Hitting the right nerve: effects of transcutaneous vagus nerve stimulation on symptoms of anxiety

Burger, A.M.

Part II

Negative Thought Intrusions

Chapter 6

Transcutaneous Vagus Nerve Stimulation Reduces Spontaneous but not Induced Negative Thought Intrusions in High Worriers

Burger AM, Van der Does W, Thayer JF, Brosschot JF, Verkuil B (2019). *Biological Psychology, 142,* 80-89. DOI: 10.1016/j.biopsycho.2019.01.014.

Abstract

Worrying is a central component of anxiety disorders. We tested whether non-invasive vagus nerve stimulation reduces negative thought intrusions in high worriers. Worry was assessed with a Breathing Focus Task, which consists of a pre-worry period, a worry induction, and a post-worry period. Ninety-seven high worriers were randomly allocated to receive transcutaneous electrical stimulation of the auricular branch of the vagus nerve at the concha (tVNS), or of the earlobe (sham stimulation) throughout the lab session. Participants who received tVNS reported significantly fewer negative thought intrusions during the pre-worry period, but the effects of tVNS after the worry induction were mixed. An exploratory analysis indicated that participants in the tVNS condition were more likely to report negative thought intrusions shortly after the worry induction, but became less likely to do so as the post-worry period went on. No effects of tVNS on RMSSD were observed. These findings provide preliminary indications that tVNS may decrease the occurrence of worrisome thoughts.

Introduction

Perseverative cognition such as worry and rumination is observed in a wide range of stress-related disorders including depression, anxiety, and burnout [265,266]. Perseverative cognition is a symptom of generalized anxiety disorder (GAD) in particular. GAD is a highly prevalent condition that is mainly characterized by excessive and uncontrollable worrying. Roughly 5% of the population suffer from GAD at some point in their life, and another 12% suffer from subthreshold GAD characterized by excessive worrying [2,267]. People suffering from GAD, as well as high worriers in general, are extremely occupied with stress-related thoughts and continuously prioritize threat-related information at the expense of safety information. Given the high prevalence of GAD and high worriers, it is crucial to understand what factors maintain worrying and to develop interventions that reduce it. Current psychological and pharmacological interventions are moderately effective, with 46% of patients assigned to cognitive behavioral therapy responding to treatment [268]. Furthermore, worrisome thoughts can be easily re-activated after successful treatment, resulting in high relapse rates [269]. Thus, it seems critical to test new interventions that might reduce worry. Besides the currently available psychological and pharmacological interventions, recent years have seen an increase in neuromodulation techniques, One such method is the non-invasive stimulation of the auricular branch of the vagus nerve, which is the method under scrutiny in this study.

In this study, we aimed to examine whether experimentally enhancing vagus nerve activity will acutely decrease worrying in a group of high worriers. Recent technological advances allow us to test this hypothesis using a non-invasive approach, by transcutaneously stimulating the auricular branch of the vagus nerve (ABVN) via the concha of the outer ear. This procedure is called transcutaneous vagus nerve stimulation or tVNS. Crucially, fMRI studies have shown that tVNS directly promotes activity in brain areas that reduce worry, including the prefrontal cortex and the anterior cingulate (for a review, see [270]). Furthermore, tVNS increases the functional connectivity between the amygdala and the prefrontal cortex in depressed patients [80]. Functional connectivity between the amygdala and the prefrontal cortex has repeatedly and robustly been demonstrated as a function of anxiety [81], and has also been linked to self-reported worry intensity in patients suffering from GAD [82–84].

Previous studies have also indicated that tVNS affects cognitive functions that rely on prefrontal activity, e.g. enhanced associative memory formation and consolidation [86,165,196] and action control [87]. Critically, tVNS promotes the ability to inhibit task-irrelevant information processing [88,89,271], a process which is strongly compromised in patients suffering from GAD [90]. Finally, the potential effect of tVNS on worrying is further illustrated by a non-randomized study that showed medium to large effect sizes of tVNS on symptoms of depression and anxiety in patients suffering from depressive disorders [91]. However, knowledge of the effects of tVNS on worry, a core

pathological component of mental disorders, is still lacking. In this study we tested if tVNS acutely decreases worry in high worriers, compared to sham – using the same stimulation procedure as in our previous studies (e.g. [88,165,196,272]).

In summary, previous studies suggest that enhancing vagus nerve activity via tVNS produces neural, cognitive and emotional effects that indicate a possible effect on worry. In this experiment, we compared the effects of tVNS to sham stimulation on worry in high trait worriers. Worry was assessed by measuring the frequency of negative thought intrusions before and after a worry induction during a Breathing Focus Task [273–275] as measurements of both spontaneous and induced worry behavior. We tested the hypotheses that high worriers who received tVNS have fewer negative thought intrusions than those who received sham stimulation, both before and after the worry induction part of the BFT. As an additional exploratory analysis, we tested the effects of tVNS on both resting levels and worry-induced reductions in heart rate variability . Heart rate variability is often used as an index of efferent vagal tone, and although tVNS is unlikely to lead to cardiac effects - due to the lateralization of cardiac input of the vagus nerve, and low intensity of stimulation [264,276] - we will still report these findings to contribute to the literature on efferent effects of stimulating the left ABVN.

Methods

Participants

Ninety-seven students (78 female, 19 male), between the ages 18-25 (M_{age} = 21.04, SD_{age} = 2.08), were included. Participants were recruited from Leiden University through pamphlets and a designated university website, specifically targeting participants who worry frequently. Participants could participate if they scored at least 45 on the Penn State Worry Questionnaire (PSWQ). The cut-off score of 45 was suggested as a highly sensitive and specific cut-off score for clinical GAD in an advertised-for population [219]. Participants suffering from epilepsy, cardiac arrhythmia or bradycardia, alcoholism, or migraines requiring medication use were excluded from the study.

