Received: 24 May 2023 | Revised: 20 October 2023 | Accepted: 20 October 2023

DOI: 10.1111/psyp.14484

PSYCHOPHYSIOLOGY SPR WILEY

ORIGINAL ARTICLE

Non-invasive vagus nerve stimulation and the motivation to work for rewards: A replication of Neuser et al. (2020, *Nature Communications*)

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Funding information
Nederlandse Organisatie voor Wetenschappelijk Onderzoek, Grant/Award Number: VI.C.181.032

Abstract

The vagus nerve is thought to be involved in the allostatic regulation of motivation and energy metabolism via gut-brain interactions. A recent study by Neuser and colleagues (2020) provided novel evidence for this process in humans, by reporting a positive effect of transcutaneous auricular vagus nerve stimulation (taVNS) on the invigoration of reward-seeking behaviors, especially for food rewards. We conducted an independent direct replication of Neuser et al. (2020), to assess the robustness of their findings. Following the original study, we used a single-blind, sham-controlled, randomized cross-over design. We applied left-sided taVNS in healthy human volunteers ($n = 40$), while they performed an effort allocation task in which they had to work for monetary and food rewards. The replication study was purely confirmatory in that it strictly followed the analysis plans and scripts used by Neuser et al. Although, in line with Neuser et al., we found strong effects of task variables on effort invigoration and effort maintenance, we failed to replicate their key finding: taVNS did not increase the strength of invigoration ($p = .62$); the data were five times more likely ($BF_{10} = 0.19$) under the null hypothesis. We also found substantial evidence against an effect of taVNS on effort maintenance ($p = .50$; $BF_{10} = 0.20$). Our results provide evidence against the idea that left-sided taVNS boosts the motivational drive to work for rewards. Our study also highlights the need for direct replications of influential taVNS studies.

KEYWORDS
effort, gut-brain, HLM, reproducibility, taVNS, tVNS

1 | INTRODUCTION

Transcutaneous auricular vagal nerve stimulation (taVNS) is a non-invasive technique that electrically stimulates the auricular branch of the vagus nerve, the nerve that connects the gut and brain through the gut-brain axis. The stimulation is applied to the outer ear, at the cymba concha, on the inner side of the tragus. taVNS has been found to activate the nucleus of the solitary tract (NTS) and brainstem arousal nuclei via afferent vagal pathways (Borgmann et al., 2021;

Federica Lucchi and Beth Lloyd contributed equally to this work.

Yakunina et al., 2017). taVNS is used as a treatment of drug-resistant epilepsy and depression (Ellrich, 2011; Liu et al., 2020) and has promising effects on other clinical outcome measures (Busch et al., 2013; Ferstl et al., 2020).

Laboratory experiments have also reported positive effects of taVNS on cognitive functions, including cognitive control, emotion recognition, associative memory, and cooperation (Fischer et al., 2018; Jacobs et al., 2015; Oehrn et al., 2022; Sellaro et al., 2018). The primary mechanism of action hypothesized to underlie these effects of taVNS is an increase in central noradrenergic activity. This assumption is based on animal studies that have found that invasive VNS increases the firing rate of locus coeruleus neurons and increases extracellular norepinephrine levels (e.g., Dorr & Debonnel, 2006; Hassert et al., 2004; Raedt et al., 2011). However, the evidence for an effect of taVNS on non-invasive physiological markers of noradrenergic activity is mixed, with some studies finding positive evidence (Giraudier et al., 2022; Lloyd et al., 2023; Sharon et al., 2021; Ventura-Bort et al., 2018) and other studies finding negative evidence (Burger et al., 2020; D'Agostini et al., 2022; Keute et al., 2019; Warren et al., 2019)—especially studies using the relatively long on/off stimulation cycles of the taVNS device most often used in laboratory experiments (NEMOS®, Cerbomed GmbH, Erlangen, Germany). Positive findings of laboratory taVNS experiments with long on/off cycles or continuous stimulation, especially those using relatively low stimulation intensities (e.g., 0.5 mA), therefore warrant scrutiny, for example using direct replication studies.

