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Transcutaneous vagus nerve stimulation does not affect attention to fearful faces in high worriers

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ABSTRACT

People suffering from chronic worries pay excessive attention to emotional information. In this study we examined whether a reduced ability to inhibit attention from fearful faces (i.e. inhibition of return; IOR) can be attributed to the low vagus nerve activity observed in high worriers. Our pre-registered hypothesis was that transcutaneous auricular vagus nerve stimulation (tVNS) would enhance IOR to fearful faces. Ninety-four students who scored above a pre-determined cut-off on the Penn State Worry Questionnaire were randomly allocated to receive either tVNS (n = 45) or sham stimulation of the earlobe (n = 49). Meanwhile, to assess IOR, they performed an emotional exogenous cueing task wherein neutral and fearful faces predicted the target location at chance level. Resting levels of HRV were also collected before stimulation onset. Results showed that levels of trait worry were associated with reduced IOR, but resting levels of HRV were not. Critically, tVNS did not affect performance on the exogenous cueing task when compared to sham stimulation. These findings did not confirm the hypothesized causal role of vagus nerve activity in maintaining disrupted IOR for emotional information. They also provide evidence that high levels of worry are associated with generally reduced IOR. This points to a clear need to understand the neurobiological basis of inhibitory problems in worriers.

1. Introduction

The ability to flexibly react to signals of threat and safety in one's environment is crucial for the survival of species. In daily life, humans are continuously faced with an abundance of potential threat and safety signals. Attentional control mechanisms, balancing stimulus driven bottom-up input and goal–directed top-down input, allow us to reflexively attend to salient information (Posner, 1990). At the same time, they also tend to inhibit attention to information that has been appraised as not salient (Aron, 2007; Houghton & Tipper, 1996; Prime, Visser, & Ward, 2006). In mood and anxiety disorders, these attentional mechanisms seem to malfunction, and threat information – at the cost of safety information – seems to be given much more weight than is required by environmental demands (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & Van Ijzendoorn, 2007; Fox, Russo, & Dutton, 2002; Macleod, Mathews, & Tata, 1986). This is manifested as perseverative cognition: a continuous worrying about possible threatening events, and problems with disengaging, or inhibiting, attention from threatening information (Verkuil, Brosschot, Gebhardt, & Thayer, 2010). In this study we examine if stimulation of the vagal nerve counteracts the inhibitory difficulties experienced by people suffering from chronic worries, as little is known about this.

According to the neurovisceral integration model (Smith, Thayer, Khalsa, & Lane, 2017; Thayer & Lane, 2000, 2009), prolonged processing of threat-related information in chronic worriers is due to a breakdown of inhibitory cortical influences on subcortical areas in the brain. At the peripheral level, this breakdown in prefrontal inhibition is reflected in low resting levels of high frequency heart rate variability (HF-HRV). Low HF-HRV can be considered as a biomarker of psychopathology (Chalmers, Quintana, Abbott, & Kemp, 2014) and is typically observed in chronic worriers (Ottaviani et al., 2016). Several studies have shown that such low HF-HRV can be attributed to reduced activity in the parasympathetic nervous system, particularly the vagus nerve that innervates the pacemaker of the heart, the sinoatrial node, and the atrioventricular node (Smith et al., 2017; Thayer & Lane, 2000, 2009). Apart from this efferent function of the vagus nerve, it mainly provides the brain with critical afferent information about the status in the viscera (Hassert, Miyashita, & Williams, 2004; Ruffoli et al., 2011). Reduced activity in this large wandering nerve is believed to indicate thwarted neurovisceral integration, impeding adaptive, flexible responses to environmental demands.

Until now, the neurovisceral integration model has received support from several correlational studies. Low resting HF-HRV has been associated with reduced cognitive control (Thayer, Hansen, Saus-Rose, &