Ethical approval for this study was given by the ethical committee of the Institute of Psychology of Leiden University (CEP #8988381492). Participants were rewarded with either 10 euros or course credit.

Instruments and Questionnaires

Breathing Focus Task

The breathing focus task (BFT) was developed by Borkovec et al. [275] and later adapted by Hirsch et al. [273,274] as a measure of spontaneous and induced worry. For a graphical overview of the BFT, see figure 1. The BFT consisted of three periods: a 5 minute pre-worry breathing focus period, followed by

a 5 minute worry induction period, and finally a 5 minute post-worry breathing focus period. During the breathing focus periods, participants were instructed to close their eyes and simply focus their attention on their breathing. Importantly, participants were not given any instructions on how to breathe in terms of technique, depth or pace. An auditory cue was presented through their headphones every 20-30 seconds for a total of 12 cues per breathing focus period. Participants were instructed to open their eyes after hearing the cue, at which time a question appeared on the computer screen, asking them whether their attention was focused on their breathing or whether they were thinking of something else at the moment they heard the tone. If participants reported that their attention was focused on 'something else', they were asked whether this was something positive, neutral or negative. Contrary to previous studies that have used the BFT [273,274], participants were not asked to provide a short description of the intrusion (e.g. "worrying about whether I'll pass my exams"), but were simply instructed to once again close their eyes and focus on their breathing.

At the end of each breathing focus period, three questions were asked to check whether the breathing focus prompts were truly representative of worrying: ''Estimate the percentage of time you were able to focus on your breathing (0% not at all – 100% all of the time)''; ''Rate how difficult you found it to focus on your breathing (0 not at all difficult – 100 extremely difficult)''; and ''Estimate the percentage of time you worried during the last 5 minutes (0% none of the time – 100% all of the time)''.

During the worry induction period, participants were instructed to worry "as they normally do" about the topic they specified beforehand. No auditory cues were presented during this period.

After the worry induction period, participants were asked to answer several questions concerning their worry behavior: ''Estimate the percentage of time that you were able to spend worrying (0% not at all – 100% all of the time)'', ''Rate how intensely you were worrying (0 not intensely at all – 100 extremely intensely)", "To what extent could you control your thoughts? (0 not at all – 100 all of the time)" [274].

Transcutaneous vagus nerve stimulation

The tVNS instrument provided electrical stimulation using two titanium electrodes, positioned on top of a silicon earplug, which was connected by a wire to a portable neurostimulator (Nemos®, Cerbomed, Erlangen, Germany). The electrodes delivered 30-second waves of electrical stimulation (0.5mA, 25Hz, 250µs), alternated by 30-second breaks. The electrodes were attached to either the cymba concha (tVNS condition) or the center of the earlobe (sham stimulation condition) of the left ear. In contrast to the cymba concha, the earlobe is not innervated by the vagus nerve [25]. We stimulated the left ear to avoid potential cardiac effects that have been related to efferent vagal fibers of the right ear [119] but not the left [121].

Questionnaires

Prior to the lab session, participants were asked to complete the Penn State Worry Questionnaire (*PSWQ*; [135,277]) at home. The PSWQ 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 [135,136].

To ascertain that there were no between-group differences prior to the experimental manipulation that could have affected responses on the BFT, participants were asked to complete four questionnaires at the start of the lab session. The questionnaires were used to assess worrying, state and trait anxiety, attentional control and ruminative thoughts.

The Generalized Anxiety Disorder-7 scale (GAD-7; [278]) is a 7-item clinical measure assessing the severity of GAD symptoms with good reliability and validity in the general as well as clinical population [279].

The State Trait Anxiety Inventory (*STAI*; [138])is a self-report questionnaire consisting of 2 versions with 20 questions each, measuring both state and trait anxiety. The STAI has shown acceptable internal consistency and validity [137,139].

The Attentional Control Scale (ACS; [280]) is a 20-item self-report measure consisting of a 9-item measure of attentional focusing and an 11-item measure of attentional shifting with good internal and predictive validity [281].

The Ruminative Response Scale (RRS; [282]) is a 22-item questionnaire assessing the engagement of participants in response to feeling sad or depressed. The RRS has been shown to have good internal consistency and moderate to high validity for predicting depression.

Side Effects

At the end of the experimental paradigm, participants were presented with a list of seven side-effects commonly reported during tVNS (ie. headache, neck pain, nausea, muscle contractions, pricking sensations, burning sensations, and general feelings of uncomfortableness). Participants were asked to rate to what extent they had experienced each of these side-effects on a scale of 1 (‘applies not at all’) to 5 (‘strongly applies to me’). As this side-effects form was only added to the experimental procedure after data acquisition had already started, 91 out of 97 participants completed this questionnaire.

Heart Rate Variability

Over the course of the experimental procedure, participants were asked to wear a chest strap with a sensor at the base of the sternum to measure cardiovascular activity through two electrodes connected to the belt (Movisens, GmbH, Karlsruhe, Germany). Raw ECG was measured at 1024Hz and

was automatically cleaned for outliers and measurements artifacts by the Movisens Data-Analyzer software, after which 60-s averages of the root of the mean square of successive differences in interbeat intervals (RMSSD) were further aggregated to 5 minute means to separate the different periods of the BFT. Contrary to high frequency HRV – an alternative measure of vagally mediated HRV -, some studies appear to show that RMSSD is hardly affected by changes in breathing patterns or movement [283,284]. LF/HF ratio was not included as a measurement of vagally mediated HRV because it remains difficult to interpret this index, based on studies that showed involvement of the vagus nerve in both high and low frequency components of heart rate variability [285].