Here we report a direct replication of a taVNS study (Neuser et al., 2020) that has attracted a lot of interest, in particular because of its focus on gut-brain interactions, which have a major influence on motivational states. Previous evidence has shown that, as an important component of the autonomic nervous system, the vagus nerve is involved in allostasis, the regulation of motivation, and energy metabolism (Burger et al., 2020; Han et al., 2018). Activation of vagal pathways can modulate reward-seeking behaviors (Han et al., 2018), and control food intake through negative feedback signals routed via the NTS (Chen et al., 2015). Thus, the vagus nerve may serve as a link between peripheral metabolic signals and central functions involved in goal-directed, allostatic behavior. To test the role of the vagus nerve in regulating motivation, Neuser and colleagues examined the effect of taVNS on the motivation to work for rewards.

The participants in Neuser et al. (2020) carried out an effort allocation task in which they were asked, on each trial, to work for a primary (food) or secondary (money) reward by vigorously pressing a button. The reward magnitude and effort required to achieve the reward (i.e., task difficulty) varied across trials. After the effort phase of each trial, participants were asked to rate their wanting of the reward at stake and their exertion level (i.e., the perceived cost of their action). The 30-s on phase of taVNS or sham stimulation was aligned with the 30-s effort phase of the trial, followed by an off phase during the subjective ratings. Neuser et al. found that taVNS boosted the invigoration of effort—that is, how quickly a participant energized effortful behavior on each trial by ramping up the frequency of button presses. In contrast, taVNS did not affect the overall effort produced on each trial (i.e., average frequency of button presses).

The critical question in our study was whether we could replicate Neuser et al.'s (2020) key finding that taVNS enhances the invigoration of effort. To ensure that we replicated the original study as closely as possible, we ran the task and analyzed the data using protocols and scripts kindly provided by Neuser and colleagues. An important difference between the studies is that unlike Neuser et al. we only tested participants with stimulation of the *left* cymba concha. In the past, the large majority of laboratory taVNS studies have only applied stimulation to the left cymba concha, because of cardiac safety concerns. Although these concerns have recently been challenged (Chen et al., 2015; Farmer et al., 2021), our ethics committee did not allow us to administer taVNS to the right cymba concha. In this context, it is important to note that the effects of left-sided and right-sided stimulation on invigoration reported by Neuser and colleagues were essentially equal in size.

Before the start of the study, we decided to stop data collection when the evidence for or against the key hypothesis would reach a Bayes factor of 8 or 0.125 (i.e., 1/8), with a maximum of 40 included participants due to resource constraints (Lakens, 2022)—note that Neuser et al. (2020) tested 41 participants with left-sided stimulation. In accordance with these criteria, we stopped data collection after including 40 participants, and reaching a Bayes factor of 0.19, reflecting *substantial* evidence for the null hypothesis (Jeffreys, 1998).

2 | METHOD

2.1 | Participants

A total of 42 participants took part in the study, which consisted of two sessions scheduled between 2 and 8 days from each other at approximately the same time of day. In one session, participants received stimulation of their left cymba conchae (taVNS condition); in the other session, their left ear lobe was stimulated (sham condition). Two participants were excluded because they missed the second session or failed to respond on many trials. The resulting final sample consisted of 40 participants (32 females [sex assigned at birth]; mean age $M = 21.30$, range 18 to 29).

All participants were right-handed and used to eating breakfast between 6 and 10 am. Exclusion criteria were having participated in any taVNS experiment in the previous month, history of cardiac conditions, neurological or psychiatric disorders, skin disorders, current use of psychoactive medication or drugs, and active implants (e.g., cochlear implant, pacemaker). Participants were asked to not consume any food or alcohol within the 12 hr before the start of the experiment. The study was approved by the ethics committee of the Institute of Psychology at Leiden University (S.T.-V3-2972).

2.2 | taVNS stimulation

A NEMOS® stimulation device was used to stimulate the left auricular branch of the vagus nerve. Two titanium electrodes placed either at the left cymba conchae (taVNS) or left earlobe (sham) transmitted electrical current with biphasic impulse frequency of 25 Hz and a stimulation duration of 30-s (Frangos et al., 2015). This device has previously been used in both clinical settings (Bauer et al., 2016; Kreuzer et al., 2012) and fundamental research (Frangos et al., 2015; Neuser et al., 2020; Sharon et al., 2021). The default setting of the device follows a 30-s on phase followed by a 30-s off phase. To replicate Neuser et al.'s procedure during the effort task, the experimenter aligned the onset of the stimulation to the start of each trial, which meant shortening the 30-s off phase.

