental approach to investigate the culmination of respiratory symptoms is the use of inspiratory resistive loads. By applying ascending inspiratory resistive loads, breathing restriction increases inducing feelings of dyspnea with increasing intensity under experimentally controlled conditions. Interestingly, and tapping into the question of whether other modulating factors may influence symptom culmination, when restricting the inspiration by resistive loads, persons show enormous interindividual differences in the way they respond to the induced feeling of dyspnea (Harver & Mahler, 1998). For example, the defensive respiratory pattern in reaction to dyspnea is altered in anxious individuals with high suffocation fear (SF) paralleling those of patients with PD. Numerous studies have demonstrated that high SF and a history of suffocation experiences is associated with automated negative evaluation of suffocation sensations, increased autonomic arousal, as well as reports of greater anxiety and panic symptoms during provocation of dyspnea (Alius et al., 2013; Eifert, Zvolensky, Sorrell, Hopko, & Lejuez, 1999; Eke & McNally, 1996; Kroeze et al., 2005; McNally & Eke, 1996; Ogliari et al., 2010). Moreover, experimental studies demonstrated that, while persons low in SF show a typical compensatory decrease in breathing frequency when breathing against an inspiratory resistive load (Harver & Mahler, 1998; Iber, Berssenbrugge, Skatrud, & Dempsey, 1982), high SF individuals were characterized by an increase in respiratory rate (Alius et al., 2013; Pappens, Smets, Van den Bergh, & Van Diest, 2012), thus increasing their exposure frequency to the loaded inspiration. Interestingly, high SF persons also reported more respiratory symptoms and rated their feelings of dyspnea as more severe or unpleasant compared to low SF persons (Alius et al., 2013; Pappens, Smets, Van den Bergh, & Van Diest, 2012).

To specifically target the question of the interplay of symptom culmination and anxiety along with its possible switch into defensive action, in the present study, respiration, anxiety, and bodily symptoms were measured in persons who reported low or high SF to increasingly restricted breathing. Increasingly obstructed breathing, that is, increasing threat originating from the respiratory system, was modulated by successively applying three inspiratory resistive loads of increasing intensity that were followed by a complete breathing occlusion, an experimental model for a suffocation experience that has been shown to be a potent unconditioned internal threat (Nardi et al., 2006; Pappens et al., 2014; Pappens, Smets, Vansteenwegen et al., 2012). Thus, the application of increasing loads was indicative of an approaching complete breathing occlusion. The present experimental design is based upon and extends previous respiratory experimental panic research (Acheson, Forsyth, & Moses, 2012; Acheson, Forsyth, Prenoveau, & Bouton, 2007; Li et al., 2006; Pappens, Smets, Vansteenwegen et al., 2012; Wan et al., 2008) in that increasing dyspnea is induced by progressive breathing restriction requiring increasing breathing effort—a condition patients with PD or obstructive respiratory diseases including asthma commonly experience in their everyday life (e.g., during panic or asthma attacks). Extending previous paradigms, we wanted to establish an experimental paradigm that also enables us to study defensive action (flight behavior). Therefore, all participants had the opportunity to terminate the exposure to resistive loads (by button press) if dyspnea became too aversive.

It was assumed that reported anxiety and intensity of panic and respiratory symptoms would increase during increasing dyspnea and that this increase would be stronger

for individuals with high SF. Based on previous studies (Alius et al., 2013), we hypothesized that, while dyspnea increases, a respiratory response would evolve in high SF persons indicated by an increased respiratory rate. In contrast to high SF persons, we expected that low SF participants would exhibit a compensatory decrease in respiratory rate. We also expected that a significant number of participants would prematurely terminate the exposure to dyspnea. In accordance with previous findings (Hamm et al., 2016; Richter et al., 2012), we predicted that a higher number of high compared to low SF persons would terminate the exposure. In line with previous studies showing a strong defensive mobilization of autonomic and respiratory responses during panic or initiation of defensive action (Löw et al., 2015; Meuret et al., 2011; Richter et al., 2012), we assumed that a strong increase in respiratory rate and anxiety would occur just prior to the termination of exposure, probably motivating such escape behavior.

2 | METHOD

2.1 | Participants

Participants were recruited from a pool of 400 university students based on their scores on the suffocation fear subscale of the Claustrophobia Questionnaire (Radomsky, Rachman, Thordarson, McIsaac, & Teachman, 2001). In a first step, potential study participants were contacted for a standardized telephone interview if they reported either low ($4 \leq$ SF) or high ($11 \geq$ SF) suffocation fear (lower and upper quartile of the screened population). Individuals who reported cardiovascular, respiratory (e.g., asthma, COPD), or neurological (epileptic or apoplectic seizures, multiple sclerosis) diseases, current or past psychotherapeutic treatment due to anxiety problems, loss of hearing, or pregnancy were excluded. Overall, 69 participants took part in the laboratory assessment. Twenty-one low SF and 20 high SF participants (18 females per group) completed the whole experimental procedure. An additional 10 low SF and 18 high SF participants (for low and high SF: 2 vs. 3 males) prematurely terminated the exposure to restricted breathing as described in the Procedure section. All participants provided written informed consent prior to the study and either received course credit or financial compensation for their participation. The study protocol was approved by the ethics committee of the German Psychological Society.

2.2 | Apparatus and materials

2.2.1 | Breathing circuit

Participants breathed through a tightly fitting soft silicone face mask (7400 series; Hans Rudolph, Inc., Kansas City, MO) connected to a rigid tube with sensors for measuring respiration. A flow sensor was mounted to the mouth port of the two-way Y-shaped nonrebreathing valve (no. 2630; Hans Rudolph, Inc.), which enabled unrestricted expiration through the expiratory port of the valve. A plastic tube (length: 2.75 m; diameter: 35 mm) was connected to the inspiratory part of the valve and mounted to the common port of a Five-Way Gatlin-Shape Inflatable-Balloon-Type valve (2440 series, Hans Rudolph, Inc.), placed in the adjacent control room. Closing and opening of the valves were controlled via VPM software triggering a pneumatic controller (2430 series, Hans Rudolph, Inc.). This system allowed a prompt and easy switching between different ports and thus between three different inspiratory resistive loads and unrestricted breathing (one port without attached load).

2.2.2 | Inspiratory resistive loads

For the induction of dyspnea, nylon flow resistors of linear type (7100 series, Hans Rudolph, Inc.) were either attached separately or combined in groups of two or more resistors (i.e., a maximum of seven resistors) to the different ports of the valve. The combination of separate resistors followed a fixed scheme resulting in the total resistance (range: 0.05–23.19 kPa/l/s). The total resistance is given by the sum of all separate loads.

2.2.3 | Breathing occlusion

Breathing occlusions (simultaneous closure of all inspiratory ports for 15 s) were manually triggered at the end of expiration as indicated by a display of the respiration curve generated by thoracic and abdominal respiration belts connected to an inductive plethysmography system (Respitrace, Q.D.C., SensorMedics, NewMedics GmbH, Öhringen, Germany).

2.2.4 | Respiratory parameters

A modified ZAN 600 system (Spire Health, Inc., Oberthulba, Germany) allowed continuous recording of respiratory flow, mouth pressure, and fractional CO_2.

2.2.5 | Subjective reports

Participants rated the intensity and unpleasantness of dyspnea as well as the severity of panic symptoms as listed in the DSM-5 during loaded breathing using a computer keyboard on the following scale: 1 (*not at all*), 2 (*slight*), 3 (*moderate*), 4 (*strong*), 5 (*very strong*), and 6 (*maximally tolerable*). Moreover, using a touchpad (Intuos PEN & Touch S, Wacom Europe GmbH, Krefeld, Germany), participants were asked to draw a line indicating their anxiety intensity as

experienced during exposure to the respiratory loads as well as during the occlusion. Rating responses were projected onto a 1.50 × 1.30 m screen in front of the subjects.

2.3 | Questionnaires

2.3.1 | Suffocation Fear Subscale (SF)

SF is a subscale of the Claustrophobia Questionnaire (CLQ; Radomsky et al., 2001) comprised of 14 items that are rated on a 5-point Likert scale ranging from 0 (*not at all*) to 4 (*extremely*). Participants rate how anxious they would feel in specific situations associated with suffocation fear. The SF subscale of the CLQ has shown excellent reliability (e.g., internal consistency $\alpha = .85$) and validity (Radomsky et al., 2001). It has been demonstrated that SF is associated with exaggerated anxious responding and active avoidance or escape (Alius et al., 2013; McNally & Eke, 1996; Radomsky et al., 2001; Richter et al., 2012). Patients with anxiety disorders reported higher SF compared to healthy controls (Hamm et al., 2016).

2.3.2 | Anxiety Sensitivity Index-3 (ASI-3)

The ASI-3 (Kemper, Ziegler, & Taylor, 2009; Taylor et al., 2007) is an 18-item measure that assesses the tendency to fear anxiety-related sensations (AS; McNally, 2002) on a 5-point Likert scale ranging from 0 (*very little*) to 4 (*very much*). The ASI-3 has demonstrated good reliability and validity (e.g., internal consistency $\alpha = .92$; Kemper, Lutz, Bahr, Ruddel, & Hock, 2012; Taylor et al., 2007). It has been shown that anxiety sensitivity predicts the occurrence of panic attacks and the onset of PD (Li & Zinbarg, 2007; Woud, Zhang, Becker, McNally, & Margraf, 2014). Moreover, it has been demonstrated that anxiety sensitivity is related to increased defensive mobilization in a variety of interoceptive threat tasks (McNally, 2002; Melzig et al., 2011).

2.3.3 | Body Vigilance Scale (BVS)

The BVS (Schmidt, Lerew, & Trakowski, 1997) is a four-item instrument that measures the attention focused on bodily sensations and perturbations rated on an 11-point Likert scale. The BVS has displayed adequate internal consistency ($\alpha = .75$). Higher BVS scores have been found in patients with PD compared to healthy controls (Olatunji, Deacon, Abramowitz, & Valentiner, 2007; Schmidt et al., 1997)

2.3.4 | State-Trait Anxiety Inventory (STAI)

The trait portion of the STAI (Laux, Glanzmann, Schaffner, & Spielberger, 1981; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) measures the general proneness to experience anxiety and perceive situations as threatening with 20 items on a 4-point Likert scale. The internal consistency typically ranges from .86 to .95 (Spielberger et al., 1983). High trait anxiety is associated with anxiety disorders (Chambers, Power, & Durham, 2004; Plehn & Peterson, 2002).

2.4 | Procedure

As illustrated in Figure 1, the experiment consisted of five consecutive parts. After all sensors were attached, the face mask was tightly and comfortably fitted using light standard headgear (Hans Rudolph, Inc.) with four elastic Velcro straps connected to the face mask. Then, the experiment proceeded as follows.

1. Determination of breath-holding time. The maximal post-expiratory breath-holding time (breath holding at functional residual capacity as suggested by Asmundson & Stein, 1994) was measured using a standardized procedure in which at the end of an expiration the examiner directed the participants to hold their breath as long as they could, while the breathing circuit was occluded. Hence, during this period no breathing was possible until the participants terminated this breathing occlusion by pressing a button.
2. Determination of detection threshold. For the determination of the individual detection threshold, inspiratory resistive loads (IRLs) were separately presented for 20 s, followed by a 20-s recovery phase. Starting with the lowest load (0.05 kPa/l/s), the load intensities were increased stepwise following an exponential curve (range in the current study: 0.05–0.78 kPa/l/s) until participants noticed any change in respiration (see also Alius et al., 2013).
3. Presentation and selection of increasing IRLs. To get acquainted with the range of possible loads, participants were asked to rate the intensity and unpleasantness of dyspnea of a mild (0.49 kPa/l/s above threshold) and a more severe load (4.90 kPa/l/s above threshold). Then, IRLs were gradually increased, again following an exponential curve. Loads were presented for 30 s, followed by a recovery phase lasting 30 s. Afterward, participants rated the intensity and unpleasantness of experienced dyspnea during IRL presentation. After an IRL was rated as evoking an unpleasantness of 6 (*maximally tolerable*), loads were not increased further. Then, participants were exposed to one breathing occlusion lasting for 15 s, followed by a recovery phase of 30 s and, again, ratings of intensity and unpleasantness of dyspnea. Finally, IRLs producing slight, strong, and maximally tolerable dyspnea were selected for the following assessment phase.
4. Repeated presentation of increasing IRLs followed by occlusion. The experiment started with a one-time only

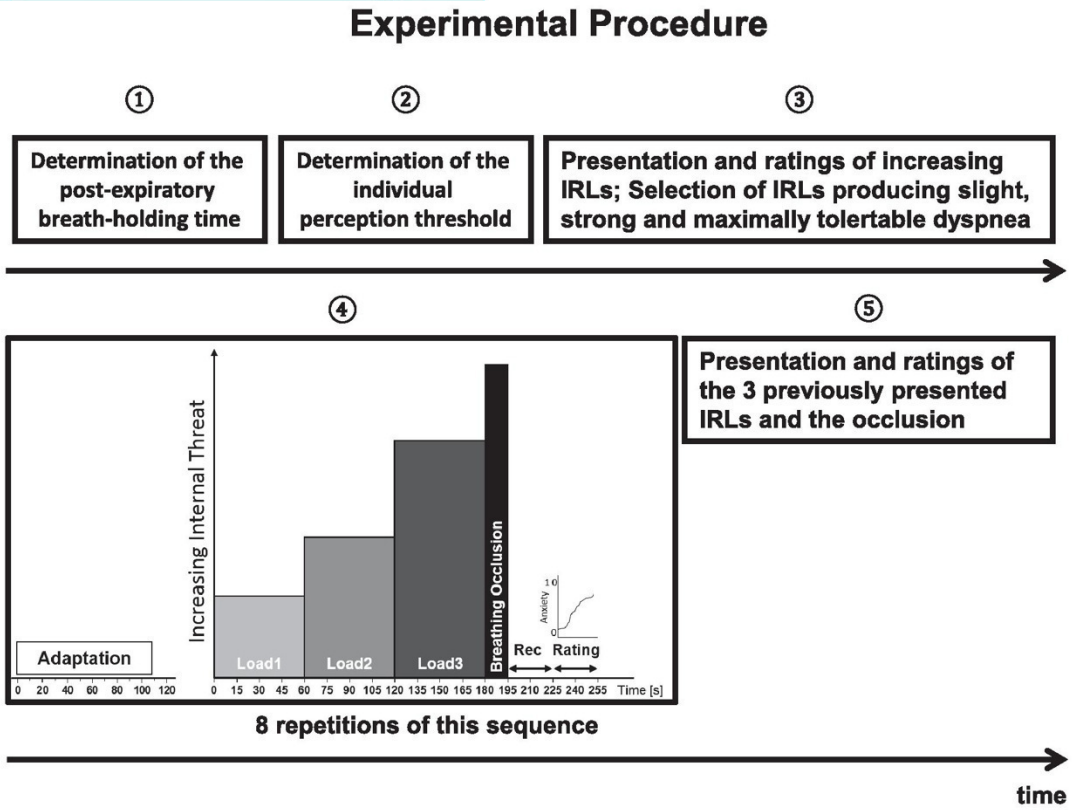

FIGURE 1 Experimental procedure of the present study. The experiment consisted of the determination of the breath-holding time (1) and the detection threshold of IRLs (2), the presentation and selection of increasing IRLs (3), the repeated presentation of increasing IRLs followed by occlusion (4), as well as the individual presentations and ratings of IRLs and occlusion (5)

adaptation phase of 110 s. Subsequently, the first trial started consisting of three loads of increasing intensity (previously rated as producing slight [Load1], strong [Load2], and maximally tolerable [Load3] unpleasant feelings of dyspnea) consecutively presented for 60 s each. Presentation of the third load was immediately followed by a postexpiratory breathing occlusion for 15 s and a 30-s recovery phase. Afterward, participants rated the intensity course of their anxiety during exposure by a line drawing on a touchpad. Overall, there were eight such trials in the experiment. Participants had the option to terminate the trial prematurely at any moment during the experiment by pressing a button (this button press led to a forward skip to the next recovery phase) or to terminate the entire experiment by calling the experimenter via intercom at any time.