Procedure

This study was part of a larger project on the effects of tVNS on worry behavior and stress-related attentional biases. This larger study was preregistered on the Open Science Framework, https://osf.io/za9mu.

Students who showed interest in the study received a link via email asking them to fill in the PSWQ online. Participants scoring 45 or higher were invited to the lab. Potential participants who scored lower than 45 were informed that they did not fulfill the criteria for participating and the questionnaire was locked for that particular IP address.

For a graphical overview of the experimental procedure, see figure 1. At the start of the experiment, after having signed an informed consent form for the experiment, participants were instructed to write down a personally relevant worry topic. Participants were instructed to wear an ECG chest strap throughout the remainder of the study. Subsequently, a 2-minute pupillometry measurement was conducted. During this baseline recording, participants were instructed to simply look at a fixation cross in the middle of a screen. 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 [115,122,144,187], a short build-up period of the effects of tVNS and sham stimulation was used during which participants were instructed to sit and relax for five minutes. During this time, a baseline recording of their heart rate was conducted. Subsequently, participants were instructed to complete several questionnaires. The questionnaires included a short demographics form, the STAI, the GAD-7, the ACS and the RRS. On average, completing the questionnaires took 15 minutes.

After completing the questionnaires, participants were instructed to complete the BFT, which consisted of two breathing focus periods separated by a worry induction, as described below. Subsequent to the BFT, participants completed a second pupillometry measurement, followed by two cognitive tasks and one final pupillometry measurement, which are not reported here.

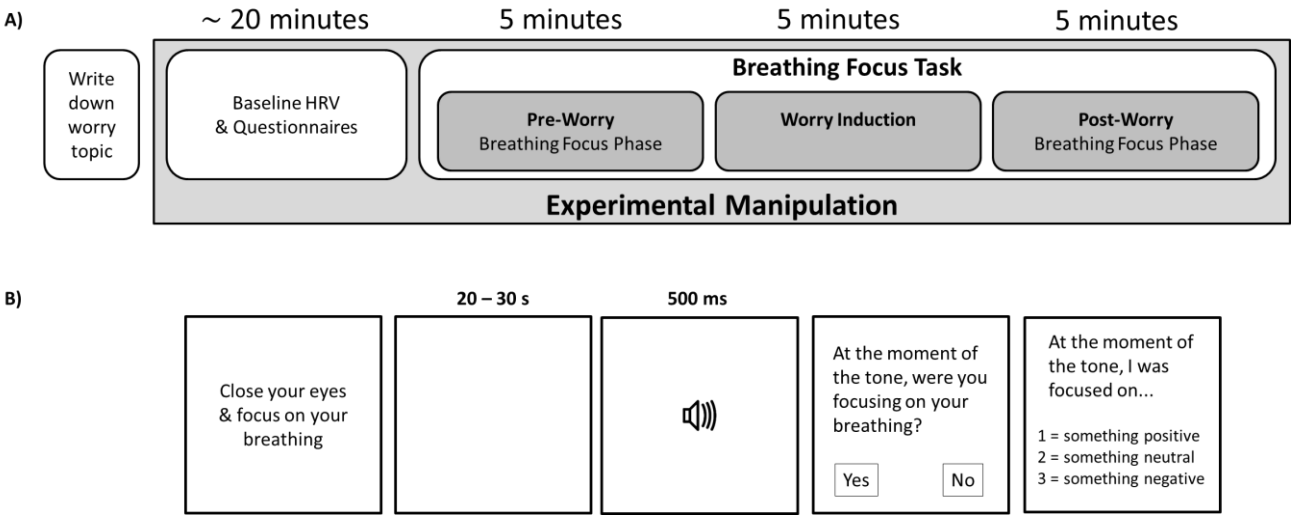

Figure 1. A) Overall experimental overview. After having signed an informed consent form, participants were asked to fill in their currently most pressing worry topic. Then, participants were randomly allocated to receive either tVNS or sham stimulation throughout the rest of the session. The Breathing Focus Task consisted of three separate phases; two Breathing Focus phases separated by a Worry Induction phase.

B) Graphical depiction of a trial during the Breathing Focus Phases. Participants were instructed to close their eyes and simply focus on their breathing. Then, after 20 or 30 seconds, they heard a soft tone prompting them to open their eyes and assess whether they were focused on their breathing at the time of the tone. If they were not, they were additionally asked to rate whether they were focused on something positive, neutral or negative. Both Breathing Focus phases consisted of 12 trials.

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 RMSSD.

Since the dependent variable of our main analysis (*number of reported negative thought intrusions*) is a count variable, using conventional statistical procedures based on a Gaussian distribution of the data would produce unreliable results. Instead, we opted to use generalized linear mixed modelling using a Poisson distribution with a log link function. The variables *Condition* (0 = Sham, 1 = tVNS) and *Time* (0 = pre-worry, 1 = post-worry) were dummy coded and included as covariates in the generalized linear mixed model. Additionally, the model included a random intercept for every individual.

To test the effects of tVNS on HRV over the course of the BFT, we performed a linear mixed model analysis with log transformed RMSSD as a dependent variable, *Time* as a categorical independent variable (pre-worry, worry induction, post-worry; pre-worry being the reference category) and Experimental Condition as a dummy-coded covariate (0 = Sham, 1 = tVNS). To test the

effects of tVNS on HRV over the course of the BFT, we performed a linear mixed model analysis with log transformed RMSSD as a dependent variable, Time as a categorical independent variable (pre-worry as a reference category) and Experimental Condition as a dummy-coded covariate (sham group as a reference category). Prior to these analyses, we assessed the need to control for medication use, caffeine intake or smoking frequency on baseline RMSSD. Spearman's rho correlations between RMSSD and these potential confounders were all non-significant (all $\rho < .20$). As we found no clear effects of any of these factors on log transformed RMSSD in our sample, we decided to analyze the data for all participants without controlling for these variables, to increase the power of our analyses.

