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Attentive immobility in the face of inevitable distal threat—Startle potentiation and fear bradycardia as an index of emotion and attention

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Abstract

During fear conditioning, a cue (CS) signals an inevitable distal threat (US) and evokes a conditioned response that can be described as attentive immobility (freezing). The organism remains motionless and monitors the source of danger while startle responses are potentiated, indicating a state of defensive hypervigilance. Although in animals vagally mediated fear bradycardia is also reliably observed under such circumstances, results are mixed in human fear conditioning. Using a single-cue fear conditioning and extinction protocol, we tested cardiac reactivity and startle potentiation indexing low-level defensive strategies in a fear-conditioned ($n = 40$; paired presentations of CS and US) compared with a non-conditioned control group ($n = 40$; unpaired presentations of CS and US). Additionally, we assessed shock expectancy ratings on a trial-by-trial basis indexing declarative knowledge of the previous contingencies. Half of each group underwent extinction under sham or active transcutaneous vagus nerve stimulation (tVNS), serving as additional proof of concept. We found stronger cardiac deceleration during CS presentation in the fear learning relative to the control group. This learned fear bradycardia was positively correlated with conditioned startle potentiation but not with declarative knowledge of CS-US contingencies. TVNS abolished differences in heart rate changes between both groups and removed the significant correlation between late cardiac deceleration and startle potentiation in the fear learning group. Results suggest, fear-conditioned cues evoke attentive immobility in humans, characterized by cardiac deceleration and startle potentiation. Such defensive response pattern is elicited by cues predicting inevitable distal threat and resembles conditioned fear responses observed in rodents.

KEYWORDS

attentive immobility (freezing), extinction, fear bradycardia, fear conditioning, startle potentiation, transcutaneous vagus nerve stimulation

1 | INTRODUCTION

Fear can be conceived as an action disposition evoked by threat-related stimuli that activates behavioral defensive strategies to ensure the organism's survival (Hamm & Flor, 2015; Lang, 1995; Lang & Bradley, 2010). These defensive strategies can broadly be separated into defensive anticipation and immobility (*freezing*) and defensive action (e.g., active avoidance or attack; Hamm, 2020; Lang & Bradley, 2010; Marks, 1987) and are dynamically executed depending upon the imminence of the threat and the available behavioral options of the organism (e.g., chance of threat avoidance), providing flexible adaptation to the situation and, hence, increased probability of survival (Fanselow, 1994; Hamm, 2020; Lang et al., 1997, 2000; Marks, 1987; Mobbs et al., 2020).

As soon as a cue signaling a possible upcoming threat is detected, the organism is engaged in a fear-related state of attentive immobility or freezing (Eilam, 2005; Hamm, 2020; Lang et al., 1997; Marks, 1987; Roelofs, 2017). Such attentive immobility is defined by increased selective attention toward the threat-signaling cue, inhibited locomotion, a tense body posture, and potentiation of the protective startle reflex (Blanchard & Blanchard, 1969; Eilam, 2005; Fanselow, 1984; Gewirtz et al., 1997; Kalin & Shelton, 1989; Kolassa et al., 2005; Leaton & Borszcz, 1985).

Animal research shows that a wide variety of species also responds with a profound phasic deceleration of the heart rate when facing such distal threat, a phenomenon for which comparative psychophysiologists have coined the term *fear bradycardia* (Campbell et al., 1997). Supporting this view, animal fear conditioning studies showed strong and positive correlations between behavioral freezing and both prolonged heart rate deceleration (Walker & Carrive, 2003) and startle potentiation (Gewirtz et al., 1997; Leaton & Borszcz, 1985). Moreover, rodent research showed that cardiac deceleration in response to threat-signaling cues is mediated by similar underlying neural substrates, that also modulate threat-related startle potentiation and behavioral freezing, involving the central nucleus of the amygdala (CeA) and its projections to the ventrolateral periaqueductal gray (vlPAG; Applegate et al., 1983; Choi & Brown, 2003; Davis, 2006; Fendt & Fanselow, 1999; LeDoux et al., 1988; Walker & Carrive, 2003).

In human psychophysiological research, heart rate deceleration has been traditionally interpreted as an index of increased orienting toward significant stimuli that carry information (Graham, 1979). Accordingly and contrary to animal research, strong cardiac decelerations elicited by a conditioned stimulus (CS) signaling the occurrence of a threat (unconditioned stimulus, US) in early human fear conditioning experiments have been interpreted as an inhibition of the habituation of the orienting reflex (Geer, 1964; Putnam et al., 1974), rather than reflecting a fear response. In fact, although in human fear conditioning studies startle responses were found to be reliably potentiated (Grillon & Davis, 1997; Hamm et al., 1993; Lipp et al., 1994, for a review see Hamm, 2015), heart rate changes have shown to vary as a function of CS-content, US-intensity, and individual response patterns, with cardiac deceleration being observed more commonly with neutral CSs, whereas cardiac acceleration was associated with fear relevant CSs and more intense USs (Dimberg, 1987; Hamm et al., 1993; Hamm & Vaitl, 1996; Hodes et al., 1985; Lipp & Vaitl, 1990; Moratti & Keil, 2005; see Lonsdorf et al., 2017 for a review).

However, by relating stimulus significance to motivational systems that serve survival functions, Bradley (2009) presented evidence that prolonged parasympathetically dominated cardiac deceleration consistently occurs during states involving increased perceptual effort, engaged during selective attention toward motivationally significant stimuli. As this is the case during monitoring sources of inevitable danger, Bradley (2009), thus, provided a link between orienting and fear (see also Bradley et al., 2018). Supporting this view, recent research found that heart rate changes in the face of a threat in fact critically vary depending upon the behavioral options at hand, along with the actually executed defensive strategy (see Krause et al., 2018; Löw et al., 2015). As demonstrated in these two studies, there was strong cardiac deceleration as well as startle potentiation, if there was no option to actively avoid an approaching threat (moderately painful stimulus; or forced breath holding), and both measures were strongest immediately prior to the delivery of the aversive stimulus, while cardiac acceleration and startle inhibition was found when the organism was beyond vigilance and engaged in vigorous defensive action (Krause et al., 2018; Lang & Davis, 2006; Lang et al., 2000; Löw et al., 2015). These data were supported by findings of Roelofs and coworkers, showing heart rate decrease during different inevitable threat conditions including fear conditioning. In these studies heart rate deceleration was associated with reduced locomotion as measured by postural sway on a stabilometric platform, supporting the view that defensive responses acquired in human fear conditioning studies might be instances of attentive freezing (Gladwin et al., 2016; Roelofs, 2017; Roelofs et al., 2010). Accordingly, recent imaging research indicated that similar neural mechanisms that underlie behavioral freezing and cardiac deceleration in rodents also apply to humans during processing of distal inevitable threats (Wendt et al., 2017).

The current study follows up on this research and aims to provide an analysis between cardiac reactivity and startle modulation during human fear conditioning. We strived for harmonizing cross-species methodology (see Haaker et al., 2019 for a detailed discussion), by applying a multiple-day single-cue fear conditioning and extinction protocol, closely adapted to animal research (see Peña et al., 2013, 2014, but also Wong & Lovibond, 2017, 2018). Such paradigm involves between-subject comparisons of conditioned responses between a fear learning group, receiving repeated presentations of a CS paired with an aversive US during an acquisition training,

TABLE 1 Demographics and body-mass-index for the experimental groups

	Fear learning group		Control group	
	Sham	**tVNS**	**Sham**	**tVNS**
N (female/male)	20 (15/5)	20 (16/4)	20 (12/8)	20 (14/6)
Age (years)	23.75 (*SD* = 3.34)	23.30 (*SD* = 4.22)	22.05 (*SD* = 3.20)	21.90 (*SD* = 2.83)
Body-Mass-Index (kg/m^2)	21.65 (*SD* = 1.89)	22.30 (*SD* = 2.00)	21.65 (*SD* = 1.89)	22.58 (*SD* = 2.24)

and a control group, receiving explicitly unpaired presentations of both stimuli (Lonsdorf et al., 2017; Rescorla, 1967). Moreover, we expanded the extinction period to investigate the extinction of defensive responding in more detail.