5. Individual presentations and ratings of IRLs and occlusion. During a final rating phase, the three previously selected loads and the breathing occlusion were presented again separately for 30 s each, followed by a 30-s recovery phase and per-load/occlusion ratings of panic and respiratory symptoms were obtained.

At the end of the laboratory session, participants completed the questionnaires mentioned above and were fully debriefed by the experimenter.

2.5 | Data reduction and analysis

Fractional CO_2 was converted to partial pressure CO_2 (PCO_2) and visually inspected together with the mouth pressure signal using BrainVision Analyzer software (version 2.0, Brain Products GmbH, Gilching, Germany). For both parameters, artifacts such as coughing were manually deleted and treated as missing values. Then, maximal mouth pressure during inspiration (P_I) and end-tidal PCO_2 were automatically detected and exported in 5-s bins. The same artifact correction procedure was conducted with volume and timing-related respiratory parameters using ANSLAB version 2.4 (Autonomic Nervous System Laboratory, University of Basel, Switzerland). Then, the tidal volume (V_T), minute ventilation (V_E), inspiratory time (T_I), expiratory time (T_E), duty cycle (T_I/T_{TOT}), respiratory rate (f_R), and inspiratory flow rate (IFR) were calculated breath by breath and

TABLE 1 Means (standard deviations) of demographic characteristics and questionnaire measures for low and high SF participants who completed or prematurely terminated exposures

	Exposures completed			Exposures terminated		
	low SF ($n = 21$)	high SF ($n = 20$)	p value	low SF ($n = 10$)	high SF ($n = 18$)	p value
CLQ-SF [0–46]	2.2 (1.3)	16.05 (5.5)	$p < .001$	2.60 (1.4)	15.06 (4.5)	$p < .001$
ASI-3 [0–72]	13.7 (8.1)	23.55 (12.4)	$p < .01$	17.50 (12.4)	29.55 (7.1)	$p < .01$
BVS [0–40]	12.6 (6.0)	17.03 (7.4)	$p < .05$	17.17 (4.9)	16.647 (7.4)	$p = .699$
STAI-Trait [20–80]	38.3 (10.0)	41.90 (10.9)	$p = .276$	34.00 (7.0)	40.44 (6.4)	$p < .05$
Age	22.8 (2.3)	22.90 (3.7)	$p = .926$	20.5 (1.8)	23.60 (4.1)	$p < .05$
Sex (female/male)	18/3	18/2	$p = 1.000$	8/2	15/3	$p = 1.000$
Weight (kg)	65.3 (12.8)	63.1 (10.0)	$p = .528$	65.6 (12.8)	71.9 (12.1)	$p = .203$
Height (cm)	169.8 (7.9)	172.3 (8.9)	$p = .350$	173.5 (9.0)	172.6 (5.7)	$p = .737$
Body mass index	22.5 (3.1)	21.2 (2.6)	$p = .152$	21.6 (2.4)	24.3 (4.7)	$p = .110$

Note. Possible questionnaire score ranges are listed in brackets. CLQ = Claustrophobia Questionnaire; ASI = Anxiety Sensitivity Index; BVS = Body Vigilance Scale; STAI = State-Trait Anxiety Inventory.

exported in weighted 5-s means. All respiratory parameters were averaged across the 60-s presentation period of each load within each trial. Averages were also calculated for the adaptation and each recovery phase. The output of the anxiety rating curve was averaged per load as well as for the occlusion phase. Similarly, the intensity of panic symptoms excluding respiratory symptoms (shortness of breath, feeling of choking, and chest pain) and the intensity of respiratory symptoms were averaged for each of the three loads as well as for the occlusion period. If trials were terminated prematurely, ratings and respiratory responses were analyzed prior to the button press moving backward from the button press in 10-s bins for the 60 s preceding the button press.

The present data were analyzed using mixed regression models as this approach incorporates the special structure of the data (trials nested within participants) and allows a flexible and powerful analysis of the repeated measures data with missing values (Blackwell, Leon, & Miller, 2006; Tabachnick & Fidell, 2007; West, 2009). For all statistical analyses, group (low SF vs. high SF) was entered as a between-subjects factor. To test for between-group baseline differences in ventilation during the adaptation phase and the perception threshold of IRLs, a model with the between-subjects factor group was run. To examine the effect of increasing intensity of IRLs on our dependent variables, mixed regression models were applied with the repeated measures factor load (1st vs. 2nd vs. 3rd, respectively, 1st vs. 2nd vs. 3rd vs. occlusion for symptoms). For participants who completed all trials, respiratory responses as well as anxiety ratings were analyzed using mixed regression models including the repeated measures factors trial (1st through 8th trial) and load (1st vs. 2nd vs. 3rd, respectively, 1st vs. 2nd vs. 3rd vs. occlusion for anxiety ratings) and their interactions.[1] Respiratory responses and anxiety ratings prior to premature terminations were analyzed using mixed models comprised of termination (1st through 8th termination) and time (seven 10-s blocks [-60 s to 0 s]) as repeated measures factors as well as their interactions.[2] The random part of the models included a person-specific intercept and repeated measures effects for trial and load (respectively, termination and time in analyses of premature terminations) with a first-order autoregressive covariance structure (homogeneous variances and correlations that decline with time). To test whether there are differences in respiratory and subjective responses between completers and persons who prematurely terminated exposure, the between-subjects factor subgroup (persons who completed exposure vs. persons who terminated

[1]No respiratory parameters could be analyzed during occlusion as there was no breathing during this phase.

[2]One participant was excluded from analyses due to technical problems with the pneumotachography system. Analyses of respiratory responses were run separately for trials with terminations during loaded breathing and trials with terminations during breathing occlusion. As there were no respiration data during breathing occlusion, terminations during occlusion could not be analyzed relative to the button press. Hence, if trials were terminated during the breathing occlusion, the last 60 s during loaded breathing were used to explore changes in respiratory responses. Thus, analyses concerning trials with terminations during occlusion included 20 participants. Analyses of terminations during loaded breathing were based on 24 participants.

exposure) was included for exploratory analyses. For exploratory analyses of differences in subjective and respiratory responses between completed and terminated exposure trials, responses prior to terminations were compared with responses observed during a matched sequence (from the same participant) which was not terminated. The statistical analyses included the repeated measures factors time (seven 10-s blocks [-60 s to 0 s]) and match (termination trials vs. nontermination trials) as well as their interactions. All overall statistical tests used a significance level of $p < .05$. All data were processed using SPSS 22.0 (SPSS for Windows, IBM).

3 | RESULTS

3.1 | Behavioral and questionnaire data

Forty-one (21 low SF and 20 high SF) participants completed all sequences of increasing restricted breathing (59.4%). Twenty-eight (10 low SF and 18 high SF) participants of the entire sample (40.6%) prematurely terminated at least one increasing loads-occlusion trial. Overall, 165 (69 trials in low SF and 96 trials in high SF persons) sequences of increasingly restricted breathing were prematurely terminated either during slight (1.2%), strong (12.7%), maximally tolerable (42.2%) loads, or the breathing occlusion (43.6%).

As indicated in Table 1, high compared to low SF persons who completed exposures reported greater anxiety sensitivity and increased vigilance to body sensations. High and low SF completers did not differ in sex, body weight, height, body mass index, trait anxiety, or age. In participants who prematurely terminated exposures, those with high SF scores were older and reported increased anxiety sensitivity and higher trait anxiety. Low and high SF participants who prematurely terminated exposures did not differ in sex, body weight, height, body mass index, and in the attention focused on bodily sensations. Overall, completers and persons who prematurely terminated exposures did not differ in their suffocation fear, trait anxiety, vigilance to body sensations, body weight, height, body mass index, age, $ts(67) < 1.89$, $ps > .064$, and sex, $\chi^2(1, N = 69) = .430$, $p = .512$. However, persons who terminated exposures reported greater anxiety sensitivity compared to completers, $t(67) = 2.46$, $p = .017$.

3.2 | Effects of increasing dyspnea in high and low SF completers

3.2.1 | Adaptation phase

High and low SF participants who completed all trials did not differ significantly in baseline levels of f_R, V_T, IFR, and P_I, $Fs(1, 39) < 2.14$, $ps > .151$. High SF individuals, however, had a lower resting end-tidal PCO_2 level, $F(1, 39) = 7.59$, $p = .009$, than low SF persons.

3.2.2 | Manipulation check for the induction of increasing dyspnea

As depicted in Figure 2a, the physical intensity of the perception threshold did not differ between high and low SF groups, $F(1, 39) = 1.57$, $p = .217$. As expected, the reported unpleasantness of dyspnea increased significantly with the physical intensity of IRL, $F(2, 74.54) = 59.75$, $p < .001$. There were no differences in physical intensity of the chosen loads and reported unpleasantness between high and low SF individuals, Load × Group, $F(2, 74.54) < 1$, $p = .58$; group,

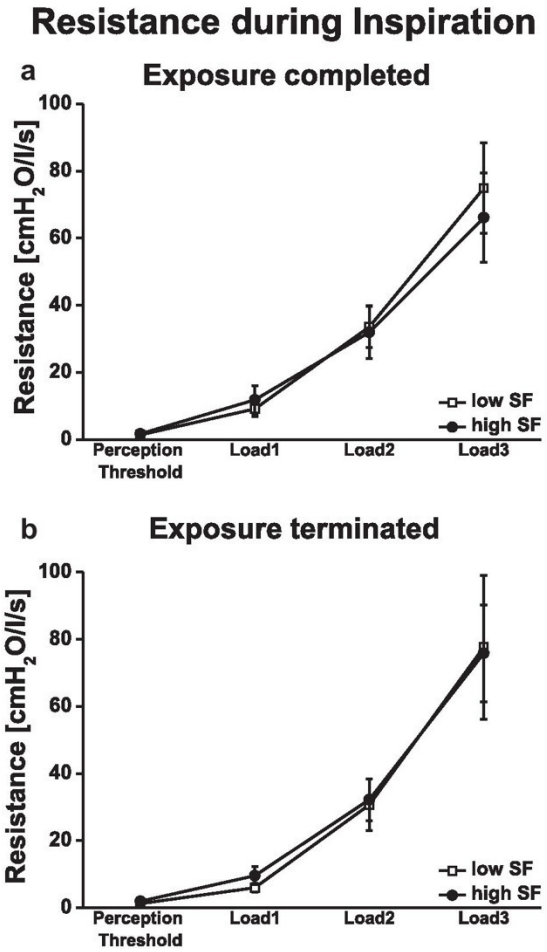

FIGURE 2 Manipulation check for the induction of increasing dyspnea in persons who completed all exposures (a) and in those who prematurely terminated exposure at least once (b). Mean physical resistance of the perception threshold and inspiratory resistive loads producing slight (Load1), strong (Load2), and maximally tolerable (Load3) unpleasant feelings of dyspnea in persons with low and high suffocation fear. Error bars represent standard errors of means

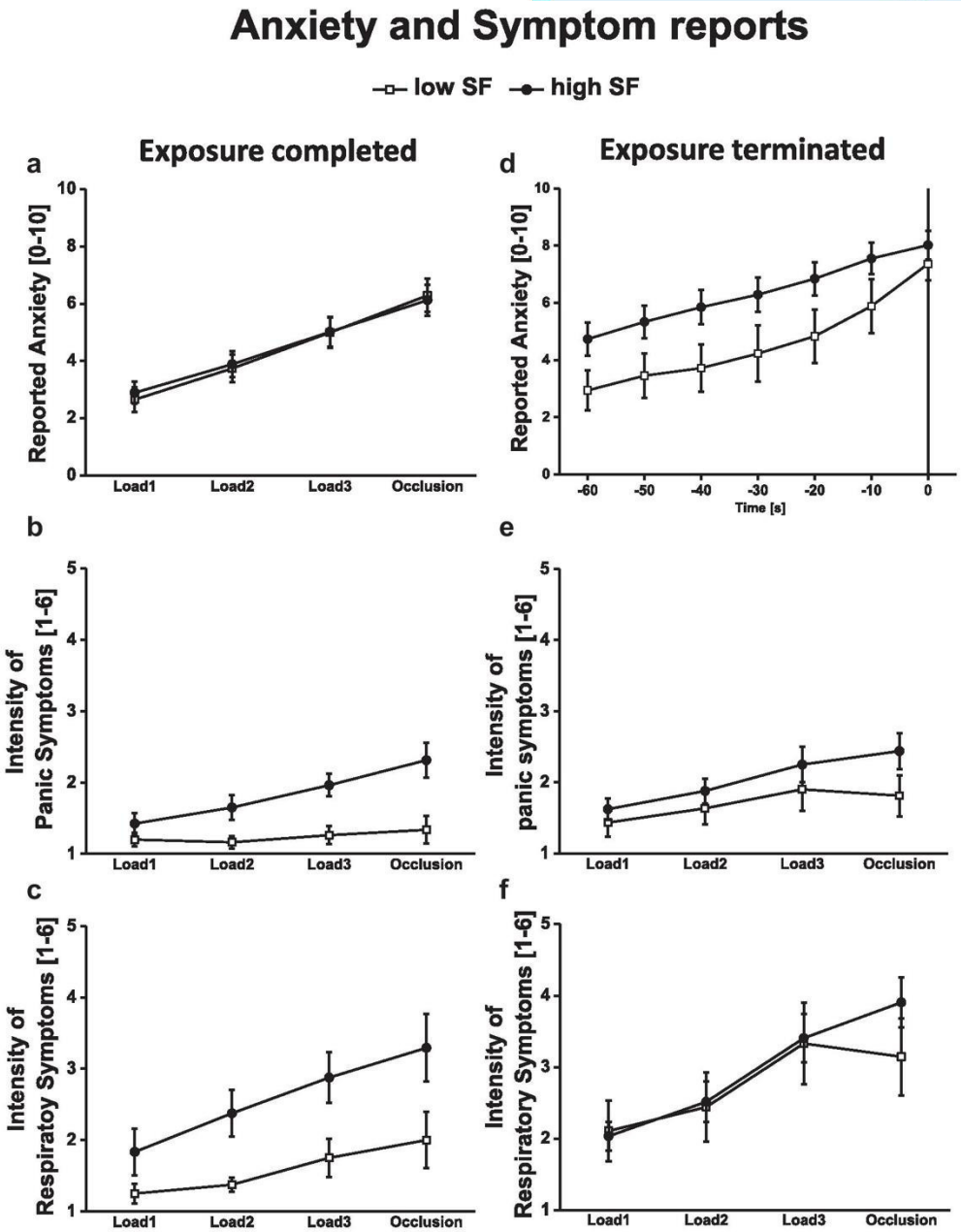

FIGURE 3 Anxiety and symptom reports for participants who completed (left) or prematurely terminated exposures (right). Means and standard errors of the reported anxiety (a), the intensity of panic (b, e), and respiratory symptoms (c, f) comparing low and high SF persons while breathing through a slight, strong, and maximally tolerable load as well as during breathing occlusion. Means and standard errors of the reported anxiety during the 60 s prior to button presses (d)

$F(1, 37.51) < 1$, $p = .835$. The inspiratory flow rate (V_T/T_I) decreased during loaded breathing, $F(2, 293.94) = 155.25$, $p < .001$, but did not differ between low and high SF persons, for all loads $Fs < 1.85$, $ps > .180$, suggesting that the stimulus intensity of IRLs was comparable between both groups.

