Mixed evidence for the potential of non-invasive transcutaneous vagal nerve stimulation to improve the extinction and retention of fear.

Burger, A.M., Verkuil, B., Fenlon, H., Thijs, L., Cools, L., Miller, H., Vervliet, B., Van Diest, I.

Burger, A.M., Verkuil, B., Fenlon, H., Thijs, L., Cools, L., Miller, H., Vervliet, B., Van Diest, I. (in press). Mixed evidence for the potential of non-invasive transcutaneous vagal nerve stimulation to improve the extinction and retention of fear. Behaviour Research and Therapy.

ABSTRACT

Extinction memories are fragile and their formation has been proposed to partially rely on vagus nerve activity. We tested whether stimulating the auricular branch of the vagus (transcutaneous VNS; tVNS) accelerates extinction and reduces spontaneous recovery of fear. Forty-two healthy students participated in a 3-day fear conditioning study, where we tested fear acquisition (day 1), fear extinction (day 2) and the retention of the extinction memory (day 3). During extinction, participants were randomly allocated to receive tVNS or sham stimulation concurrently with each CS presentation. During the acquisition and retention phases, all participants received sham stimulation. Indexes of fear included US-expectancy, startle blink EMG and skin conductance responses. Results showed successful acquisition and extinction of fear in all measures. tVNS facilitated the extinction of declarative fear (US expectancy ratings), but did not promote a stronger retention of the declarative extinction memory. No clear effects of tVNS on extinction and retention of extinction were found for the psychophysiological indexes. The present findings provide tentative indications that tVNS could be a promising tool to improve fear extinction and call for larger scale studies to replicate these effects.

Keywords: transcutaneous vagus nerve stimulation, fear extinction, memory, anxiety.

Highlights

- First study to assess effects of tVNS on psychophysiological indices of fear extinction in humans.
- tVNS accelerates declarative fear extinction.
- tVNS does not affect declarative fear retention.
- No clear evidence for effects of tVNS on psychophysiological fear extinction.

Abbreviations

tVNS – transcutaneous vagus nerve stimulation

INTRODUCTION

Fear is an evolutionarily adaptive response to actual or potential harm that predisposes the body towards a defensive reaction (Fendt & Fanselow, 1999). The acquisition of fear is strongly dependent on the process of Pavlovian conditioning (Indovina, Robbins, Nunez-Elizalde, Dunn, & Bishop, 2011; Lissek et al., 2005; Mineka & Zinbarg, 2006): When a neutral stimulus (conditioned stimulus, CS) is contingently paired with an inherently aversive stimulus (unconditioned stimulus, US), the CS will start to elicit a learned or conditioned fear response (CR). This Pavlovian conditioning of fear is most often adaptive as it allows an individual to learn from aversive experiences. However, it can also lead to pathological anxiety. For example, in recent years it has become clear that patients with anxiety disorders and stress-related disorders including post-traumatic stress disorder have difficulties extinguishing the learned fear response (for a recent meta-analysis, see Duits et al., 2015). That is, when the CS is no longer followed by a US, anxiety patients show prolonged fear responses in the absence of clear threat. This finding is in line with studies showing that exposure therapy, the treatment of choice for most anxiety and trauma-related disorders (Mark E Bouton, Mineka, & Barlow, 2001; Hofmann, 2007), is only moderately effective (Stewart & Chambless, 2009). Understanding the neurobiological mechanisms behind fear and safety learning is therefore crucial in order to improve the treatment of anxiety and trauma-related disorders.

Knowledge about the neurobiological underpinnings of fear learning is accumulating. During situations of imminent threat, the body initiates a fight-flight-response, consisting of a cascade of bodily reactions that allow appropriate responding to the stressor. Of particular importance to fear learning, the appraisal of danger or threat leads to the release of peripheral epinephrine (Mcgaugh & Roozendaal, 2002), which activates beta-adrenergic receptors on the afferent vagus nerve. When this afferent information reaches the nucleus of the solitary tract, noradrenergic projection neurons in the locus coeruleus (LC) are activated and release norepinephrine (NE) in several cortical and subcortical brain regions that support memory formation (McGaugh, 2002). Due to this increased release of NE, fear memories are more strongly consolidated and subsequently more easily remembered than neutral memories (Cahill & Mcgaugh, 1998).

Meta-analyses have indicated that this system of learning new and emotional memories is thwarted during extinction learning (Duits et al., 2015; Lissek et al., 2005). Experimental studies have found that the consolidation of extinction memory could be enhanced by utilizing the same mechanism through which a fear memory attains its privileged position in memory storage. For example, promoting NE release in cortical and limbic structures through the use of yohimbine, an alpha2-adrenoreceptor, has the potential to facilitate fear extinction (Mueller & Cahill, 2010).

Unfortunately, yohimbine increases the release of central NE by increasing peripheral adrenal activity. Therefore, the use of yohimbine in patients is not warranted, as it may increase arousal which may have anxiety-provoking effects in anxiety patients (Cain, Blouin, & Barad, 2004). Especially in patients with panic disorder, increased peripheral arousal during exposure therapy may have iatrogenic effects and strengthen the fear memory instead of establishing an extinction memory.

More recently, stimulation of the vagus nerve (VNS) has been proposed as a non-pharmacological alternative to enhance extinction memory through the increase of noradrenergic transmission (Peña, Engineer, & McIntyre, 2013). Low levels of vagus nerve activity – as measured by vagally-mediated heart rate variability - have been observed in anxiety patients (Chalmers, Quintana, Abbott, & Kemp, 2014; Friedman, 2007). Furthermore, higher levels of vagus nerve activity have been associated with increased ability for safety learning and inhibition of conditioned fear responses (Pappens et al., 2014; Wendt, Neubert, Koenig, Thayer, & Hamm, 2015). Contrary to yohimbine, VNS is unrelated to peripheral adrenergic activity (Hassert, Miyashita, & Williams, 2004) and has repeatedly been found to have anxiolytic effects (e.g., Fang et al., 2015; George et al., 2008; Rong et al., 2016). Electrical stimulation of the vagus nerve leads to activation of the noradrenergic projection neurons in the LC, which causes NE to be released in the brain (Fanselow, 2013; Grimonprez, Raedt, Baeken, Boon, & Vonck, 2015). In line with this, several studies have reported on memory-enhancing effects of VNS in animals as well as in humans (for a review, see Vonck et al., 2014). Specifically, studies in rats have repeatedly underlined the importance of the vagus nerve on the extinction of fear. For instance, cutting the afferent vagal nerve fibers attenuated extinction learning in rats (Klarer et al., 2014). By contrast, stimulating the vagus nerve in rats led to enhanced extinction learning (Alvarez-Dieppa, Griffin, Cavalier, & Mcintyre, 2016; Peña et al., 2014, 2013), but only when VNS was conducted during and not after the extinction phase (Peña et al., 2013). Due to the invasive nature of VNS, research on potential effects of vagus nerve stimulation on fear extinction in humans has been limited.

In the past decade, non-invasive ways of stimulating the vagus nerve in humans have been developed, commercialized and approved for clinical use in epileptic and depressive patients (Ben-Menachem, Revesz, Simon, & Silberstein, 2015). Evidence indicates that implanted VNS and transcutaneous stimulation of the auricular branch of the vagus nerve stimulate similar brain structures (Frangos, Ellrich, & Komisaruk, 2014). In line with this, recent studies have documented a range of effects of tVNS in humans, including an enhancement of associative memory and memory of emotional events (Jacobs, Riphagen, Razat, Wiese, & Sack, 2015). Critically, tVNS has been found to promote inhibitory processes, which might be compromised in anxiety patients, such as inhibitory control (Beste et al., 2016; Sellaro, Leusden, & Colzato, 2015) and – at the neural level – the

functional connectivity between the right amygdala and the dorsolateral prefrontal cortex (Liu et al., 2016). We have previously examined the effects of tVNS on fear extinction and retention. These preliminary findings suggested that tVNS accelerates the formation of declarative extinction memories in healthy humans (Burger et al., 2016), although we found no evidence for an enhanced consolidation of the extinction memory, as reflected by the lack of significant differences in explicit fear on the retention test 24 hours later. The paradigm that was used failed to elicit differential fear conditioning on psychophysiological indices of fear, and thus we were unable to assess potential effects tVNS may have on psychophysiological fear responses.

The present study therefore aimed to further investigate effects of tVNS during extinction training in healthy humans with another type of paradigm. First, to ensure fear learning, the present study used an electrocutaneous stimulus as US, as opposed to the auditory US used in our previous study. Second, acquisition, extinction, and retention of extinction were tested on three separate days, ensuring sufficient time for both the acquisition and extinction memories to consolidate. Furthermore, in contrast to Burger and colleagues (Burger et al., 2016), we now specifically paired the extinction learning trials with tVNS, which yielded the strongest effects in the animal studies by Pena et al (Peña et al., 2013). Our main hypotheses were that tVNS would accelerate the extinction of both declarative and psychophysiological fear responses. Additionally, we hypothesized that tVNS would increase the consolidation of extinction memories, contrary to what was found in our previous study (Burger et al., 2016) but in line with animal studies on the effects of VNS on fear extinction (Peña et al., 2013).

METHODS

Participants

Forty-two healthy students from the University of Leuven (16 men and 26 women; age range: 20 – 36 years) participated in the experiment.[1] In return they received a financial compensation of 70 euros and a one in three chance to win a cinema ticket after completion of the entire experiment. Participants between the ages of 18 and 50 could participate in this study. Exclusion criteria included self-reported current or past psychiatric, cardiac or neurological disorders, use of psychopharmacology or any medication that affects autonomic nervous functioning (e.g., beta-blockers) and pregnancy.

The study was approved by the Medical Ethical Committee of the University of Leuven. Additionally, this study has been preregistered at ClinicalTrials.gov under NCT02113306.

Experimental Design

The experiment consisted of three sessions, run on separate days: acquisition on day 1, extinction training on day 2, and a test of retention of extinction on day 3[2]. The time in between sessions was 24 hours.