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Johnsen, 2009) and inhibitory performance in particular (Krypotos, Jahfari, van Ast, Kindt, & Forstmann, 2011; Park, Van Bavel, Vasey, & Thayer, 2012). Park and colleagues found that individual differences in resting levels of HRV were associated with inhibition of return (IOR). IOR is the phenomenon where people tend to inhibit their attention from locations that they have previously attended to, in order to explore novel locations: they show 'inhibition of return' to the previously attended location (Lupiáñez, Klein, & Bartolomeo, 2006). IOR is a relatively stable and adaptive phenomenon observed when people are performing exogenous cueing tasks. However, Park et al. (2012) observed that a diminished IOR in response to emotional cues (fearful faces) was present in participants low in resting HF-HRV, indicating that these participants were more inclined to return their attention to locations where threatening (emotionally salient) information had been presented. This diminished IOR for emotional cues has also been observed in samples that are known to demonstrate low HF-HRV (i.e., highly worrying and anxious participants (Fox et al., 2002; Verkuil, Brosschot, Putman, & Thayer, 2009)). It remains unknown however if these findings points towards a possible causal involvement of the vagus nerve in the IOR phenomenon. Therefore, we aimed to manipulate vagus nerve activity in order to test if the vagus nerve plays a causal role in maintaining the bias as observed in high worriers.

Recent developments in neurostimulation techniques allow us to stimulate the vagus nerve non-invasively, via its auricular branch (Ventureyra, 2000). Transcutaneous stimulation of the auricular branch of the vagus nerve is accompanied by similar brain activation patterns as invasive vagus nerve stimulation, thereby providing evidence for the validity of this stimulation (Badran et al., 2018; Frangos, Ellrich, & Komisaruk, 2015; Kraus et al., 2007; Yakunina, Kim, & Nam, 2017). Several recent studies have shown that transcutaneous vagus nerve stimulation (tVNS), compared to placebo stimulation of the earlobe, has acute effects on cognitive tasks that depend on inhibition. That is, tVNS was associated with becoming more careful (slowed responses) after having made a mistake (post-error slowing; Sellaro et al., 2015). Additionally, tVNS improved performance on a task where multiple actions had to be performed in a specific temporal order (action cascading; Steenbergen et al., 2015). The underlying mechanism of these cognitive effects of tVNS are believed to be the increases in levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the brain and increased activity in the locus coeruleus-noradrenalin system (Capone et al., 2015; Chen & Williams, 2012; Ruffoli et al., 2011). Noradrenalin is a neurotransmitter that is, among other processes, involved in cognitive control (Aston-Jones & Cohen, 2005). The vagus nerve projects onto the nucleus of the solitary tract, which in turn innervates the locus coeruleus in the brainstem (and this is the primary center that provides the brain with noradrenalin). Invasive vagus nerve stimulation in animals indeed leads to increases in central noradrenalin (Grimonprez et al., 2015). In terms of functionality, higher tonic activity in the locus coeruleus-noradrenalin system is believed to promote exploration and scanning of the environment (Aston-Jones & Cohen, 2005). By increasing activity in this system, tVNS might promote the search for targets at unexplored spatial locations (eg., in a exogenous cueing task), at the expense of attending to locations that have already been attended to. Importantly for the purpose of this study is the previously observed association between levels of noradrenalin (measured indirectly as the size of the pupil during cue presentation) and the magnitude of the IOR effect (Gabay, Pertzov, & Henik, 2011). Stronger IOR was related to larger pupil-derived noradrenergic activity. We therefore hypothesize that tVNS will enhance the IOR effect, especially for emotional cues (fearful faces) as IOR for these stimuli is reduced in high worriers.

Using tVNS to affect IOR for emotional cues would not only be of theoretical importance as a further test of the neurovisceral integration model. It would add to the studies that examine the clinical applications of tVNS. Several preliminary studies suggest that a prolonged period of tVNS reduces depressive and anxiety symptoms in depressed patients (Hein et al., 2013; Rong et al., 2016). It could be possible that these antidepressant effects partly depend on changes in cognitive bias preceding the mood effects. This would be similar to one of the proposed working mechanisms of antidepressant medication, where acute effects are observed on cognitive bias, and mood improvements are only apparent after a couple of weeks (Harmer, Goodwin, & Cowen, 2009). To date, no study has addressed whether tVNS affects the processing of emotionally salient information in people vulnerable for mood and anxiety disorders.

Here, we test if the reduced ability to inhibit attention to negative emotional faces, typically observed in high worriers, can be attributed to low vagus nerve activity. Our pre-registered hypothesis was that tVNS would enhance IOR for fearful faces, compared to sham stimulation. In addition, we tested if we could replicate previous findings showing that low resting levels of HRV and high levels of trait worry are related to reduced IOR for negative emotional cues.