Between-groups differences on the side-effects ratings were not normally distributed. Therefore, we calculated between-group differences in side-effect severity by using Yates corrected Chi Square analyses.

Cohen's *d* effect sizes were calculated using the formula $d = \frac{\mathrm{b}}{\text{pooled SD}}$, where *b* denotes the regression coefficient of the corresponding effect and *SD* corresponds to the pooled within-group standard deviation [225]. Analyses were performed in SPSS version nr. 23.

Results

Descriptive Statistics

As shown in table 1, there were no significant differences between the groups in resting levels of RMSSD and the questionnaire scores. The average score on the PSWQ for both the tVNS and the control group corresponded to the 90th percentile of the general population and the 30th percentile of a GAD-patient population [142]. Likewise, the average score on the GAD-7 fell within the range of mild to moderate clinical anxiety, which is in the 90th percentile of the general population ($M_{\text{GAD-7}}$ =3.0,[279]).

Compared to the general population, participants in the current study also scored above average on state and trait anxiety (STAI; [143]). Similarly, compared to general student populations, participants scored above average on rumination (RRS; [286]), and below average on perceived attentional control (ACS; [287]).

On average, participants reported 1.25 (*SD* = 1.35) negative thought intrusions during the pre-worry period of the BFT. This number increased to an average of 1.80 (*SD* = 1.67) during the post-worry period.

Table 1. Descriptive statistics.

	Sham (*N* = 49)		tVNS (*N* = 48)		
	M	*SD*	*M*	*SD*	*p*
PSWQ	60.53	7.70	62.25	7.56	.27
GAD-7*	9.13	4.31	8.83	5.01	.76
STAI State	45.65	9.61	43.18	9.52	.21
STAI Trait	48.85	9.32	48.86	10.47	.99
RRS	50.69	12.25	48.94	13.24	.50
ACS	46.42	9.12	47.85	7.38	.40
Resting RMSSD	42.28	25.6	43.66	27.16	.81

Note. *N_{sham} = 40/ N_{tVNS} = 40 for the GAD-7. This questionnaire was added after data acquisition had already started as an additional check to ensure that the current sample consisted of high worriers.

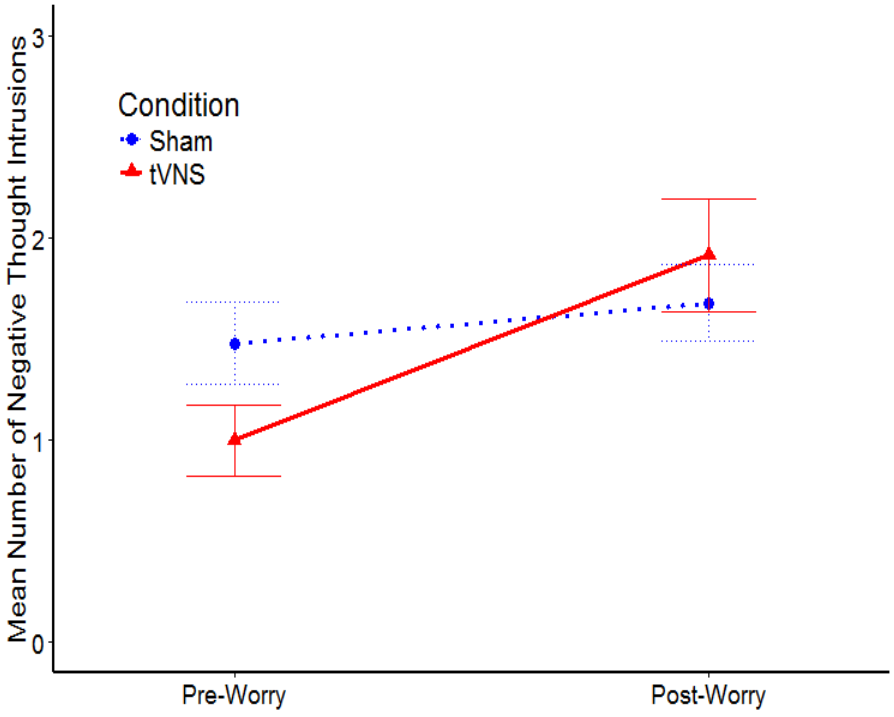

Figure 2. Line graph of average number of negative thought intrusions for every experimental condition before and after worry induction. Error bars denote ± 1 standard error.

Effects of tVNS on Negative Thought Intrusions

Figure 2 shows the mean number of negative thought intrusions per condition. During the pre-worry period, participants who received tVNS reported significantly fewer negative thought intrusions than participants in the sham condition (M_{tVNS} = 1.0 (*SD* = 1.2), M_{Sham} = 1.5 (*SD* = 1.4)), as reflected in the significant main effect of Condition, *b* = -.48 (.23), *t*(190) = -2.10, *p* = .037, *d* = -.36. After the worry induction, participants who received tVNS reported a stronger increase in negative thought intrusions than participants in the sham condition, as indicated by a significant Time*Condition interaction, *b* = .52 (.18), *t*(190) = 2.85, *p* = .005, *d* = .29. The number of negative thought intrusions reported by participants in the sham condition did not change significantly after the worry induction, as reflected by a non-significant main effect of Time, *b* = .13 (.12), *t*(190) = 1.10, *p* = .30, *d* = .08. Participants in the tVNS condition reported a slightly higher number of negative thought intrusions than participants in the sham condition after the worry induction (M_{tVNS} = 1.9 (*SD* = 1.9), M_{Sham} = 1.7 (*SD* = 1.4), see figure 2). To test whether the between-group difference during the post-worry period was significant, we performed a subsequent analysis where the post-worry period was used as the reference category. This analysis confirms that the between-group difference during the post-worry period is not significant, as reflected by the main effect of Condition, *b* = .05 (.21), *t*(190) = .22, *p* = .83, *d* = .03.