In the tVNS condition (*N* = 21, 11 women and 10 men), participants received tVNS stimulation on the concha of the left ear during extinction training (day 2), and sham stimulation on the left ear on day 1 (acquisition) and 3 (retention of extinction). The 'sham' condition (*N* = 21, 15 woman and 6 men) received sham stimulation on the left ear on all 3 days.

Stimuli and materials

Stimuli

[1] The current study was part of a larger study. Halfway through data collection, a second control group was added that included a context shift during day 2, comparable to the tVNS condition. In contrast to the first control condition, participants in this condition received sham stimulation to their right ear on the second day. However, participants in this second control group reported significantly lower US expectancy ratings to the CS+ during the acquisition phase compared to both the tVNS group and the first control group. For this reason, we concluded that the participants in this second control group were not comparable to the participants who were recruited from the beginning of the study. The data of the second control group is not included in this manuscript but can be requested alongside the data for the other two experimental groups from the first author.

[2] After the retention phase, participants were also subjected to a reacquisition phase and a generalization phase. These phases were added to the experimental paradigm for exploratory reasons and are beyond the scope of the current study. Exploratory analyses of these experimental phases are added as supplementary material to this manuscript. All data related to these exploratory analyses can be requested from the first author.

Two geometrical figures presented on a computer screen with a black background, served as the CSs: a blue-colored triangle (width: 27.5 cm, height: 20.5 cm) and a yellow-colored circle (width: 22 cm). CS allocation was counterbalanced so that half of the participants received the blue triangle as the CS+ and half received the yellow circle as the CS+.

The order of CS+ and CS- presentations was semi-randomized; the restriction used implied that no more than 3 trials of the same type in a row were allowed. Each CS was presented for 30 seconds, followed by a 40 second inter trial interval (ITI). Stimulation (sham) with the tVNS device occurred concurrently with each CS for 30 seconds.

An electrocutaneous stimulus served as the unconditional stimulus (US). Two electrodes were placed on the inside of the non-dominant leg, right underneath the knee, about 2,5 cm apart. An electrical stimulus generator, producing a bipolar constant current (DS5 Isolated Bipolar Constant Current Stimulator), generated a 500 ms stimulus that was individually tailored with a calibration procedure on day 1 (see procedure section). The mean stimulation given was 6.3 mA (range 2.0 mA - 9.9 mA).

Acoustic startle probes (95 dB, 50 ms with near instantaneous rise time) were presented binaurally through headphones. Two acoustic startle probes were presented during each CS and each ITI. Startle probes occurred at a random time within the following two time windows: 4-8, and 16-23 after CS and ITI onset.

tVNS and sham stimulation

Transcutaneous vagus nerve stimulation was conducted using the NEMOS® stimulator unit (Cerbomed, Erlangen, Germany). Stimulation was programmed to coincide with the presentation of every CS. Active stimulation consisted of 250µs monophasic square wave pulses at 25Hz. During tVNS, the stimulator is fitted on the concha of the left ear, an area of the ear that is 100% innervated by the vagus nerve (Peuker & Filler, 2002). During sham stimulation, the tVNS device was fitted on the earlobe of the left ear. However, the sham position of the electrode did not fit in the ear of 3 participants in the sham group, and so for these participants the electrode was placed on the concha of the left ear and set at an intensity of 0.1 mA to avoid having any effects on the vagus nerve.

Stimulation intensity was set at 0.5 mA, but was lowered if the participant experienced the stimulation to be painful. 7 out of 21 participants in the tVNS condition and 8 out of 21 in the sham condition received an adjusted intensity. Specifically, in the tVNS condition 1 participant received 0.2mA, 3 received 0.3mA and 3 received 0.4mA. One participant in the tVNS condition considered intensities higher than 0.1mA to be painful and was excluded from analyses, as an intensity of 0.1mA

has been found to not affect vagus nerve activity (Clark et al., 1998; Clark, Naritoku, Smith, Browning, & Jensen, 1999). In the sham condition, 2 participants received 0.1mA, 1 received 0.3mA and 2 received 0.4mA.

Measurements

US expectancy

A custom-made dial knob allowed participants to continuously rate how much they expected the painful US to occur (US-expectancy) during the experiment. Participants were instructed to continuously indicate their US-expectancy using the dial knob. The dial's scale ranged from 0 ("I am positive that the electric shock is not coming now") to 100 ("I am positive that the electric shock is coming now"). The analogue output signal was digitized and stored at 10 Hz. US-expectancy ratings were averaged across the 30 s for each CS and each trial.

Skin Conductance Response

The skin conductance response (SCR) was measured using standard Ag/AgCl electrodes (1 cm diameter) filled with K-Y gel lubricant on the palm of the non-dominant hand (Scerbo, Freedman, Raine, Dawson, & Venables, 1992). The skin on the palm of the non-dominant hand was cleaned with a disposable hypo-allergenic wipe before the start of the procedure. Afterwards, the electrodes were placed 2 cm apart. A constant 0.5 V was maintained by a Coulbourn skin conductance coupler (LabLinc v71-23). This signal was digitized and stored at 100 Hz. SCR were calculated by subtracting the mean skin conductance level (SCL) during 2 s prior to stimulus onset from the maximum SCL during 6 s following CS onset. SCRs with a value below 0.01 µSiemens were set at 0, as such low values are generally accepted to reflect a non-response (Dawson, Schell, Filion, & Berntson, 2007). Skin conductance responses were log transformed to reduce skewness of the data.

Eye blink startle response

Activity of the *orbicularis oculi* electromyographic activity (EMG) in response to the acoustic startle probe was measured using three Ag/AgCl Sensormedics electrodes (0.25 cm diameter) filled with Microlyte gel. After the skin was cleaned with a disposable hypo-allergenic wipe to reduce any potential inter-electrode resistance, two electrodes were placed just below the left eye, and one electrode was placed at the center of the forehead. A Coulbourn isolated bioamplifier with bandpass filter (Lablinc v75-04; 13 Hz-500Hz) was used to amplify the signal. This was then rectified online and smoothed out using a Coulbourn multifunction integrator (LabLinc v76–23 A) with a time constant of 50 ms. The EMG signal was digitized at 1000 Hz from 500ms before the onset of the auditory startle probe until 1000 ms after probe onset.

Eye blink startle EMG responses were calculated by subtracting the mean baseline (0 to 20 ms after probe onset) from the peak value found in the 21-175ms time window after probe onset. EMG signals with artifacts (e.g., excessive noise from muscular activity prior to the startle probe) were rejected from analysis and defined as missing. The average percentage of rejected responses per participant was 10%. Non-rejected startle responses were averaged per trial and subsequently standardized into T-scores for every individual (Blumenthal et al., 2005).

Electrocardiogram

The electrocardiogram (ECG) was obtained using three standard Ag/AgCl electrodes (1 cm diameter) filled with electrolyte and placed on the thorax across the heart: two electrodes were placed below the left and right clavicle, one electrode was placed on the left lower rib cage. The signal was sampled at 1000 Hz and transduced, amplified and filtered through a Coulbourn S75-04 Isolated Bioamplifier. Low frequencies were cut off at 10 Hz, high frequencies at 1 kHz.

The signal was visually inspected and artifacts were manually corrected. Interbeat intervals were extracted from the filtered signal, from which HR and the root mean square of the successive differences (RMSSD) between heart rates were calculated.

A seven-minute baseline recording of every participant's RMSSD level was conducted at the start of every testing day to assess participants' vagally-mediated HRV and to check for possible differences in baseline vagal tone.

Self-reports

Prior to the first session, participants were asked to fill in several questionnaires to check for possible differences between the groups in terms of sensitivity to fear and pain prior to having received the experimental manipulation.

The Pain Catastrophizing Scale (PCS) (Crombez & Vlaeyen, 1996; Sullivan, Bishop, & Pivik, 1995) was administered to assess how individuals experience pain. The PCS consists of 13 items scored on a 5-point scale.

Anxiety sensitivity, or the fear of anxiety-related bodily sensations, was measured using a Dutch translation of the Anxiety Sensitivity Index – 3 (ASI-3; De Jong, 2008; Taylor et al., 2007). The ASI-3 consists of 18 items that are scored on a 5-point scale, with higher scores indicating more anxiety sensitivity.

The Fear of Pain Questionnaire (FPQ-III) (Peters et al., 2002; McNeil & Rainwater, 1998) consists of 30 items that are scored on a 5-point scale ranging from “no fear at all” to “extreme fear”.

After each session, the participants had to fill in the Positive And Negative Affect Schedule (PANAS; Engelen et al., 2006; Watson, Clark & Tellegen, 1988). The PANAS consists of two mood scales, one that measures positive affect (PA scale) and one that measures negative affect (NA scale).

Procedure

The first session started with participants providing informed consent and completing the questionnaires. Following this, participants, seated in front of a computer, were fitted with all electrodes (psychophysiological measures, tVNS, and electrodes for electrocutaneous stimulation). The intensity of the electric shock was individually calibrated in the first session only, to an intensity that was “moderately painful and demanding some effort to tolerate”. Participants were informed that this level of intensity, could be used in all three sessions. Then, the intensity of the tVNS was determined. The starting intensity was at 0.1 mA and stopped until 0.5 mA was reached. If an intensity was considered painful by the participant, the intensity was lowered until it was no longer regarded as painful. On each day, prior to the experimental procedure, participants sat quietly for a 7 min ECG-baseline measurement. Following this, headphones were put on and participants were exposed to twelve acoustic probes in order to habituate them to the probe.

Day 1: Fear Acquisition. Participants received twelve CS+ and twelve CS- trials. The CS+ was reinforced with the US (electrocutaneous stimulus) in 75% of the trials. The US occurred unpredictably in the first 8- 12 second time interval of the CS+ trial, and 67% of these reinforced trials also had a second US in the last 23 - 27 second time interval of the CS+ trial. Thus, participants received a total number of fifteen electrocutaneous stimuli during the acquisition phase. All participants received sham stimulation during this phase.

Day 2: Fear Extinction. The extinction phase consisted of twenty unreinforced presentations of the CS+ and CS-. Participants were randomized to receive either tVNS or sham stimulation during this phase.