2. Methods

2.1. Participants

This study was part of a larger study to assess the effects of tVNS on worry behavior as well as stress-related attentional biases. This larger study has been preregistered on the Open Science Framework, https://osf.io/za9mu. Results from the effects of tVNS on spontaneous and induced worry are reported in Burger et al. (under review). For this study, ninety-seven chronically worrying students from Leiden University, between the ages 18–25, were recruited. Participants could only participate in this study if they scored at least 45 on the Penn State Worry Questionnaire (PSWQ). A cut-off score of 45 ensured a highly sensitive selection of chronic worriers in an advertised-for population (Behar, Alcaine, Zuellig, & Borkovec, 2003). Sample size was based upon the larger aim of the study to detect medium sized effects (Cohen's $d = 0.50$) when comparing the tVNS to the sham conditions with a power of .80 and a one-tailed alpha of .05.

Participants with self-reported bradycardia, cardiac arrhythmia, cardiac diseases, significant head trauma, pregnancy, drug use, neurological or psychiatric disorders were excluded from participating in this study. Ethical approval for this study was given by the local ethical committee. Participants were rewarded with 10 euros or partial course credit for participating in this study.

Procedure Prior to being invited to participate in this study, participants received a link via email asking them to fill in the PSWQ online. Participants that scored 45 or higher on the PSWQ were invited to participate in our study. In case participants scored lower than 45, researchers received a confirmation that the participant had not fulfilled the study criteria and the questionnaire was locked for that particular IP address, to ensure participants could not retake the questionnaire. Participants were subsequently informed that they did not fulfill the criteria for participating in the study. At the start of the experiment, participants were instructed to wear an ECG chest strap throughout the remainder of the study. Then, a 2-min baseline recording of pupil size was conducted. During this baseline recording, participants were instructed to simply look at a fixation cross in the middle of a screen. Thereafter, a 5-min baseline recording of HRV was conducted. During this baseline participants watched a short relaxing movie, without sound (an excerpt from 2001: A Space Odyssey). Afterwards, the tVNS device was attached to the participant's left ear, stimulating either the concha (tVNS) or the earlobe (sham stimulation). Since not much is known about the temporal latency of the effects of tVNS, participants were instructed to complete several questionnaires prior to the experimental tasks, in order to account for a short build-up period of the effects of tVNS (Hassert et al., 2004). Filling in the questionnaires took roughly 15 min. After filling in the questionnaires, participants were instructed to complete a Breathing Focus Task (Burger al., submitted). Subsequent to the BFT, participants completed a second pupillometry

measurement, followed by an attentional blink task and the exogenous cueing task. Finally, participants were instructed to complete one final pupillometry measurement. The results of the pupillometry and attentional blink task are beyond the scope of this article and will be described elsewhere.

3. Instruments and questionnaires

3.1. Exogenous cueing task

To measure IOR, we used an exogenous cueing task adapted from Park et al. (2012). Pictures of 132 faces (66 with fearful expressions and 66 with neutral facial expressions; 33 women and 33 men with each expression) were used as cue stimuli. We used 120 faces (60 fearful and 60 neutral faces) for experimental trials and 12 faces for practice trials. All cue pictures were black-and-white (256 gray levels), and contrast and brightness were adjusted to maintain constancy across different face sets. Each face was enclosed in a circular frame to exclude non-facial features (e.g., hair). The target that the participants had to localize was a black dot with a diameter of 0.5 cm. The cue and target stimuli were presented inside two light grey boxes that were continuously present on the computer screen. These boxes were 5 cm high by 3.0 cm wide and were displayed 2.25 cm to the left and the right of a central fixation point (shape: +). All stimuli were presented on a Dell computer with a 17″ Dell LCD monitor (resolution: 1280 * 1024). Each trial started with a fixation point, which was presented at the center of the screen for 800 ms. A schematic face cue was then presented for 300 ms in either the left or the right box. This cue was then blanked out and 200 ms later the central cross was presented in bold type for 300 ms. The initial fixation display was then presented for 160 ms. Following this, the target was presented in the lower half of either the left or the right box for 33 ms. Subsequently, the initial fixation display was presented until the participant responded (or until 2000 ms elapsed). This resulted in a cue-target onset asynchrony (SOA) of 960 ms. We used an intertrial interval of 1000 ms. Each participant completed 12 practice trials, followed by 120 experimental trials. Fifty percent (60) of the experimental trials were valid (i.e., the target appeared in the same box as the cue), and 50% (60) were invalid (i.e., the target appeared in the opposite box to the cue). Fearful and neutral face cues each appeared 30 times on valid trials and 30 times on invalid trials. The probability of any particular cue appearing in the left- and right-hand side boxes was equal, as was that of the types of faces. All participants were seated roughly 65 cm from the computer screen. From this viewing distance, the boxes measured 2.6° horizontally and 4.4° vertically. The middle of these boxes was located at a distance of 3° from the fixation point. They were told that the position of the cue did not predict the location of the target and therefore they should ignore the cue and keep their eyes focused on the centre of the screen and respond as quickly and as accurately as possible. The participant's task was to respond to the target which appeared either on the left or the right hand location by pressing the “Z” on the keyboard when the target was located on the left hand side of the screen and the “M” when the target was located on the right hand side of the screen. A standard QWERTY keyboard was used.