With regard to the retrospective worry assessments, we found no effects of tVNS on the time spent worrying during the breathing focus periods, either before (*p* = .92) or after the worry induction (*p* = .80). No effect of tVNS was observed on any of the other retrospective assessments (see Table 2).

Exploratory Analyses

To test whether the effect of tVNS on thought intrusions is specific to negative intrusions, or whether it reflects a reduction in mind wandering in general, we performed additional exploratory analyses to test whether tVNS also reduced the number of positive and neutral thought intrusions reported by participants. For both neutral and positive thought intrusion frequency, we found no effects of time, indicating that the number of positive or neutral intrusions were not affected by the worry induction. Additionally, neutral and positive thought intrusion frequency were not affected by tVNS in either breathing focus period, all *p* > .05.

Finally, we explored possible time-dependent effects of tVNS during the post-worry period. Visual inspection of the data suggested that during the post-worry period, participants in the tVNS condition were more likely to report negative thought intrusions at the start of the post-worry period, and became progressively less likely to report such thought intrusions as the period went on (see figure 3). To test this possible time-dependent effect of tVNS on negative thought intrusions, we conducted a logistic generalized linear mixed model analysis. To account for the differences in slopes between

the pre- and post-worry phase, we performed a piecewise regression analysis and included two independent *Time* variables in the model. The first Time variable, $Time_{pre\text{-}worry}$, is a continuous variable that counts the number of pre-worry probes that have been presented (ranging between 0 and 11 in the pre-worry phase, 11 throughout the post-worry phase). The second Time variable, $Time_{post\text{-}worry}$, is a continuous variable that counts the number of post-worry probes that have been presented (0 throughout the pre-worry phase, ranging between 1 and 12 during the post-worry phase). The model also includes Condition (0 = Sham, 1 = tVNS) and Phase (0 = pre-worry, 1 = post-worry) as dummy-coded covariates. The generalized linear mixed model included main effects of both Time variables, Condition, and Phase. Additionally, $Time_{pre\text{-}worry}$*Condition, $Time_{post\text{-}worry}$*Condition and Phase*Condition interactions were included in the model.

Over the course of the pre-worry phase, participants in both conditions showed a slight increase in the probability of reporting negative thought intrusions, as reflected by the main effect of $Time_{pre\text{-}worry}$, b = 0.07 (0.03), $t(2200) = 2.53$, $p = .011$, OR = 1.08 [1.02 – 1.15]. In line with the main analysis, the probability of reporting negative thought intrusions during the pre-worry phase was lower for participants in the tVNS condition, although this difference was no longer significant in this analysis, b = -0.73 (0.41), $t(2200) = -1.76$, $p = .078$, OR = 0.48 [0.21 – 1.09]. The linear increase in reported negative thought intrusions throughout the first breathing focus period did not differ between participants in the tVNS and sham conditions ($p = .97$).

As can be seen in figure 3, participants in the sham condition did not display a significant increase in the probability of reporting negative thought intrusions (main effect of Phase, $p = .82$). However, compared to participants in the sham condition, the proportion of participants in the tVNS condition that reported negative thought intrusions increased significantly from the end of the pre-worry phase to the start of the post-worry phase, as indicated by a significant Condition*Phase interaction, b = 1.71 (0.49), $t(2200) = 3.52$, $p < .001$, OR = 5.51 [2.13 – 14.25]. There was no significant decrease in the proportion of participants who reported negative thought intrusions in the sham condition, as reflected by the main effect of $Time_{post\text{-}worry}$ (p = .29). However, there was a significant Condition*$Time_{post\text{-}worry}$ interaction, b = -0.14 (.05), $t(2200) = -2.74$, $p = .006$, OR = .87 [.79 - .96], indicating that the proportion of participants reporting negative thought intrusions in the post-worry phase showed a significantly stronger reduction in the tVNS condition than in the sham condition.