Day 3: Retention of Extinction. Participants received six unreinforced presentations of the CS+ and CS-. All participants received sham stimulation during the retention tests.

Statistical analyses

Differences between experimental conditions on the questionnaire and baseline HRV data were analyzed using independent samples *t*-tests.

We used multilevel mixed model analyses to assess whether the conditioning procedure resulted in successful fear learning in our participants in terms of both self-reports and psychophysiological outcomes. When we found significant response differentiation between CS- and CS+ trials on a measurement modality during acquisition, we continued to use multilevel mixed model analyses to analyze the effects of tVNS during the extinction and retention phases.

All multilevel mixed models were created using maximum likelihood modelling. We allowed intercepts to vary randomly across participants, but did not add random slopes to avoid overfitting our models. We did, however, model the heterogeneous AR1 autoregressive covariance structure of trials for within each experimental phase by specifying the nestedness of every trial within CStypes (or ITI, for startle probe responses) within individual participants.

Mixed model analyses are a similar but more flexible approach to data analysis than repeated measures analyses, which seem to have become the standard for fear conditioning research. Specifically, mixed model analyses can incorporate a more detailed clustering of the data and therefore provide a more accurate fit of the covariance structure of the data (Gueorguieva & Krystal, 2011). Additionally, mixed model analyses do not use list wise deletion when encountering missing data, which means there is no more need to aggregate trials to avoid the risk of missing data.

The independent variable Time, signifying trial number within each session, was group mean centered around the first trial of every phase. Experimental Condition was dummy coded (0 = Sham, 1 = tVNS) and treated as an interval variable. CStype was also dummy coded (0 = CS-, 1 = CS+) and treated as an interval variable when the dependent variable was either US expectancy rating or SCR. When EMG responses were the dependent variable, CStype had three levels: CS-, CS+ and ITIs. For these analyses, CStype was coded so that the CS+ was the reference variable to enable the comparisons between CS+ and CS- and CS+ and ITI.

In all models, the learning curve was fitted using a linear and a loglinear curve to account for non-linear learning rates (Burger et al., 2016). Either component was removed when this would result in a higher model fit as indexed by the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). AIC and BIC are methods of estimating model fit that can both be interpreted using similar methods of model fit approximations, only differing in the extent to which they penalize models with more parameters (generally, BIC favors parsimonious models more strongly than the AIC; Burnham & Anderson, 2004). For both AIC and BIC, smaller criterion values

indicate a better model fit, and thus the models with the smallest values for AIC and BIC were selected. In some cases, AIC and BIC showed discordant results in model fit preferences when selecting between a model using both time effects or just one. In these cases, the more parsimonious model was selected to improve interpretability of the main variables of interest.

All analyses concerning the effects of tVNS on extinction and retention learning are reported as one-tailed tests as the hypotheses we tested were directional and based on previous studies (Burger et al., 2016; Peña et al., 2013). Specifically, we aim to test whether tVNS accelerates the extinction of fear memories and strengthens the retention of these extinction memories. Analyses that were not focused on the effects of tVNS but assess the fear conditioning process were tested using two-tailed tests.

All analyses were performed using SPSS v23. Graphs were created using the ggplot package in R.

RESULTS

Participants

Data of one participant was excluded from analyses because the participant considered tVNS intensities higher than 0.1mA to be painful. Data from two other participants were excluded because they were unable to learn the CS-US contingency – specifically, they had higher US-expectancies for the CS- than for the CS+ in the last two trials of the acquisition phase. Furthermore, SCR data of two participants were missing because an electrode broke. Startle EMG data from four participants were excluded from analyses, because three participants were defined as non-responders (these participants showed startle responses after fewer than 33% of the startle probes), and data from yet one other participant was extremely noisy. As such, the final datasets were n = 39 for US-expectancy (n_{tVNS} = 19, n_{sham} = 20), n = 37 for SCR (n_{tVNS} = 18, n_{sham} = 19), and n = 35 for startle EMG (n_{tVNS} = 18, n_{sham} = 17).

As displayed in table 1, participants in the tVNS and sham condition did not differ significantly on background variables that may affect fear conditioning and fear extinction. Participants' scores on the PCS, ASI-III and FPQ-III were comparable to norm scores from healthy college students or community samples (Osman et al., 2000, 2010; Wijk & Hoogstraten, 2006).

Table 1. Descriptive statistics. Mean scores on baseline variables with standard deviations presented between brackets.

		Sham	tVNS	*p*
Day 1	PCS	18.05(8.13)	18.29(8.71)	.93
	ASI-III	15.20(9.56)	14.57(8.61)	.83
	FPQ-III	59.55(16.77)	66.10(16.77)	.19
	PA	31.93(6.43)	33.06(6.21)	.62
	NA	18.87(5.36)	17.56(4.46)	.47
	RMSSD	34.72(26.41)	45.00(30.36)	.27
	HR	83.29(10.77)	77.47(13.20)	.15
Day 2	PA	31.33(6.60)	32.75(6.50)	.55
	NA	16.93(3.97)	17.06(4.67)	.94
	RMSSD	30.90(21.50)	43.27(28.17)	.14
	HR	81.83(11.77)	78.85(9.67)	.40

Day 3	PA	31.87(5.13)	33.00(4.75)	.63
	NA	17.00(4.75)	16.94(5.08)	.97
	RMSSD	37.42(31.17)	40.05(25.53)	.78
	HR	81.50(12.85)	81.66(12.90)	.97

Note: *N* = 39. PCS = Pain Catastrophizing Scale, ASI-III = Anxiety Sensitivity Index III, FPQ-III = Fear of Pain Questionnaire III, PA = Positive Affect, NA = Negative Affect, RMSSD = Root mean square of successive differences between heart rates, HR = Heart Rate.

Acquisition

US Expectancy

As depicted in figure 1A (left column), participants showed clear signs of differential fear learning on US expectancy ratings during the acquisition phase. Importantly, participants showed a clear differential acquisition of fear, as indicated by a significant interaction between LogTrial*CStype (b = 23.06, $t(92.06) = 10.41$, $p < .001$; see also Table 2). There was a significant decrease in US expectancy ratings for CS- trials (LogTrial, b = -13.70, $t(92.06) = -8.75$, $p < .001$).

We found no significant main or interaction effects of Condition on US expectancy ratings (all ps > .05). Thus, there were no significant between group differences in the declarative acquisition of fear prior to the experimental manipulation.

Startle EMG

A significant main effect of time showed that startle responses decreased overall during the acquisition phase, which is indicative of a general habituation to the startle probe, b = -3.85, $t(276.64) = -4.24$, $p < .001$ (see Table 3). Yet, participants' startle responses during the acquisition phase reflected a clear differential fear learning, as depicted in figure 1B. The significant interaction between LogTrial*CStype_{CS-}, indeed indicated that there was a stronger decline in startle response for CS- trials compared to CS+ trials, b = -4.36, $t(274.02) = -3.42$, $p = .001$.

Similarly to the US expectancy ratings, there were no main or interaction effects of Condition (all ps > .05).

SCR

The differential learning curve for SCR is depicted in figure 1C (left column). The model that provided the strongest model fit for SCR did not include either a Trial or a LogTrial effect, indicating that there was no distinct *learning curve* present in the SCR data during the acquisition phase. However, participants did show a significant differentiation in SCR between CS+ and CS- trials, which is indicative of differential fear conditioning (main effect of CStype, b = .09, t(227.90) = 4.80, p < .001; see Table 4).

In accordance with our expectations, we found no significant effects of Condition for SCR on either CS- or CS+ trials during the acquisition phase (both p > .05).

Extinction

US Expectancy

As depicted in figure 1A (middle column), participants in both conditions had initially higher US expectancies during CS+ trials than during CS- trials (main effect of CStype, b = 47.33, t(161.82) = 10.27, p < .001; see Table 2), indicating successful retrieval of the fear memory at the start of the extinction phase. Extinction of fear was also successful for all participants: participants showed a stronger decrease in expectancy ratings for CS+ trials than for CS- trials over the course of the extinction phase, CStype*LogTrial, b = -15.59, t(181.22) = -8.54, p < .001.

To test whether tVNS facilitated extinction learning, the CStype*LogTrial*Condition interaction was examined. This interaction was not significant (p = .94), indicating that there was no overall difference between the experimental conditions in differential learning rates during the extinction phase. However, the Condition*CStype interaction was significant, b = -12.60, t(181.22) = -1.91, p = .03. The latter interaction indicates that participants in the tVNS condition reported significantly lower differentiation between CS+ and CS- trials during the extinction phase, compared to sham (see Table 2 – an initial difference of 12.60).

As can be observed in Figure 1A, successful extinction had already occurred halfway during the extinction phase, in both conditions. We therefore tested more specifically whether any differences in the learning curves could be observed during these trials during which learning actually took place. To do so, we selected only the first 10 CS+ and CS- trials of the extinction phase and ran the mixed model analyses again. There was no significant CStype*LogTrial*Condition effect (p = .22), nor was there a Condition*CStype interaction (p = .07). By contrast, if we included only the first CS+ 10 trials, we did find a significant Condition*Logtrial effect, b = -11.73, t(83.18) = -1.93, p = .03, indicating a faster deceleration of US expectancy ratings for CS+ trials in the tVNS condition.

Startle EMG

As shown in figure 1B (middle column), participants in both conditions had higher startle responses for CS+ trials than for CS- trials at the start of the extinction phase (main effect of $CStype_{CS-}$, b = -9.06, t(441.28) = -4.67, p < .001; see Table 3), indicating successful retrieval of the fear memory acquired during the acquisition phase. Subsequently, there was a faster decline in startle EMG for CS+ trials compared to CS- trials ($CStype_{CS-}$*LogTrial, b = 2.69, t(500.27) = 3.31, p < .001), which indicates a successful fear extinction. This is also clearly visible in figure 1B: at the end of the extinction procedure, both the tVNS and the sham condition no longer show an elevated startle response to the CS+ compared to the CS-.