3.2. Transcutaneous vagus nerve stimulation

Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive method of electrically stimulating the afferent auricular branch of the vagus nerve located at the cymba conchae. In this study, we used a tVNS instrument that provides electrical stimulation using two titanium electrodes, positioned on top of a silicon earplug, which are connected by a wire to a portable neurostimulator (Nemos®, Cerbomed, Erlangen, Germany). The electrodes deliver 30-s waves of electrical stimulation (0.5 mA, 25 Hz, 250 μs) to the concha of the left outer ear (Peuker & Filler, 2002), alternated by 30-s breaks. In the sham condition, the electrodes are connected to the center of the earlobe instead of the concha. In contrast to the concha, the earlobe is not innervated by the vagus nerve (Peuker & Filler, 2002).

3.3. Instruments

3.3.1. Trait worry

The *Penn State Worry Questionnaire* (PSWQ (Meyer, Miller, Metzger, & Borkovec, 1990; Bart; Verkuil, Brosschot, & Thayer, 2007); is a 16-item self-report questionnaire that assesses the duration and uncontrollability of worry. The PSWQ has demonstrated high reliability, high temporal stability and substantial validity in the assessment of trait-worry (Meyer et al., 1990; Verkuil et al., 2007).

3.3.2. Heart rate variability

Over the course of the experimental procedure, participants were asked to wear a chest strap with a sensor worn at the base of the sternum to measure cardiovascular activity through two electrodes connected to the belt (Movisens, Gmhb, Karlsruhe, Germany). Raw ECG was measured at 1024 Hz and was automatically cleaned for outliers and measurements artifacts by the Movisens Data-Analyzer software, after which 60-s averages of the high frequency heart rate variability (HF-HRV in ms^2) were calculated for further analyses. These were aggregated to a 5 min resting baseline average.

4. Statistical analyses

To test for possible confounding baseline differences between participants in the tVNS and sham group, we conducted independent samples t-tests on test scores of all questionnaires. Similarly, we tested baseline differences in resting levels of HF-HRV.

The main hypothesis that tVNS would affect IOR was tested using a multilevel analysis with maximum likelihood estimation. Reaction times on the different trials were nested within participants. Reaction time was the dependent variable. Condition was the between subjects factor. Valence and validity were the within subjects factors. We allowed the intercepts of the models to vary randomly. Inclusion of random slopes did not improve model fit and were therefore not included. In subsequent models, HF-HRV and PSWQ scores (grand mean centered) were entered as additional independent variables.

All main analyses were performed in SPSS, version 23. Two tailed tests were conducted with alpha set to 0.05.

5. Results

5.1. Descriptive statistics

Ninety-four participants performed the exogenous cueing task (N_{sham} = 49, N_{tVNS} = 45). Of all trials, 0.4% had to be excluded because RTs were < 150 ms or > 1000 ms. On 0.6% of the trials (72 of 11233 trials) an error was made, and RTs on these trials were also excluded. Due to some recording errors, there were 3 missing values for the HF-HRV baseline assessment. In addition, in both conditions two outliers were identified (> 3 SDs from the mean) that were excluded from the HF-HRV analyses (inclusion yielded similar results).