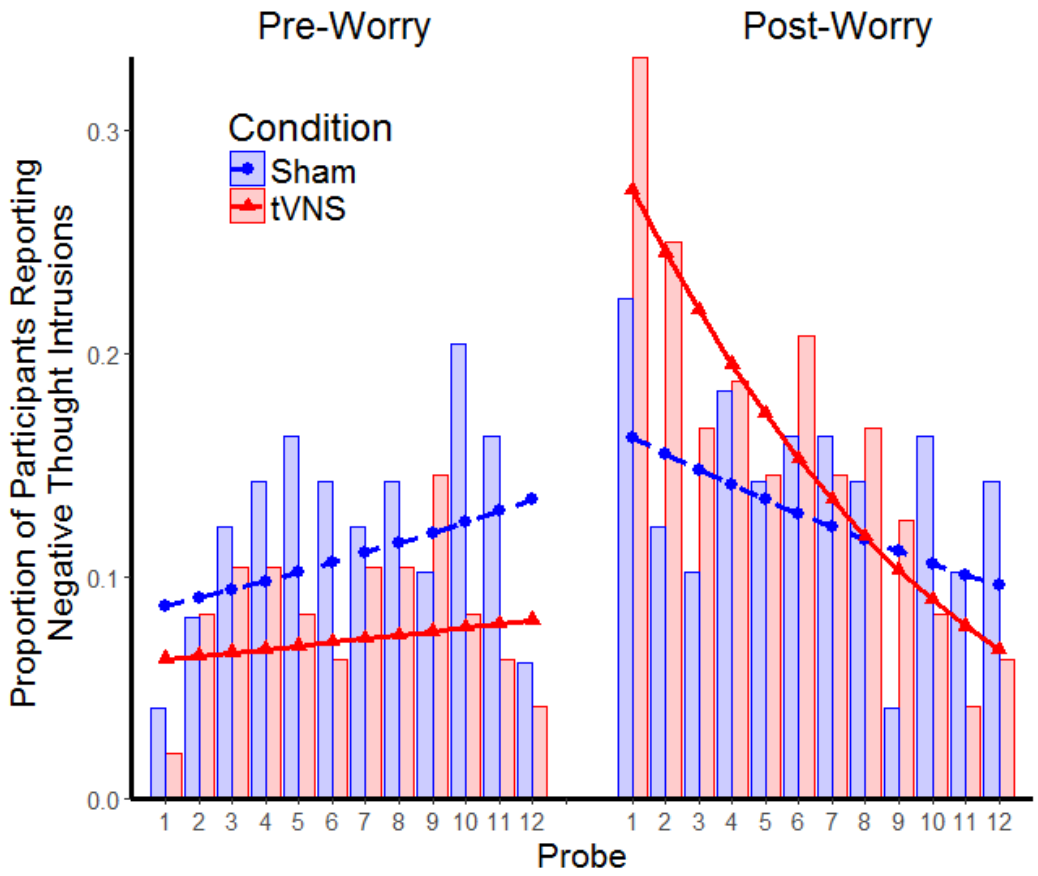

Figure 3. The bars denote the proportion of reported negative thought intrusions for every sound probe taken before and after the worry induction for participants in the sham (blue bars) and tVNS (red bars) condition. The lines depict the estimated marginal means of the logistic mixed model analysis for the sham (blue line) and tVNS (red line) condition.

Worry Induction

As displayed in table 2, the worry induction successfully led to a clear increase in time spent worrying compared to the first breathing focus period, b = 37.39 (2.88), $t(96)$ = 13.00, p < .001, d = 1.63. Additionally, participants spent more time worrying during the post-worry period compared to the pre-worry period, b = 6.95 (2.19), $t(96)$ = 3.17, p = .002, d = .29. There was no difference between conditions in the time that participants spent worrying, their perceived control over their worrying, or their worry intensity (all p > .05). Thus, the initial between-group difference in the number of negative thought intrusions during the post-worry period could not be attributed to a difference in the worry experienced during the worry induction between conditions.

Table 2. Mean self-report measures for tVNS and Sham stimulation conditions during pre- and post-worry breathing focus periods and the worry induction.

	Pre-worry			Worry Induction			Post-worry		
	Time spent worrying	Difficulty focusing on breathing	Time focused on breathing	Time spent worrying	Control over thoughts	Worry Intensity	Time spent worrying	Difficulty focusing on breathing	Time focused on breathing
tVNS	27.3%(25.0%)	46.4(28.5)	58.5%(23.7%)	66.0%(22.0%)	53.5(20.1)	52.7(21.5)	35.0%(24.5%)	58.5(25.7)	49.1%(25.3%)
Sham	28.5%(23.3%)	51.4(24.7)	50.5%(23.6%)	64.7%(22.0%)	49.4(20.8)	50.9(19.9)	34.8%(22.8%)	60.3(22.1)	48.1%(20.7%)

Note. Percentages denote percentage of time spent on an activity within the five minute period. Standard deviations are presented between brackets.

Heart Rate Variability

We explored the effects of tVNS on changes in log-transformed RMSSD over the course of the BFT. A significant decrease in RMSSD was observed during the worry induction and the post-worry period compared to the pre-worry period (main effect of Time, $F(2, 92.77) = 8.17$, $p < .001$). Regardless of experimental group, participants displayed a clear reduction in HRV during the worry induction, b = -.11 (.03), $t(90.00) = -4.04$, $p < .001$, $d = -.20$ (see figure 4). Participants showed a partial recovery of their HRV during the post-worry period, but HRV scores were still significantly lower than in the pre-worry period, $b = -.07$ (.03), $t(102.63) = -2.31$, $p = .023$, $d = -.13$. tVNS did not significantly affect the cardiac response to the worry induction ($p = .99$, $d < .01$) nor did it affect HRV during either of the breathing focus periods ($p_{\text{worry induction*Condition}} = .44$, $d = .05$, $p_{\text{post-worry*Condition}} = .83$, $d = .02$).