In contrast to our expectations, there were no differences between the conditions in the differential learning rates during the extinction phase, as indicated by the non-significant $CStype_{CS-}$*LogTrial*Condition (CS+ versus CS- trials) and $CStype_{ITI}$*LogTrial*Condition (CS+ versus ITI trials) interactions (both *p*s > .05). However, there was a generally accelerated decrease in startle response for both CSs and ITI in the tVNS group compared to the sham group (Condition*LogTrial interaction, b = -2.72, t(495.02) = -3.27, p < .001). As can be seen in figure 1, this accelerated decrease in startle responding is likely driven by the higher startle EMG response displayed by participants in the tVNS condition at the start of the extinction phase. The tVNS indeed started out with higher startle responses, as indicated by the main effect of Condition, b = 7.00, t(441.28) = 3.48, which was in the unexpected direction (p = .99).

SCR

As depicted in figure 1C (middle column), participants' SCR reflected a significant differentiation between CS+ and CS- trials at the start of the extinction phase, indicating a retention of the initial fear memory (main effect of CStype, b = .14, t(251.93) = 2.78, p = .006; see table 4). Subsequently, as indicated by the significant LogTrial * CStype interaction (b = -.05, t(301.44) = -2.39, p = .02), participants showed a stronger decrease in SCR for CS+ trials than for CS- trials over the course of the extinction phase, indicating a successful extinction of fear in both groups.

In contrast to our main hypotheses, there was no main effect of Condition, nor did we find any interaction effects of Condition (all *p*s > .05), indicating that tVNS did not affect the extinction of fear as reflected by SCR.

Retention

US Expectancy

As shown in figure 1A (right column), participants reported higher US expectancies for CS+ trials than for CS- trials at the start of the retention phase (main effect of CStype, b = 23.97, t(80.75) = 4.30, p < .001; see table 2). Again, there was a clear differential re-extinction curve, where participants had stronger decreases in US expectancy ratings for CS+ trials than for CS- trials as indicated by the CStype*LogTrial interaction, b = -10.84, t(128.42) = -3.29, p = .001. This differential learning indicates that renewed declarative extinction learning took place in both groups during the retention phase.

In contrast to our main hypotheses, Condition did not affect the return of declarative fear, nor did it affect extinction learning rates during the retention phase (all ps > .05).

Startle EMG

As can be seen in figure 1B (right column), participants' EMG responses during CS+ and CS- trials are elevated compared to during ITI at the start of the retention phase. Indeed, although there was no differentiation in EMG responses between CS+ and CS- trials at the start of the retention phase, startle responses during CS+ trials were significantly larger than during ITI ($CStype_{ITI}$, b = -5.54, t(198.02) = -2.28, p = .02; see table 3). The lack of differentiation in startle responses between CS+ and CS- trials could possibly indicate a generalization of the initial fear memory. Over the course of the retention phase, participants displayed a renewed overall decrease in startle responses (LogTrial, b = -5.19, t(240.43) = -4.10, p < .001), possibly reflecting a renewed habituation to the startle probe. There was no renewed differential startle extinction learning ($CStype_{ITI}$*LogTrial and $CStype_{CS-}$*LogTrial both p > .05).

Similarly to the extinction phase, there were no differences between the conditions in the differential learning rates during the retention phase, as indicated by the non-significant $CStype_{CS-}$*LogTrial*Condition and $CStype_{ITI*}$LogTrial*Condition interactions (both ps > .05). However, there was – similar to the startle responses during extinction - an accelerated decrease in startle responding in the tVNS group (Condition*LogTrial interaction, b = -3.34, t(238.06) = -1.82, p = .04), possibly reflecting an accelerated habituation to the startle probe.

SCR

Participants showed a small, non-significant differential return of fear in SCR during the retention test (main effect of CStype, b = .05, t(93.24) = 1.66, p = .10; see table 4). Similarly to the Acquisition phase, the model that provided the strongest model fit for SCR was a model that did not include

either a Time or a LogTrial effect, indicating that participants did not show a clear learning curve during the retention phase.

We found no significant differences between the experimental conditions on overall SCR, as indicated by the main effect of Condition (p = .65). There was, however, a significant Condition*CStype interaction, b = -.07, t(93.24) = -1.73, p = .04), indicating that compared to the sham condition, participants in the tVNS condition had lower differential SCRs during the retention test. However, consistent with the absence of a main effect of CStype in the main analysis, when we performed an exploratory mixed model analysis on the effects of LogTrial and CStype for each experimental condition separately, participants in neither condition showed a significant differential fear response as indexed by a main effect of CStype (both p > .05). Thus, although the initial analysis revealed that participants in the tVNS condition showed a smaller differential SCR to the CS+ than participants in the sham condition, this result should be interpreted with caution, as participants in neither group showed a significant differential fear response as reflected by SCR during the retention phase.

Table 2. Regression weights and standard errors for mixed model analyses predicting US expectancy ratings in Acquisition, Extinction and Retention phases.

Predictor	Acquisition	Extinction	Retention
Intercept	50.87(3.29)***	28.01(4.10)***	22.83(5.29)***
CStype	4.14(4.22)	47.33(4.61)***	23.97(5.58)***
LogTrial	-13.70(1.57)***	-5.53(1.29)***	-5.79(2.33)*
LogTrial*CStype	23.06(2.22)***	-15.59(1.83)***	-10.84(3.30)**
Condition	-2.06(4.72)	6.10(5.88)	4.45(7.58)
Condition*LogTrial	.97(2.24)	-1.88(1.85)	-.25(3.30)
Condition*CStype	-2.01(6.04)	-12.60(6.60)*	.48(7.99)
Condition*LogTrial*CStype	2.02(3.17)	4.30(2.62)	-1.51(4.72)

Note. Reference category for CStype is the CS- trial type. All analyses on the effects of tVNS were conducted using one-sided hypothesis tests. *p < .05, **p < .01, ***p < .001.

Table 3. Regression weights and standard errors for mixed model analyses predicting EMG in Acquisition, Extinction and Retention phases.

Predictor	Acquisition	Extinction	Retention
Intercept	66.05(1.89)***	66.19(1.40)***	56.39(1.98)***
$CStype_{CS-}$	1.65(2.48)	-9.06(1.94)***	-2.19(2.44)
$CStype_{ITI}$	-6.06(2.48)*	-16.00(1.93)***	-5.54(2.43)*
LogTrial	-3.85(.90)***	-7.77(.57)***	-5.19(1.27)***

LogTrial*CStype$_{CS-}$	-4.36(1.28)***	2.69(.81)***	.37(1.78)
LogTrial*CStype$_{ITI}$	-1.78(1.28)	5.46(.81)***	1.83(1.78)
Condition	-.44(2.73)	7.00(2.04)^{t}	4.29(2.87)
Condition*CStype$_{CS-}$	-2.41(3.60)	-1.96(2.81)	1.48(3.53)
Condition*CStype$_{ITI}$	1.03(3.60)	-5.75(2.80)^{t}	-4.68(3.53)
Condition*LogTrial	.57(1.31)	-2.72(.83)***	-3.34(1.83)*
Condition*LogTrial*CStype$_{CS-}$	.93(1.85)	.95(1.18)	-.70(2.59)
Condition*LogTrial*CStype$_{ITI}$	-1.35(1.85)	1.76(1.17)	2.59(2.59)

Note. Reference category for CStype is the CS+ trial type. All analyses on the effects of tVNS were conducted using one-sided hypothesis tests. Regression weights denoted by ^{t} reflect variables in the regression model that were significant, but in the non-hypothesized direction. *p < .05, **p < .01, ***p < .001.

Table 4. Regression weights and standard errors for mixed model analyses predicting SCR in Acquisition, Extinction and Retention phases.

Predictor	Acquisition	Extinction	Retention
Intercept	.21(.04)***	.27(.06)***	.29(.05)***
CStype	.09(.02)***	.14(.05)**	.05(.03)
LogTrial	-	-.01(.02)	-
LogTrial*CStype	-	-.05(.02)*	-
Condition	.02(.06)	-.02(.08)	-.03(.07)
Condition*CStype	-.01(.03)	-.05(.07)	-.07(.04)*
Condition*LogTrial	-	-.01(.02)	-
Condition*LogTrial*CStype	-	.02(.02)	-

Note. Reference category for CStype is the CS- trial type. All analyses on the effects of tVNS were conducted using one-sided hypothesis tests. *p < .05, **p < .01, ***p < .001.