3.2.3 | Anxiety ratings

As depicted in Figure 3a, both groups reported a gradual increase in anxiety intensity starting from Load1 up to the occlusion, $F(3, 154.28) = 82.63$, $p < .001$; Load × Group, $F(3, 154.28) < 1$, $p = .792$. There was no significant difference in the anxiety ratings

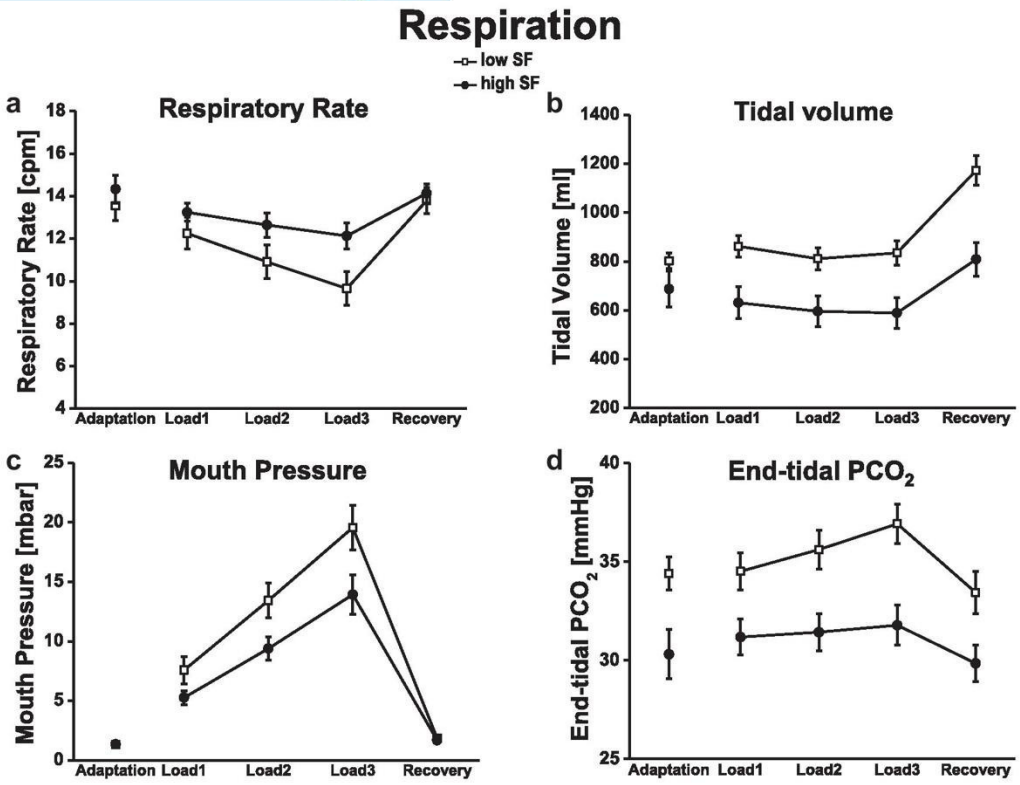

FIGURE 4 Respiratory response to increasing dyspnea in persons with low and high suffocation fear. Means and standard errors of respiratory rate (a), tidal volume (b), mouth pressure (c), and end-tidal PCO_2 (d) during the adaptation phase, increasing unpleasant feelings of dyspnea, and the recovery phase

between high and low SF individuals, $F(1, 40.81) < 1$, $p = .985$. Interestingly, the reported anxiety increased with increasing number of repetitions of exposure, $F(7, 214.69) = 5.40$, $p < .001$; Trial × Group, $F(7, 214.69) < 1$, $p = .903$; Trial × Load × Group, $F(21, 542.59) = 1.27$, $p = .188$.

3.2.4 | Respiratory rate

Low SF participants showed continuous decrease in f_R during increasingly loaded breathing, $F(2, 215.62) = 30.66$, $p < .001$. In contrast, persons with high SF did not show this reduction of their breathing frequency, Load × Group, $F(2, 215.32) = 4.88$, $p = .008$; Load × Trial × Group, $F(14, 459.45) < 1$, $p = .681$ (see Figure 4a). The group difference was found to be particularly pronounced while breathing through strong and maximally tolerable loads, Load2: group $F(1, 45.90) = 2.95$, $p = .093$; Load3: group $F(1, 46.09) = 6.23$, $p = .016$. SF scores positively correlated with breathing frequency in high SF individuals during Load2 and 3, $r_{Spearman} = .320–.368$, $ps < .049$ (two-tailed), respectively.

3.2.5 | Tidal volume

Participants with high as compared to low SF showed an overall reduced tidal volume during loaded breathing, $F(1, 41.80) = 8.42$, $p = .006$; Trial × Group, $F(7, 354.91) < 1$, $p = .638$ (see Figure 4b). Moreover, low and high SF completers comparably decreased their tidal volume during increasingly loaded breathing, $F(2, 216.45) = 4.22$, $p = .016$; Load × Group, $F(2, 216.45) < 1$, $p = .546$.

3.2.6 | Mouth pressure

In accordance with the applied physical resistance, the mouth pressure increased from Load1 to Load2 and 3, $F(2, 261.36) = 182.51$, $p < .001$. Although the low and high SF persons did not differ during adaptation phase (see above), the increase of mouth pressure during Load1, 2, and 3 was steeper in persons with low compared to high SF (see Figure 4c), Load × Group, $F(2, 261.36) = 4.72$, $p = .010$; Load × Trial × Group, $F(14, 429.90) = 1.06$, $p = .393$.

3.2.7 | End-tidal PCO_2

Supporting the findings from the adaptation phase, participants with high SF had a generally lower end-tidal PCO_2 during loaded breathing in comparison to persons with low SF (see Figure 4d), $F(1, 36.90) = 7.52$, $p = .010$. Low SF participants showed a small (2 mmHg) but significant rise in end-tidal PCO_2 during increasingly loaded breathing, $F(2,$

221.19) = 50.24, $p < .001$. In high SF individuals, PCO_2 did not change significantly, $F(2, 221.19) = 3.02$, $p = .051$; Load × Group, $F(2, 221.19) = 13.77$, $p < .001$; Load × Trial × Group, $F(14, 433.99) = 1.56$, $p = .230$.

Analyses of additional respiratory parameters are summarized in Table S1 (see online supporting information).

3.2.8 | Symptom ratings

The intensity of reported panic symptoms increased continuously from Load1 to the occlusion, $F(3, 112.70) = 13.78$, $p < .001$. Higher SF scores were associated with greater intensity and number of reported panic symptoms for periods of breathing through strong and maximally tolerable loads as well as during occlusion: $r_{Spearman} = .429–.494$, $ps < .006$ (two-tailed). Accordingly, when the analysis was limited to those participants with SF scores 1 *SD* above or below the mean ($M = 10.46 \pm SD = 8.64$, $n = 8$ per SF), only participants with high SF showed an increase in the intensity of panic symptoms, $F(3, 38.37) = 7.93$, $p < .001$. In contrast, low SF individuals reported similar panic symptom severity across all three loads and during the occlusion (see Figure 3b), $F(3, 38.37) < 1$, $p = .750$; Load × Group, $F(3, 38.37) = 2.78$, $p = .054$. Reported respiratory symptoms increased from Load1 to occlusion, $F(3, 112.84) = 24.54$, $p < .001$. Again, SF was positively correlated with the reported intensity of respiratory symptoms for strong and maximally tolerable loads and occlusion: $r_{Spearman} = .282–.328$, $ps < .075$ (two-tailed). As indicated for panic symptom severity, again, individuals scoring 1 *SD* above the mean on the SF scale reported significantly more intense respiratory symptoms compared to low SF individuals (see Figure 3c), $F(1, 13.80) = 6.64$, $p = .022$.

3.3 | Exploratory analyses of differences between completers and persons who prematurely terminated exposure

There were no differences in baseline levels between persons who completed exposures and those who prematurely terminated exposures, $Fs < 3.45$, $ps > .067$. Comparisons between completers and participants who prematurely terminated exposures revealed no significant differences in the physical intensity of the perception threshold, $F(1, 66) < 1$, $p = .661$, and the IRLs, $Fs < 1$, $ps > .605$. Persons with premature terminations showed a comparable pattern of respiratory responses and anxiety ratings during completed exposure trials as observed in low and high completers.[3] Moreover, persons who prematurely terminated exposure reported an overall higher number and intensity of panic symptoms as well as a higher intensity of respiratory symptoms compared to completers, $Fs > 7.52$, $ps < .009$.

3.4 | Premature termination of increasing dyspnea

3.4.1 | Adaptation phase

High and low SF participants who prematurely terminated exposures did not differ significantly in baseline levels of V_T, IFR, end-tidal PCO_2, and P_I, $Fs(1, 25) < 2.48$, $ps > .128$. However, high compared to low SF individuals who prematurely terminated exposures had a slightly higher breathing frequency, $F(1, 25) = 4.43$, $p = .046$.

3.4.2 | Manipulation check for the induction of increasing dyspnea

In persons who terminated the exposure of increasingly restricted breathing at least once, the physical intensity of the perception threshold did not differ between those who reported high and low SF, $F(1, 25) = 1.31$, $p = .263$. The physical intensity of IRL significantly increased while the reported unpleasantness of dyspnea of the selected loads increased from slight to strong to maximally tolerable, $F(2, 58.78) = 38.41$, $p < .001$. There were no differences in physical intensity between participants who reported low or high SF, Load × Group, $F(2, 58.78) < 1$, $p = .943$; group, $F(1, 27.29) < 1$, $p = .929$. The inspiratory flow rate during loaded breathing decreased in both SF groups, load $F(2, 53.88) = 18.60$, $p < .001$; group, $F(1, 10.33) = 1$, $p = .384$; Load × Group, $F(2, 54.64) < 1$, $p = .867$.

3.4.3 | Anxiety ratings

Participants reported a continuous increase in anxiety prior to terminations, $F(6, 146.71) = 40.79$, $p < .001$. As depicted in Figure 3d, high compared to low SF persons reported higher anxiety levels at the beginning of the sequence (i.e., 60 s prior to the termination) but not immediately before the button press, Time × Group, $F(6, 146.71) = 2.21$, $p = .045$; group, $F(1, 29.11) = 2.09$, $p = .159$. Interestingly, overall anxiety ratings rose with increasing number of terminations, $F(7, 179.60) = 3.48$, $p = .002$.

3.4.4 | Respiratory rate

The 60 s prior to premature terminations were characterized by a surge in respiratory rate (see Figure 5a), $F(6, 149.31) = 2.80$, $p = .013$, that did not differ between both SF groups,

[3]In participants who prematurely terminated exposures at least once, 3 low and 10 high SF individuals completed at least one sequence of exposure.

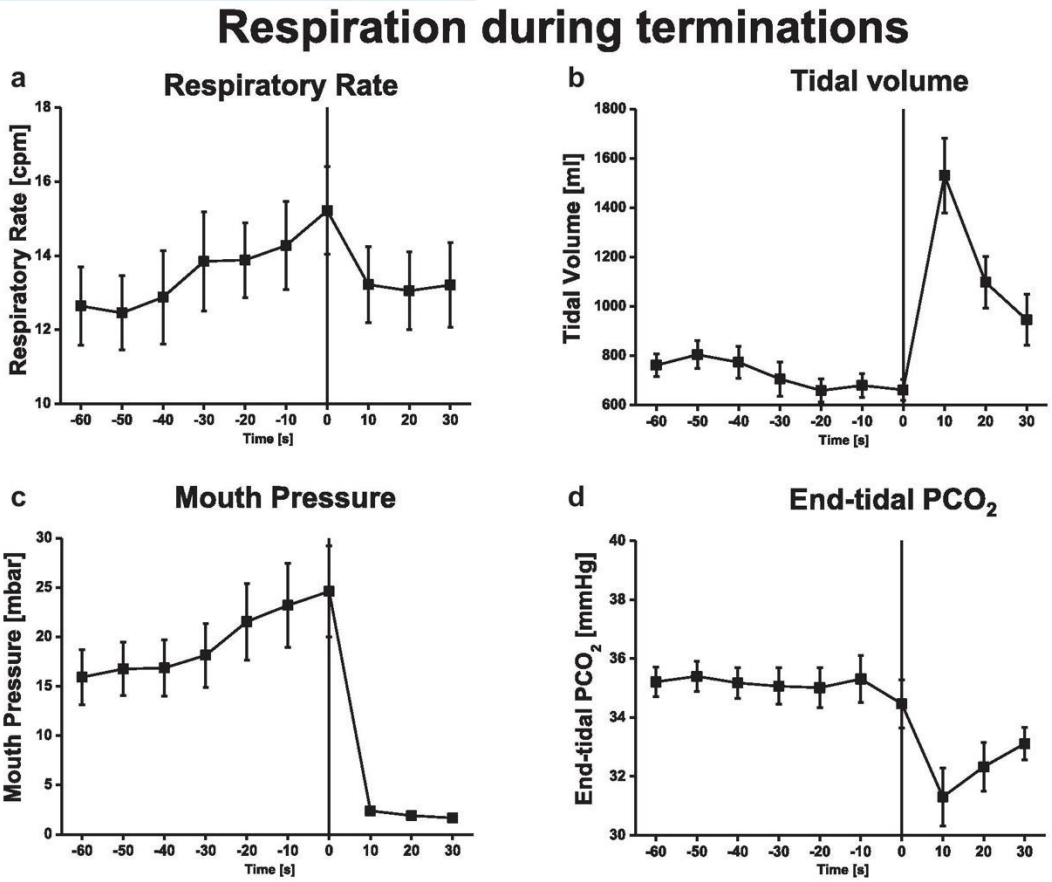

FIGURE 5 Respiratory responses prior to premature terminations of increasingly restricted breathing. Means and standard errors of respiratory rate (a), tidal volume (b), mouth pressure (c), and end-tidal PCO_2 (d) 60 s before, during, and 30 s after button presses

Time × Group, $F(6, 149.31) < 1$, $p = .523$; Time × Terminations × Group, $F(42, 249.44) = 1.18$, $p = .216$. The breathing frequency did not differ between both SF groups, $F(1, 20.85) = 1.67$, $p = .210$. The increase in respiratory rate was already present during loaded breathing although exposure was terminated only during occlusion, $F(6, 148.37) = 3.50$, $p = .003$; Time × Group, $F(6, 149.31) < 1$, $p = .523$.

3.4.5 | Tidal volume

In low and high SF participants, tidal volume decreased during the last 60 s before premature terminations (see Figure 5b), $F(6, 134.49) = 2.37$, $p = .033$; Time × Group, $F(6, 134.49) < 1$, $p = .523$. There were no overall level differences in tidal volume between low and high SF persons, $F(1, 134.49) = 1.17$, $p = .322$. When trials were terminated during occlusion, the decrease in tidal volume was already exhibited during the last stage of loaded breathing, $F(6, 139.33) = 2.90$, $p = .011$; Time × Group, $F(6, 139.33) = 1.02$, $p = .416$.

3.4.6 | Mouth pressure

As depicted in Figure 5c, there was an increase in mouth pressure 60 s prior to terminations, $F(6, 149.28) = 5.30$, $p < .001$. There were no overall level differences in mouth pressure between low and high SF participants, $F(1, 17.09) < 1$, $p = .774$; Time × Group, $F(6, 149.28) < 1$, $p = .667$. The increase in mouth pressure was already present during loaded breathing when exposure was terminated only during occlusion, $F(6, 149.02) = 12.34$, $p < .001$; Time × Group, $F(6, 146.24) < 1$, $p = .532$.