We hypothesized, that human laboratory participants of the fear learning group would show stronger cardiac deceleration during the presentation of a conditioned stimulus compared with controls, as they are suggested to function at a stage of attentive immobility with easy escape blocked (e.g., by social compliance; Lang et al., 2000). More specifically, we presumed that such fear bradycardia is primarily expressed in stronger *prolonged* cardiac deceleration late during the CS presentation, which is suggested to reflect increased sensory intake or stimulus anticipation (e.g., an aversive US; Hodes et al., 1985), but not in early cardiac deceleration, which has been viewed as a transient detecting response indexing stimulus registration (Bradley, 2009; Graham, 1987; Hodes et al., 1985). Moreover, such prolonged fear bradycardia was expected to be significantly correlated to behavioral low-level correlates of attentive freezing, that is being related to increased potentiation of the startle reflex. Importantly, as primitive thalamic projections to the amygdala are particularly involved in the expression of fear during single-cue conditioning protocols, we expected that this correlation is stronger than the association between bradycardia and declarative knowledge of CS-US contingency, which has been suggested to require higher order cortical involvement (for a review see LeDoux, 1995). Additionally, defensive responding and, thus, freezing has shown to decrease during an extinction training, presumably due to reduction of CeA activity by inhibitory projections from the ventromedial prefrontal cortex (vmPFC) and the basolateral amygdala (BLA; Amano et al., 2010; Ehrlich et al., 2009; Gewirtz et al., 1997; Milad & Quirk, 2012). Thus, we hypothesized that late cardiac deceleration would extinguish in the fear learning group. Animal research has indicated that the stimulation of the vagus nerve may facilitate such extinction, possibly due to increasing noradrenergic activation of the BLA and vmPFC by way of its afferent projections to the locus coeruleus noradrenergic system (Mueller & Cahill, 2010; Peña et al., 2013, 2014). Transcutaneous vagus nerve stimulation (tVNS), involving the non-invasive stimulation of the exclusively vagally innervated left cymba conchae, is presumed to lead to an activation of afferent fibers of the left auricular vagus nerve and has shown to similarly increase activity in both the amygdala and vmPFC, correspondingly resulting in promoted fear extinction in humans when applied during extinction training (Burger et al., 2016, 2017, 2018; Frangos et al., 2015; Peuker & Filler, 2002; Szeska et al., 2020). Thus, we used transcutaneous vagus nerve stimulation as an additional proof of concept in our paradigm and expected that tVNS would promote the extinction of prolonged cardiac deceleration during extinction training by facilitating inhibition of human neural freezing circuitry. Importantly, attentive immobility as well as fear bradycardia are suggested to be primarily parasympathetically dominated defensive responses (Campbell et al., 1997; Roelofs, 2017). Thus, we hypothesized that a potential tVNS-induced attenuation of cardiac deceleration would be driven by an inhibition of parasympathetic control of the heart, rather than by an increase in sympathetic nervous activity, as indexed by the skin conductance level (SCL).

2 | METHOD

2.1 | Participants

The study included 80 participants, primarily students of the University of Greifswald (*M* = 22.75, *range* = 18 to 34 years; 57 women; see Table 1 for further information). During a phone interview, all participants reported to be in the desired age range (18–35 years), to have a body-mass-index in normal range (18.5 kg/m^2 to 27 kg/m^2), and to be free from any previous or current medical or mental condition, which would have been associated with an affection of any of the outcome variables or would have contraindicated the use of tVNS (i.e., cochlear implants or pregnancy, checked by a pregnancy test). The sample and data set is the same as has been reported by Szeska et al., (2020). Each participant gave her/his informed consent and received either monetary reward (34 €) or partial course credits. The study was approved by the ethical committee of the German Society for Psychology ("Deutsche Gesellschaft für Psychologie; DGPs").

2.2 | Stimulus materials

Figure 1a gives an overview of the used stimulus materials. All visual stimuli were presented on a 24-inch computer monitor

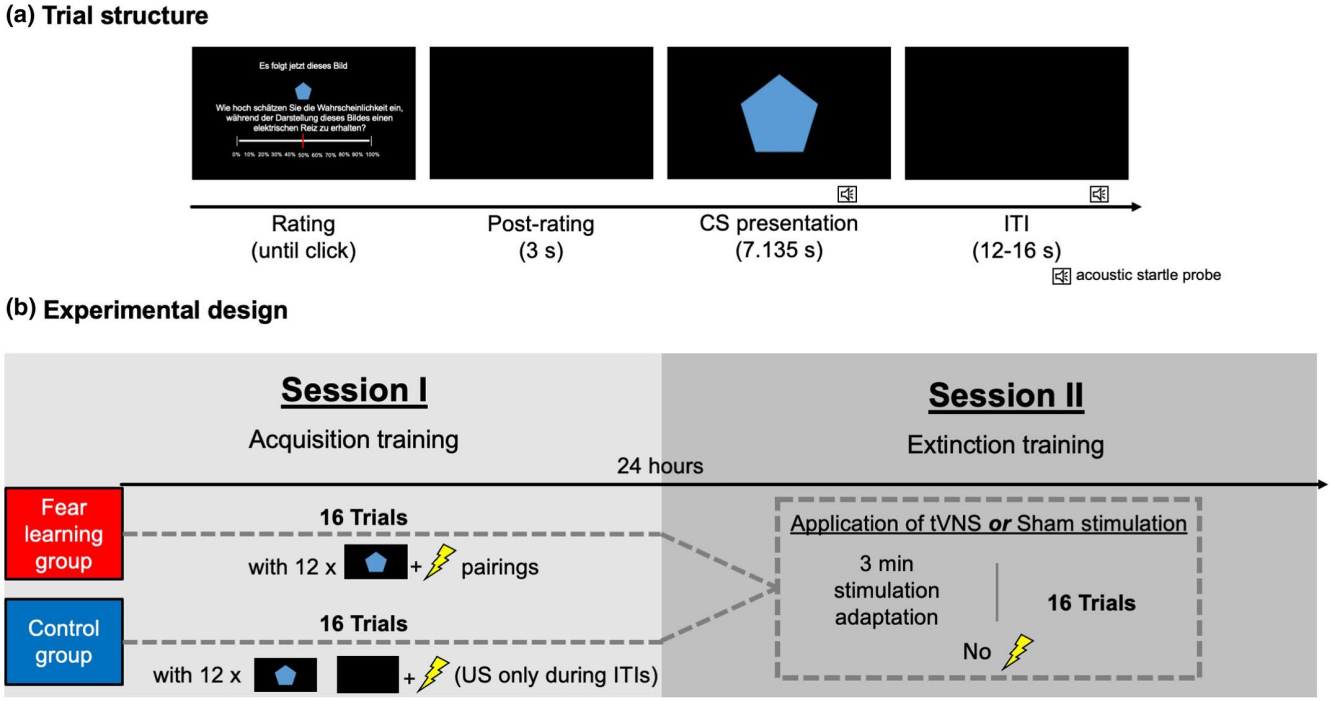

FIGURE 1 (a) Trial structure during the single-cue fear conditioning and extinction paradigm. Each trial began with a CS-US contingency rating, where the CS (blue pentagon) was previewed in smaller size, and participants were instructed to rate the probability, that this cue would be followed by the US during the upcoming CS presentation in full size (English translation of the German instruction: "Next, this picture will follow. How likely do you think is it to receive an electrical shock during the upcoming presentation of this picture?"). Three seconds after completing the rating, the cue was presented in full size on the screen, ensuring that physiological fear responses were not affected by any parallel cognitive evaluation task. (b) Schematic presentation of the analyzed experimental sessions. The acquisition and extinction training consisted of 16 trials each. The fear learning group received paired presentations of the CS and US in 12 of the 16 trials (75% CS-US contingency) during acquisition (i.e., four CS presentations without US), whereas individuals of the control group received 16 presentations of the CS and 12 shocks during the inter-trial interval (ITI) so that CS and US were explicitly unpaired (0% CS-US contingency). Extinction started with a 3 min adaption period to the stimulation device (for both sham stimulation and tVNS, respectively). During extinction, 16 CSs were presented in both groups without any US. Half of the fear learning and control group underwent the extinction training under the influence of tVNS, whereas the other half received a sham stimulation of the earlobe. Throughout each experimental session, acoustic startle probes were presented during the presentations of the CS and during the ITIs

(1,024 × 768 pixel resolution) 1.45 m in front of the participant. The CS was a blue pentagon on a black background, which was displayed for 7.135 s, whereas a black screen, presented for 12, 14, or 16 s ($M = 14$ s), served as inter-trial interval (ITI).

An unpleasant, individually adjusted electrical shock with a duration of 625 ms, consisting of 125 single pulses, each with a duration 2 ms and a 3 ms break between pulses was applied by an S-48K stimulator (Grass instruments, West Warwick, RI, USA) and was used as US. Importantly, there was no significant difference between the tVNS and sham condition in adjusted US intensity ($M_{tVNS} = 3.41$ mA, $SD = 1.53$; $M_{sham} = 3.44$ mA, $SD = 1.47$; Stimulation and Stimulation × Group, all $Fs < 1.09$, all $ps > .30$).

A binaurally presented 95 dB(A) burst of white noise with a duration of 50 ms and an instant rise/fall time (<1 ms), presented by AKG K66 headphones, was used as acoustic startle probe to elicit the startle eyeblink response.

The device for transcutaneous vagus nerve stimulation (CMO2, Cerbomed, Erlangen, Germany) was applied during session 2 (extinction training) at the left auricle with two titanium electrodes positioned in either of two locations: In the tVNS condition, the electrodes were positioned at the cymba conchae, which is exclusively innervated by the auricular branch of the vagus nerve (ABVN), whereas in the sham condition, electrodes were placed in the center of the earlobe, which is free of vagal innervation because of being innervated by the great auricular nerve (GAN; Peuker & Filler, 2002). Electrical stimulation was delivered during the stimulation adaptation period (3 min), as well as throughout the following extinction training (session 2; approximately 10 min) with a pulse width of 200–300 μs at a rate of 25 Hz, applying a 30 s ON and 30 s OFF procedure. Ensuring the activation of either the ABVN or GAN, participants were required to individually adjust the stimulation intensity at the beginning of session 2 to be clearly perceivable, but below the pain

threshold (see also 2.3.2). Importantly, the mean stimulation intensity did not differ between the tVNS and sham condition ($M_{tVNS} = 2.28$ mA, $SD = 1.13$; $M_{sham} = 2.53$ mA, $SD = 1.11$; stimulation and stimulation × group, all Fs < 1.31, all ps > .25).