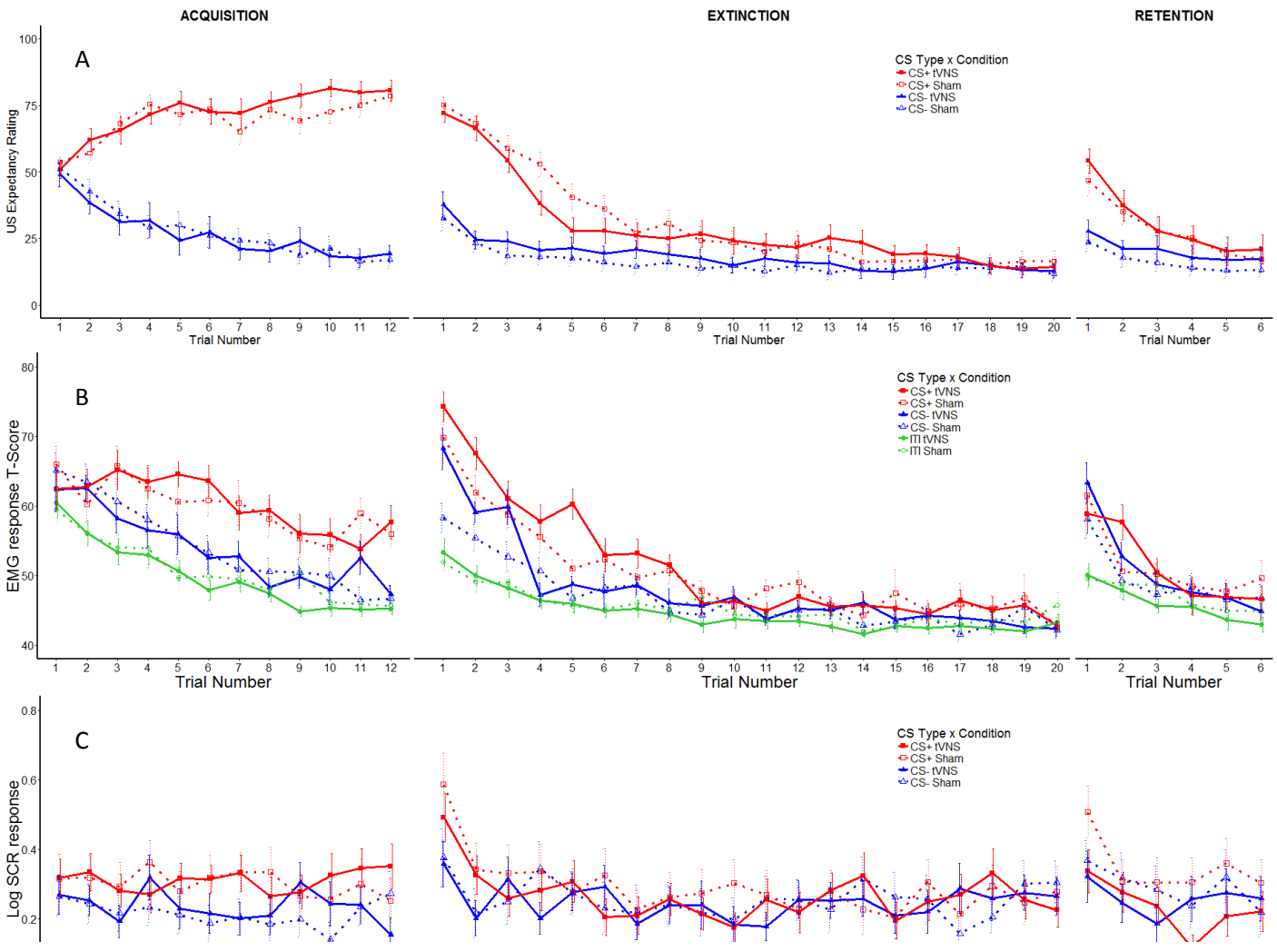


Figure 1. Overview of results for the acquisition (left), extinction (middle) and retention (right) phases of the study. The figure shows mean responses per trial for US expectancy ratings (A), EMG (B) and SCR (C). Error bars indicate ±1 standard error confidence intervals.

DISCUSSION

Non-invasive stimulation of the vagus nerve has been proposed as a promising tool to improve the rate and consolidation of extinction learning (Alvarez-Dieppa et al., 2016; Burger et al., 2016; Peña et al., 2014, 2013). In this study, we found evidence that tVNS accelerated the extinction of declarative fear. This finding is in line with findings from prior animal studies showing accelerated extinction of fear in rats (Alvarez-Dieppa et al., 2016; Peña et al., 2014, 2013), as well as our previous study where we found that tVNS accelerated declarative fear extinction in humans (Burger et al., 2016). However, we found no indication of an enhanced consolidation of declarative extinction memory during the retention test 24 hours later. These results are also in line with findings from our previous study, where we also found no effects of tVNS on declarative fear retention (Burger et al., 2016). However, the present study did not find clear effects of tVNS on physiological indices of fear during either the extinction or the retention phase. Taken together, the results of the current study point towards a positive effect of tVNS on the declarative but not the physiological extinction of fear. The accelerated declarative fear extinction learning found in this study may be caused by the effects of VNS on NE concentration in the PFC as well as limbic areas such as the amygdala and hippocampus (Hassert et al., 2004; Peña et al., 2014). Increased NE levels have been found repeatedly as a result of VNS in animal literature (e.g. Dorr & Debonnel, 2006; Follesa et al., 2007). Similarly, VNS has been associated with increased activity in the LC, the main hub for NE synthesis, in humans (Desbeaumes et al., 2015; Frangos et al., 2014). NE is involved in the formation and consolidation of new memories by increasing the excitation and synaptic plasticity of the target neurons (for a review, see Mueller & Cahill, 2010). A recent study provided additional insights into the molecular mechanisms by which VNS speeds up extinction learning. In this study with rats, VNS promoted plasticity by increasing levels of the protein kinase CMKII and decreasing expression of Arc protein (Alvarez-Dieppa et al., 2016). This amount of change in the level of proteins, induced by stimulating the vagus, was not observed in the sham group, and was only reached in a group of non-stimulated rats that received *extended* extinction training, lasting *five times longer.* This indicates that VNS could indeed speed up extinction learning. From a clinical point of view, an accelerated declarative fear extinction could be very promising, since populations that receive exposure therapy often have difficulties extinguishing fear primarily at the start of the extinction phase due to heightened fear expression – a phenomenon called *fear load* (Norrholm et al., 2011, 2015). Accelerating extinction learning in this pivotal early phase may have large consequences for the effectiveness of exposure treatments, for example by reducing treatment dropout for patients suffering from PTSD, which occurs frequently during the early stages of treatments (Kehle-Forbes, Meis, Spoont, & Polusny, 2016).

A visual inspection of figure 1A indicated that the effects of tVNS on declarative fear extinction were mainly visible in the first half of the extinction phase. Therefore, we performed exploratory analyses to assess the differential and non-differential fear extinction in this subset of the data. TVNS was only associated with an accelerated declarative extinction curve when non-differential fear extinction was assessed, that is to say when CS- trials were not included in the statistical model. This discrepancy is unlikely to indicate that the effect of tVNS on CS+ is genuinely non-differential: in line with our hypotheses, figure 1A clearly shows that the US expectancy ratings of participants in the tVNS condition decrease more quickly for CS+ trials and do not show such a pattern for CS- trials. Instead, the difference between these models is likely to reflect a lack of robustness of our statistical models, as a consequence of this study's small sample size and the relatively modest effect that tVNS had on US expectancy ratings. Clearly, although the effects of tVNS on declarative fear extinction seem positive, they call for larger scale studies to replicate these effects.

Despite the accelerated extinction of declarative fear, there was no evidence for an enhanced retrieval of the declarative extinction memory during the retention phase. These results suggest that the encoding or acquisition of the extinction memory was affected by the stimulation, but the subsequent consolidation and retrieval of that memory was not affected by stimulation. In this respect, the findings from the current study differed from the animal studies conducted by Peña and colleagues (2013), who repeatedly found effects of tVNS paired with extinction training on the subsequent test day. Although this finding may reflect a true effect where tVNS affects the speed of encoding but not the subsequent consolidation of memories in humans, this contrasting finding may also be due to a characteristic of this experiment itself. Specifically, due to the high number of extinction trials, both groups may have had a chance to create a strongly consolidated extinction memory by the end of the extinction phase. As can be clearly seen in figure 1, participants in both conditions had finished learning the CS-noUS association halfway through the extinction phase, leaving more than half the session to further consolidate the extinction memory. In future studies, researchers could consider including fewer extinction trials to avoid potential ceiling effects in the consolidation of extinction memories. Alternatively, in anxious populations, fear memories are more resistant to extinction and there is a greater risk of a return of fear (Lissek et al., 2005). Thus, future research may benefit from focusing on populations with subclinical or clinical anxiety to further elucidate the effects of tVNS on the retention of extinction memories.

The current study is the first to report on the effects of tVNS on psychophysiological indices of fear in humans. In contrast to our previous study, participants in this study showed clear differential fear learning during the fear acquisition phase, as indexed by both SCR and startle EMG.

However, in contrast to the declarative indices of fear extinction, psychophysiological indices of fear extinction were not affected by tVNS. The accelerated decrease in startle responding during the extinction and retention phases for participants in the tVNS condition likely reflected a general habituation pattern instead of a clear fear extinction curve, since the accelerated curve was not specific to CS+ trials and may have partially been caused by the increased initial startle response during the extinction phase, leaving more 'room for improvement'. Skin conductance responses were not significantly affected during the extinction phase. Participants in neither condition showed a differential skin conductance response during the retention phase, and thus the small but significant effect of tVNS on differential skin conductance responses is too preliminary to interpret and may be just a chance finding. Clearly, these results are puzzling and call for larger, more highly powered replication studies.

To speculate, the discrepancy in the effects of tVNS on declarative and psychophysiological indices of fear may be in line with the two-factor account of emotional memory proposed by Phelps (2004). In short, this theory proposes that distinct aspects of fear are controlled by at least two independent memory systems: The first is linked to the amygdala and is mainly involved in the processing of the emotional load of the event, whereas the second is linked to the hippocampus and specializes in forming declarative memories of the event (Phelps, 2004). Although these two memory systems often interact with each other, studies in patients with damage to either brain area have revealed that either memory system also operates independently (e.g. Bechara et al., 1995; LaBar, LeDoux, Spencer, & Phelps, 1995). Thus, since tVNS mainly affects the declarative extinction of fear, this could possibly indicate that tVNS leads to more prominent changes in activity of the hippocampal complex, and less so in the amygdala. This explanation of increased hippocampal activity after tVNS is strongly in line with animal studies that have shown increased NE activity and increased cellular proliferation in the hippocampus after VNS (Biggio et al., 2009; Dorr & Debonnel, 2006; Revesz, Tjernstrom, Ben-Menachem, & Thorlin, 2008). However, recent neuroimaging studies of tVNS in humans stand in contrast to this speculation. These studies suggest that tVNS may lead to a decrease in hippocampal activity while people are resting (Frangos et al., 2014; Kraus et al., 2007). It is possible that the hypothesized increased activation of the hippocampal complex is only apparent when participants are actively learning and creating new declarative memories. Clearly, more research needs to be done to assess the effects of tVNS on brain activity during emotional learning.

There were several large differences in the experimental designs between this study and our previous study (Burger et al., 2016). The current study issued a 24h period between the acquisition and extinction phases to allow for a stronger consolidation of the fear memory. Additionally, the timing of tVNS or sham stimulation was programmed to coincide with the presentation of the CS,

similar to previous animal studies (Peña et al., 2014, 2013). Finally, the current study used a painful electrocutaneous shock instead of a loud noise as a US. Despite these differences in experimental designs, the rates of declarative extinction learning in this study and in our previous study are strikingly similar for both the tVNS and the sham stimulation conditions, possibly suggesting that the efficacy of tVNS is not conditional on any of these factors. It also suggests that the timing of the tVNS (unpaired with extinction trials as in (Burger et al., 2016), or paired as in the current study) might not be of crucial importance. Indeed, animal studies have shown that even short stimulation periods lead to prolonged increases in NE levels (Hassert et al., 2004), and thus tVNS is likely to affect the attentional processing of stimuli regardless of the exact timing of the stimulation.