3.4.7 | End-tidal PCO_2

Both SF groups did not change their end-tidal PCO_2 prior to terminations (see Figure 5d), $F(6, 126.58) < 1$, $p = .472$; Time × Group, $F(6, 126.58) = 1.17$, $p = .328$. Low and high SF persons did not differ in the level of end-tidal PCO_2, $F(1, 19.04) < 1$, $p = .881$. Similarly, end-tidal PCO_2 did not change during loaded breathing in trials that were terminated

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during breathing occlusion, $F(6, 67.86) = 1.41$, $p = .223$; Time × Group, $F(6, 65.64) = 1.04$, $p = .407$.

Analyses of additional respiratory parameters are summarized in Table S2–S4 in the supporting information.

3.5 | Symptom ratings in persons who prematurely terminated exposure

The intensity of reported panic symptoms gradually increased from Load1 to occlusion (see Figure 3e), $F(3, 74.68) = 8.18$, $p < .001$. Higher SF scores were associated with greater intensity and number of reported panic symptoms for all loads and the breathing occlusion: $r_{Spearman} = .485–.665$, $ps < .011$ (two-tailed).

Reported respiratory symptoms increased from Load1 to occlusion (see Figure 3f), $F(3, 74.38) = 13.04$, $p < .001$. Again, SF was positively correlated with the reported intensity of respiratory symptoms for strong and maximally tolerable loads and the occlusion: $r_{Spearman} = .379–.591$, $ps < .052$ (two-tailed).

3.6 | Exploratory analyses of differences in subjective and respiratory responses between termination trials and matched nontermination trials

3.6.1 | Anxiety ratings

Levels of reported anxiety did not differ between termination trials and matched trials that were not terminated (see Figure S1), match, $F(1, 104.44) = 2.55$, $p = .114$; Match × Time, $F(6, 72.93) = 1.13$, $p = .356$.

3.6.2 | Respiratory rate

The respiratory rate was higher during termination trials compared to matched nontermination trials (see Figure S2), match, $F(1, 17.13) = 7.92$, $p = .012$, and match, $F(1, 24.13) = 12.10$, $p = .002$ for analyses of terminated occlusion trials and terminated load trials, respectively.

3.6.3 | Tidal volume

Tidal volume was overall decreased during termination trials as compared to during matched nontermination trials when exposure was terminated during occlusion, $F(1, 22.25) = 6.59$, $p = .018$, but not when terminated during loaded breathing (see Figure S3), $F(1, 34.00) = 1.95$, $p = .172$.

3.6.4 | Mouth pressure

As depicted in Figure S4, there were no differences in mouth pressure when comparing termination trials with matched nontermination trials, match, $F(1, 26.16) = 2.15$, $p = .154$, and match, $F(1, 113.56) < 1$, $p = .473$, for analyses of terminated occlusion trials and terminated load trials, respectively.

3.6.5 | End-tidal PCO_2

There were no differences in PCO_2 when comparing termination trials with matched nontermination trials (see Figure S5), match, $F(1, 21.79) = 1.13$, $p = .261$, and match, $F(1, 31.55) < 1$, $p = .533$, for analyses of terminated occlusion trials and terminated load trials, respectively.

4 | DISCUSSION

The current study presented an experimental design for exploring dynamic changes of respiratory response pattern during the culmination of dyspnea. In addition, the study was targeted at exploring how suffocation fear modulates the dynamics of this respiratory response pattern. Exposure to successively increasing inspiratory loads was associated with increasingly unpleasant feelings of dyspnea and comparable flow rates in all groups (low and high SF completers and persons who terminated exposure), suggesting that the experimental manipulation was equally aversive for all participants. Moreover, the perception thresholds for feelings of dyspnea did not differ between groups. Individuals with high fear of suffocation exhibited higher respiratory rates that evolved with increasing severity of dyspnea. Increased respiratory rates were associated with reports of more intense panic symptoms even when respiratory symptoms were excluded. In contrast, persons with low SF showed a compensatory decrease in breathing frequency with increasing load intensity and, accordingly, reported less intense panic symptoms than the high SF group. We demonstrated that premature terminations of exposure to increasing loads or occlusions were preceded by an increase in respiratory rate and a decrease in tidal volume in high as well as low SF persons. This response pattern was not found for nontermination trials that were matched in time to the analysis periods of the termination trials.

4.1 | Characterization of defensive mobilization to increasing dyspnea in relation to SF

Previous studies have demonstrated that high SF participants show an increase in respiratory rate or ventilation during exposure to loaded breathing (Alius et al., 2013; Pappens,

Smets, Van den Bergh, & Van Diest, 2012). The present study replicated and extended these findings by demonstrating the dynamics of this pattern in accordance with increasing feeling of dyspnea. A higher breathing frequency in high SF participants only became apparent when dyspnea became more severe and the threat more acute, that is, when the perceived risk of suffocation became more imminent. In accordance with evidence showing excessive breathing (e.g., increases in respiratory rate) during imagination of suffocation as well as anticipatory anxiety and provocation of feared bodily sensations (Alius et al., 2013; Masaoka & Homma, 1997, 2001; Melzig et al., 2011; Pané-Farré et al., 2015; Pappens, Smets, Van den Bergh & Van Diest, 2012; Van Diest et al., 2001, 2005), the present finding suggests that dyspnea predicting impending risk of suffocation elicits a defensive respiratory pattern in high SF persons that is indicated by increased respiratory rates. These findings are in line with assumptions derived from the modern learning theories of PD (Bouton et al., 2001; Hamm et al., 2014) that predict that internal body sensations might become sufficient to trigger respiration changes that might spiral into defensive action. In fact, active defense behavior was observed in several participants. In line with these data, it has been demonstrated that the level of SF in a group of panic disorder patients predicted the frequency of avoidance and escape behavior as well as increased panic symptom severity and anxiety during a situation of entrapment (Hamm et al., 2016; Richter et al., 2012).

A higher breathing frequency during obstructed breathing has been suggested to worsen the feelings of dyspnea and discomfort due to increased exposure frequency to the resistive load (Alius et al., 2013; Kikuchi et al., 1992; Pappens, Smets, Van den Bergh, & Van Diest, 2012). This enhanced afferent input from peripheral respiratory receptors (e.g., from chest wall, airways, and lung mechanoreceptors or vagal stretch receptors) is processed in the insula, limbic system, and sensorimotor cortex, inducing dyspnea and generating new motor output (Gigliotti, 2010; Harver & Mahler, 1998; Parshall et al., 2012; von Leupoldt et al., 2009). The increased afferent information and the efferent motor output exceeds the expected and demanded ventilatory response to achieve or maintain adequate levels of ventilation and gas exchange, a process that may also contribute to the experience of dyspnea (Burki, 2010; Gigliotti, 2010; Nishino, 2011; Parshall et al., 2012). Interestingly, increased breathing also leads to airways cooling and drying that may amplify the dyspnea and may set the stage for the culmination of symptoms (Meuret & Ritz, 2010). In the present study, the observed breathing pattern was accompanied by a greater intensity of panic symptoms. However, it is important to note that more severe dyspnea or panic symptoms may have led to increases in respiratory rate.

During loaded breathing, low compared to high SF persons showed an increased tidal volume that was obtained through higher mouth pressure (Harver & Mahler, 1998). In contrast, persons who fear suffocation exhibited a reduced mouth pressure associated with a decreased volume per breath (Harver & Mahler, 1998; Shipherd, Beck, & Ohtake, 2001). This suggests that high SF persons may have decreased their workload of the respiratory muscles to avoid the unpleasant respiratory sensations associated with a higher muscle tension (Ritz, Meuret, Bhaskara, & Petersen, 2013). Interestingly, this pattern, which may be interpreted as being indicative of a behavioral avoidance strategy, did not alleviate the intensity of bodily sensations. Persons with high SF even reported more intense respiratory and panic symptoms during increasing dyspnea as compared to low SF persons, supporting the role of increased breathing in the escalation of symptoms (Alius et al., 2013).

4.2 | Characterization of active defense behavior in low and high SF persons

As already described, anxiety and dyspnea may culminate in defensive action, such as escape or active avoidance behavior. The examination of spontaneous panic attacks or escapes from a situation of entrapment in patients with PD revealed that, at the peak of increasing threat, defensive response patterns are characterized by a strong mobilization of autonomic and respiratory responses (Meuret et al., 2011; Richter et al., 2012). In the present study, respiratory response mobilization during initiation of defensive action was assessed in an experimentally controlled setting. Strikingly, in the present study, terminations were characterized by a strong mobilization of respiratory responses as indexed by an increase in respiratory rate. This respiratory pattern prior to defensive action might indicate that increasing activation of the brain's defense circuit prompted increasing mobilization of responses in preparation of effective action (Fanselow, 1994).

Interestingly, there were no differences in respiratory responses just prior to terminations between low and high SF persons. Thus, these findings suggest that the flight-related adjustment of respiration pattern might be an innately determined defensive behavior evolutionarily relevant to protect the organism against life-threatening conditions (e.g., a possible suffocation; Bolles, 1970; Fanselow, 1994). That is, increasing dyspnea signaling increasing risk of suffocation elicits exaggerated defensive activation and might trigger a compensatory adjustment of respiration (i.e., respiratory rate) to prevent possible suffocation. However, the surge in respiratory mobilization (i.e., the increase in respiratory rate) might under specific conditions be disadvantageous as it possibly leads to a marked worsening of the feelings of dyspnea and discomfort. In fact, as found in our study, the elicited respiratory pattern may increase the severity of symptoms or

vice versa, which in turn leads to greater defensive activation spiraling into defensive action. Interestingly, it has been suggested that mild respiratory perturbations may lead to initiation of compensatory overbreathing, which in turn elicits bodily symptoms leading to increased defensive activation (Bouton et al., 2001; Klein, 1993; Ley, 1996; Melzig et al., 2011; Meuret, Ritz, Wilhelm, & Roth, 2005; Wollburg, Roth, & Kim, 2011). According to the suffocation false alarm theory proposed by Klein (1993), it may be assumed that the perceived risk of suffocation during increasingly severe dyspnea triggers an oversensitive suffocation alarm monitor eliciting panic attacks or active flight from dyspnea and initiating an increase in respiration to prevent suffocation. This interpretation is in agreement with the learning theory of PD in that respiratory sensations indicating suffocation (e.g., dyspnea) might act as elicitors of anxious apprehension and prompt changes of respiration that spiral into panic attacks or defensive action (Bouton et al., 2001).

The present findings of dynamic adjustments in respiratory responses to increasing intensity of interoceptive threat cues (increasing dyspnea) are also in line with animal as well as human data showing dynamic changes in defensive response adjustments depending upon the proximity of an approaching external threat (e.g., a predator or a painful event; Fanselow, 1994; Fanselow & Lester, 1988; Kozlowska, Walker, McLean, & Carrive, 2015; Lang, Bradley, & Cuthbert, 1997; Löw, Lang, Smith, & Bradley, 2008). For such approaching external threats, a translational model—the threat imminence model (or defense cascade model; Blanchard & Blanchard, 1989; Fanselow, 1994; Hamm et al., 2014, 2016)—has been proposed describing the dynamic changes of defensive behavior in relation to the proximity of external threat. In case of unpleasant interoceptive cues, proximity might not be the crucial dimension since the origin of the threat is already inside the body. The current data suggest that increase of intensity of felt bodily symptoms might shape defensive response mobilization in a comparable dynamic way (Hamm et al., 2014, 2016). In the current study, we only looked at the dynamic changes in respiration and defensive action as a function of threat intensity. Future research should also measure other indices of defense circuit activation using this paradigm and consider the conceptual confounding of threat proximity and threat intensity.

Interestingly, in a recent analysis of repeated premature terminations of exposure to increasing dyspnea, we found that the first premature termination of exposure was preceded by a strong surge of autonomic arousal and an inhibition of the startle reflex (Benke, Krause, Hamm, & Pané-Farré, 2017). However, autonomic, reflex, and brain responses changed as behavioral response pattern switched from terminating to preventing (i.e., from escaping to avoiding) a breathing occlusion during repetitive terminations. In contrast to existing evidence (Benke et al., 2017; Delgado, Jou, LeDoux, & Phelps, 2009; Lovibond, Saunders, Weidemann, & Mitchell, 2008; Vervliet & Indekeu, 2015), we did not observe changes in defensive mobilization of respiratory responses prior to repetitions of terminations. Thus, mobilization of respiration prior to defensive action is prompted irrespective of the learning history as the adjustment of respiration might be a substantial defensive behavior to prevent a possible suffocation.

Importantly, while respiratory rate increased prior to terminations, reported anxiety increased before terminations, but this increase in anxiety was also observed in nontermination trials. Such a discordance between verbal reports and physiological or behavioral indices of anxiety has frequently been observed (see Cook, Melamed, Cuthbert, McNeil, & Lang, 1988; Rachman & Hodgson, 1974). Possibly, subjective reports of anxiety are more related to the negative appraisal or misinterpretation of body sensations than to the actual physiological respiratory symptoms. From the viewpoint of a two-system framework of anxiety, it has been proposed that defensive response mobilization and subjective feelings of anxiety in the presence of threat are mediated by two different neural circuits (LeDoux & Pine, 2016). Thus, defensive response mobilization and subjective feelings of anxiety may diverge in the face of threat (LeDoux & Pine, 2016).

4.3 | Limitations of the current study

The present study aimed at exploring how the dynamics of human defense behavior to increasingly intense interoceptive threat is altered in anxious individuals (high SF persons). So far, research on interoceptive threat typically focused on persons who fear anxiety-related symptoms (high anxiety sensitive persons) as there is evidence that anxiety sensitivity is associated with increased defensive mobilization to bodily sensations. There is scarce evidence regarding the role of SF in anxious responding to interoceptive threat. However, the current results encourage a further evaluation of effects of SF on the dynamics of defensive response mobilization to interoceptive threat. It needs to be noted that the sample is predominantly composed of female undergraduates limiting the generalization of the result. However, higher rates of females are typically observed in anxious populations, for instance, in those who report high SF (Alius et al., 2013; Kroeze et al., 2005). Moreover, the repeated and extended exposure to inspiratory loads might have led to respiratory muscle fatigue, which may have affected the way participants coped with the resistive loads. Although the observed breathing pattern persisted until the end of the experiment, future studies should assess respiratory muscle fatigue to exclude possible effects of fatigue on respiratory responses.

4.4 | Conclusion and future directions

In the current study, we established and validated an experimental paradigm exploring the dynamic of defensive responses during increasing intensity of interoceptive threat. The presented data show how defensive responding changes with increasing intensity of an interoceptive threat. We demonstrated that persons who fear suffocation responded with enhanced breathing frequency when feelings of dyspnea increase and predict an upcoming occlusion. This breathing pattern was associated with increasingly unpleasant respiratory symptoms, suggesting that increased breathing frequency augments dyspnea and thereby increases the intensity of perceived and reported panic symptoms. We also observed active defense behavior that was preceded by a strong defensive mobilization of respiratory responses prior to defensive action. As the current data are also in line with assumptions that can be derived from the interoceptive learning account of PD, it needs to be explored whether fear of suffocation might be a risk factor that modulates learning processes and therefore facilitates the development of PD. Future clinical studies using a longitudinal design and assessing a broader range of defensive responses and brain activation should more precisely examine the role of suffocation fear in the development of PD.