2.3 | Experimental design and procedure

Figure 1 provides a linear depiction of the trial structure (a), the experimental design and procedure (b). We used a 2 × 2 between-subject design to test our hypotheses, with Group (fear learning vs. control group) and Stimulation (tVNS vs. sham stimulation) as between-subject factors. Consequently, eligible participants were allocated to one of four conditions: a fear learning group receiving tVNS ($n = 20$), a fear learning group receiving sham stimulation ($n = 20$), a control group receiving tVNS ($n = 20$), and a control group receiving sham stimulation ($n = 20$). The allocation to either of the four conditions was randomized and single-blind sham controlled.

Participants were seated in a dimly lit, sound-attenuated room during each experimental session. Sensors for physiological recording as well as the electrodes to deliver the electrical shock at the non-dominant hand's wrist were attached prior to any experimental manipulation. Each session began with a startle habituation phase, during which six acoustic startle probes were presented (inter-stimulus intervals of 7, 9, 10, 6, and 8 s; $M = 8$ s; duration: 84 s), ensuring the adaptation of startle magnitudes to a stable baseline.

2.3.1 | Acquisition training (session 1)

Prior to acquisition training, participants underwent a shock workup, during which the experimenter individually adjusted the US intensity following a standardized protocol to a level, which the participant perceived as clearly unpleasant but not painful. The workup consisted of a number of sample shocks, starting at an intensity of 2.0 mA. After each shock administration, participants were asked to rate the shock intensity on a continuous 5-point visual analog scale, ranging from "1 (not painful/annoying)" to "5 (very painful/annoying)." After each rating, the shock intensity was increased to finally achieve an intensity that was rated as "4 (unpleasant/quite annoying)." As soon as the shock was rated as "4 (unpleasant/quite annoying)," the shock workup was terminated and the respective shock intensity was used for the experiment (see also Klumpers et al., 2010).

After the shock workup and just before the acquisition training, all participants were instructed, that the CS, the US and acoustic startle probes may be presented at any time, with no explicit information given with regard to the CS-US contingencies.

During the acquisition training, all participants received 16 presentations of the CS. In the fear learning group, the CS was paired with the aversive US in 12 of the 16 trials (6.5 s after CS onset; 75% CS-US contingency) to induce a reliable and robust conditioned fear response while increasing its resistance to extinction. By contrast, the control group received explicitly unpaired presentations of CS and US, which was delivered 12 times only during the inter-trial intervals (3, 4, 5, 6, 7, 8, 11, or 12 s after ITI onset, $M = 6.98$ s; 0% CS-US contingency). Startle probes were delivered during the CS in 12 out of 16 trials for both groups either at 4.5, 5 or 5.5 s (four trials for each probe time) after the CS onset arranged in eight experimental orders, participants were randomly assigned to. Moreover, 12 startle probes were presented during the inter-trial intervals (ITIs). By using a minimum interval of 1 s between startle probe and US onset, we ensured that the presentation of both stimuli was not confounded in any of the experimental groups.

2.3.2 | Extinction training (session 2)

The second experimental session took place 24 ± 4 hr after the acquisition training. After the electrodes for physiological assessment and US-application were refitted, the tVNS/sham stimulation device was positioned at the participants' left ear in the desired location and a tVNS/sham stimulation workup began, where participants were instructed to set the stimulation intensity to be clearly perceivable, but without being painful. Following the same protocol as Ventura-Bort and colleagues (2018), adjustment started at an intensity of 0.1 mA and after each up- or down-adjustment of 0.1 mA participants were asked to rate their subjective sensation of the stimulation intensity on a visual 11-point scale, ranging from "nothing (0)," "light tingling (3)," "strong tingling (6)" to "painful (10)". The workup lasted until a "strong tingling" sensation of 8 was reported by the participant, after which a full 30 s ON and 30 s OFF stimulation protocol was run in order to provide an experience of the stimulation, as it would be during the extinction training (see also Ventura-Bort et al., 2018). Only if the participants still rated the sensation as 8 after the protocol, the adjusted stimulation intensity would be used for the extinction training—otherwise the workup went on until that point was reached.

Subsequently, participants were informed that the upcoming second experimental session would begin with a 3-min period to adapt to the stimulation (either tVNS or sham). No other stimuli (startle probes or CSs) were presented during this period. Participants were further instructed that after the adaptation period any of the stimuli might be presented, that have also been presented during session 1. Again, no explicit information was given with regard to the CS-US contingencies. During extinction

training, the CS was presented 16 times without any US. Presentation of acoustic startle probes was similar to order of the acquisition training.

2.4 | Assessments and data reduction

2.4.1 | Electrocardiogram (ECG; Heart rate)

The ECG was measured using an Einthoven Lead II setup with two electrolyte filled (Marquette Hellige, Freiburg, Germany) standard Ag/AgCl electrodes (8 mm diameter). Using a Coulbourn system, the raw signal was filtered with an 8–13 Hz band-pass filter and amplified by the factor 2000. ECG data were digitally sampled at 400 Hz and artifact corrected using ANSLAB (v. 2.4; Autonomic Nervous System Laboratory, University of Basel, Switzerland), and subsequently converted to heart rate in beats per minute for every half-second of the sampling period (Graham, 1978). Finally, allowing to quantify baseline-independent cardiac responding during the CS, heart rate during the CS was subtracted from base period heart rate (mean of the first two half-seconds after CS onset) for every half-second after the CS onset for the full duration of CS presentation (14 data points for the 7.135 s CS duration). These half-second bins were averaged across all trials for each experimental session and additionally for each half of the extinction training to analyze the time course of extinction learning.

As conditioned cardiac responses have shown to follow a triphasic course, we additionally identified average peaks of early cardiac deceleration (*D1*, slowest half-second between 1 and 2 s after CS onset), acceleration (*A1*; fastest half-second between 2 and 5 s after CS onset), and late cardiac deceleration (*D2*; slowest half-second between 5 and 7 s after CS onset) for each experimental session adapted from the rules of Gatchel and Lang (1973). Average peaks of the heart rate responses are expressed in beats per minute change scores, deviated from the base period (Δ bpm).

2.4.2 | Electromyography (EMG; Startle eyeblink response)

We measured the eyeblink component of the startle response, elicited by the acoustic startle probe, by recording the electromyographic activity of the orbicularis oculi muscle underneath the left eye by using two electrolyte filled (Marquette Hellige, Freiburg, Germany) Ag/AgCl miniature surface electrodes (3 mm diameter, Sensormedic, Yorba Linda, CA, USA), which were attached on the skin over the muscle. The EMG signal was amplified by a Coulbourn S75-01 amplifier and filtered with a 30 Hz high-pass and a Kemo LEM-VBf8-03 400 Hz low-pass filter (smoothing the rectified signal with a time constant of 10 ms). Moreover, a notch filter (50 Hz) was used. The signal was digitally sampled at a rate of 1,000 Hz between 100 ms before and 400 ms after the startle probe onset. Startle eyeblink responses were scored semi-automatically with a computer program, identifying blink onset and peak amplitude (Globisch et al., 1993). Each detected startle eyeblink response was additionally visually inspected for artifacts (Blumenthal et al., 2005) and manually corrected if necessary. Only blinks were scored as valid startle responses, which started 20–120 ms after the startle probe onset and peaked within 150 ms, with a minimum amplitude of 1.954 μV. If no blink was detected, the trials were scored as zero responses. Based on previously published guidelines, we set trials as missing if clear movement artifacts, excessive baseline activity, or artifacts due to tVNS/sham stimulation were found (Blumenthal et al., 2005). For acquisition training (session 1), 0.5% were scored as zero responses ($M = 0.15$), and 2.2% of all probed trials were set as missing ($M = 0.66$). For the extinction training (session 2), 0.3% of all probed trials were scored as zero responses ($M = 0.09$), and 31.2% were set as missing ($M = 9.38$; higher rate of missings due to tVNS/sham stimulation-induced noise). After scoring, raw blink magnitudes were *z*-transformed and finally *T*-standardized ($50 + (z \times 10)$) individually for each participant to control for individual differences in overall startle magnitude. Finally, we computed the mean startle potentiation (difference of *T*-transformed CS startle and *T*-transformed ITI startle magnitude) for each experimental session.