Mean level of HF-HRV power was slightly above the norms reported by Nunan et al. (Nunan, Sandercock, & Brodie, 2010). As HF-HRV was non-normally distributed a log transformation was successfully applied, which was used in the reported multilevel analyses.

5.1.1. tVNS, vagally mediated HF-HRV and inhibition of return

Mean reaction times are presented in Table 1. To test if tVNS affected RTs on the cueing task, a multilevel analysis on the mean RTs, with Condition, Valence and Validity as independent variables was conducted. Results of this analysis are presented in Table 2. A main effect of Validity was observed (B = −9.25, SE = 2.19, *t*

Table 1
Basic descriptive statistics.

	Condition					
	Sham			tVNS		
	N	M	SD	N	M	SD
RT neutral valid	49	332.06	36.99	45	340.68	45.70
RT neutral invalid	49	320.71	35.53	45	325.72	39.18
RT fear valid	49	331.35	33.85	45	338.20	45.24
RT fear invalid	49	324.21	38.52	45	328.24	47.71
Baseline HF-HRV power (ms^2)	45	851.81	762.72	42	870.60	704.91
PSWQ	49	60.53	7.70	44	62.60	7.49
Age	48	20.79	2.02	43	21.19	2.12

Note: HF-HRV = high frequency heart rate variability; PSWQ = Penn State Worry Questionnaire.

Table 2
Results of the multilevel analysis on reaction times per condition, valence and validity.

	B	SE	df	*t*	*p*
Intercept	327.08	5.39	94	60.66	.00
Valence	1.40	2.19	282	0.64	.52
Validity	−9.25	2.19	282	−4.22	.00
Condition	6.13	7.79	94	0.79	.43
Valence x Validity	4.20	4.38	282	0.96	.34
Condition x Valence	−1.38	3.17	282	−0.43	.66
Condition x Validity	−3.21	3.17	282	−1.01	.31
Condition x Valence x Validity	0.80	6.33	282	0.13	.90

(282) = −4.22, $p < .001$), indicating faster responses at invalid trials compared to valid trials. No effects of Valence were observed. No main effect of Condition was observed, nor any significant two-way interactions. The hypothesized Condition x Valence x Validity interaction was also not significant, thereby not confirming the hypothesis that tVNS affects IOR to fearful faces. Thus, an IOR effect was observed, that was not affected by the presentation of the emotional cues, nor by the application of tVNS (see Table 1).

We additionally explored if individual differences in resting levels of HF-HRV moderated the potential effects of tVNS. Resting HF-HRV was entered as an additional independent variable to the previous model. There was no direct relation between resting HF-HRV and IOR, as neither the HF-HRV x Validity interaction, nor the HF-HRV x Valence x Validity interaction was significant ($ps > .13$). However, the HF-HRV x Condition x Valence x Validity interaction was significant (B = 37.67, SE = 14.62, $p = .011$). This indicated that the effect of tVNS on RTs was dependent on participants' resting HF-HRV. To examine this four-way interaction, we calculated cue validity indices for each cue type by subtracting RTs on valid trials from those on invalid trials (negative values thereby represent stronger IOR). These cue validity indices are presented in Fig. 1. Independent t-tests showed that the cue validity index for neutral trials differed between the sham and tVNS condition, but only for those with higher levels of HF-HRV (i.e., scoring above the median), $t(42) = 2.254$, $p = .030$, Cohen's $d = 0.68$). However, when using Bonferroni corrections, there were no significant associations between the cue validity indices and HF-HRV levels, when examined per condition.

5.1.2. Trait worry and inhibition of return

A similar analysis was performed to explore if PSWQ scores would moderate the hypothesized effects of tVNS on IOR. The four-way interaction was not significant however, B = −0.09, SE = 0.82, t (282) = −0.11, $p = .907$.

A basic premise of the current study was that high levels of trait worry would be associated with reduced IOR to fearful faces. To test this premise, a separate multilevel model was fit with PSWQ scores, Valence and Validity as independent variables (disregarding the non-significant effect of Condition). Results are presented in Table 3. A significant PSWQ x Validity was observed (B = 0.48, SE = 0.21, t (282) = 2.35, $p = .016$). The PSWQ x Valence x Validity interaction was not significant (B = 0.78, SE = 0.41, $t(282) = 1.90$, $p = .06$). As can be observed in Fig. 2, the group of participants with the highest levels of trait worry levels showed slower RTs to invalid trials relative to the valid trials, i.e., a decrease of the IOR effect.