The current study has several limitations. First, the limited sample size reduces the statistical power of our analyses, thereby increasing the risk of type II errors. Secondly, we decided to decrease the stimulation intensity of the tVNS device if participants felt uncomfortable with the intensity set at 0.5mA. The stimulation intensity was adjusted for 7 out of 19 participants in the tVNS condition. Although this approach towards determining the optimal stimulation intensity has also been used in previous research (e.g. Frangos et al., 2014), the decreased stimulation intensities may have also negatively affected the efficacy of the stimulation procedure for these participants. Clearly, too little is currently known about what effects stimulation parameters may have on the efficacy of noninvasive VNS. Parametric studies on basic behavioral outcomes (e.g. associative memory, mood, inhibitory control) are clearly needed. Thirdly, a limitation of tVNS studies in general is that there is currently no reliable, non-invasive measure to assess whether tVNS has successfully increased the activation of the vagus nerve. One possible alternative would be to test the hypothesized working mechanism through which tVNS affects learning and memory. For example, future studies could include assessments of alpha amylase or pupil dilation as indirect measures of NE (e.g. Laeng, Sirois, & Gredeback, 2012; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004), to see whether changes in NE levels predict extinction rates for tVNS.

One final important limitation of this particular study is the potentially confounding role that the context switch has had on extinction learning. One could argue that participants in the tVNS condition received a context switch on the second day due to the stimulator being switched from the ‘sham’ to the ‘active’ position (ie. stimulation of the concha instead of the earlobe). By contrast, participants in the sham condition received sham stimulation on all three days. We tried to control for these effects by recruiting an additional control group post-hoc, in which participants received a context switch on the second day by applying sham stimulation to the right ear instead of the left ear. Unfortunately, participants that were recruited for that purpose appeared not to be comparable to the participants in the initial two groups, as reflected in their significantly lower US expectancy

ratings during the acquisition phase, prior to the context switch. Because of our inability to compare the tVNS condition to a control group that was also exposed to a context switch, we cannot exclude the possibility that the accelerated extinction in the tVNS condition was confounded by the context switch that occurred on day 2. Although fear memories are generally believed to be context independent (e.g. Bouton & King, 1983; Thomas, Larsen, & Ayres, 2003), specific studies on the effects of context switches on the rate of extinction learning (measured using US expectancy ratings) are scarce and less consistent. For example, Effting and Kindt (2007; experiment 1) reported a marginally faster extinction learning rate in a group that experienced a context switch during extinction, but did not replicate this accelerated extinction learning in a subsequent experiment (Effting & Kindt, 2007, experiment 2), although less discrimination between the CSs was observed on the first extinction trial in the context switch groups. In addition, Sjouwerman, Niehaus, and Lonsdorf, 2015 reported that a context switch did not facilitate extinction learning when assessed using US expectancy ratings (facilitation was observed only when assessed using SCRs), although US expectancy ratings to both CS types were reduced after the context switch. Given these preliminary data a careful interpretation of the results in this study is still warranted. More research is clearly needed on the effects of context switches on the rates of extinction learning, which is crucial for a wide range of studies that aim to boost this process. Conversely, one of the major problems facing exposure therapy interventions and their experimental counterparts is the context-dependency of extinction- and safety-memories (for a review, see Bouton, Westbrook, Corcoran, & Maren, 2006), indicating that the switch from tVNS back to sham stimulation on day three (retention testing) may have also lead to a stronger return of fear, possibly causing us to underestimate the effects of tVNS on the retention of extinction memories. Future studies may avoid these limitations by applying tVNS or sham stimulation only during extinction, and withholding any stimulation during both the acquisition and retention phases. That way, all participants receive a context switch during extinction, regardless of experimental condition. In fact, this approach was used in our previous study (Burger et al., 2016), in which effects of tVNS on extinction curves were found that were very similar to the ones reported in the current study. The similarity in results found in this study and in our previous study suggest that it is unlikely that the effects found in the current study are driven solely by an unwanted context switch.

In this study, we found indications that tVNS may positively affect learning and memory in a fear extinction paradigm. These results clearly call for more large scale studies to assess the effects of tVNS on fear-related learning processes, including extinction and retention learning.

Acknowledgments

This work was supported by the following research grants: a KU Leuven grant (PF/10/005) to the Centre for Excellence of Generalization Research in Ill Health and Psychopathology (IVD); the ‘‘Asthenes’’ long-term structural funding (METH/15/011) - Methusalem grant by the Flemish Government (IVD); a Veni Grant 451-14-013 from NWO, the Netherlands (BartV); a postdoctoral research fellowship grant from the Research Foundation Flanders (HCM); a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme (PIOR-GA-2013-6277743; to BramV). Special thanks go to Lisse Van Duppen for her assistance with processing the ECG data.

REFERENCES

Alvarez-Dieppa, A. A. C., Griffin, K., Cavalier, S., & Mcintyre, C. K. (2016). Vagus nerve stimulation enhances extinction of conditioned fear in rats and modulates Arc protein, CaMKII, and GluN2B-containing NMDA receptors in the basolateral amygdala. *Neural Plasticity*, 1–19.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double Dissociation of Conditioning and Declarative Knowledge Relative to the Amygdala and Hippocampus in Humans. *Science*, *269*(August), 1115–1118.

Ben-Menachem, E., Revesz, D., Simon, B. J., & Silberstein, S. (2015). Surgically implanted and non-invasive vagus nerve stimulation: A review of efficacy, safety and tolerability. *European Journal of Neurology*, *22*(9), 1260–1268. http://doi.org/10.1111/ene.12629

Beste, C., Steenbergen, L., Sellaro, R., Grigoriadou, S., Zhang, R., Chmielewski, W., … Colzato, L. (2016). Effects of Concomitant Stimulation of the GABAergic and Norepinephrine System on Inhibitory Control – A Study Using Transcutaneous Vagus Nerve Stimulation. *Brain Stimulation*. http://doi.org/10.1016/j.brs.2016.07.004

Biggio, F., Gorini, G., Utzeri, C., Olla, P., Marrosu, F., Mocchetti, I., & Follesa, P. (2009). Chronic vagus nerve stimulation induces neuronal plasticity in the rat hippocampus. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *12*(9), 1209–1221. http://doi.org/10.1017/S1461145709000200

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, *42*(1), 1–15. http://doi.org/10.1111/j.1469-8986.2005.00271.x

Bouton, M. E., & King, D. a. (1983). Contextual control of the extinction of conditioned fear: tests for the associative value of the context. *Journal of Experimental Psychology. Animal Behavior Processes*, *9*(3), 248–265. http://doi.org/10.1037/0097-7403.9.3.248

Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A Modern Learning Theory Perspective on the Etiology of Panic Disorder. *Psychological Review*, *108*(1), 4–32. http://doi.org/10.1037//0033-295X.108.1.4

Bouton, M. E., Westbrook, R. F., Corcoran, K. A., & Maren, S. (2006). Contextual and Temporal Modulation of Extinction: Behavioral and Biological Mechanisms. *Biological Psychiatry*, *60*(4), 352–360. http://doi.org/10.1016/j.biopsych.2005.12.015

Burger, A., Verkuil, B., Van Diest, I., Van Der Does, W., Thayer, J., & Brosschot, J. (2016). The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans. *Neurobiology of Learning and Memory*, *132*, 49–56. http://doi.org/10.1016/j.nlm.2016.05.007

Burnham, K. P., & Anderson, R. P. (2004). Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods & Research*, *33*(2), 261–304. http://doi.org/10.1177/0049124104268644

Cahill, L., & Mcgaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends in Neurosciences*, *2236*(1997), 22983–22986.

Cain, C. K., Blouin, A. M., & Barad, M. (2004). Adrenergic Transmission Facilitates Extinction of Conditional Fear in Mice. *Learning & Memory*, *11*(2), 179–187. http://doi.org/10.1101/lm.71504

Chalmers, J., Quintana, D., Abbott, M., & Kemp, A. (2014). Anxiety Disorders are Associated with Reduced Heart Rate Variability: A Meta-Analysis. *Frontiers in Psychiatry*, *5*(July), 1–11. http://doi.org/10.3389/fpsyt.2014.00080

Clark, K., Naritoku, D., Smith, D., Browning, R., & Jensen, R. (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects, *2*(1).

Clark, K., Smith, D., Hassert, D., Browning, R., Naritoku, D., & Jensen, R. (1998). Posttraining electrical stimulation of vagal afferents with concomitant vagal efferent inactivation enhances memory storage processes in the rat. *Neurobiology of Learning and Memory*, *70*(3), 364–373. http://doi.org/10.1006/nlme.1998.3863

Dawson, M. E., Schell, A. M., Filion, D. L., & Berntson, G. G. (2007). The Electrodermal System. In J. T. Cacioppo, L. G. Tassinary, & G. Berntson (Eds.), *Handbook of Psychophysiology* (pp. 157–181). Cambridge: Cambridge University Press. http://doi.org/10.1017/CBO9780511546396.007

Desbeaumes, V., Lespérance, P., Nguyen, D. K., Fournier-gosselin, M., Richer, F., Hospitalier, C., & Université, D. (2015). Effects of vagus nerve stimulation on pupillary function. *International Journal of Psychophysiology*, *98*(3), 455–459. http://doi.org/10.1016/j.ijpsycho.2015.10.001

Dorr, A. E., & Debonnel, G. (2006). Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *The Journal of Pharmacology and Experimental Therapeutics*, *318*(2), 890–898. http://doi.org/10.1124/jpet.106.104166.and

Duits, P., Cath, D. C., Lissek, S., Hox, J., Hamm, A. O., Engelhard, I. M., … Baas, J. M. P. (2015). Updated meta-analysis of classical fear conditioning in the anxiety disorders. *Depression and Anxiety*, *32*, 239–253. http://doi.org/10.1002/da.22353

Effting, M., & Kindt, M. (2007). Contextual control of human fear associations in a renewal paradigm. *Behaviour Research and Therapy*, *45*(9), 2002–2018. http://doi.org/10.1016/j.brat.2007.02.011

Fang, J., Rong, P., Hong, Y., Fan, Y., Liu, J., Wang, H., … Kong, J. (2015). Transcutaneous Vagus Nerve Stimulation Modulates Default Mode Network in Major Depressive Disorder. *Biological Psychiatry*, *79*(4), 1–8. http://doi.org/10.1016/j.biopsych.2015.03.025

Fanselow, M. S. (2013). Fear and anxiety take a double hit from vagal nerve stimulation. *Biological Psychiatry*, *73*(11), 1043–4. http://doi.org/10.1016/j.biopsych.2013.03.025

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews*, *23*, 743–760.