2.4.3 | Shock expectancy ratings

Before each CS presentation, participants were required to rate their expectancy to receive an US during the upcoming CS on a continuous 11-point visual analog scale (ranging from "0%" to "100%") by shifting a red cursor and pushing the left mouse button (see Figure 1a). During this rating, the CS was presented in smaller size above the line rating. This procedure is very much comparable to clinical practice during exposure-based treatments, during which patients are asked to rate the likelihood, that their central concern might become true (e.g., fainting), before the exposure exercise begins (see Hollandt et al., 2020). There was no time restriction for completing the rating. After the rating was completed, a three second post-rating period (black screen) followed. After the post-rating period, the CS was displayed in full size on the screen. Thus, we ensured that physiological responses evoked by the CS were not affected by a parallel cognitive evaluation task. Equivalently to the startle responses, we computed the mean shock-expectancy rating separately for each experimental session as an index of declarative knowledge of CS-US contingency.

2.4.4 | Skin conductance level

The SCL was measured from the hypothenar eminence of the palmar surface of the participant's non-dominant hand to provide an index of sympathetic nervous activity. Two Ag/AgCl electrodes (8 mm diameter) were filled with a 0.05 M sodium chloride electrolyte medium before attachment. The signal was amplified by a Coulbourn S71-22 skin conductance coupler, which provided a constant current of 0.5 V across the two electrodes, sampled at a rate of 10 Hz and processed with a resolution of 0.01 μS. SCL for every half-second after the CS onset for the full CS duration (14 points for analysis during the 7.135 s stimulus presentation) was subtracted from the base period SCL (mean of the first two half-seconds after CS onset) and averaged across all trials for the extinction training, thus using the same scoring procedure as for heart rate.

2.4.5 | Statistical analyses and Figure creation

We analyzed the course of conditioned cardiac responses during each session (acquisition training and extinction training) using linear mixed models with only fixed effects included (see Bagiella et al., 2000; Duricki et al., 2016). On the one hand, such linear mixed regression models advantageously also include participants with missing values, whereas on the other hand it is possible to model the error covariance structure in a way that best fits the data, providing higher statistical power of analysis (Bagiella et al., 2000; Duricki et al., 2016). We created all linear mixed regression models using restricted maximum likelihood estimation, to include all available data (Duricki et al., 2016), and modeled the error covariance structure of the repeated measurements by specifying a first-order autoregressive covariance structure with heterogeneous variances (ARH1). This type of covariance structure was chosen because it provided the best fit to the sample data according to Akaike's information criterion while being parsimonious in parameter estimation, thus following recommendations of Duricki and colleagues (2016). *Group* (fear learning vs. control group) and *Stimulation* (tVNS vs. sham stimulation) served as between-subject factors, and *Time* (14 half-second bins during the CS presentation) served as within-subject factor. Moreover, to specifically examine the impact of the extinction training on cardiac responding, we compared cardiac waveforms during the first half with cardiac waveforms during the second half of the extinction training, by additionally including the within-subject factor *Half* into analyses.

If significant differences between average, heart rate curves were found across groups during acquisition or extinction, univariate analyses of variances were carried out, analyzing between-subject differences between average peaks of cardiac decelerative (*D1* and *D2*) and accelerative response components (*A1*) of phasic heart rate changes during each session with *Group* and *Stimulation* as between-subject factors. In a second step, we compared cardiac peak component scores between the first and second half of extinction to analyze the impact of the extinction in more detail. We used the linear mixed regression models as described above, with *Group* and *Stimulation* as between-subject factors and *Half (first vs. second)* as within-subject factor. To examine the potential influence of startle probes on late cardiac deceleration (*D2*) during acquisition and extinction (probed vs. non-probed trials), we did additional analyses including the within-subject factor *Probe (probed vs. non-probed)*.

Moreover, we computed Spearman rank correlations between cardiac response components and mean startle potentiation as well as mean shock expectancy ratings for each experimental session to evaluate the association between heart rate changes, startle potentiation, and CS-US shock expectancy ratings. In a second step, we tested whether these correlations were significantly different following the procedure recommended by Meng and colleagues (Meng et al., 1992). We also calculated correlations during extinction for each of the four experimental groups to assess the influence of the stimulation conditions and the learning experience on the correlational pattern (see Eid et al., 2011 for the procedure used to compare independent correlations).

Changes in SCL during the extinction training were analyzed as an index of sympathetic nervous activity by using linear mixed regression models as described above, with *Group* (fear learning vs. control group) and *Stimulation* (tVNS vs. sham stimulation) as between-subject factors and *Time* (14 half-second bins) serving as within-subject factor.

Partial eta-squared was computed following recommendations by Lakens (2013). Bonferroni correction was applied, when relevant. All statistical analyses were conducted using IBM SPSS Statistics 25. Microsoft Excel and Microsoft PowerPoint were used for Figure creation.

3 | RESULTS

3.1 | Acquisition training

The overall heart rate response to the CS during acquisition training in the fear learning (red line) and the control group (blue line) is depicted in panel (a) of Figure 2. All participants showed a significant heart rate deceleration to the CS (Time, $F(13,320.25) = 19.58$, $p < .001$, $\eta^2_p = .44$; Figure 2a). However, a significant time by group interaction indicated stronger cardiac deceleration in the fear learning relative to the control group prior to the delivery of the US (Time × Group, $F(13,320.25) = 3.68$, $p < .001$, $\eta^2_p = .13$; significant group differences 6–7 s after CS onset). Correspondingly, although

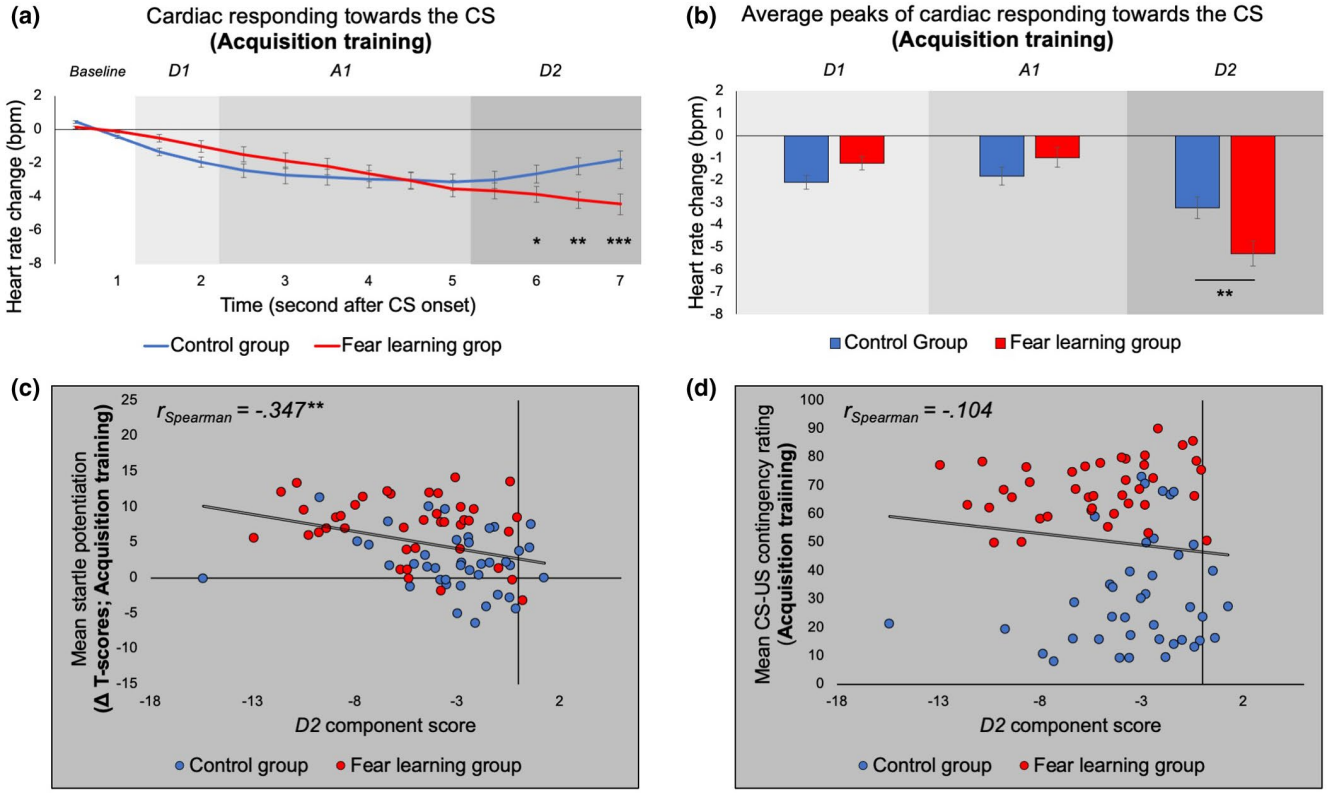

FIGURE 2 (a) Heart rate change after CS onset during the acquisition training for the fear learning (red line) and control group (blue line), averaged across all 16 acquisition trials, depicted in half-second bins. (b) Average peaks of cardiac response components during the acquisition training for the fear learning (red bars) and control group (blue bars). *D1* indicates the mean slowest half-second between 1 and 2 s after CS onset. *A1* indicates the mean fastest half-second between 2 and 5 s after CS onset. *D2* indicates the mean slowest half-second between 5 and 7 s after CS onset. (c) Scatter plot of mean startle potentiation (standardized [T-scores] startle magnitudes elicited during the CS minus standardized [T-scores] startle magnitudes elicited during the ITI, averaged across all probed trials) as a function of *D2* component score variation during the acquisition training for the fear learning (red dots) and control group (blue dots). (d) Scatter plot of mean CS-US contingency rating (averaged across all trials) as a function of *D2* component score variation during the acquisition training for the fear learning (red dots) and control group (blue dots). For all graphs: Time windows for the analyses of the average peaks of cardiac response components are depicted in different gray scales ranging from light (*D1*) to medium (*A1*) and dark gray (*D2*). Error bars, when depicted, represent standard error of the mean. Asterisks indicate statistical significance of correlations with $*p \leq .05$, $**p \leq .01$, and $***p < .001$