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SUPPORTING INFORMATION

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Dynamics of defensive response mobilization during repeated terminations of exposure to increasing interoceptive threat

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Dynamics of defensive response mobilization during repeated terminations of exposure to increasing interoceptive threat

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ABSTRACT

Resistant avoidance behaviors play a crucial role in the maintenance of anxiety disorders and are therefore central targets of therapeutic interventions. In the present study, the development of avoidance behavior was investigated in 24 healthy participants who repeatedly prematurely terminated the exposure to increasing interoceptive threat, i.e., the feeling of dyspnea induced by increasing inspiratory resistive loads that were followed by the ultimate threat, a short breathing occlusion. Physiological responses and subjective anxiety preceding terminations were compared to matched intervals of a matched control group ($N = 24$) who completed the exposure. Initially, participants terminated during the ultimate threat, i.e., during occlusion. This first termination was preceded by a strong surge in autonomic arousal and reported anxiety. Startle reflex and the P3 component of event-related brain potentials to startle probes were strongly inhibited, indicating preparation for defensive action. With repetitive terminations, individuals successively terminated earlier, avoiding exposure to the occlusion. This avoidant behavior was accompanied by alleviated autonomic arousal as compared to the first termination. In addition, no indication of physiological response preparation was found implying that the avoidance behavior was performed in a rather habitual way. Matched controls did not show any indication of a defensive response surge in the matched intervals. In matched controls, no changes in physiological response patterns were detected while anxiety levels increased with repetitions. The present results shed new light on our understanding of the motivational basis of avoidance behavior and may help to refine etiological models, behavioral analysis and therapeutic strategies in treating anxiety disorders.

1. Introduction

The avoidance of a threat (e.g., pain, suffocation) is an adaptive instrumental defense behavior to protect the individual from life-threatening consequences, thus ensuring the adaptation to changing environmental conditions (Skinner, 1953; Hamm and Weike, 2005; Cain and LeDoux, 2008). However, if avoidance behaviors become too dominant they may impair psychosocial functioning and quality of life (Barlow, 2002; American Psychiatric Association, 2013). In fact, maladaptive changes in behavior that prevent exposure to or terminate confrontation with a perceived threat are one of the core features of a wide spectrum of mental disorders (Craske et al., 2009; American Psychiatric Association, 2013; Krypotos et al., 2015). These maladaptive behaviors (e.g., avoiding eye contact or taking medication) are typically persistent and inflexible in nature, automatically elicited by threat-related cues (e.g., body sensations or phobic objects), and thus performed in a habit-like manner (Dickinson, 1985; Gillan et al., 2016c; LeDoux et al., 2016). Most importantly, avoidance behavior is often not adaptive and consistently performed even though expected negative outcomes and environmental conditions may have changed (Dickinson, 1985; LeDoux et al., 2016). Of clinical importance is that in patients with anxiety disorders persistent avoidance prevents the disconfirmation of central concerns about the consequences (e.g., the mental representation of the unconditioned stimulus) of a specific situation. As such, avoidance plays a key role in preventing extinction of a learned association and maintaining anxiety and irrational fears (Barlow, 2002; Mineka and Zinbarg, 2006; Craske et al., 2008; Helbig-Lang and Petermann, 2010).

In modern exposure-based therapies, the prevention of safety-seeking behaviors including avoidance and escape is a key prerequisite to facilitate extinction (Barlow et al., 2004; Craske et al., 2014; Pittig et al., 2016). Persistent avoidance, therefore, interferes with extinction learning - one central mechanism of exposure based therapies (Powers et al., 2004; Craske et al., 2008; Lovibond et al., 2009; Helbig-Lang and Petermann, 2010). Possibly, resistant avoidance behavior accounts for the relatively high rates of dropouts or refusals, nonresponders and

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relapses in exposure-based therapies (Craske et al., 2006; Gloster et al., 2013; Fernandez et al., 2015). As such, it becomes clear that a comprehensive analysis of human avoidance behavior and its underlying mechanisms and motivational basis is of high relevance and could help to enhance the effectiveness of exposure therapy.

Early animal data, as well as recent findings from humans, suggest that avoidance behaviors can persist following fear extinction (Solomon et al., 1953; Vervliet and Indekeu, 2015) suggesting that fear might initiate instrumental avoidance behavior but might be less important for its maintenance (see LeDoux et al., 2016 for a review). It has been demonstrated that as rodents start to exert behavioral control over a threat (e.g., show instrumental avoidance responses) defensive fear responses (e.g., freezing) elicited by the threat-predicting cues will diminish (see Campese et al., 2016 for a review). Moreover, there is increasing evidence that different neural networks are involved in regulating freezing and defensive action (Amorapanth et al., 2000; Choi et al., 2010; Ramirez et al., 2015). The switch from reactive responses to instrumental defensive action is assumed to be coordinated by the prefrontal cortex (infralimbic prefrontal cortex) that actively inhibits central amygdala mediated expression of conditioned freezing and thus facilitates defensive action (Martinez et al., 2013; Moscarello and LeDoux, 2013). Finally, when avoidance behavior is performed repeatedly, defensive actions may become inflexible, stimulus-triggered and automatic, i.e., become amygdala-independent defensive habits (Campese et al., 2016; LeDoux et al., 2016).

The findings in animals are consistent with recent human brain imaging data suggesting that the amygdala, prefrontal cortex, and striatum are involved in avoidance learning (Schlund et al., 2010; Schlund and Cataldo, 2010; Schlund et al., 2011; Levita et al., 2012; Schlund et al., 2013; Collins et al., 2014; Boeke et al., 2017). Indeed, in addition there is evidence from human research demonstrating that autonomic arousal decreases during avoidance learning (Lovibond et al., 2008; Delgado et al., 2009; Vervliet and Indekeu, 2015; Boeke et al., 2017). While these data are promising, in most studies individuals are instructed or trained specifically to exhibit avoidance behavior. In contrast, although highly clinically relevant, there are almost no data on spontaneously occurring avoidance behavior and its maintenance in humans. The present study therefore aimed at characterizing defensive behaviors, physiological arousal, and reported anxiety associated with spontaneously occurring repeated termination of exposure to a threat.

In the present study, we used an interoceptive threat increasing in intensity because such threat bears high relevance for a variety of anxiety and health problems. For example, bodily symptoms may spiral into panic and may elicit defensive action in persons with panic disorder (Goodwin et al., 2005; Kessler et al., 2006; Pané-Farré et al., 2013; Pané-Farré et al., 2014). In our study, the increasing interoceptive threat was established by evoking increasing feelings of dyspnea using increasing respiratory loads to impede inspiration and a complete breathing occlusion, a model for a suffocation experience that has been shown to be a potent unconditioned internal threat (Nardi et al., 2006; Pappens et al., 2012; Pappens et al., 2014). Participants were provided with a response button that they could press (during the presentation of increasing loads and the occlusion) to terminate the trial. In the present analysis we explored (1) at which threat intensity (increasing loads vs. occlusion) participants terminated the exposure, (2) how the behavioral pattern, (3) reports of anxiety, (4) physiological responses and brain stem reflex measures as well as (5) startle probe evoked brain potentials as an index of selective attention changed with repetitions of premature terminations of the exposure sequences. To control for the possibility that changing response patterns during repeated terminations could be the result of the mere repetitions of exposure to increasing interoceptive threat, defensive responses prior to terminations were compared to responses during matched control intervals of individuals who completed all exposure sequences.

Based on previous findings and clinical observations, we assumed that after the initial defensive action at the ultimate threat level (e.g., during occlusion) successive defensive actions would be initiated increasingly earlier at lower threat levels. We also predicted that repetitive defensive actions would be accompanied by different autonomic response patterns. We expected that the first termination would be motivated by a strong fear response elicited at the highest threat level, characterized by a surge in sympathetic arousal (increased heart rate and skin conductance level) (Richter et al., 2012; Hamm et al., 2016), as would be predicted by Mowrer's two-factor model (Mowrer, 1939). In contrast, no such strong autonomic responses were expected during later premature terminations supporting animal data and initial evidence in humans, that the maintenance of avoidance is not motivated by fear and therefore not accompanied by strong autonomic indices of fear (Lovibond et al., 2008; Delgado et al., 2009; Campese et al., 2016). In matched control persons, we predicted that there would be no increase in autonomic arousal during the first and subsequent matched control intervals. Besides autonomic measures, we also assessed the modulation of the startle response – an additional rather low-level brain stem measure of fear (see Hamm, 2015 for a review) prior to exposure terminations.

There is evidence showing that if individuals have the option to actively avoid exposure to a threat by performing a motor task (button press), startle response magnitudes are inhibited during the acute preparation for action (Löw et al., 2008; Richter et al., 2012; Löw et al., 2015; Wendt et al., 2017). This inhibition of the startle blink magnitudes was associated with a sharp drop of the probe-elicited P3 component of the evoked brain potentials, suggesting that attentional resources are allocated to the visual cue that signals the critical time window for the initiation of the avoidance response, thus reducing the selective attention to the irrelevant secondary acoustic startle probe (see Löw et al., 2015). Based on these results, we expected an inhibition of the startle eyeblink response and a reduction of the P3 component of the ERP to the acoustic probe stimuli prior to initial defensive action as a result of binding of attentional resources in the context of response preparation. In contrast, we expected that repetitive avoidance would be performed rather automatically or in a habit-like manner, thus not requiring allocation of attentional resources to facilitate the preparation and initiation of the behavioral response (Solomon et al., 1953; Lovibond, 2006; Ilango et al., 2014; Krypotos et al., 2015; Gillan et al., 2016c; LeDoux et al., 2016). As such, we assumed that the startle eyeblink responses would no longer be inhibited and the probe-evoked P3-component would no longer be reduced.

2. Methods and materials

2.1. Participants

Participants were recruited from a pool of 400 university students. Exclusion criteria were cardiovascular, respiratory (e.g., asthma, COPD), or neurological (e.g., epileptic or apoplectic seizures, multiple sclerosis) diseases, current or past psychotherapeutic treatment for anxiety problems, hearing impairment, or pregnancy. Overall, 69 participants took part in the laboratory assessment. Twenty-eight participants prematurely terminated the exposure to the restricted breathing at least once as described in the procedures section. The sample included in this analysis consisted of those 24 individuals who *repeatedly* (more than once) terminated the exposure. Verbal reports of anxiety and physiological responses of repeated terminations were compared with matched exposure sequences from 24 control individuals matched for age, sex, and level of suffocation fear who completed all experimental procedures. A description of the group characteristics is presented in Table 1, indicating that the groups did not differ by age, sex, body weight, height, body mass index, trait anxiety, anxiety sensitivity, suffocation fear, agoraphobic cognitions, fear of bodily sensations or the vigilance to body sensations. All participants provided written informed consent prior to the study and either received course credit or

Table 1
Means and standard deviations of demographic characteristics and questionnaires for persons who prematurely terminated exposure and matched controls.

	Premature terminations	Exposure completed	*p*-Value
CLQ – SF [0–46]	10.4 (7.7)	10.8 (8.6)	$p = 0.874$
ASI – 3 [0–72]	24.0 (11.0)	20.5 (12.2)	$p = 0.296$
BSQ [1–5]	2.4 (0.6)	2.2 (0.7)	$p = 0.481$
ACQ [1–5]	1.6 (0.4)	1.6 (0.4)	$p = 0.658$
BVS [0–40]	15.8 (5.5)	15.9 (6.7)	$p = 0.965$
STAI - trait [20–80]	38.3 (7.8)	42.8 (11.4)	$p = 0.121$
Age	22.8 (3.8)	23.2 (3.4)	$p = 0.691$
Sex (female/male)	20/4	20/4	$p = 1.000$
Weight (kg)	68.5 (12.6)	67.6 (12.7)	$p = 0.803$
Height (cm)	173.5 (6.9)	172.3 (8.6)	$p = 0.570$
Body mass index	22.8 (4.2)	22.7 (3.3)	$p = 0.937$

Note: CLQ: Claustrophobia Questionnaire; ASI: Anxiety Sensitivity Index; BVS: Body Vigilance Scale; STAI: State-Trait Anxiety Inventory; BSQ: Body Sensations Questionnaire; ACQ: Agoraphobic Cognition Questionnaire; possible questionnaire score ranges are listed in parentheses.

financial compensation (20 €) for their participation. The study protocol was approved by the ethics committee of the German Psychological Society.

2.2. Materials and measurements

2.2.1. Breathing circuit

Participants breathed through a tightly fitting face mask (7400 series; Hans Rudolph, Inc., Kansas City, MO) connected to a rigid tube with sensors for measuring respiration. A flow sensor was mounted to the mouth port of the two-way y-shaped non-rebreathing valve (no. 2630; Hans Rudolph, Inc.) which enabled unrestricted expiration. A plastic tube (length: 2.75 m; diameter: 35 mm) connected the inspiratory port of the y-valve to the common port of a Five-Way Gatlin-Shape™ Inflatable-Balloon-Type™ valve (2440 series, Hans Rudolph, Inc.), placed in the adjacent control room. Closing and opening of the 4 ports of this valve was controlled via VPM software triggering a pneumatic controller (2430 series, Hans Rudolph, Inc.). This system allowed a prompt and easy switching between three different inspiratory resistive loads, unrestricted breathing, and total occlusion (all ports closed).

2.2.2. Inspiratory resistive loads (IRL)

For the induction of dyspnea, nylon flow resistors of linear type (7100 series, Hans Rudolph, Inc., range: 0.5–236.5 $cmH_2O/l/s$) were attached to three ports of the valve.

2.2.3. Breathing occlusion

Breathing occlusions of 15 s duration were manually triggered at the end of expiration as indicated by visual display of the respiration curve generated by thoracic and abdominal respiration belts connected to an inductive plethysmography system (Respitrace, Q.D.C., SensorMedics, NewMedics GmbH, Öhringen, Germany).

2.2.4. Startle stimulus

The startle probes, 50 ms bursts of broadband white noise (rise/fall time $<$ 1 ms), were presented binaurally with an intensity of 95 dB(A) through AKG K-66 headphones.

2.2.5. Subjective reports

Using a computer keyboard, participants rated the experienced intensity and unpleasantness of dyspnea as well as the anxiety and severity of panic symptoms as listed in the DSM-5 during loaded breathing and occlusion on the following scale: 1 (not at all), 2 (slight), 3 (moderate), 4 (strong), 5 (very strong), and 6 (maximally tolerable). Moreover, using a touchpad (Intuos PEN & Touch S, Wacom Europe GmbH, Krefeld, Germany) participants were asked to draw a line indicating the course of their subjective anxiety during exposure to restricted breathing in a coordinate system which was labeled "time" on the x-axis and "anxiety intensity" on the y-axis (0 – 10). Rating options were projected onto a 1.50 × 1.30 m screen in front of the participants.

2.2.6. Physiological recordings

To measure the eyeblink component of the startle response, electromyographic (EMG) activity was recorded with two electrolyte-filled (Marquette Hellige, Freiburg, Germany) Ag/AgCl miniature surface electrodes (Sensormedic, Yorba Linda, CA) attached over the orbicularis oculi muscle beneath the lower left eyelid. The amplification of the raw EMG signal was realized using a Coulbourn S75-01 amplifier. The signal was filtered using a 30 Hz high-pass and a Kemo KEM-VBF8-03400 Hz low-pass filter. Digital sampling at a rate of 1000 Hz was carried out via a 12-bit A/D converter starting 100 ms before the onset of the startle stimulus and lasting 400 ms following the startle probe.

An electroencephalogram (EEG) was recorded with Ag/AgCl electrodes (8 mm diameter; Marquette Hellige) filled with EC2 Genuine Grass Electrode Cream (West Warwick, RI) and placed at Pz, Cz, and Fz according to the international 10–20 system. All channels were referenced to Pz and re-referenced offline to a linked ear lobe reference (two linked Ag/AgCl ear-clip electrodes). Vertical and horizontal eye movements (electrooculogram, EOG) were registered with two electrolyte-filled (Hellige electrode cream) Ag/AgCl electrodes (8 mm diameter; Marquette Hellige) placed above and on the right side of the right eye. Electrode impedance was kept below 20 kΩ. Both the EOG and the EEG were amplified (20,000-fold for EEG and 2000 for EOG, resp.) using a 12-channel Isolated Bioelectric AC/DC Amplifier System (San Diego Instruments, San Diego, CA) with a time constant of 1 s and a low-pass filter of 35 Hz. The signals were digitally sampled at 250 Hz.