6. Discussion

The present study was set-up to test if manipulating vagus nerve activity would facilitate inhibition of return for emotional information in high worriers. Although we did observe that higher levels of trait worry were associated with reduced IOR, we could not confirm our hypothesis that tVNS would affect IOR to fearful faces. Yet, an exploratory analysis suggested that tVNS enhanced IOR, but only for neutral information and only in those participants that were already high in resting levels of HRV.

Combined the findings do not speak in strong favour of tVNS as a tool to modulate thwarted emotional attentional processes in high worriers; at least not with the current stimulation parameters. We also could not confirm the previously observed relation of reduced IOR for fearful faces in people low in HRV (Park et al., 2012). However, in the study by Park et al. the reduced IOR for fearful faces seemed most pronounced when the pictures of the fearful faces had been filtered, leaving only the high spatial frequency aspects intact. Such high spatial frequency filtered pictures are believed to be processed mainly by (prefontal) cortical areas in the brain (eg., fusiform and occipital gyrus). One could argue that is still possible that vagus nerve activity affects the IOR phenomenon, but maybe only specifically for pictures that require the highest levels of prefrontal processing. Still, as both subcortical and cortical pathways are involved in the processing of broad spectral pictures (Vuilleumier, Armony, Driver, & Dolan, 2003) - used in the current study - an enhancement of the IOR effect would still be expected. Interestingly, a recent MRI study suggested that tVNS does not affect activity in the fusiform and occitipal gyri (Yakunina et al., 2017). This suggests that tVNS will at least not affect IOR by affecting brain areas required for the perception of the emotional faces.

The exploratory finding that the effect of tVNS on RTs was dependent on participants' resting HF-HRV can only be tentatively reflected upon, since this finding was not significant when correcting for multiple testing. If future research is able to replicate this finding, then it could suggest that tVNS promotes IOR for neutral faces in people that are already high in parasympathetic control. It remains debatable whether promoting enhanced IOR for neutral information in this group of people would be adaptive. On the one hand, being able to ignore distracting neutral information for the benefit of enhanced task performance would seem beneficial. On the other hand, neutral information can be considered ambiguous information, and it may even be adaptive to pay prolonged, instead of shortened, attention to ambiguous information, for the sake of making correct interpretations of this information (i.e., 'erring on the side of caution'). All in all, it remains to be established if tVNS could be more effective in people with low resting HRV levels, especially since our sample size was not aimed at detecting moderators of the hypothesized main effect of tVNS. Additionally, with respect to the final sample size, we were able to detect significant differences between the conditions that were larger than Cohen's $d = 0.34$ (Lakens, 2017).

It is also possible that with the current stimulation parameters, we were not able to increase activity in the locus-coeruleus-noradrenalin system sufficiently to affect attentional inhibition. Little is known about the effects of different stimulation parameters, let alone a dose-response relation between stimulation, noradrenalin and cognition. In previous studies, cognitive effects that supposedly rely on noradrenalin were obtained using the same stimulation parameters, but an obvious