Before participants performed the task, we carried out a work-up procedure to achieve a stimulation intensity that corresponded to a "mild pricking" (Neuser et al., 2020). Participants were repeatedly asked to rate the received intensity of the stimulation on a 10-point visual analogue scale ("How strong do you perceive pain induced by the stimulation?" ranging from 0, "no sensation" to 10, "strongest sensation imaginable"). Stimulation started at an amplitude of 0.1 mA and was increased in steps of 0.1–0.2 mA until a rating of 4, corresponding to a "mild pricking" sensation, was reached. Note that this procedure differs from the preferred method of limits, in which ascending and descending staircases are typically used in conjunction. The resulting stimulation intensities ($M_{taVNS} = 1.36$ mA; range = 0.3 to 2.8 mA; $M_{sham} = 1.84$ mA; range = 0.6 to 4.4 mA) were similar to those reported by Neuser et al. ($M_{taVNS} = 1.28$ mA; $M_{sham} = 1.85$ mA. Participants then proceeded with the effort allocation task.

2.3 | Effort allocation task

Task scripts for administering the effort allocation task were provided by Neuser and colleagues (2020) and were run using the Psychophysics Toolbox v3 (Brainard, 1997; Kleiner et al., 2007) in MATLAB R2018b. During the task, participants were required to exert effort by repeatedly pressing a button with their right index finger on an Xbox 360 controller joystick (Microsoft Corporation, Redmond, WA), to obtain either monetary or food rewards. Like Neuser et al., we measured the frequency of button presses as an indicator of physical effort. At the beginning of each trial, participants were presented with the reward that could be obtained on that trial (Figure 1). This could be either food, represented by a picture of one or more cookies, or money, represented by a picture of one or more coins. The number of coins and cookies could be either one or several, indicating the magnitude of the reward on a given trial. Only one item corresponded to 1 point per second, whereas several items corresponded to 10 points per second.

After 1 s, a blue ball inserted in a tube was shown on the screen. Participants were instructed to continually press a button with their right index finger to move the ball vertically up the tube and keep it above a red line. Depending on the level of difficulty for that trial, the height of the red line was adjusted. The difficulty was manipulated across trials (either 75% [low difficulty] or 85% [high difficulty] of the individual maximum frequency) with the order of difficulty counterbalanced across participants. At the start of each trial, the difficulty level was made clear by the height of the red bar. The longer the ball was kept above the line, the more reward points a participant earned on that trial. For every second, the ball was above the red line, signaled by the ball changing color from dark blue to light blue, reward points were added, and the new total was displayed in the top right corner of the screen.

Following the effort phase of a trial (30 s), two visual analogue scales with a range from 0 to 100 and a default setting of 50 were displayed on the screen. Participants were asked to rate their level of exertion and their wanting of the reward on the preceding trial. Although we used a task script including task instructions provided by Neuser et al. (2020), we noticed that our participants decided to not move away the marker from the default setting of 50 on a much larger percentage of the trials than the participants tested by Neuser et al. (current study: exertion 19.4%, wanting 14.5%; Neuser et al.: exertion 1.8%, wanting 1.7%). All English instructions that were used in the task for the current study are publicly available here: https://github.com/bethlloyd/neuser_replication. When we discussed this discrepancy with Neuser and colleagues, we learnt that their participants had received instructions on how to use the visual analogue scales in an experiment that immediately preceded the effort allocation task and that was published elsewhere after we collected our data (Müller et al., 2022). Because this large percentage of ratings of