Skin conductance was recorded from the hypothenar eminence on the palm of the participants' non-dominant hand using two Ag/AgCl standard electrodes (8 mm diameter, Marquette Hellige) filled with a 0.05 M sodium chloride electrolyte medium. A constant DC voltage of 0.5 V was applied across electrodes (attached 15 mm apart) by a Coulbourn S71–22 skin conductance coupler that processed the signal with a resolution of 0.01 μS. The DC voltage amplified signal was continuously sampled at 10 Hz by a 12-bit A/D-converter.

Electrocardiogram (ECG) was measured with electrolyte filled Ag/AgCl standard electrodes (Marquette Hellige) placed in an Einthoven-II-setup. The raw ECG signal was amplified and filtered through a 0.1–13 Hz band-pass filter using a Coulbourn S75-01 bioamplifier. The digital sampling rate was set to 100 Hz.

2.3. Procedure

Following the attachment of the breathing mask and all sensors, the experiment proceeded as follows.

(1) *Determination of breath-holding time*. The maximal post-expiratory breath-holding time (breath-holding at functional residual capacity as suggested by Asmundson and Stein, 1994) was determined using a standardized procedure. At the end of an expiration, the examiner signaled via a computer screen to hold the breath as long as possible, while the breathing circuit was occluded. Hence, during this period no breathing was possible until the participants terminated the breathing occlusion by button press which automatically initiated opening of the inspiratory port.
(2) *Determination of load detection threshold*. Next, for the determination of the individual's detection threshold of loaded breathing, IRLs were separately presented for 20 s, each followed by a 20 s recovery phase and stepwise increased until participants noticed any change in respiration (for a detailed description see Alius et al., 2013).
(3) *Presentation and selection of increasing IRLs*. Subsequently, to get acquainted with the range of possible loads, participants were asked

to rate the intensity and unpleasantness of dyspnea elicited by presentation of a mild (5 cmH2O/l/s above threshold) and a more severe load (50 cmH2O/l/s above threshold). In the next step, inspiratory resistive loads were gradually increased following an exponential curve. Loads were presented 30 s and each followed by a recovery phase lasting 30 s. After each load presentation participants rated the intensity and unpleasantness of experienced dyspnea. After an IRL was rated with an unpleasantness of 6 (= maximally tolerable) loads were not further increased. Finally, participants were exposed to one post-expiratory breathing occlusion lasting for 15 s, and, again, ratings of intensity and unpleasantness of dyspnea were obtained.

(4) *Repeated presentation of increasing IRLs followed by occlusion*. The assessment phase started with a one-time-only startle habituation phase (110 s) during which 8 startle probes were presented to reach a stable baseline for startle response magnitudes. Then, three loads of increasing intensity (previously rated as producing slight [load1], strong [load2] and maximally tolerable [load3] unpleasant feelings of dyspnea) were consecutively presented for 60 s each. During each load, three startle probes were presented with a randomized inter-stimulus-interval varying between 10 s and 30 s. Presentation of the third load was immediately followed by a post-expiratory breathing occlusion for 15 s and a 30s recovery phase. Two startle probes were presented during occlusion and recovery, respectively, with an inter-probe-interval of 5–20 s. Then, participants were asked to draw the course of anxiety they experienced during the exposure trial. The described load-occlusion-recovery sequence (trial) was repeated eight times.

Participants were informed that during the sequences of increasing loads and the occlusion they could press a termination button if, at any point in time, they were unable to tolerate the exposure any longer. However, it was stressed to the participants that it is crucial for the experiment to complete the sequences. A button press immediately terminated the presentation of loads or an occlusion and initiated a forward skip to the next recovery and anxiety-rating phase. From there, the experimental procedure (next sequence) was resumed normally. Thus, as eight load-occlusion sequences were presented, participants were able to terminate up to eight times at maximum. Note: Participants also had the option to terminate the entire experiment by calling the experimenter via intercom at any time.

(5) *Individual presentations and ratings of IRLs and occlusion*. During a final rating phase, the three previously selected loads and the breathing occlusion were presented separately for 30 s each, followed by a 30 s recovery phase and per-load/occlusion ratings of panic and respiratory symptoms were obtained.

At the end of the laboratory session, participants completed the Anxiety Sensitivity Index – 3 (ASI-3, Taylor et al., 2007), the Claustrophobia Questionnaire (CLQ, Radomsky et al., 2001), Body Sensations Questionnaire (BSQ, Chambless et al., 1984), the Agoraphobic Cognition Questionnaire (ACQ, Chambless et al., 1984), the State-Trait Anxiety Inventory (STAI, Spielberger et al., 1983) and the Body Vigilance Scale (BVS, Schmidt et al., 1997), and were fully debriefed by the experimenter.

2.4. Data reduction and analysis

The raw EMG signal was filtered off-line with a 60 Hz high-pass filter, rectified and smoothed using a 1st-order low-pass filter with a time constant of 10 ms. Then, the startle eyeblinks were scored using a computer program (Globisch et al., 1993) that identified blink onset and peak amplitude. Only trials in which blinks started during 20–100 ms after delivery of the startle probe and reached their peak amplitude within 150 ms were scored as valid startle responses. If no blink was detected in the defined time window the trials were scored as zero responses. Trials were rejected and treated as missing values if there was excessive baseline activity or movement artifacts. Digital values were converted to μV and then exported. To remove inter-individual variability not related to the experimental manipulation, for further analyses all values were transformed to T-scores ($M = 50$, $SD = 10$) as recommended by the guidelines for human startle eyeblink studies (Blumenthal et al., 2005).

EEG and EOG signals were filtered offline using a 0.1–35 Hz bandpass filter and a 50 Hz notch filter. The EEG signal was then corrected for vertical and horizontal eye movements using the Gratton-Coles algorithm (Gratton et al., 1983). Epochs of 700 ms (including a 100 ms pre-stimulus baseline) were extracted relative to startle probe onset, baseline corrected, and excluded from further analysis whenever the maximum-minimum difference of the EEG activity was larger than 100 μV. Additionally, all data were visually inspected and epochs with movement artifacts or technical failures were excluded. EEG analysis was processed with Brain Vision Analyzer 2.0 (Brain Products, Munich, Germany). The P3 component of the ERP was determined as the mean activity between 170 and 310 ms.

Digital values of skin conductance level (SCL) were converted to μS and exported in half-second means.

The ECG signal was visually inspected, movement artifacts set to missing, and misplaced R-wave triggers were corrected using ANSLAB version 2.4 (Autonomic Nervous System Laboratory, University of Basel, Switzerland). Inter-beat-intervals were calculated, converted to heart rate (HR in bpm), and exported in half-second bins.

Data from persons who prematurely terminated exposures were analyzed time-locked to the button press. Reported anxiety, as well as physiological data, were analyzed for 90 s preceding the button press averaged in blocks of ten seconds, thus providing 10 data points prior to the premature termination of the trial. Blink magnitudes were averaged into blocks across three acoustic probes. Proximal response magnitudes were calculated as the mean of responses to three probes immediately prior to premature termination and distal response magnitudes were averaged over the preceding three probes presented during the load. Evoked brain potentials to the acoustic probes were analyzed using the same logic. Physiological and verbal report data were only analyzed for trials from which data were available for at least 90 s (at least 6 probe stimuli) prior to premature termination. Due to technical problems, analyses of startle responses and ERPs were based on data from 21 participants per group. The physiological and subjective data from matched control persons were analyzed applying the same trial and timing information used for analyses of premature termination trials.

Linear mixed models were chosen for the statistical analyses as this approach corresponds to the special structure of the data (trials nested in participants and missing data points) and allows a flexible and powerful analysis of the repeated-measures data with missing data points (Blackwell et al., 2006; Tabachnick and Fidell, 2007; West, 2009). In persons who prematurely terminated exposure and matched control persons, changes in reported anxiety and physiological responses prior to premature terminations or during matched control intervals were analyzed with linear mixed models including the repeated measures factors *proximity* (ten 10s–blocks [from -90s to 0 s], resp., two blocks [distal vs. proximal] for startle responses and ERP analyses), *trial* (first to eighth termination, resp., first to eighth matched interval) and the between-subject factor *group* (persons who terminated exposure vs. matched completer) as well as their interactions. For the analyses of the P3 component of the probe-evoked potentials electrode *location* (Pz vs. Cz vs. Fz) was entered as an additional repeated measures factor. The random part of the models included a person-specific intercept and repeated-measures effects for proximity and number of terminations (and location for ERP data) with a first-order autoregressive covariance structure (homogeneous variances and correlations that decrease with time). To check whether the experimental manipulation was successful in the present study, the change in

physical intensity of IRLs as well as in anxiety and panic symptom intensity during increasing loads and the occlusion was analyzed using a mixed-model ANOVA with the repeated-measures factor *load* (first vs. second vs. third load, resp., first vs. second vs. third load vs. occlusion for anxiety and symptom ratings) and the between-subject factor *group*. To evaluate whether with increasing number of terminations the behavioral pattern changed, i.e., whether the frequency of terminations decreased during occlusion and increased during loaded breathing, an ANOVA was run with the repeated-measures factor *number of terminations* (first to eighth termination). Whenever physiological and verbal report data significantly changed prior to terminations or during loads as indicated by a significant main effect of proximity or load, linear and quadratic trends were analyzed to test for significant increases in physiological or verbal report data. All statistical tests used a significance level of $p < 0.05$. Whenever necessary, a Greenhouse-Geisser correction was applied. All data were processed using SPSS 22.0 (SPSS for Windows, IBM).

3. Results

3.1. Manipulation check

In persons who prematurely terminated exposure and matched controls, the physical intensity of inspiratory resistive loads increased while the reported unpleasantness of dyspnea of the selected loads increased from slight to strong to maximally tolerable, $F(2, 92) = 72.00$, $p < 0.001$, Group × load $F(2, 92) = 2.71$, $p = 0.106$, group $F(2, 92) < 1$, $p = 0.495$, linear and quadratic trend $p < 0.001$. In both groups anxiety and symptom intensity continuously increased from load1 to occlusion, load $F(3, 138) = 39.81, p < 0.001$, Group x Load $F(3, 138) = 1.02$, $p = 0.372$, linear trend: $p < 0.001$, quadratic trend: $p = 0.076$ and load $F(3, 138) = 50.96$, $p < 0.001$, Group × load $F(3, 138) = 1.13$, $p = 0.324$, linear trend: $p < 0.001$, quadratic trend: $p = 0.286$ for anxiety and symptom ratings, respectively. However, persons who prematurely terminated exposures reported overall higher anxiety and symptom intensity as compared to matched controls, group $F(1, 46) = 5.88$, $p = 0.019$ and group $F(1, 46) = 3.87$, $p = 0.055$ for anxiety and symptom ratings, respectively.

3.2. Behavioral data

As depicted in Fig. 1, the frequency of terminations during the occlusion decreased with repeated trials, $F(7, 98) = 5.14$, $p < 0.001$, $\eta_p^2 = 0.27$, $\chi^2(7) = 28.20$, $p < 0.001$, linear trend: $p < 0.001$, quadratic trend: $p = 0.617$. Instead, participants more frequently terminated the trials already during the loaded breathing period.

3.3. Anxiety ratings

As depicted in Fig. 2,[1] both groups reported a continuous increase in the intensity of anxiety during the analyzed 90 s interval of termination or matched control trials, $F(9, 327.71) = 18.99$, $p < 0.001$, Group x Proximity $F(9, 327.71) = 1.09$, $p = 0.373$, linear trend: $ps < 0.001$ and quadratic trend: $ps > 0.262$ for both groups. Moreover, in both groups, the reported intensity of anxiety increased with the number of termination and matched control trials, respectively, $F(7, 927.64) = 6.82, p < 0.001$, Group x Trial $F(7, 927.64) < 1, p = 0.521$, linear and quadratic trends for both groups: $ps < 0.004$. Persons who prematurely terminated exposure reported a higher anxiety intensity immediately prior to the first and second termination as compared to during matched points in time of the matched control persons, e.g., at 0 s: group $Fs > 6.84$, $ps < 0.011$. Interestingly, autonomic indices of fear did not correspond with the verbal report data and showed a different pattern.

3.4. Autonomic arousal

As depicted in the upper left panel of Fig. 3, the pattern of SCL differed between persons who prematurely terminated exposure and matched controls, Group x Trial $F(7, 263.90) = 2.65$, $p = 0.012$. In persons who terminated exposure, the increase in SCL prior to premature terminations significantly changed with increasing number of terminations, $F(63, 712.12) = 4.83$, $p < 0.001$. There was a strong increase in SCL across the 90s prior to the first termination of exposure, $F(9, 603.07) = 4.19$, $p < 0.001$, linear trend: $p < 0.001$, quadratic trend: $p = 0.005$, while the second, $F < 1$, $p = 0.950$, and subsequent terminations were not preceded by significant changes in skin conductance level, $Fs < 1$, $ps > 0.870$. In contrast, there was no significant change in skin conductance during the first matched trial, $F < 1$, $p = 0.995$, nor did SCL change during subsequent matched control intervals (see right upper panel of Fig. 3), $Fs < 1, ps > 0.722$.

Supporting electrodermal data, persons who prematurely terminated exposure and matched control persons showed a different pattern of changes in heart rate, Group x Proximity x Trial $F(63, 1425.25) = 1.32$, $p = 0.052$. In persons who prematurely terminated exposure, heart rate increased significantly prior to the first termination (see lower left panel of Fig. 3), $F(9, 940.48) = 3.44$, $p < 0.001$, linear trend: $p < 0.001$, quadratic trend: $p = 0.023$. However, this increase in heart rate diminished with increasing number of terminations, $F(63, 985.46) = 1.33$, $p = 0.049$. Supporting the skin conductance data heart rate did not change significantly prior to the second, $F(9, 958.04) = 1.61$, $p = 0.107$, and subsequent terminations, $Fs < 1.50$, $ps > 0.144$. In contrast, in matched completers, heart rate did not change during the first matched control intervals, $F < 1, p = 0.555$, or during subsequent trials, $Fs < 1.88$, $ps > 0.070$.

3.5. Probe-evoked potential[2]

As depicted in Fig. 4, the pattern of the probe-evoked P3 amplitudes differed between persons who terminated exposure and matched controls, Group x Proximity x Trial $F(7, 709.89) = 4.62$, $p < 0.001$. The probe-evoked P3 amplitudes decreased significantly immediately prior to the first premature termination (see left panel of Fig. 4), $F(1, 224.92) = 25.88$, $p < 0.001$. However, this pattern changed with repeated terminations, Proximity x Trial $F(7, 474.17) = 11.90$, $p < 0.001$. There was no significant reduction of probe-evoked P3 amplitudes prior to the second, $F(1, 224.92) = 1.02$, $p = 0.314$, or subsequent terminations, $Fs < 2.53$, $ps > .112$. In contrast to the pattern found in persons who terminated exposure, in matched controls the P3 amplitudes did not decrease during the first matched non-termination trial, $F(1, 305.55) < 1$, $p = 0.366$, nor did P3 amplitudes change during subsequent trials, $Fs < 2.60$, $ps > 0.107$.

3.6. Startle response magnitudes

In line with the probe-evoked P3 data, startle response magnitudes decreased just before the first premature termination of exposure (see left panel of Fig. 5), $F(1, 244.89) = 7.42$, $p = 0.007$. Interestingly, increased inhibition of the startle response (proximal minus distal to the button press) was significantly correlated with reported anxiety just prior to the first termination, $r = -0.687$, $p = 0.001$. Again, startle response magnitudes did not change prior to the second, $F(1,$

[1] As reported for the subjective and physiological data, the response pattern did not change between the second and eighth termination. For a clear and consistent presentation of the data and effects found in the present study, only data from the first, second and eighth termination were depicted in Fig. 2 and all following figures. Data from all terminations are depicted in Fig. S1 to S4 (see online Supporting information).