no significant differences were found between the fear learning and control group in early decelerative (*D1*: Group, $F(1,76) = 3.94$, $p = .051$; Figure 2b) or accelerative cardiac peak components (*A1*: Group, $F(1,76) = 1.95$, $p = .167$; Figure 2b), participants of the fear learning group showed increased *late* cardiac decelerative peaks (*D2*) compared with participants of the control group (Group, $F(1,76) = 7.66$, $p = .007$, $\eta^2_p = .09$; Figure 2b). Although cardiac deceleration was overall smaller in probed relative to the non-probed trials (Probe, $F(1,76) = 5.79$, $p = .018$, $\eta^2_p = .07$; Figure S1a), such effect was significantly smaller in the fear learning group (Probe × Group, $F(1,76) = 5.42$, $p = .023$, $\eta^2_p = .07$; Figure S1b). As predicted, overall correlation between late heart rate deceleration (*D2*) and fear potentiated startle was significant ($r_{Spearman}$ (80) = −.347, $p = .002$). By contrast, no significant overall correlation between cardiac deceleration and CS-US expectancy ratings was observed ($r_{Spearman}$ (80) = −.104, $p = .358$; Figure 2c,d). Further testing revealed that the correlation between late cardiac deceleration (*D2*) and startle potentiation was significantly stronger than the correlation between the *D2* component and CS-US contingency ratings ($z = -1.80$, p(one-tailed) = .036). By contrast, neither early decelerative (*D1*) nor accelerative cardiac peak responses (*A1*) were significantly associated with startle potentiation (*D1*: $r_{Spearman}$ (80) = .010, $p = .933$; *A1*: $r_{Spearman}$ (80) = −.091, $p = .420$) or CS-US expectancy ratings (*D1*: $r_{Spearman}$ (80) = .196, $p = .086$; *A1*: $r_{Spearman}$ (80) = .085, $p = .454$). As expected, no effects of stimulation were found during the acquisition training, as no stimulation was yet applied (all Fs < .66, all ps > .427).

3.2 | Extinction training

During the extinction training 24 hr later, all participants continued to show a significant heart rate deceleration in response

to the CS, indicating that the CS has acquired relevance to the participants (Time, $F(13,319.59) = 19.25$, $p < .001$, $\eta^2_p = .44$; Figure 3a,b). Yet, the fear learning group displayed significantly stronger heart rate deceleration relative to controls during the presentation of the conditioned stimulus, particularly during the late phase of the CS (Time × Group, $F(13,319.59) = 3.27$, $p < .001$, $\eta^2_p = .12$; Figure 3a,b). As expected, tVNS significantly attenuated prolonged cardiac deceleration but only in subjects of the fear learning group, whereas cardiac reactivity was unaffected by stimulation in controls (Group × Stimulation, $F(1,99.43) = 5.81$, $p = .018$, $\eta^2_p = .65$; Time × Group × Stimulation, $F(13,319.59) = 2.99$, $p < .001$, $\eta^2_p = .11$; Figure 3a,b).

In the sham condition, fear learning group participants showed significantly stronger overall cardiac deceleration relative to controls (Group, $F(1,48.87) = 16.33$, $p < .001$, $\eta^2_p = .25$; Figure 3a), again with strongest deceleration during the late phase of CS-processing (Time × Group, $F(13,97.45) = 4.32$, $p < .001$, $\eta^2_p = .37$; Figure 3a; significant group differences 2.5–7 s after CS onset). Accordingly, although both groups of the sham condition did not differ in early deceleration (*D1*; Group, $F(1,38) = 1.79$, $p = .189$; Figure 3c), the fear learning group displayed lower accelerative and stronger late decelerative peak responding relative to controls (*A1*: Group, $F(1,38) = 21.24$, $p < .001$, $\eta^2_p = .36$; *D2*: Group, $F(1,38) = 14.51$, $p < .001$, $\eta^2_p = .28$; Figure 3c). Importantly, these between-group differences in the sham condition were significantly stronger at the beginning of the experimental session and declined throughout the extinction training (Half × Group, $F(1,212.68) = 5.47$, $p = .020$, $\eta^2_p = .03$; Figure 4a,b), which resulted from extinguished heart rate deceleration in fear learning group participants (Half, $F(1,112.34) = 3.49$, $p = .064$, $\eta^2_p = .03$), rather than from increased deceleration in controls (Half, $F(1,95.06) = 1.92$, $p = .169$). Accordingly, although early decelerative peak responding remained stable in all sham-stimulated subjects (*D1*: Half × Group, $F(1,38) = .76$, $p = .388$; Figure 4e), fear learning group participants showed lower cardiac accelerative and

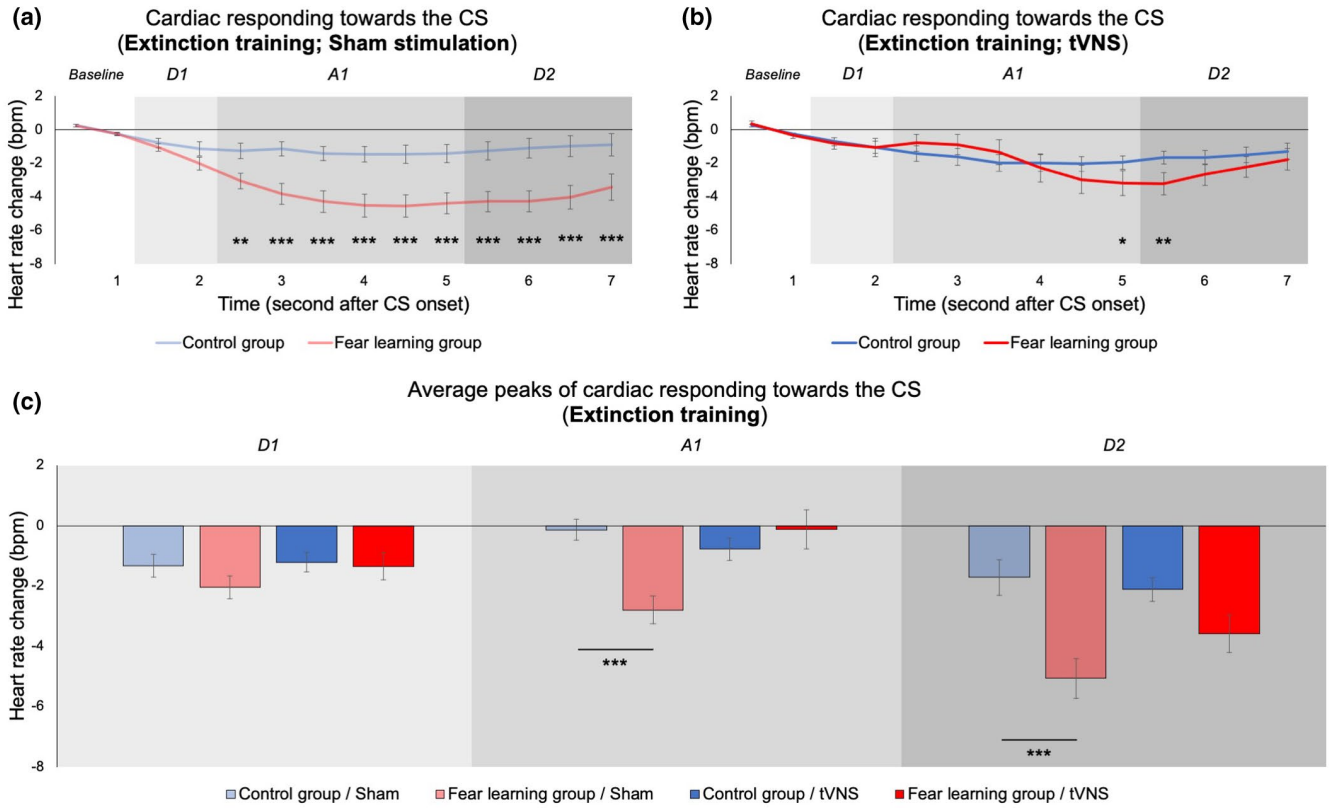

FIGURE 3 (a) Heart rate change after CS onset during the extinction training in the sham condition, averaged across all 16 trials, depicted in half-second bins for the fear learning (light red line) and control group (light blue line). (b) Heart rate change after CS onset during the extinction training in the tVNS condition, averaged across all 16 trials, depicted in half-second bins for the fear learning (red line) and control group (blue line). (c) Average peaks of cardiac response components during the extinction training for both the fear learning and control group in the sham condition (light red and light blue bars) and tVNS condition (red and blue bars). *D1* indicates the mean slowest half-second between 1 and 2 s after CS onset. *A1* indicates the mean fastest half-second between 2 and 5 s after CS onset. *D2* indicates the mean slowest half-second between 5 and 7 s after CS onset. For all graphs: Time windows for the analyses of the average peaks of cardiac response components are depicted in different gray scales ranging from light (*D1*) to medium (*A1*) and dark gray (*D2*). Error bars, when depicted, represent standard error of the mean. Asterisks indicate statistical significance of correlations with $*p \leq .05$, $**p \leq .01$, and $***p < .001$