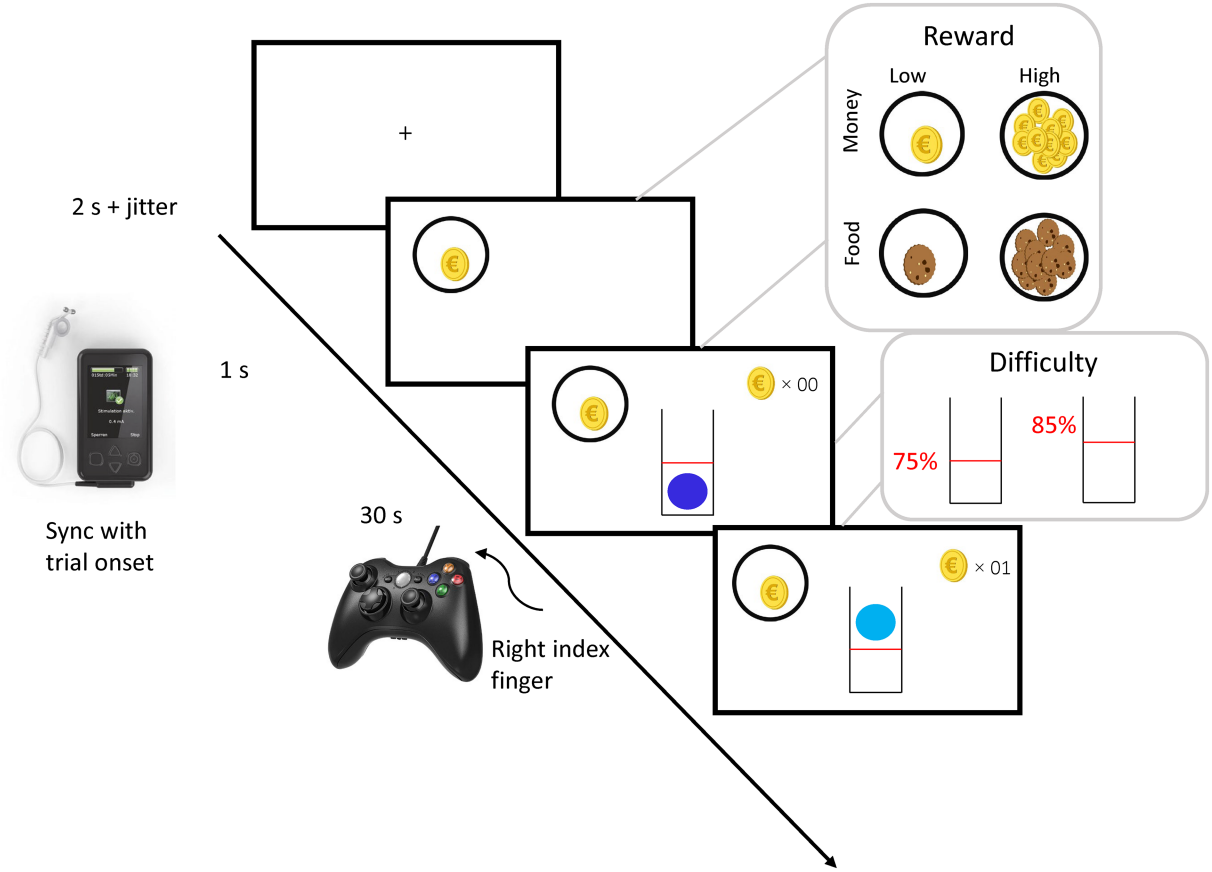

FIGURE 1 Schematic summary of the effort allocation task (Neuser et al., 2020).

50 substantially biased the correlations between invigoration/maintenance and exertion/wanting, we decided to remove these trials before computing the correlations (see Figure S1 for the rating distribution from both studies).

The main experiment consisted of 48 trials interrupted by two short breaks of 15 s each. Before starting the task, participants were shown instructions, which emphasized that the task was too difficult to always keep the ball above the red line. Participants were encouraged to always hold the controller in the same way and to take breaks at their convenience to recover during trials, so that they could try to exceed the threshold again. At the end of the task, participants were shown the total amount of food and monetary rewards earned, with their conversions into euros and calories.

2.4 | Experimental procedure

The design of the current study was single-blind, within-subjects, randomized, and counterbalanced across conditions. Both sessions started between 7:30 am and 10:10 am, as in Neuser et al. (2020). However, most of the participants were scheduled to start at either 8 am or 9:30 am. All participants were tested by the same experimenter. Participants were told they could drink water ad libitum during the experiment and that the earned points would be converted into calories and money at the end of the session.

Next, participants practiced the effort allocation task for ~5 min. During this training phase, the maximum frequency of button presses was estimated for each participant. A complete description of the practice phase can be found in Neuser et al.