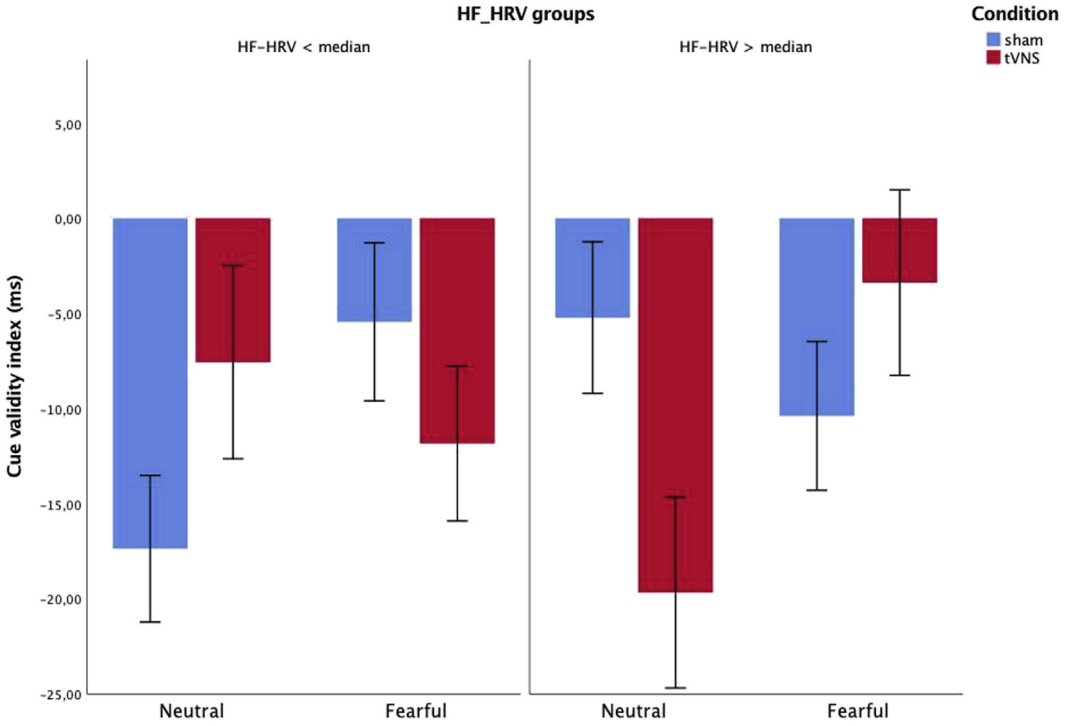

Fig. 1. Cue validity scores on the exogenous cueing task (± 1 SE), split per Condition and HF-HRV level (left panel = low HF-HRV, right panel = HF-high HRV), with cue type groups on the x-axis.

Table 3
Results of the multilevel analysis on RTs by trait worry, valence and validity.

	B	*SE*	*df*	*t*	*p*
Intercept	330.02	3.90	94	84.51	.00
Valence	0.74	1.56	282	0.47	.64
Validity	−10.79	1.56	282	−6.92	.00
Valence x Validity	4.59	3.12	282	1.47	.14
PSWQ	0.12	0.51	94	0.24	.81
PSWQ x Valence	0.12	0.21	282	0.58	.57
PSWQ x Validity	0.48	0.21	282	2.35	.02
PSWQ x Valence x Validity	0.78	0.41	282	1.90	.06

Note: PSWQ = Penn State Worry Questionnaire.

difference is that these previous studies included only healthy participants, and we selectively included high worriers. It is possible that in these worriers, tonic activity in the locus coeruleus-noradrenalin system is already moderate or even high. Further increasing activity in this system could possibly lead to either a ceiling effect, or could induce distractibility (Aston-Jones & Cohen, 2005). However, the first is unlikely given that the reduced IOR in the highest worriers was not affected by tVNS. Enhanced distractibility would be most visible in a general slowing down of reaction times, and this is not what we observed.

Another limitation of the current study is that although we intended to stimulate the afferent vagus nerve via its auricular branch, there currently exists no clear biomarker showing that the stimulation indeed enhanced vagus nerve activity. We can therefore not completely rule out the possibility that individual differences in the innervation of the auricle existed that led to suboptimal stimulation in some participants. However, in the light of our previous findings using tVNS and the MRI studies that have been performed (Badran et al., 2018; Frangos et al., 2015; Kraus et al., 2007; Yakunina et al., 2017), this possibility might be unlikely. A further limitation of this study was that the order of the tasks was not counterbalanced and that the exogenous cueing task was administered after two other tasks. These tasks involved instructed worrying and an attentional blink task, which could have taxed attentional resources, possibly affecting the efficacy of tVNS. We cannot completely rule of this possibility. However, in between the tasks pupillometry took place, where participants could let their minds wander, instead of focusing on a task. In addition, when compared to previous work with this task (Park et al., 2012), the participants of the current study showed faster reaction times and less errors. This seems to rule out the option that attentional resources were depleted. Still, even though the current procedure might have also added to the ecological validity of the study (where in daily life our attentional resources are frequently taxed), it remains important to keep this limitation in mind.