Follesa, P., Biggio, F., Gorini, G., Caria, S., Talani, G., Dazzi, L., … Biggio, G. (2007). Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Research*, *1179*, 28–34. http://doi.org/10.1016/j.brainres.2007.08.045

Frangos, E., Ellrich, J., & Komisaruk, B. R. (2014). Non-invasive Access to the Vagus Nerve Central Projections via Electrical Stimulation of the External Ear: fMRI Evidence in Humans. *Brain Stimulation*, *8*(3), 624–636. http://doi.org/10.1016/j.brs.2014.11.018

Friedman, B. H. (2007). An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. *Biological Psychology*, *74*(2), 185–99. http://doi.org/10.1016/j.biopsycho.2005.08.009

George, M. S., Ward, H. E., Ninan, P. T., Pollack, M., Nahas, Z., Anderson, B., … Ballenger, J. C. (2008). A pilot study of vagus nerve stimulation (VNS) for treatment-resistant anxiety disorders. *Brain Stimulation*, *1*(2), 112–21. http://doi.org/10.1016/j.brs.2008.02.001

Grimonprez, A., Raedt, R., Baeken, C., Boon, P., & Vonck, K. (2015). The antidepressant mechanism of action of vagus nerve stimulation: Evidence from preclinical studies. *Neuroscience & Biobehavioral Reviews*, *56*, 26–34. http://doi.org/10.1016/j.neubiorev.2015.06.019

Gueorguieva, R., & Krystal, J. H. (2011). Move over ANOVA. *Arch Gen Psychiatry*, *61*(April 2004), 310–317. http://doi.org/10.1001/archpsyc.61.3.310

Hassert, D. L., Miyashita, T., & Williams, C. L. (2004). The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. *Behavioral Neuroscience*, *118*(1), 79–88. http://doi.org/10.1037/0735-7044.118.1.79

Hofmann, S. G. (2007). Enhancing exposure-based therapy from a translational research perspective. *Behaviour Research and Therapy*, *45*(9), 1987–2001.

Indovina, I., Robbins, T. W., Nunez-Elizalde, A. O., Dunn, B. D., & Bishop, S. J. (2011). Fear-Conditioning Mechanisms Associated with Trait Vulnerability to Anxiety in Humans. *Cell*, 563–571. http://doi.org/10.1016/j.neuron.2010.12.034

Jacobs, H. I. L., Riphagen, J. M., Razat, C. M., Wiese, S., & Sack, A. T. (2015). Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiology of Aging*, *36*(5), 1860–1867. http://doi.org/10.1016/j.neurobiolaging.2015.02.023

Kehle-Forbes, S. M., Meis, L. A., Spoont, M. R., & Polusny, M. A. (2016). Treatment Initiation and Dropout From Prolonged Exposure and Cognitive Processing Therapy in a VA Outpatient Clinic. *Psychological Trauma: Theory, Research, Practice, and Policy*, *8*(1), 107–114.

Klarer, M., Arnold, M., Günther, L., Winter, C., Langhans, W., & Meyer, U. (2014). Gut vagal afferents differentially modulate innate anxiety and learned fear. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(21), 7067–76. http://doi.org/10.1523/JNEUROSCI.0252-14.2014

Kraus, T., Hösl, K., Kiess, O., Schanze, A., Kornhuber, J., & Forster, C. (2007). BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *114*(11), 1485–93. http://doi.org/10.1007/s00702-007-0755-z

LaBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *J.Neurosci.*, *15*(October), 6846–6855. Retrieved from http://www.jneurosci.org/content/15/10/6846.full.pdf

Laeng, B., Sirois, S., & Gredeback, G. (2012). Pupillometry: A Window to the Preconscious? *Perspectives on Psychological Science*, *7*(1), 18–27. http://doi.org/10.1177/1745691611427305

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. a, Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research and Therapy*, *43*(11), 1391–424. http://doi.org/10.1016/j.brat.2004.10.007

Liu, J., Fang, J., Wang, Z., Rong, P., Hong, Y., Fan, Y., … Kong, J. (2016). Transcutaneous vagus nerve stimulation modulates amygdala functional connectivity in patients with depression. *Journal of Affective Disorders*, *205*, 319–326. http://doi.org/10.1016/j.jad.2016.08.003

McGaugh, J. L. (2002). Memory consolidation and the amygdala: A systems perspective. *Trends in Neurosciences*. http://doi.org/10.1016/S0166-2236(02)02211-7

Mcgaugh, J. L., & Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain James L McGaugh and Benno Roozendaal. *Current Opinion in Neurobiology*, *12*, 205–210.

Mineka, S., & Zinbarg, R. (2006). A Contemporary Learning Theory Perspective on the Etiology of Anxiety Disorders. *American Psychologist*, *61*(1), 10–26. http://doi.org/10.1037/0003-066X.61.1.10

Mueller, D., & Cahill, S. P. (2010). Noradrenergic modulation of extinction learning and exposure therapy, *208*, 1–11. http://doi.org/10.1016/j.bbr.2009.11.025

Norrholm, S., Glover, E., Stevens, J., Fani, N., Galatzer-levy, I., Bradley, B., … Jovanovic, T. (2015). Fear load : The psychophysiological over-expression of fear as an intermediate phenotype associated with trauma reactions. *International Journal of Psychophysiology*, *98*(2), 270–275. http://doi.org/10.1016/j.ijpsycho.2014.11.005

Norrholm, S., Jovanovic, T., Olin, I., Sands, L., Bradley, B., & Ressler, K. (2011). Fear Extinction in Traumatized Civilians with Posttraumatic Stress Disorder : Relation to Symptom Severity. *BPS*, *69*(6), 556–563. http://doi.org/10.1016/j.biopsych.2010.09.013

Osman, A., Barrios, F. X., Gutierrez, P. M., Kopper, A., Merrifield, T., & Grittmann, L. (2000). The Pain Catastrophizing Scale : Further Psychometric Evaluation with Adult Samples, *23*(4).

Osman, A., Gutierrez, P. M., Smith, K., Fang, Q., Lozano, G., Devine, A., … Fang, Q. (2010). The Anxiety Sensitivity Index – 3 : Analyses of Dimensions , Reliability Estimates , and Correlates in Nonclinical Samples The Anxiety Sensitivity Index – 3 : Analyses of Dimensions , Reliability Estimates , and Correlates in Nonclinical Samples, *3891*(November 2016). http://doi.org/10.1080/00223890903379332

Pappens, M., Schroijen, M., Sütterlin, S., Smets, E., Van Den Bergh, O., Thayer, J. F., & Van Diest, I. (2014). Resting heart rate variability predicts safety learning and fear extinction in an interoceptive fear conditioning paradigm. *PloS One*, *9*(9), e105054. http://doi.org/10.1371/journal.pone.0105054

Peña, D. F., Childs, J. E., Willett, S., Vital, A., McIntyre, C. K., & Kroener, S. (2014). Vagus nerve stimulation enhances extinction of conditioned fear and modulates plasticity in the pathway from the ventromedial prefrontal cortex to the amygdala. *Frontiers in Behavioral Neuroscience*, *8*(September), 327. http://doi.org/10.3389/fnbeh.2014.00327

Peña, D. F., Engineer, N. D., & McIntyre, C. K. (2013). Rapid remission of conditioned fear expression with extinction training paired with vagus nerve stimulation. *Biological Psychiatry*, *73*(11), 1071–7. http://doi.org/10.1016/j.biopsych.2012.10.021

Peugh, J. L. (2010). A practical guide to multilevel modeling. *Journal of School Psychology*, *48*(1), 85–112. http://doi.org/10.1016/j.jsp.2009.09.002

Peuker, E. T., & Filler, T. J. (2002). The nerve supply of the human auricle. *Clinical Anatomy (New York, N.Y.)*, *15*(1), 35–7. http://doi.org/10.1002/ca.1089

Phelps, E. a. (2004). Human emotion and memory: Interactions of the amygdala and hippocampal complex. *Current Opinion in Neurobiology*, *14*(2), 198–202. http://doi.org/10.1016/j.conb.2004.03.015

Revesz, D., Tjernstrom, M., Ben-Menachem, E., & Thorlin, T. (2008). Effects of vagus nerve stimulation on rat hippocampal progenitor proliferation. *Experimental Neurology*, *214*(2), 259–265. http://doi.org/10.1016/j.expneurol.2008.08.012

Rohleder, N., Nater, U. M., Wolf, J. M., Ehlert, U., & Kirschbaum, C. (2004). Psychosocial stress-induced activation of salivary alpha-amylase: An indicator of sympathetic activity? *Annals of the New York Academy of Sciences*, *1032*, 258–263. http://doi.org/10.1196/annals.1314.033

Rong, P., Liu, J., Wang, L., Liu, R., Fang, J., Zhao, J., … Kong, J. (2016). Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: A nonrandomized controlled pilot study. *Journal of Affective Disorders*, *195*, 172–179. http://doi.org/10.1016/j.jad.2016.02.031

Scerbo, A. S., Freedman, L. W., Raine, A., Dawson, M. E., & Venables, P. H. (1992). A major effect of recording site on measurement of electrodermal activity. *Psychophysiology*, *29*(2), 241–246. http://doi.org/10.1111/j.1469-8986.1992.tb01693.x

Sellaro, R., Leusden, J. Van, & Colzato, L. (2015). Transcutaneous vagus nerve stimulation (tVNS) enhances post-error slowing. *Accepted for Publication in Journal of Cognitive Neuroscience*, *27*(11), 2126–2132.