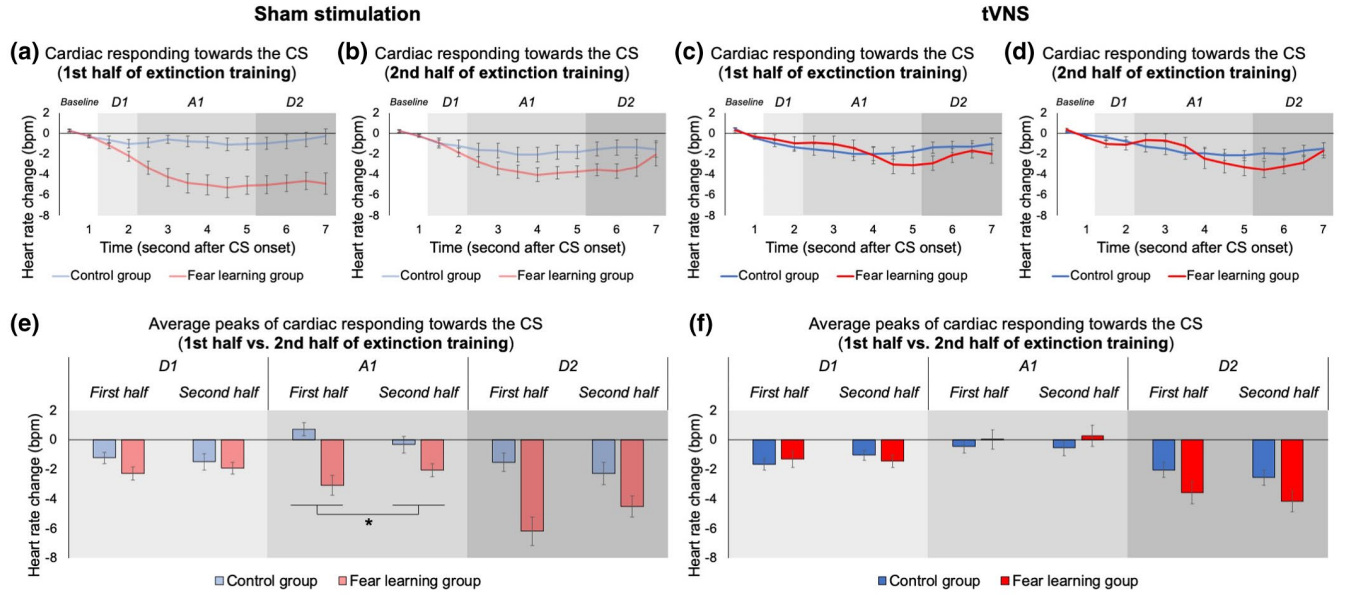

FIGURE 4 (a) and (b) Heart rate change after CS onset during the first half (a) and second half (b) of the extinction training in the sham condition, averaged across all 16 trials, depicted in half-second bins for the fear learning (light red line) and control group (light blue line). (c) and (d) Heart rate change after CS onset during the first half (a) and second half (b) of the extinction training in the tVNS condition, averaged across all 16 trials, depicted in half-second bins for the fear learning (red line) and control group (blue line). (e) and (f) Average peaks of cardiac response components during the first half (e) and second half (f) of the extinction training for both the fear learning and control group in the sham condition (light red and light blue bars, respectively) and tVNS condition (red and blue bars, respectively). *D1* indicates the mean slowest half-second between 1 and 2 s after CS onset. *A1* indicates the mean fastest half-second between 2 and 5 s after CS onset. *D2* indicates the mean slowest half-second between 5 and 7 s after CS onset. For all graphs: Time windows for the analyses of the average peaks of cardiac response components are depicted in different gray scales ranging from light (*D1*) to medium (*A1*) and dark gray (*D2*). Error bars, when depicted, represent standard error of the mean. Asterisks indicate statistical significance of correlations with $*p \leq .05$, $**p \leq .01$, and $***p < .001$

stronger late decelerative peaks during the first half of extinction, which both appeared to decline throughout the session (*A1*: Half × Group, $F(1,38) = 4.78$, $p = .035$, $\eta^2_p = .11$; *D2*: Half × Group, $F(1,38) = 4.08$, $p = .051$, $\eta^2_p = .09$; Figure 4e).

In contrast to the sham condition, the application of tVNS abolished differences in overall cardiac responding between the fear learning and control group during the extinction training (Group, $F(1,50.87) = .36$, $p = .554$; Figure 3b), which was evident right from the beginning of the experimental session (heart rate curves: Half × Group, $F(1,187.37) = .03$, $p = .854$; Figure 4b,c; peak components: Half × Group, all $Fs < 1.930$, all $ps > .172$; Figure 4f). Nevertheless, the fear learning group still displayed stronger cardiac deceleration during the late phase of the CS processing (Time × Group, $F(13,179.93) = 2.15$, $p = .014$, $\eta^2_p = .14$; significant group differences 5–5.5 s after CS onset; Figure 3b). Accordingly, we observed no significant group differences in early decelerative peaks (*D1*; $F(1,38) = .06$, $p = .807$) or cardiac acceleration (*A1*; $F(1,38) = .78$, $p = .382$), whereas we found a trend for stronger late decelerative peaks in the fear learning group relative to controls in the tVNS condition (*D2*: $F(1,38) = 3.89$, $p = .056$, $\eta^2_p = .09$; Figure 3c).

Further analyses revealed that the abolished group differences in the tVNS condition did not result from attenuated early or late decelerative peak responding (*D1*: Stimulation × Group, $F(1,76) = .57$, $p = .454$; *D2*: Stimulation × Group, $F(1,76) = 2.69$, $p = .105$; Figure 3c), but from elevated cardiac acceleration in vagally stimulated fear learning group subjects compared with the sham condition (*A1*: Stimulation × Group, $F(1,76) = 12.47$, $p = .001$, $\eta^2_p = .14$; Figure 3c). Thus, although both active and sham-stimulated subjects of the fear learning group displayed significant cardiac deceleration immediately prior to the US (*D2* component or late cardiac deceleration), tVNS resulted in a significantly delay of such increased cardiac deceleration due to transient cardiac acceleration 2 s after CS onset. As no differences between stimulation conditions were found in SCL change during the CS presentation (all $Fs < 1.66$, all $ps > .070$; Figure 5a,b), our data indicate that such elevated cardiac acceleration was not accompanied by an increase in sympathetic nervous activity.

As during acquisition, late cardiac deceleration (*D2*) was overall reduced during probed compared with no probed trials (Probe, $F(1,76) = 5.40$, $p = .023$, $\eta^2_p = .07$; Figure S2a) – an effect that did not differ between fear learning and control participants (Probe × Group, $F(1,76) = .048$, $p = .827$; Figure S2b).

As expected, stronger prolonged cardiac deceleration continued to be significantly correlated with increased startle potentiation (*D2*: $r_{Spearman}(80) = -.376$, $p < .001$;