One could argue that the cut-off of 45 that was used to select high worriers was still quite low. Other studies have used cut-off scores of 56 or 62 to select for high worriers (20% of our sample scored below 56). The latter higher scores are best used to differentiate between people suffering from GAD versus those suffering from other anxiety disorders (Startup & Erickson, 2006). In this study, the average score on the PSWQ was around 60, which is comparable to clinical samples and suggests that average levels of pathological worry were indeed high compared to general samples. Finally, it is important to stress that the exogenous cueing task was administered after two other tasks. Although we found an IOR effect, the current null effects of tVNS on IOR have to be interpreted with this procedural context in mind.

The observed relation between trait worry and reduced IOR is partly consistent with previous studies that have observed reduced IOR for negative emotional information in people high in trait anxiety and trait worry (Fox et al., 2002; Verkuil et al., 2009). However, in these studies the reduced IOR was observed in response to emotionally salient cues, and here we observed a generally reduced IOR effect. This might be due to the use of a sample of selected high worriers; previous studies included healthy students that scored on the full range of worry and anxiety. Additionally, whereas previous studies used angry, schematic faces as cues, here we used photographs of neutral and fearful faces instead. Interestingly, the reduced IOR was already observed for neutral faces. Although it remains to be established how chronic worriers interpret these faces, these findings are consistent with the recently

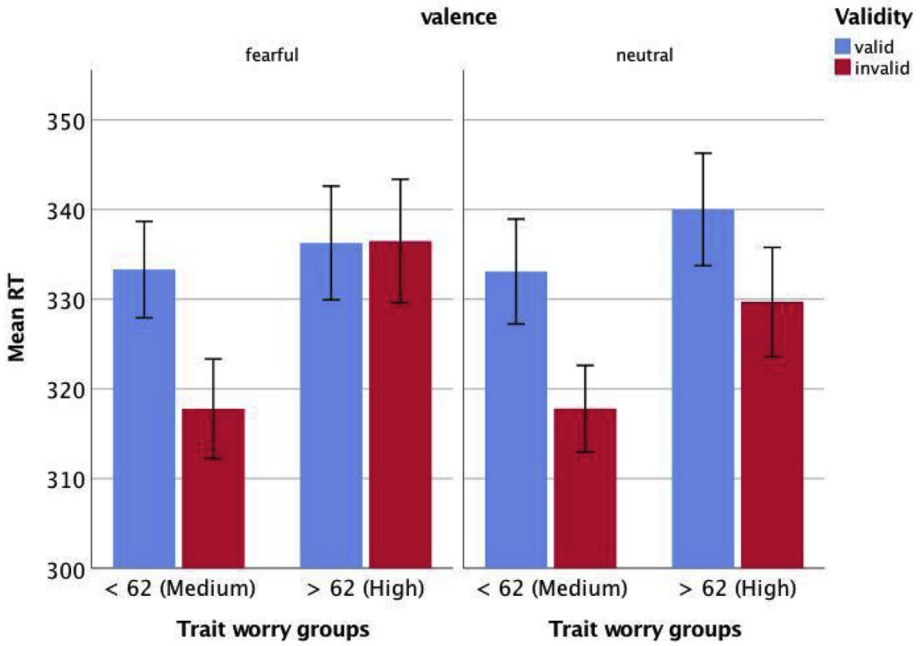

Fig. 2. Mean RTs (± 1 SE) on the exogenous cueing task, split for valence and different levels of trait worry (median split groups for illustration purposes).

proposed Generalized Unsafety Theory of Stress (GUTS (Brosschot, Verkuil, & Thayer, 2015, 2017, 2018); that predicts that chronic worriers have difficulties in recognizing signals of safety, such as neutral faces.

In conclusion, in the current study, with a sample of high worriers, tVNS did not affect the IOR phenomenon when compared to sham stimulation. There was also no association between resting levels of vagally mediated HRV and IOR, which is in contrast with previous work. However, tVNS did enhance IOR to neutral faces in participants high in resting HRV, although this finding has to be confirmed by future studies. In addition, the data showed an association between higher levels of trait worry and reduced IOR, suggestive of inhibitory problems in pathological worriers. Future studies are clearly warranted to address the neurobiological underpinnings of these inhibitory problems.

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Declarations of interest

None.

Data statement

Data, syntax and the preregistration for this study can be retrieved via: https://osf.io/4hsjy/

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brat.2018.12.009.

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