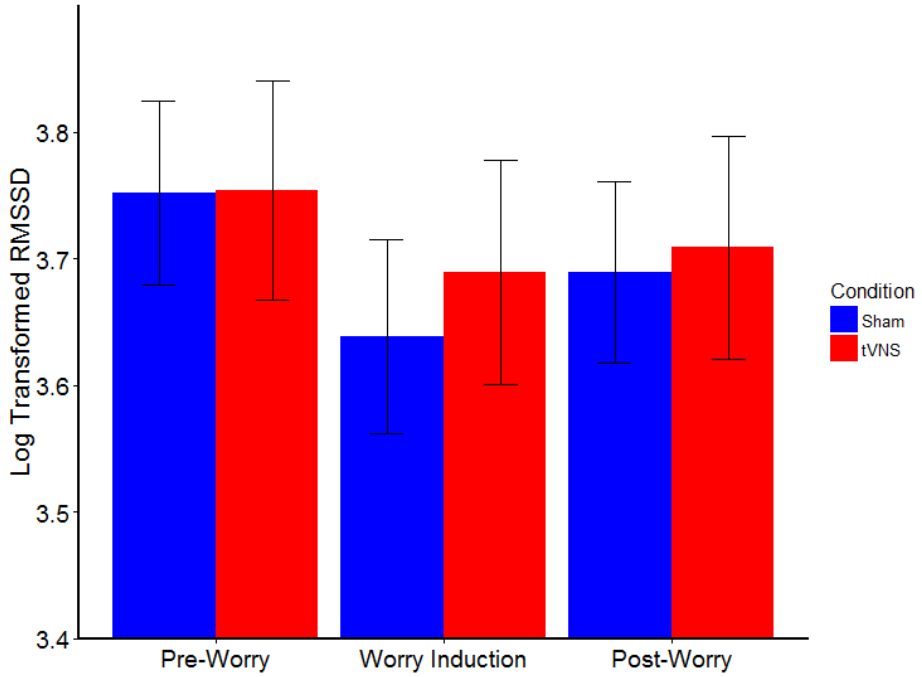

Figure 4. Log transformed RMSSD scores measured throughout the BFT (Error bars denote between-subjects standard errors). Participants in both conditions showed a decrease in RMSSD during the worry induction phase. During the post-worry period, RMSSD scores were still reduced. There were no significant effects of tVNS on RMSSD levels.

Side Effects

Participants in the tVNS condition reported more burning sensations ($Median_{tVNS}$ = 2.5 (Interquartile Range or IQR = 2 - 3), $Median_{Sham}$ = 1 (IQR = 1-2); $\chi^2(1) = 8.22$, $p = .004$) and more stinging sensations ($Median_{tVNS}$ = 4 (IQR = 3-4), $Median_{Sham}$ = 3 (IQR = 2-4); $\chi^2(1) = 7.97$, $p = .005$) as a result of stimulation compared to participants who received sham stimulation. There were no significant differences between groups on any of the other side-effects that were assessed (all $p > .08$).

Discussion

We tested whether short-term transcutaneous stimulation of the vagus nerve reduces worrying in a population of high worriers. Participants were randomized to receive either tVNS or sham stimulation during a Breathing Focus Task (BFT). The BFT measures the frequency of negative thought intrusions over two five minute periods as an index of worry propensity ('spontaneous' and 'induced' worry) [275]. Participants who received tVNS reported fewer negative thought intrusions during the pre-worry period. However, after a worry induction period, participants who received tVNS no longer significantly differed from participants who received sham stimulation in the amount of negative thought intrusions they reported during the post-worry period. We did observe an unexpected higher proportion of participants reporting negative thought intrusions in the tVNS group immediately after the start of post-worry period, which declined more rapidly over the course of the post-worry period than in the sham group.

Participants in the tVNS condition reported significantly fewer negative thought intrusions during the pre-worry breathing focus period. These results are in line with experimental studies indicating that tVNS has acute effects on cognitive processes, including inhibitory control [89,164], associative learning [165,196], and mood [123]. Additionally, these results correspond with treatment studies which have shown that tVNS may affect anxiety symptoms in patients suffering from major depressive disorders [91,92]. The results also seem to be in line with the neurovisceral integration model, which suggests that vagus nerve activity is associated with increased inhibitory control [64], which is believed to be impaired in chronic worriers and may contribute to the perceived uncontrollability of worrying [288].

We can only speculate on the neurobiological mechanisms underlying the effect of tVNS on worrying. Previous studies have found that stimulating the vagus nerve leads to increased functional connectivity between the PFC and the amygdala [50,80], which has been found to be attenuated in high worriers [82–84]. Alternatively, resting state fMRI studies have repeatedly, although not consistently, shown that tVNS is related to reduced activity in areas related to the Default Mode Network (DMN), notably the anterior cingulate gyrus, precuneus, and superior medial frontal gyrus (for an overview of tVNS effects on resting-state fMRI, see [270]). The DMN is thought to play an important role in self-referential thought and worry [73,83,289]. The reduction in negative thought intrusions during the pre-worry period could therefore be attributed to changes in activity in the DMN. However, stimulation of the earlobe (the sham stimulation condition in this experiment) has shown similar decreases in activity in these areas (possibly through the activation of the great auricular nerve) and no consistent significant differences in deactivation patterns were found in these areas between

participants who receive tVNS and participants who receive sham stimulation [92,270]. These results may indicate that earlobe stimulation may have also decreased the activation of the DMN and thereby the amount of negative thought intrusions.

After an explicit instruction to 'worry as you normally do' for 5 minutes, there was no longer an effect of tVNS on negative thought intrusions during the subsequent breathing focus period. An exploratory analysis of responses on a probe level within the post-worry period revealed that participants in the tVNS condition initially reported negative thought intrusions more frequently, although this difference between groups was not statistically significant. By contrast, the proportion of participants who reported negative thought intrusions decreased more rapidly in the tVNS condition than in the sham condition.

Despite being a group of high worriers, participants reported a relatively low amount of negative thought intrusions on average. Indeed, when compared to a study that tested participants without excessive worry complaints [290], participants in our current study reported a similar amount of negative thought intrusions during the post-worry period, and only reported slightly more negative thought intrusions in the pre-worry period. Previous studies in high worriers and GAD patients report higher average numbers of thought intrusions during either period of the BFT (high worriers: pre-worry = 2.3, post-worry = 3.4, [273]; GAD patients: pre-worry = 3.2, post-worry = 3.8, [274]). A methodological difference between the current study and previous studies that have employed the BFT is that these previous studies often asked participants to immediately give a short description of the content of their reported thought intrusions. Possibly this request to articulate thought intrusions may have increased the probability of new thought intrusions occurring at subsequent prompts. Alternatively, the relatively low number of negative thought intrusions reported in both groups in this study may have been affected by a reduction in DMN activity caused by both tVNS and stimulation of the earlobe, as mentioned above.