Next, we placed the taVNS device on the left ear of the participant, with the electrodes making contact with the cymba conchae (taVNS) or left earlobe (sham). To increase conductance of the electrodes, the participant was instructed to wipe their left ear with an alcohol pad. Moreover, two cotton rings were soaked with electrode contact fluid and applied on the taVNS device. Next, we assessed the stimulation intensity for each participant as described above. Then, participants completed the effort allocation task, which lasted ~40 min. When asked at the end of each session which stimulation condition they were assigned to, participants were not able to guess better than chance (correct guesses: 48.5%).

At the end of each session, after completing the task, participants received their breakfast: a chocolate bar, milk, and cereals according to the number of calories earned. After the second session, participants also received their monetary compensation: a fixed amount of

22.50€ or course credits + the wins of both sessions. On average, participants won 562.52 kcal and €6.07 per session.

2.5 | Motivation indices and mixed-effects models of stimulation effects

To calculate the two motivational aspects, invigoration slope and effort maintenance, we used MATLAB R2021a and the original analysis scripts received from Neuser et al. (2020), in which the behavioral data were divided into work segments and rest segments. Like Neuser et al., we calculated the invigoration of effort by estimating the slope of the transition between the relative frequency of button presses during a rest segment and their initial plateau during the subsequent work segment (MATLAB findpeaks function). The maintenance of effort was calculated as the average frequency of button presses during a trial. In this way, we estimated how much effort participants produced over time.

In accordance with Neuser et al. (2020), invigoration slope and effort maintenance were moderately correlated, $r = .28$, 95% CI [0.25, 0.31]. The test–retest reliabilities of the two measures were high; the correlations across participants between session 1 and session 2 were 0.85 for invigoration and 0.92 for effort maintenance. To assess the effects of taVNS on invigoration and effort maintenance, single-trial estimates of the two variables were entered into two separate univariate mixed-effects models. Invigoration slope and effort maintenance were predicted as outcomes using the following dummy-coded predictors: stimulation (sham, taVNS), reward type (food, money), reward magnitude (low, high), difficulty (easy, hard), the interaction between reward magnitude and difficulty, and the interactions between stimulation and all other predictors. To account for the order of stimulation, we included stimulation order (taVNS first, sham first) at the participant level. To account for interindividual variance, we included intercepts and slopes as random effects. To examine the correlation between invigoration slope and effort maintenance with subjective ratings, we used mixed-effects models to predict either invigoration or effort maintenance as the outcome with wanting and exertion ratings as the predictors.

2.6 | Statistical threshold and software

Since the current study was an attempt at direct replication, our statistical thresholds and scripts for preprocessing data were identical to those used by Neuser et al. (2020). We used a two-tailed $\alpha \leq 0.05$ for the analysis of our primary research question of whether taVNS modulates invigoration slope or effort maintenance across conditions (i.e., the main effect of stimulation). Mixed-effects analyses were conducted with HLM v7 (Raudenbush et al., 2011) and ImerTest in R (Kuznetsova et al., 2017). To determine the relative evidence for one hypothesis over the other (i.e., whether or not taVNS facilitates motivational aspects of goal-directed behavior such as invigoration or effort maintenance), we calculated Bayes factors (BFs) using one-sided Bayesian *t* tests based on order-corrected individual estimates of all stimulation effects (calculated using ordinary least squares). To do that, we used the default Cauchy prior $r = .707$ in JASP v0.9 (Team, 2019). Results were also plotted with R v3.4.0 (i.e., ggplot; (R Core Team, 2014)).

3 | RESULTS

3.1 | Effects of task variables on invigoration and effort maintenance

We first tested whether we found the same effects of task conditions on invigoration slope and effort maintenance as found by Neuser et al. (2020). In line with Neuser et al., participants were quicker to invigorate behavior when they were working for larger rewards, $b = 4.32$, 95% CI [1.6, 7], $t(38) = 3.1$, $p = .004$, Cohen's $d = 0.49$, $BF_{10} = 10.54$ (Figure 2a; Table S1), while invigoration did not differ between food and money trials, $b = 1.97$, 95% CI [−4.75, −0.81], $t(38) = 1.38$, $p = .17$, Cohen's $d = 0.22$, $BF_{10} = 0.43$ (Figure 2c). Unlike Neuser et al., we did not find an effect of difficulty on invigoration, $b = -1.69$, 95% CI [−3.38, 0.94], $t(38) = -1.26$, $p = .22$, Cohen's $d = -0.2$, $BF_{10} = 0.53$: participants were not slower to invigorate when the difficulty level was higher. Lastly, like Neuser and colleagues, we found significant associations between invigoration and wanting ratings, $t(38) = 3.74$, $p < .001$, fixed-effects estimate = 0.15, and between invigoration and exertion ratings, $t(38) = 0.45$, $p = .65$, fixed-effects estimate = 0.018 (Figure 2e).