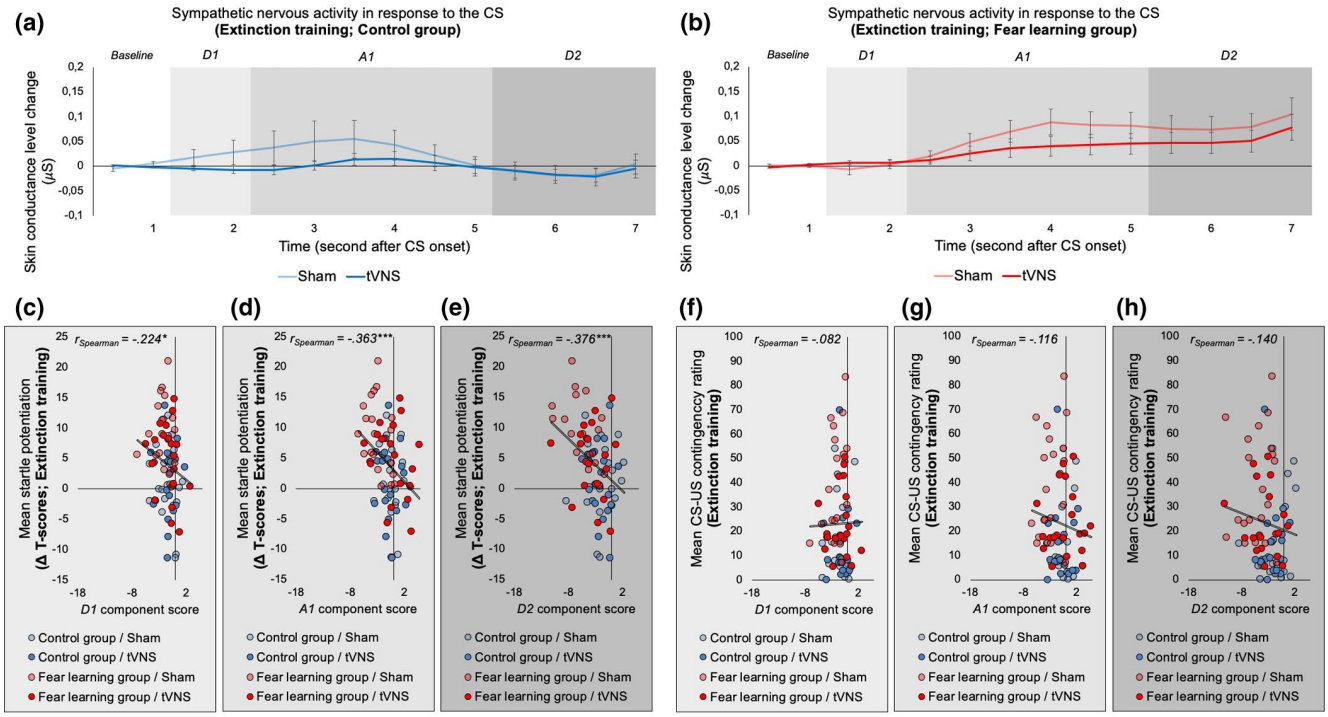

FIGURE 5 (a) and (b) Skin conductance level change after CS onset during the extinction training, averaged across all 16 trials, depicted in half-second bins for the tVNS (blue) and sham condition (light blue) of the control group (a) and fear learning group (b). (c), (d), and (f) Scatter plot of mean startle potentiation (standardized [T-scores] startle magnitudes elicited during the CS minus standardized [T-scores] startle magnitudes elicited during the ITI, averaged across all probed trials) as a function of *D1* component score variation (c), *A1* component score variation (d) and *D2* component score variation (e) during the extinction training for both the fear learning and control group in the sham (light red and light blue dots, respectively) and tVNS condition (red and blue dots, respectively). (f), (g), and (h) Scatter plot of mean CS-US contingency rating (averaged across all trials) as a function of *D1* component score variation (f), *A1* component score variation (g), and *D2* component score variation (h) during the extinction training for both the fear learning and control group in the sham (light red and light blue dots, respectively) and tVNS condition (red and blue dots, respectively). For all graphs: Time windows for the analyses of the average peaks of cardiac response components are depicted in different gray scales ranging from light (*D1*) to medium (*A1*) and dark gray (*D2*). Error bars, when depicted, represent standard error of the mean. Asterisks indicate statistical significance of correlations with $*p \leq .05$, $**p \leq .01$, and $***p < .001$

Figure 5e, but not with CS-US expectancy ratings (*D2*: $r_{Spearman}$ (80) = −.116, p = .305; Figure 5h) in the overall analysis, and the *D2* was significantly stronger related to startle potentiation than to CS-US contingency ratings during the extinction training (z = −1.99, p(one-tailed) = .023; Figure 5e,h). Analyses for the separate groups showed that the association between *D2* and startle potentiation was not significant and not modulated by tVNS in the control group (control group/tVNS: $r_{Spearman}$ = −.245, p = .298; control group/sham: $r_{Spearman}$ = .165, p = .486; z = −1.215, p(one-tailed) = .112), whereas it was significant in fear learning group participants in the sham condition (fear learning group/sham: $r_{Spearman}$ = −.627, p = .003; Figure 6a). As expected, this significant correlation was abolished by tVNS (fear learning group/tVNS: $r_{Spearman}$ = −.146, p = .539; Figure 6b; between group comparison of both correlations was significant z = −1.718, p(one-tailed) = .043).

In contrast to the acquisition training, however, we also found that earlier onset of cardiac deceleration (i.e., lower early decelerative and accelerative component scores between 1 and 5 s after stimulus onset) was significantly correlated with increased startle potentiation during the CS (*D1*: $r_{Spearman}$ (80) = −.224, p = .046; A1: $r_{Spearman}$ (80) = −.363, $p < .001$; Figure 5c,d), whereas not being related to CS-US expectancy ratings (*D1*: $r_{Spearman}$ (80) = −.082, p = .471;*A1*: $r_{Spearman}$ (80) = −.140, p = .216; Figure 5f,g). However, comparing both correlations for significant differences, there were no significant differences for D1 (z = −1.06, p(one-tailed) = .144; Figure 5c,f), whereas the correlation between *A1* and startle potentiation was significantly stronger than the correlation between *A1* and shock expectancy ratings (z = −1.72, p(one-tailed) = .043; Figure 5d,g).

4 | DISCUSSION

Prolonged cardiac deceleration has commonly been interpreted as an index of increased orienting toward motivationally significant stimuli (Bradley, 2009), but not necessarily also indexing defensive responding and fear (see Lonsdorf

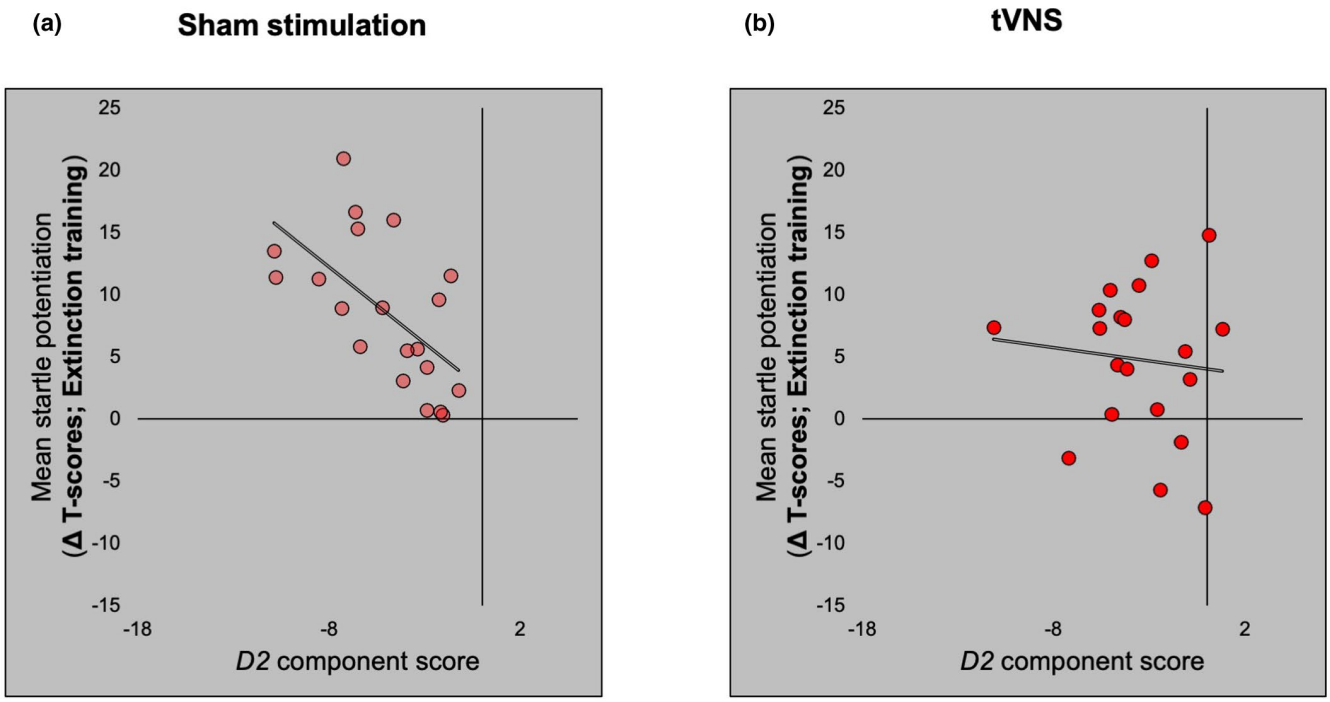

FIGURE 6 (a) and (b) Scatter plot of mean startle potentiation (standardized [T-scores] startle magnitudes elicited during the CS minus standardized [T-scores] startle magnitudes elicited during the ITI, averaged across all probed trials) as a function of *D2* component score variation during extinction training for the fear learning group receiving sham stimulation (a) and tVNS (b). Asterisks indicate statistical significance of correlations with $*p \leq .05$, $**p \leq .01$, and $***p < .001$

et al., 2017 for a review). However, previous research has consistently found cardiac deceleration during attentive immobility (freezing), a defense strategy toward distal threats when easy escape is blocked (Hamm, 2020; Krause et al., 2018; Löw et al., 2015; Marks, 1987; Roelofs, 2017). Following up on this research, we investigated cardiac reactivity as an index of defensive responding by applying a single-cue multiple-day human fear conditioning and extinction protocol. Transcutaneous vagus nerve stimulation (tVNS)—a non-invasive brain stimulation technique which has proven to facilitate the reduction of defensive responding during extinction compared with a sham stimulation of the earlobe (Burger et al., 2016, 2017, 2018; Szeska et al., 2020)—was used during extinction training as an additional proof of concept. We found stronger cardiac deceleration during the late phase of a conditioned stimulus predicting an approaching threat. Such conditioned "fear bradycardia" (Campbell et al., 1997) was significantly associated with potentiation of the startle response—a low level protective brain stem reflex (Davis, 2006), suggesting that human fear conditioning evokes a defensive response pattern that can best be characterized as attentive immobility, a defensive strategy observed in animals when withdrawal from danger is not possible or helpful (Marks, 1987). TVNS promoted the extinction of this conditioned defensive response pattern including a decoupling of the autonomic and protective reflex indices of the conditioned response.