[2] No significant interactions were found involving the factor location, all $Fs < 1.03$ and $ps > 0.421$.

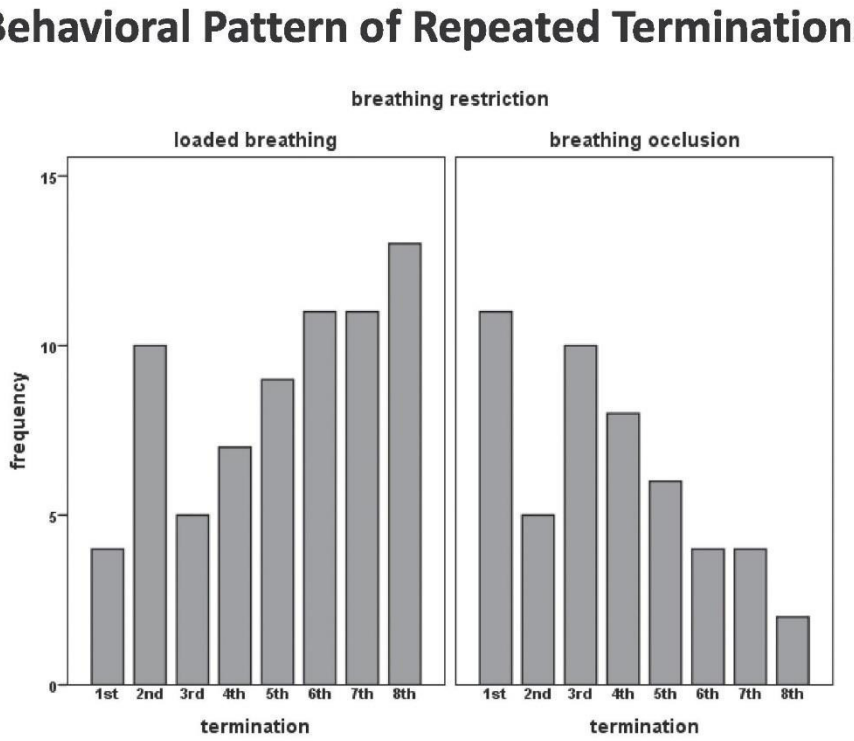

Fig. 1. The frequency of terminations either during loaded breathing or during a total breathing occlusion in the course of repeated terminations.

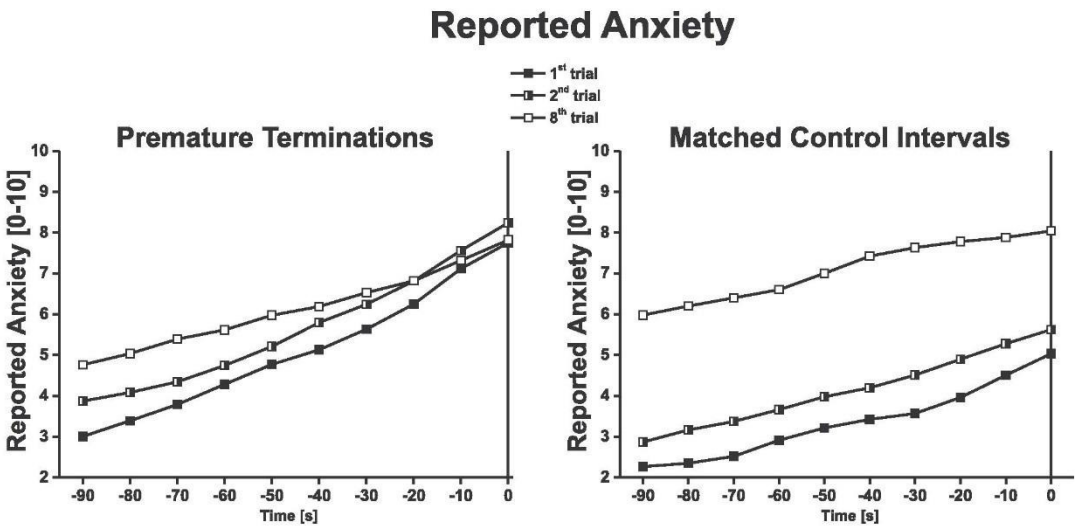

Fig. 2. Means of the reported anxiety during the 90s prior to the 1st, 2nd and 8th termination of increasing interoceptive threat (left panel) and during matched control intervals in matched controls (right panel).

244.89) < 1, $p = 0.567$, or subsequent terminations, $Fs < 1.04$, $ps > 0.309$. In accordance with the probe-evoked P3 amplitudes, in matched control subjects, no changes were observed in startle response magnitudes during matched time intervals (see right panel of Fig. 5), $Fs < 2.58$, $ps > 0.109$.

4. Discussion

The present study examined changes in defensive behavior during repeated premature terminations of exposure to an increasing interoceptive threat. In accordance with early animal data, we observed that with repeated terminations participants ceased the exposure to increasing interoceptive threat at increasingly lower threat levels.

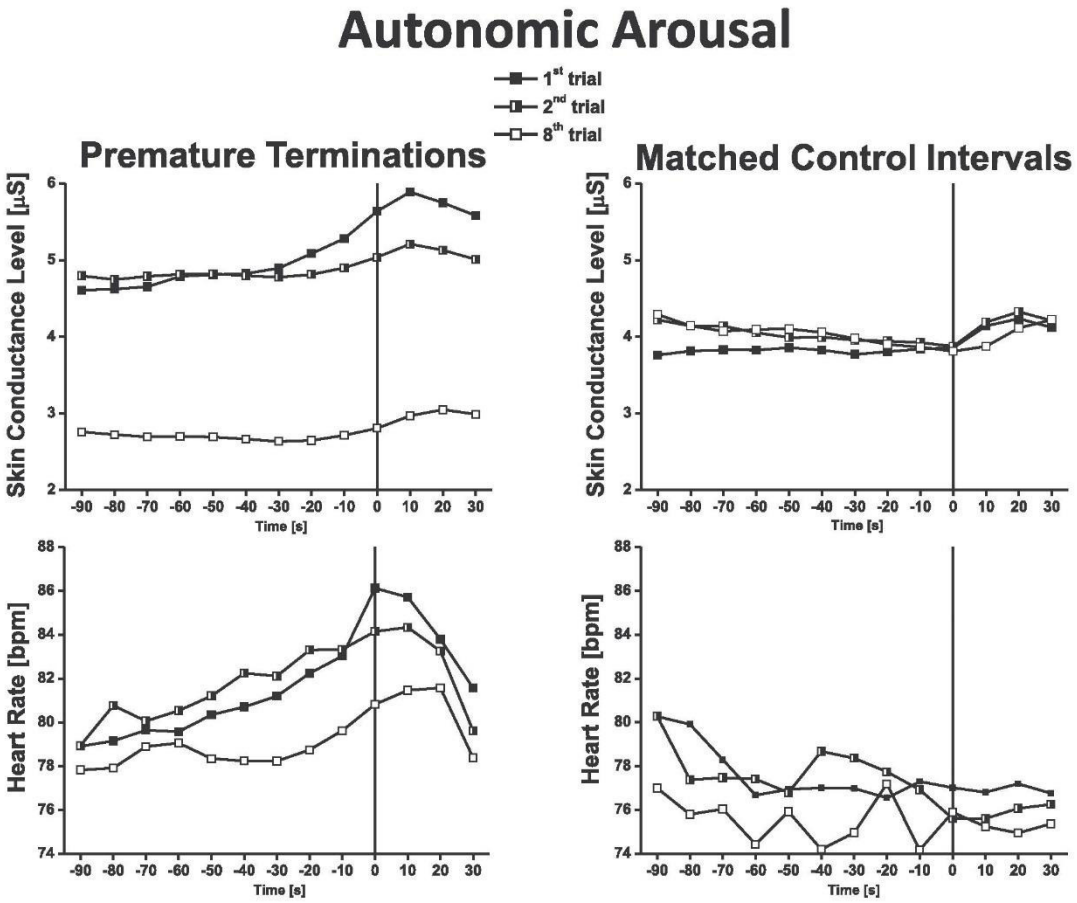

Fig. 3. Changes in autonomic arousal during the 1st, 2nd and 8th termination (left panel) or matched control trial (right panel). Mean skin conductance level (upper panel) and heart rate (lower panel) 90s before, during, and 30s after the button press.

Associated with these behavioral changes, autonomic and reflex measures of defensive behaviors changed from preparation for defensive action to habitual behavior. These changes in defensive patterns went along with corresponding changes in selective attention as indexed by evoked brain potentials. The first termination typically occurred at the highest level of threat (i.e., during occlusion) and was preceded by a surge of sympathetic arousal and an increase in reported anxiety. In addition, we observed a strong inhibition of the startle response magnitudes just prior to the premature termination supporting previous data from Löw et al. (2015). Moreover, attention to irrelevant and interfering cues in the threat environment was blocked as indicated by a reduced P3-component of the evoked potentials to the probe stimuli. A fundamentally different pattern of defensive reactivity emerged with repetition of premature terminations. The behavioral pattern changed in that participants terminated the exposure earlier in the sequences of increasing interoceptive threat, i.e., at lower levels of threat intensity, preventing the occurrence of the full occlusion. With increasing number of repetitions of premature terminations, we did not observe any increases in autonomic arousal or changes in blink magnitudes. Moreover, selective attention as indexed by the probe-P3 amplitudes was no longer modulated proximal to the button press. Importantly, this response pattern emerged exclusively in persons who prematurely terminated exposure but not in matched control persons, suggesting that the observed effects were not due to either the physiological state at the specific time of termination (i.e., during occlusion in the first termination) or the repeated confrontation with increasing interoceptive threat.

4.1. Defensive response patterns during the first termination of interoceptive threat

On a behavioral level, individuals terminated the first exposure of increasing threat at the highest threat level, i.e., the total breathing occlusion. This initial termination was preceded by strong increases in skin conductance level and heart rate. In contrast, this increase was not observed in matched control persons. These results are in line with observations in a subgroup of patients with panic disorder who escaped from a situation of entrapment during a standardized behavioral avoidance test (Richter et al., 2012; Hamm et al., 2016). These patients also showed a strong increase in heart rate and skin conductance level steadily increasing one minute prior to escape.

Startle magnitudes were inhibited prior to the first termination but not during the matched control interval, i.e., not just because of the present occlusion at this point of time. A similar relative inhibition of the startle reflex was also observed in panic disorder patients just prior to escape from entrapment (Richter et al., 2012). This decrease was even stronger for patients who escaped from the dark, narrow chamber during a self-reported panic attack (see Hamm et al., 2016). This

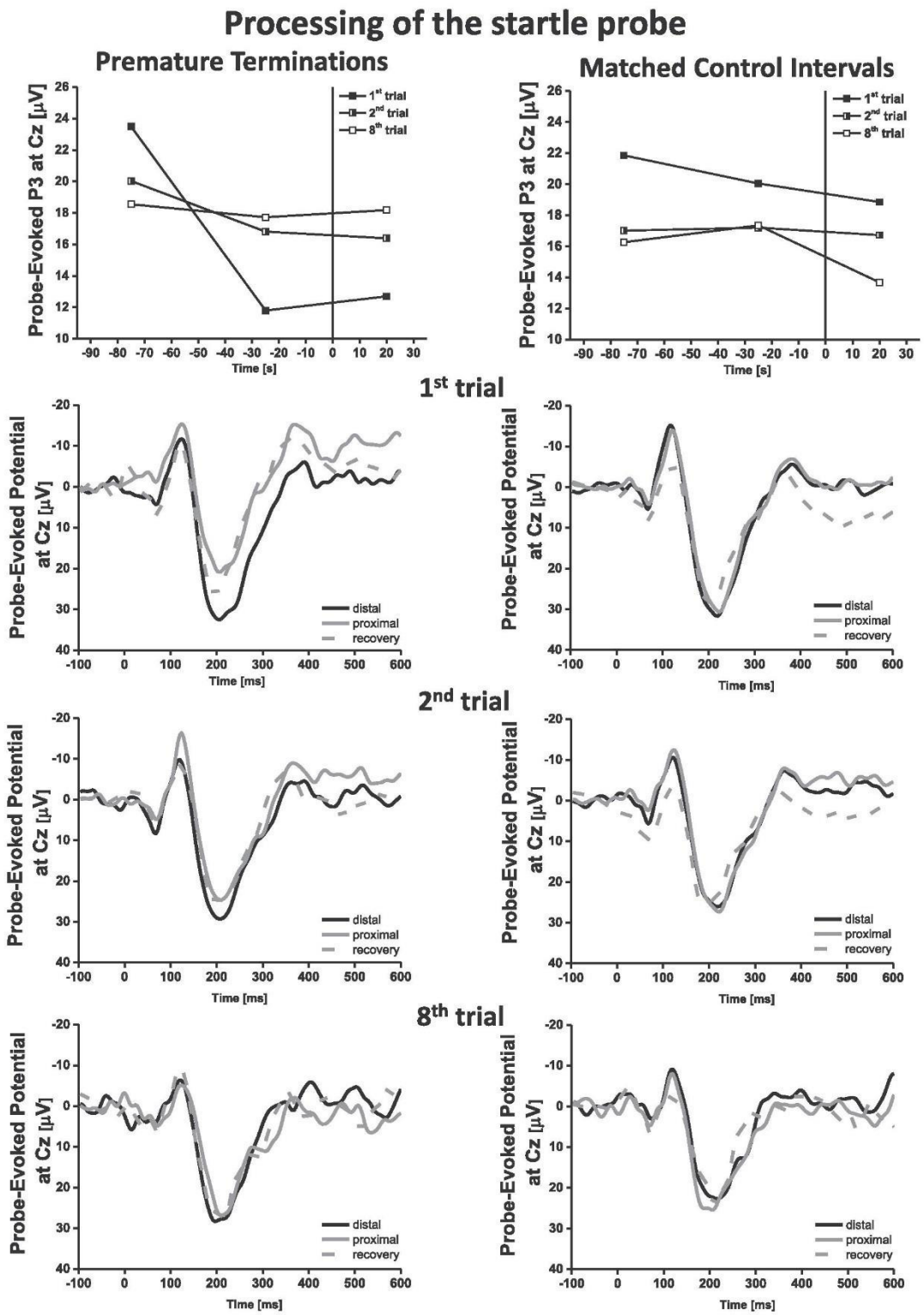

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Fig. 4. Event-related potentials evoked by startle probes distal and proximal to the button press of the 1st, 2nd, and 8th termination of exposure or matched control interval. Grand average waveforms (lower panels) and mean amplitude of the probe-evoked P3 component (upper panel) for Cz in persons who prematurely terminated exposure (left panel) and in matched control persons (right panel).