In line with Neuser et al. (2020), participants showed higher effort maintenance when more reward was at stake, $b = 4.4$, 95% CI [2.24, 6.56], $t(38) = 4.01$, $p < .001$, Cohen's $d = 0.63$, $BF_{10} = 84.34$, while their effort dropped significantly when reward was more difficult to obtain, $b = -1.83$, 95% CI [−3.66, −0.36], $t(38) = -2.45$, $p = .019$, Cohen's $d = -0.39$, $BF_{10} = 1.79$ (Figure 2b; Table S2). Furthermore, participants exerted more effort to obtain large rewards when the difficulty level was high, indicated by a reward magnitude × difficulty interaction, $b = 0.95$, 95% CI [0.23, 1.65], $t(38) = 2.65$, $p = .012$, Cohen's $d = 0.42$, $BF_{10} = 2.96$ (Figure 2b). Unlike Neuser et al., we found higher effort maintenance when food as opposed to money was the reward at stake, $b = 1.46$, 95% CI [0.46,

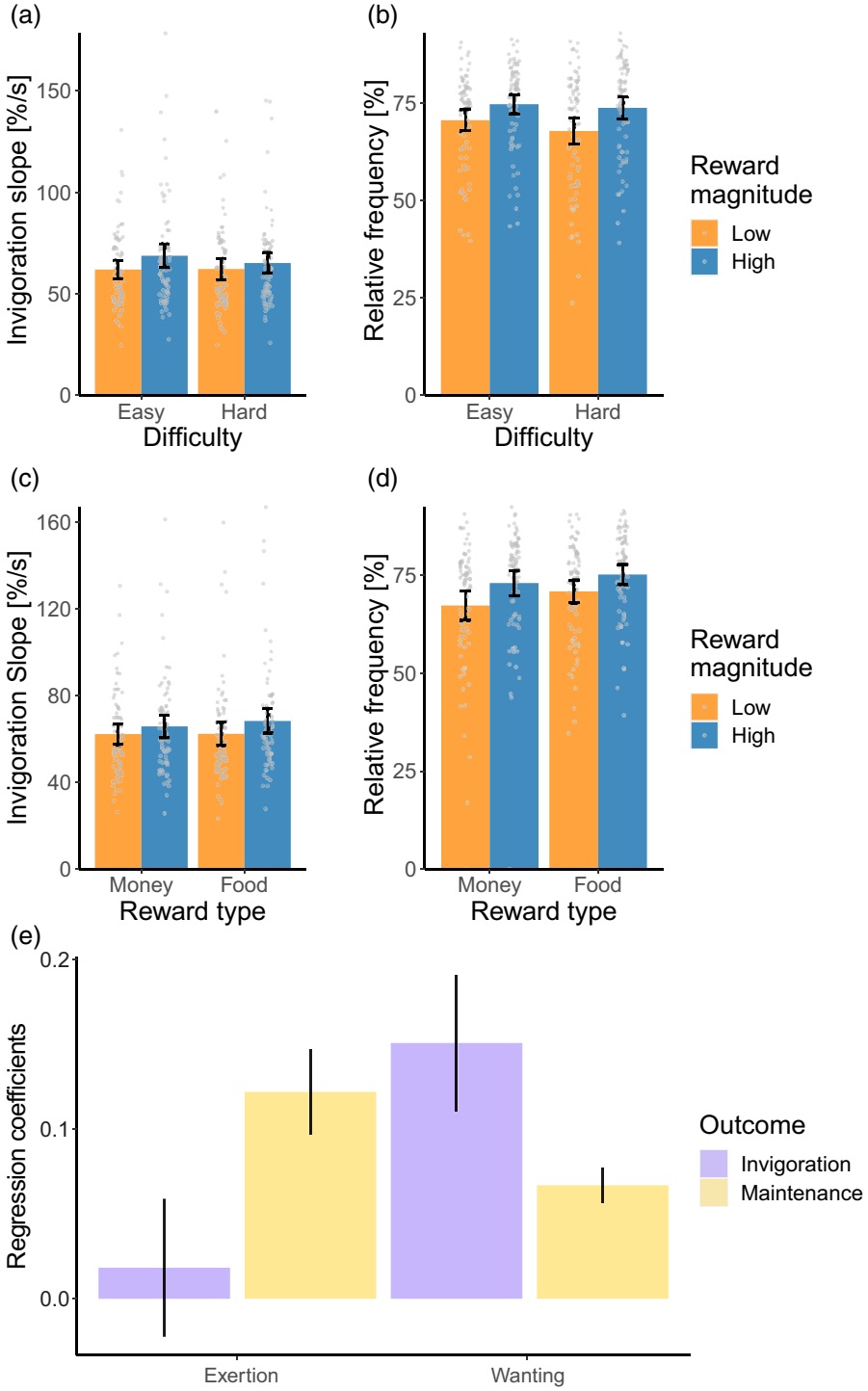

FIGURE 2 Invigoration is associated with reward magnitude while effort maintenance is linked with reward magnitude, trial difficulty, and reward type. Gray dots indicate participant means per condition; error bars refer to 95% confidence intervals at the trial level (a–d). Bars indicate the fitted coefficients from the mixed models, and error bars indicate the standard error of the mean (e). %/s, button press rate.

2.46], $t(38) = 2.84$, $p = .007$, Cohen's $d = 0.45$, $BF_{10} = 5.88$ (Figure 2d), suggesting that food had a higher incentive value. In line with Neuser et al., effort maintenance was associated with ratings of wanting, $t(38) = 6.33$, $p < .001$, fixed-effects estimate = 0.067, and exertion, $t(38) = 4.88$, $p < .001$, fixed-effects estimate = 0.122 (Figure 2e).

3.2 | Effects of taVNS on invigoration and effort maintenance

Having replicated most of the behavioral effects reported by Neuser et al., we next wanted to address the critical question of whether taVNS boosted invigoration. We