In fact, the presentation of the conditioned stimulus evoked prolonged heart rate deceleration during both acquisition and extinction training in all participants, regardless of whether they underwent a fear conditioning protocol (fear learning group) or not (control group). Previous studies consistently found such prolonged cardiac deceleration in contexts involving perceptual processing and, thus interpreted heart rate deceleration as an index of increased orienting toward informative or motivationally significant stimuli (Bradley, 2009; Graham, 1979; Graham & Clifton, 1966; Lacey & Lacey, 1970). Accordingly, our data indicate that both groups showed increased orienting toward relevant stimuli, either threat- (fear learning group) or safety-signaling (control group).

As expected, both groups did not differ in the early decelerative component of CS-evoked cardiac changes (*D1*; initial 2 s after stimulus onset), supporting previous notions that this component of conditioned cardiac responding might be considered as a reflexive index of stimulus registration occurring in response to any low- or moderate-intensity stimulus regardless of repetition or motivational significance (Bradley, 2009; Graham, 1987). Correspondingly, this early decelerative responding did not correlate with startle potentiation or shock expectancy ratings during conditioning, did not extinguish and was not affected by tVNS.

Importantly, however, both groups in fact differed in the strength of *prolonged* cardiac deceleration. The fear learning

group displayed stronger cardiac deceleration compared with controls, which was more evident later during CS presentation (*D2* component) both during acquisition and extinction. The strength, and during extinction also earlier onset, of this heart rate deceleration was positively correlated with the potentiation of the startle response. In animal studies, heart rate deceleration correlated with behavioral freezing (Walker & Carrive, 2003), and in two other studies behavioral freezing was associated with startle potentiation in rats (Leaton & Borszcz, 1985; Plappert et al., 1993). In humans, mean cardiac deceleration during anticipation of shock was associated with mean decrease in body sway (Gladwin et al., 2016). The data of the current experiment, thus, complement the picture in showing a significant relationship between heart deceleration and startle potentiation and suggest that in humans—like in other animals – the same pattern of attentive immobility is evoked during the anticipation of a mild but aversive, inevitable unconditioned stimulus. Supporting this view, our data show that cardiac deceleration was strongest immediately prior to the delivery of the inevitable threat, thus mirroring findings from Löw et al. (2015) and Krause et al. (2018), which suggested that attentive immobility becomes stronger with increasing imminence of an inevitable threat. Moreover, during conditioning such late cardiac deceleration has shown to be particularly robust despite the effects of the presented startle probes, which previously have found to cause transient heart rate acceleration (Chen et al., 2014; Cook et al., 1992).

Furthermore, as the association between heart rate deceleration and startle potentiation was stronger compared with the correlation between cardiac deceleration and CS-US expectancy ratings, the correlational pattern of the data implies that cardiac deceleration and startle potentiation are both indices of the same defensive response strategy that is activated independently of the explicit declarative knowledge of the exact contingencies. In fact, this is in line with previous animal research, showing that cardiac deceleration in the face of an inevitable threat is driven by subcortical projections from the central nucleus of the amygdala (CeA) to the midbrain ventrolateral periaqueductal gray (vlPAG), which are also involved in the mediation of behavioral freezing and startle potentiation during threat processing (Applegate et al., 1983; Choi & Brown, 2003; Davis, 2006; Fendt & Fanselow, 1999; LeDoux, 1995; LeDoux et al., 1988; Walker & Carrive, 2003). This view is further supported by genetic research, showing increased threat-induced connectivity between the amygdala and PAG along with stronger heart rate deceleration during the presentation of a threat-predicting CS in carriers of the short allelic variant of the 5-HTTLPR (serotonin transporter-linked polymorphic region; Schipper et al., 2019). Individuals carrying this genetic variant also showed increased potentiation of the startle response during fear conditioning relative to l-allele carriers (Lonsdorf et al., 2009).

Animal research further showed that the CeA is inhibited by the basolateral amygdala and ventromedial prefrontal cortex during extinction learning, resulting in successive attenuation of attentive immobility – a process, which may be facilitated by stimulation of vagal afferents (Amano et al., 2010; Dunsmoor et al., 2015; Ehrlich et al., 2009; Peña et al., 2013, 2014). In sham-stimulated subjects, consequently, cardiac deceleration declined throughout extinction, although it did not fully extinguish, indicating a well consolidated fear memory which can possibly be attributed to partial CS reinforcement during initial fear conditioning (Hilton, 1969; Lonsdorf et al., 2017) and following a 24 hr memory consolidation period until extinction training began (Norrholm et al., 2008). TVNS, on the other hand, resulted in a faster attenuation of cardiac deceleration early during extinction, reflecting both its memory enhancing and anxiolytic effects (Noble et al., 2019) and also abolished the significant correlation between cardiac deceleration and startle potentiation. Importantly, the attenuation of cardiac deceleration was not accompanied by elevated levels of sympathetic nervous activity, as indicated by the SCL changes during extinction. These data suggest that the extinction of cardiac deceleration probably results from inhibition of parasympathetic heart rate control, driven by inhibition of the CeA-PAG neural freezing circuit—a process which may further be facilitated by tVNS. Previous research proposed that the defensive response pattern during attentive immobility (freezing) is primarily parasympathetically dominated (Roelofs, 2017) and, thus, our data again foster the view that cardiac deceleration functions as an autonomic expression of such defensive responding.

Importantly, cardiac responses to threat-signaling stimuli might therefore critically distinguish between different modes of fear-related defensive responding. As previous research indicated and is supported by our study, distal and inevitable threats elicit a defensive response pattern of parasympathetically dominated attentive immobility defined by fear bradycardia, during which orienting to and monitoring the source of danger is the best strategy to ensure survival, as it allows optimal preparation for defensive action in case threatening confrontation becomes increasingly imminent (see Roelofs, 2017). However, if the threat-signaling cue has become sufficiently imminent and defensive action is required to ensure survival, the defensive response pattern switches and the organism is under sympathetic control, defined by cardiac acceleration to support flight or fight (Cannon, 1929; Eilam, 2005; Lang et al., 1997, 2000; Lang & Davis, 2006; Roelofs, 2017). Moreover, using cues that are more complex or motivationally significant (i.e., facial expressions or pictures of snakes and spiders) might also activate cardiac acceleration (Hamm et al., 1993; Hodes et al., 1985) whereas

simple geometric figures as used in the current experiment evoke a constant cardiac deceleration in anticipation of the shock. More importantly, although both attentive immobility (freezing) and active withdrawal are defensive states accompanied by the feeling of fear (see Hamm, 2020 and Mobbs et al., 2009), the switch from parasympathetic fear bradycardia to sympathetic cardiac acceleration might therefore mark a transition of defensive strategies. We, thus, want to encourage future research on human fear to include threat-related heart rate changes into analyses, which possibly yield valuable information about the actual defensive state of the organism.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTION

Christoph Szeska: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Visualization; Writing-original draft; Writing-review & editing. **Jan Richter:** Conceptualization; Methodology; Writing-review & editing. **Julia Wendt:** Conceptualization; Methodology; Writing-review & editing. **Mathias Weymar:** Conceptualization; Methodology; Writing-review & editing. **Alfons O. Hamm:** Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Writing-review & editing.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Figure S1. (a) Mean *D2* component score (slowest half-second between 5 and 7 s after CS onset) during the acquisition training, depicted for trials during which no startle probe was administered (non-probed trials) and for trials during which startle probes were delivered (probed trials). (b) Mean *D2* component score for non-probed and probed trials for both the control group (blue bars) and the fear learning group (red bars) during the acquisition training. Error bars, when depicted, represent standard error of the mean. Asterisks indicate statistical significance of correlations, with * for $p \leq .05$, ** for $p \leq .01$ and *** for $p < .001$

Figure S2. (a) Mean *D2* component score (slowest half-second between 5 and 7 s after CS onset) during the extinction training, depicted for trials during which no startle probe was administered (non-probed trials) and for trials during which startle probes were delivered (probed trials). (b) Mean *D2* component score for non-probed and probed trials for both the control group (blue bars) and the fear learning group (red bars) during the extinction training. Error bars, when depicted, represent standard error of the mean. Asterisks indicate statistical significance of correlations, with * for $p \leq .05$, ** for $p \leq .01$ and *** for $p < .001$

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