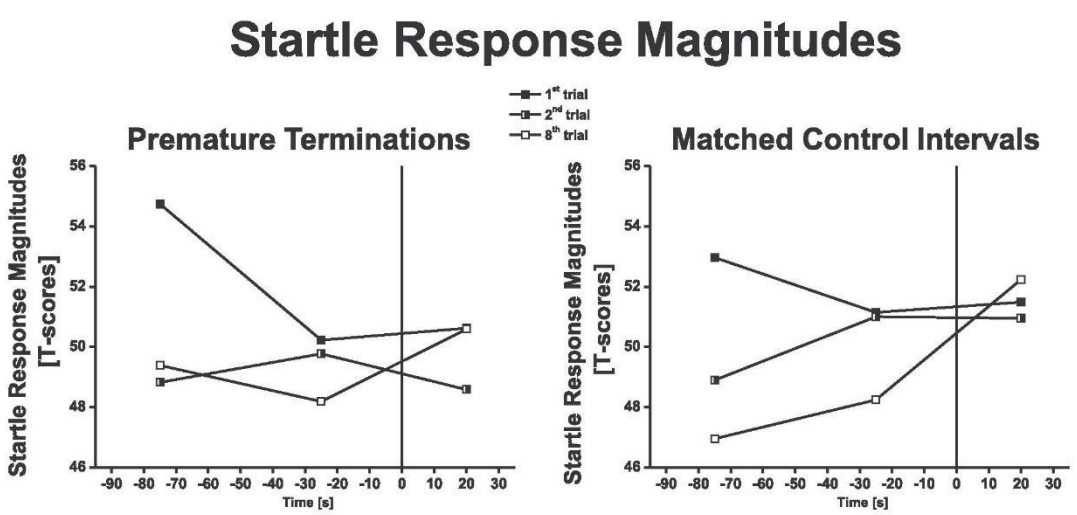

Fig. 5. Startles response magnitudes distal (responses to three startle probes) and proximal (responses to three startle probes) to the 1st, 2nd, and 8th premature termination of exposure by button press (left panel). Startle response magnitudes during matched control trials in matched controls (right panel).

corroborates animal data demonstrating that startle responses were relatively inhibited during cues predicting very intense foot shocks as compared to cues associated with mild shocks (Walker et al., 1997; Walker and Davis, 1997). In line with this evidence, we observed a stronger inhibition of the startle response when participants reported higher anxiety immediately prior to the first termination of exposure. Alternatively, this pattern of startle inhibition can be interpreted as an indicator of active response preparation that requires allocation of attentional resources to interoceptive threat cues and thus decreases available resources for processing of the auditory startle probe. This assumption goes in line with cross-modality experiments which demonstrated that startle response magnitudes were decreased when attention was allocated to stimuli that diverged from the sensory modality of the startle-eliciting probe (Anthony and Graham, 1985; Filion et al., 1998; Alius et al., 2014; Benke et al., 2015). Interestingly, the inhibition of blink magnitudes was associated with a linear increase in heart rate, replicating previous research (Löw et al., 2008; Löw et al., 2015). According to the cardiac-somatic coupling hypothesis (Obrist, 1981), we interpret the strong increase in autonomic arousal as a preparation for an effective motor response during defensive action.

In contrast to the matched control interval, the P3 component to probes presented prior to the first premature termination was significantly reduced. A similar attenuation of probe-evoked P3 amplitudes has been observed in picture viewing paradigms where affective foreground stimuli capture attentional resources thus limiting resources available for processing of the startle probe (Schupp et al., 1997; Cuthbert et al., 1998). Moreover, this pattern has also been described during exposure to interoceptive threat (Alius et al., 2014), unpredictable as well as predictable threat (Nelson et al., 2015) or while individuals were preparing for a motor response to actively avoid presentation of a threat (Löw et al., 2015). Thus, during the preparation of active avoidance attentional resources to the irrelevant acoustic stimuli are blocked and may be allocated to the interoceptive threat stimuli (including dyspnea and autonomic symptoms) to promote initiation of effective avoidance.

4.2. Defensive behavior during repeated terminations of interoceptive threat

With increasing repetitions of terminations, participants terminated the exposure sequences at successively lower threat levels (i.e., already during lower intensities of inspiratory resistive loads), avoiding the occurrence of the ultimate threat (i.e., the complete breathing occlusion). This change in defensive behavior is similarly observed in animal as well as in human avoidance learning experiments (Solomon and Wynne, 1953, 1954; Krypotos et al., 2014). From a learning perspective, one could argue that increasing dyspnea possibly became an indicator of the occurrence of the complete breathing occlusion and thus defensive action was initiated already at lower intensities of dyspnea to prevent the further increases in dyspnea (Lovibond, 2006; Pappens et al., 2012).

According to the two-factor theory postulated by Mowrer (1939), terminations of stimuli predicting imminent danger are negatively reinforced by the reduction of the conditioned fear response, which in turn would lead to the maintenance of the avoidance behavior (Mowrer and Lamoreaux, 1946; Mowrer, 1951). Based on this theory, several animal and human studies have investigated whether avoidance behavior is motivated by fear. In a line of influential studies, dogs learned to avoid shocks, however, no signs of fear (e.g., defecation, pupillary dilatation, suppression of operant appetitive behavior) were observed while avoidance behavior was maintained (Solomon et al., 1953; Solomon and Wynne, 1954; Kamin et al., 1963; Starr and Mineka, 1977; Mineka and Gino, 1980). Similarly, signs of fear were absent in humans who learned to effectively avoid a shock (Lovibond et al., 2008; Delgado et al., 2009; Lovibond et al., 2009; Vervliet and Indekeu, 2015). The present study extended these findings for spontaneously occurring avoidance behavior that was initiated by strong indices of fear but was maintained in the absence of any indices for physiological arousal.

The current data support previous data suggesting that fear responses might be an important motivator for the initiation of avoidance while alternative processes might be involved in the maintenance of avoidance behavior (for review see Krypotos et al., 2015). For example, cognitive (expectancy) accounts of avoidance learning stressed that

propositional knowledge is acquired about the presentation or omission of an aversive stimulus if no action is emitted or an avoidance response is performed (Seligman and Johnston, 1973; Lovibond, 2006). The present results are in line with these accounts predicting a reduction in fear once the expectancy of experiencing a breathing occlusion decreases after successful initiation of avoidance behavior (Lovibond et al., 2008). Following this perspective, persistent avoidance behavior is maintained – without any signs of fear - as individuals do not experience a disconfirmation of their expectancies regarding the presentation of the threat if no avoidance response is performed (Lovibond, 2006).

More importantly, Solomon et al. (1953) reported that the avoidance behavior of dogs was not only persistent but also became stereotyped, i.e., the behavior was performed in a habitual way. Interestingly, the chronic and repetitive avoidance behavior in patients with anxiety disorders, which is commonly performed in an inflexible and automatic rather than in a goal-directed way often resembles such stereotypical behaviors. Thus, avoidance behavior might be rather conceptualized as a (stimulus-driven) habit (Gillan et al., 2016c) than as a fear motivated behavior. This might explain why avoidance behavior persists while the reinforcing qualities of the response outcome (e.g., fear reduction) diminish. Most importantly, it has been suggested that the transition from defensive action to defensive habits is characterized by a shift in activated underlying neural circuits (LeDoux et al., 2016). This view is supported by experimental studies in humans showing that active avoidance is mediated by similar neural circuits involved in habit formation (e.g., the dorsal striatum) (Delgado et al., 2009; Schlund et al., 2013; Collins et al., 2014; Ilango et al., 2014).

It has been suggested that habit-like performances do not require planning or organization, thus demanding low processing capacity in contrast to goal-directed behavior (Evans and Stanovich, 2013; Wood and Runger, 2016). In contrast to the first termination, we did not find any reductions in the probe-evoked P3 amplitudes during the repeated terminations. This may indicate that later terminations did not require any reallocation of attentional resources. Instead, the motor avoidance responses might have been evoked rather automatically by the dyspnea signaling the upcoming occlusion not affecting attention to the acoustic probe stimuli. It is to note that formation of habit-like avoidance behavior not only evolve from extended training or repetitions of responses but might also result from dysfunctional goal-directed control over actions (Gillan et al., 2014; Gillan et al., 2015). In fact, this failure in goal-directed control may show early during avoidance learning as observed in the present study (Gillan et al., 2015).

The present data are also in line with cognitive accounts of avoidance learning supporting the view that less cortical processing resources are captured by avoidance performances once propositional knowledge about the relationship between the avoidance performance and the omission of an expected breathing occlusion is acquired (Lovibond, 2006). However, the rapid shift in attentional and startle response pattern might be mediated by decision-making processes. The decision to terminate exposure might initially depend on the appraisal of the increasing interoceptive threat which requires more cortical capacity for processing of the interoceptive threat cues. Once the decision was made to terminate subsequent exposures, the occurrence of the ultimate threat, i.e., a possible suffocation, would be definitively prevented. Thus, the appraisal and processing of interoceptive cues was no longer necessary. Moreover, as startle inhibition in rodents was only evident during cues associated with highly intense shocks (Walker et al., 1997; Walker and Davis, 1997) the observed startle inhibition might have disappeared once participants terminated the exposure sequence at lower threat intensities during repeated defensive actions.

4.3. Avoidance and self-reported anxiety

Although participants exhibited alleviated physiological arousal the retrospectively reported anxiety increased prior to repeated terminations and the overall anxiety level even increased upon subsequent terminations. An increase in the reported intensity of anxiety was also observed within and across matched control intervals of matched control persons. Thus, in both groups reported anxiety seemed to be associated with the expectations and therefore more related to the central concerns about what could ultimately happen than to the physiological arousal symptoms. This would explain why verbal reports and physiological or behavioral indices of fear are often discordant (Rachman and Hodgson, 1974; see Cook et al., 1988). From the viewpoint of a two-system framework of fear, it has been proposed that defensive responding and subjective feelings of fear and anxiety in the presence of threat were mediated by two different neural circuits (LeDoux and Pine, 2016). Thus, it becomes clear that defensive responding and reported feelings of fear diverge in the face of threat (LeDoux and Pine, 2016). Interestingly, the maximal reported anxiety was higher during initial termination trials compared to during control intervals in matched control persons.

4.4. Implications for exposure-based therapy

Exposure-based therapies aim at disconfirming patients' central concerns about the expected threat and establishing a new inhibitory learning association (e.g., dyspnea does not necessarily predict imminent suffocation) (Craske et al., 2006; Vervliet et al., 2013; Craske et al., 2014). This inhibitory learning process might be impaired by habit-like avoidance behaviors (Craske et al., 2008; Lovibond et al., 2009; Vervliet and Indekeu, 2015). However, it has been reported that the elimination of avoidance behavior is challenging in exposure therapy (Helbig-Lang and Petermann, 2010; Vervliet and Indekeu, 2015). Response prevention is typically recommended as an effective technique to mitigate avoidance behavior. However, avoidance behavior and threat beliefs often return after response prevention in the context of fear extinction training (Lange, 2016; Rodriguez-Romaguera et al., 2016; Lovibond et al., 2009; Bravo-Rivera et al., 2015; Vervliet and Indekeu, 2015).

For optimizing exposure the expectancy model of avoidance suggests to alleviate anxiety and avoidance by reducing the subjectively perceived costs of harmful outcomes (reinforcer devaluation) (Lovibond, 2006). However, since avoidance behaviors can be resistant to extinction, insensitive to reinforcer devaluation, inflexible and performed automatically (Gillan et al., 2016b; Gillan et al., 2016c; Wood and Runger, 2016), it might be helpful to initiate a shift from habit-like avoidance performances to goal-directed control (Quinn et al., 2010; Wood and Runger, 2016). As a first step, it could be useful to identify idiosyncratic habitual avoidance behaviors via behavioral experiments. To change habits, it has been demonstrated that the inhibition or control of habit responses might be facilitated by vigilant monitoring of responses (Quinn et al., 2010), forming implementation intentions (if-then plans) (Gollwitzer, 1999; Karsdorp et al., 2016), contexts change (Thrailkill and Bouton, 2015) or enacting actions oppositional to patients' fear action tendencies (Wolitzky and Telch, 2009). Implementing these techniques in exposure-based therapy might alleviate habit-based avoidance behaviors which in turn may facilitate inhibitory learning and prevent relapses. Of course, the effectiveness of the use of these interventions in exposures-based therapy warrants experimental proof.

4.5. Limitations

The current study aimed at examining dynamical changes of defensive response mobilization during repeated defensive action. Importantly, participants were not instructed to terminate exposures of increasing interoceptive threat but spontaneously expressed this response pattern. As a consequence, the presented analyses are based on a self-selected population, i.e., avoidance behavior was not experimentally varied and randomized within and across participants. This limits the interpretation of the data and thus the generalization of the results

in understanding the processes involved in avoidance learning. In future research, an experimentally-controlled design would help to resolve these limitations. Although defensive response mobilization changed even after a very few terminations, future research should consider to include more exposure trials to specifically investigate the formation of avoidance behavior. In contrast to previous studies investigating avoidance learning with exteroceptive stimuli (e.g., pictures, shocks), aversive interoceptive stimuli, i.e. different intensities of dyspnea, were used in the current experiment. Thus, the observed effects in the present study might be specific for avoidance learning by interoceptive threat and may not be generalized to avoidance behavior in the context of an exteroceptive threat. Future research should include both interoceptive and exteroceptive threats to reveal possible differences in defensive response mobilization during avoidance learning. In the current study, participants were matched for trait anxiety and thus did not differ in measured anxiety-related questionnaire data. However, it has been shown that individual differences including compulsive behavior and intrusive thoughts, neuroticism or distress tolerance affect avoidance learning or habit formation which might be responsible for avoidance behavior or the different response profiles in avoiders and matched controls in the present study (Lommen et al., 2010; Krypotos et al., 2015; Gillan et al., 2016a; Vervliet et al., 2017). Thus, future studies ought to include these specific factors to reveal possible individual differences.

5. Conclusion

In the present study, we observed naturally occurring repetitive terminations of exposure to increasing interoceptive threat in humans. The study design proved useful to assess changes in defensive response patterns preceding repeated terminations of threat. In contrast to commonly used paradigms investigating avoidance, the present results did not originate from a classical avoidance conditioning task with consecutive instructed avoidance but rather resulted from naturally occurring terminations in an ecologically valid and translational paradigm. In contrast to the initial defensive action which might be conceptualized as an escape response, subsequent defensive actions were performed at lower threat levels to prevent the occurrence of the ultimate threat. Persistent defensive actions were characterized by the absence of physiological arousal, elevated anxious apprehension and in comparison to the initial defensive action an increased availability of cortical processing capacity. The data support the view that fear might not be a primary motivator of avoidance behavior. In contrast, the present results support the view that avoidance behavior might be better conceived as a habit like behavior that, however, prevents violation of contingency expectancies that is important for extinction learning to occur. Further research should focus on the underlying mechanisms and the factors that possibly set the stage for developing habitual avoidance behavior. To foster our understanding of these processes and mechanisms the results need to be replicated in a larger sample as well as in patients with mental disorders (e.g., anxiety or pain disorder). We proposed techniques that might facilitate extinction of habitual avoidance, thus augmenting the effects of exposure-based therapy. However, the impact of these techniques in exposure therapy warrants experimental proof.

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The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpsycho.2017.09.013.

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Appendix D: List of Publications

Krause, E., **Benke, C.**, Koenig, J., Thayer, J. F., Hamm, A. O., & Pané-Farré, C. A. (under review). Dynamics of defensive response mobilization to approaching external vs. interoceptive threat.

Benke, C., Alius, M. G., Hamm, A. O., & Pané-Farré, C. A. (in revision). Cue and context conditioning to respiratory threat: Effects of suffocation fear and implications for the etiology of panic disorder.

Benke, C., Krause, E., Hamm, A. O., & Pané-Farré, C. A. (2017). Dynamics of defensive response mobilization during repeated terminations of exposure to increasing interoceptive threat. *Int J Psychophysiol.* doi: 10.1016/j.ijpsycho.2017.09.013

Benke, C., Hamm, A. O., & Pané-Farré, C. A. (2017). When dyspnea gets worse: Suffocation fear and the dynamics of defensive respiratory responses to increasing interoceptive threat. *Psychophysiology*, *43*(1), 174. doi: 10.1111/psyp.12881

Benke, C., Blumenthal, T., Modeß, C., Hamm, A., & Pané-Farré, C. (2015). Effects of anxiety sensitivity and expectations on the modulation of the startle eyeblink response during a caffeine challenge. *Psychopharmacology*, *232*(18), 3403–3416. doi: 10.1007/s00213-015-3996-9

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