Sjouwerman, R., Niehaus, J., & Lonsdorf, T. B. (2015). Contextual change after fear acquisition affects conditioned responding and the time course of extinction learning – Implications for renewal research. *Frontiers in Behavioral Neuroscience*, *9*(December), 1–9. http://doi.org/10.3389/fnbeh.2015.00337

Smith, S. M., & Vela, E. (2001). Environmental context-dependent memory: A review and meta-analysis. *Psychonomic Bulletin & Review*, *8*(2), 203–220. http://doi.org/10.3758/BF03196157

Stewart, R. E., & Chambless, D. L. (2009). Cognitive – Behavioral Therapy for Adult Anxiety Disorders in Clinical Practice : A Meta-Analysis of Effectiveness Studies, *77*(4), 595–606. http://doi.org/10.1037/a0016032

Thomas, B. L., Larsen, N., & Ayres, J. J. B. (2003). Role of context similarity in ABA, ABC, and AAB renewal paradigms: Implications for theories of renewal and for treating human phobias. *Learning and Motivation*, *34*(4), 410–436. http://doi.org/10.1016/S0023-9690(03)00037-7

Vonck, K., Raedt, R., Naulaerts, J., De Vogelaere, F., Thiery, E., Van Roost, D., … Boon, P. (2014). Vagus nerve stimulation…25 years later! What do we know about the effects on cognition? *Neuroscience and Biobehavioral Reviews*, *45C*, 63–71. http://doi.org/10.1016/j.neubiorev.2014.05.005

Wendt, J., Neubert, J., Koenig, J., Thayer, J., & Hamm, A. (2015). Resting heart rate variability is associated with inhibition of conditioned fear. *Psychophysiology*, *52*, 1161–1166. http://doi.org/10.1111/psyp.12456

Wijk, A. J. Van, & Hoogstraten, J. (2006). Dutch translation of the Fear of Pain Questionnaire : Factor structure , reliability and validity, *10*, 479–486. http://doi.org/10.1016/j.ejpain.2005.06.008

SUPPLEMENTARY

METHODS

Directly after the retention test, participants were presented with one unreinforced CS- trial and one reinforced CS+ trial. Subsequently, we tested the re-acquisition of fear as a result of the renewed CS-US contingency. We also tested the generalization of the fear memory, by presenting one more reinforced CS+ trial, one unreinforced CS-, three unreinforced generalized stimuli that looked similar to the CS+ (GS+) and three unreinforced trials that were similar to the CS- (GS-), presented in a semi-randomized order.

To test the effects of tVNS on the reacquisition of fear, we will focus on the changes in fear responses between the first and second CS+ and CS- trials. The generalization of fear will be tested in a separate analysis using only the three GS+ and GS- trials.

RESULTS

Reacquisition of fear

US expectancy ratings

Participants' US expectancy ratings for the CS+ were significantly higher than for the CS- at the start of the reacquisition phase, even before the CS+ had been reinforced once again (main effect of CStype, $b = 14.16$, $t(35.46) = 3.44$, $p = .002$). After the first reinforced CS+ trial, US expectancy ratings for the subsequent CS+ trial increased significantly (CStype*Trial, $b = 35.36$, $t(76.00) = 5.52$, $p < .001$), whereas US expectancy ratings for the CS- trial did not (main effect of Trial, $b = 5.58$, $t(76.00) = 1.23$, $p = .22$).

There were no between-group differences in US expectancy ratings at the start of the reacquisition phase prior to the CS+ being reinforced. Experimental condition also did not affect the reacquisition of declarative fear, as indexed by the non-significant CStype*Trial*Condition interaction ($p = .64$).

EMG

Participants did not show differential startle responding at the start of the reacquisition phase, as reflected by the non-significant main effects of $CStype_{ITI}$ and $CStype_{CS-}$ (both $p > .05$). Participants showed a clear reacquisition of differential fear, as reflected by the significant increase in startle

responding to the second CS+ trial (main effect of Time, b = 10.07, t(105.16) = 4.40, p < .001). By contrast, startle responses to the CS- did not increase after the first trial ($CStype_{CS-}$*Time, b = -8.37, t(103.59) = -2.60, p < .001), nor did the startle response to the ITI ($CStype_{ITI}$*Time, b = -13.49, t(103.59) = -4.20, p < .001).

Participants in the tVNS condition had a lower startle response during the first reinforced CS+ trial compared to the sham condition, as shown by the main effect of Condition, b = -4.57, t(110.56) = -2.04, p = .02. At this point, participants were unaware of the renewed CS-US contingency, and thus this effect could suggest a difference in the extended retention of fear between conditions. The non-significant Condition*Trial interaction indicates that there are no differences between conditions on fear potentiated startle responses during the second reinforced CS+ trial, b = 4.75, t(104.96) = 1.50, p = .94.

SCR

The model that provided the strongest model fit for SCR was a model that did not include a Time effect, indicating that the renewed CS+ reinforcement did not lead to a clear increase in SCR magnitude. Participants did, however, display a larger SCR for CS+ trials than for CS- trials, b = .12, t(37.00) = .02. This differential responding was not significantly affected by Condition (both Condition and Condition*CStype, p > .05).

Fear Generalization

US expectancy ratings

Participants rated GS+ stimuli as being more likely to be followed by a shock than GS- trials (main effect of CStype, b = 16.03, t(56.45) = 3.07, p = .003). Participants' US expectancy ratings decreased in subsequent trials as reflected by the main effect of Trial, b = -9.45, t(135.86) = -4.25, p < .001, and this decrease in US expectancy ratings was irrespective of GStype (GStype*Trial, p = .76).

There were no between-group differences in the generalization of declarative fear, nor in the subsequent extinction rate of the generalized fear response (all p > .05).

EMG

Participants showed differential startle responses to GS+ trials compared to ITIs as reflected by the main effect of $CStype_{ITI}$, b = -7.11, t(122.13) = -3.40, p = .001, but not compared to GS- trials, p = .52. Indeed, participants showed overall increased fear responses to both novel stimuli. There was no

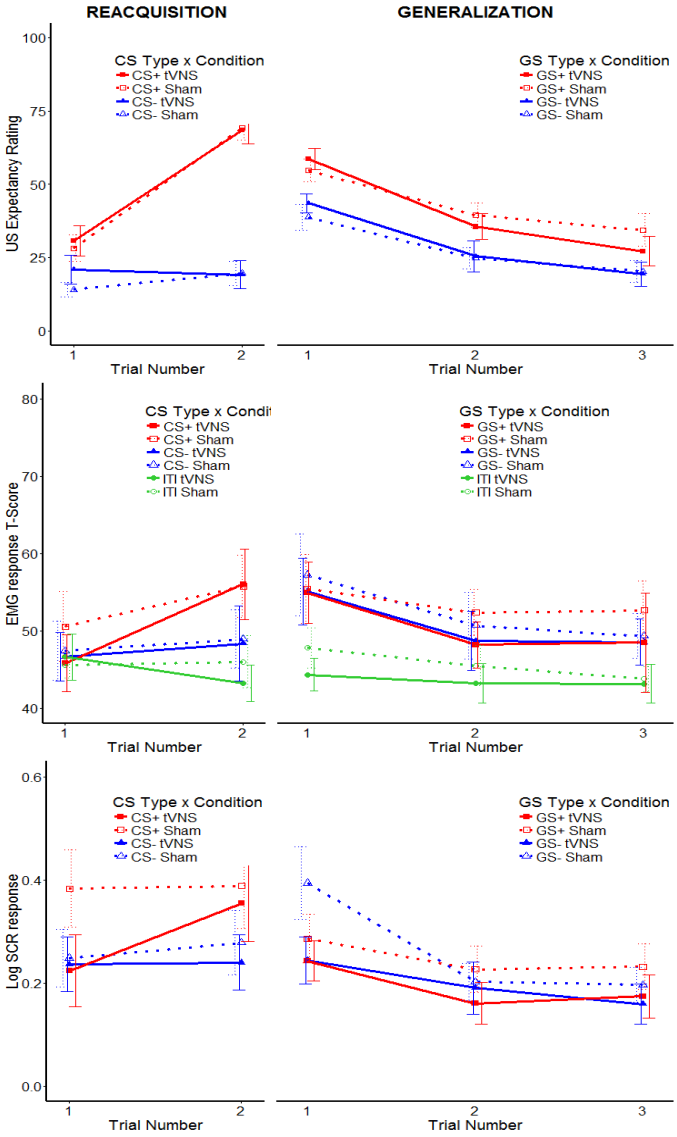
effect of Time (p = .22), and no differential learning curve for GS trials during subsequent trials (both $CStype_{CS-}$*Time and $CStype_{ITI}$*Time, p > .05).

Condition did not affect the generalization of the fear potentiated startle response, nor did it affect the extinction rate of startle responses to the generalized stimuli (all ps > .05).

SCR

Participants showed a trend towards increases in SCR magnitude to GS+ trials compared to GS- trials as reflected in the main effect of CStype, b = .12, t(104.22) = 1.84, p = .07. Subsequently, there was a stronger decline in SCR magnitude for GS+ trials as indicated by the significant CStype*Time interaction, b = -.10, t(176.33) = -1.97, p = .05, indicating an extinction of fear for the generalized CS+ trials.

Condition did not affect the generalization of the SCR magnitude, nor did it affect the extinction rate of SCR to the generalized stimuli (all ps > .05).


REACQUISITION
GENERALIZATION
US Expectancy Rating
EMG response T-Score
Log SCR response
Trial Number
CS Type x Condition
GS Type x Condition
CS+ tVNS
CS+ Sham
CS- tVNS
CS- Sham
GS+ tVNS
GS+ Sham
GS- tVNS
GS- Sham
ITI tVNS
ITI Sham