As participants were not required to articulate the contents of their thought intrusions, we cannot be certain that their negative thought intrusions always represented worry episodes. Indeed, the lack of significant effects of tVNS on retrospective worry assessments raises concerns about the validity of this assessment. On the other hand, there was a high correlation (.67) between the post-hoc worry assessment and negative intrusions reported during the breathing focus phases, indicating that 45% of the variance in negative intrusions can be explained by inter-individual differences in post-hoc worry assessment. This percentage is remarkably high considering the differences between what is being measured (repeated dichotomous point assessments compared to retrospective assessment of total worry duration) and confirms that the negative thought intrusions reported by participants reflect online assessments of worry frequency during the breathing focus phases.

The results of the current study may have been influenced by demand characteristics of the experimental procedure. During the worry assessments we asked our participants to report how long they had worried before and after a worry induction. Obviously, participants were aware of experimenter expectations. However, no other way exists to check the manipulation, and demand effects would affect both conditions. Our main outcome measure, negative thought intrusion frequency, may not have been impervious to demand characteristics, either. One could argue that in the present circumstances, with participants who were aware that they had been selected for being high worriers, the BFT may have acted as an implicit and unintentional thought suppression task. A high worrier may interpret the instruction 'focus on your breath only' as 'do not engage in worrying'. Although participants were not instructed to suppress worry, they were asked to report transgressions from the breathing focus instructions. If the BFT should indeed be interpreted as an unintentional thought suppression task, the increased proportion of participants in the tVNS condition who reported negative thought intrusions at the start of the post-worry period could reflect a stronger rebound effect, which in turn could be due to the stronger attentional control during the initial breathing focus ('thought suppression') period. Future studies should focus on using alternative paradigms to see whether this effect of tVNS on attentional control translates to worry behavior in general.

An additional limitation of the current study is that the sensations reported after tVNS and sham stimulation were not completely identical. Participants receiving tVNS reported more stinging and burning sensations than those reporting sham stimulation, which is in line with previous reports [240,291]. It remains unknown whether and in what way these differences in physical sensations may have affected performance on the BFT. On the one hand, these sensations may have distracted participants from potential negative thought intrusions. On the other hand, the sensations could have induced negative thought intrusions related to the physical sensations. To control for this second possibility, future research could consider reinstating the BFT protocol used in previous studies where the specific content of the thought intrusions are probed [273,274].

A final potential limitation of the current study was that the baseline HRV assessments, as well as the baseline questionnaires, were conducted during the ramp-up period of tVNS. Therefore, we cannot rule out that tVNS may have affected either of these measurements. Future studies should strongly consider administering these baseline measurements prior to the ramp-up period of tVNS to ensure that baseline effects cannot be affected by stimulation.

Corresponding to earlier studies that found a relation between vagal tone as indexed by HRV and worrying [73], participants showed a significant reduction in HRV during the worry induction period, which only partially recovered during the second breathing focus period. As could be expected from the stimulation parameters used in this study, there were no significant differences between the experimental conditions during any of the breathing focus phases, nor in the worry induction phase.

The finding that stimulating the vagus nerve does not subsequently affect HRV, the most widely used index of vagal tone, might strike some readers as a paradoxical finding. However, as mentioned in the introduction, this seemingly contradictory finding can easily be explained by the fact that in this study we stimulated the left branch of the vagus nerve. The left and right vagus nerve differentially innervate the heart, with the right side preferentially innervating the sinoatrial node and the left side innervating the atrioventricular node [15]. Stimulation of the sinoatrial node leads to stronger decreases in heart rate, which is reflected by stronger bradycardia after right-sided VNS [292]. In fact, due to this asymmetric cardiac innervation, invasive VNS has traditionally stimulated solely the left vagus nerve to avoid bradycardia. The lack of cardiac effects can also be explained by taking the different fiber types of the vagus nerve into account. Specifically, tVNS is likely to stimulate primarily thick myelinated *afferent* A-fibers. By contrast, cardiac effects of the vagus nerve are primarily determined by *efferent* B-fibers, which are thin myelinated efferent fibers that have a higher conduction threshold [11]. Specifically, studies in anesthetized dogs – which provide a good model for human stimulation thresholds due to the similarity in nerve diameter and total number of axons – showed that the stimulation intensity used in this study would be sufficient to stimulate *A*-fibers (threshold 0.4 mA), but not *B*- or *C*-fibers (3.8 and 17 mA, respectively; [276]).

One could argue that activation of afferent vagal fibers should still lead to significant cardiac effects indirectly by increasing prefrontal activity and increasing the functional connectivity between the prefrontal cortex and the amygdala. However, given the relatively small effect that experimental worry inductions have on HRV in subthreshold GAD participants [75,293], and the small effect size of tVNS on negative thought intrusions found in the current study, it seems likely that the current study did not have the statistical power to detect such an effect.

Thus, importantly, the lack of cardiac effects found in this study does not invalidate tVNS as a method of activating the vagus nerve. In fact, most manufacturers actively attempt to circumvent possible cardiac side-effects. This does pose an interesting new challenge however: there is a clear need for a sensitive biomarker of vagal activity, specifically a marker that activates when low intensity stimulation activates only the A-fibers of the vagus nerve. Possible candidates for such a measure of vagal activity may include the pupillary light reflex, pupil dilation or EEG measures such as the P300 as a measure of vagal effects on noradrenergic transmission [294,295].

To conclude, the current study showed that short-term tVNS may ameliorate spontaneously occurring worry in high worriers. However, the effects of tVNS after an explicitly induced worry period are mixed. In an exploratory analysis, we observed an unexpected higher proportion of participants in the tVNS group reporting negative thought intrusions immediately after the start of the post-worry period, which declined more rapidly over the course of the post-worry period than in the sham group. As such, the current study provides partial confirmation that activation of the vagus nerve may actively

reduce worrying. These results provide interesting indications for the validity of tVNS as an intervention for worry-related psychopathology.