found no effect of stimulation (taVNS vs. sham) on invigoration, $b = 0.92$, 95% CI [−2.71, 4.56], $t(38) = 0.5$, $p = .62$, Cohen's $d = 0.08$ (Figure 3a). Importantly, a Bayesian analysis revealed substantial evidence for the null hypothesis, $BF_{10} = 0.19$. Figure 4 shows how the BF evolved with each additional participant and suggests that it is unlikely that data from additional participants would result in accumulated evidence supporting the alternative hypothesis. We also did not find the anticipated interaction between stimulation and reward type, $b = -1.37$, 95% CI [−4.82, 2.1], $t(38) = -0.78$, $p = .44$, Cohen's $d = 0.12$, $BF_{10} = 0.23$—a food-specific effect of taVNS on invigoration—which in Neuser et al. was fully driven by left-sided stimulation. The impact of taVNS on invigoration was not influenced by any task factors ($ps > .08$, all $BF_{10} < 0.99$; Figure 5a).

Like in Neuser et al. (2020), there was no effect of taVNS on effort maintenance, $b = 0.48$, 95% CI [−0.91, 1.87], $t(38) = 0.68$, $p = .50$; Cohen's $d = 0.12$, $BF_{10} = 0.20$; Figure 3b), and the effect of taVNS on effort maintenance was not modulated by reward magnitude, difficulty or reward type ($ps > .35$, all $BF_{10} < 0.25$; Figure 5b).

In a final and complementary analysis of the data, we used a prior based on the posterior distribution obtained from Neuser et al. (2020) (median = 0.291, CI [0.07, 0.51]), asking whether the current effect size is equal to that found by Neuser and colleagues (H_0) or whether it is not equal (Verhagen & Wagenmakers, 2014). Using this prior—a normal distribution with a mean of 0.291, and an estimated standard of .166 resulted in a $BF_{10} = 2.0$, indicating that the data are two times more likely under the alternative hypothesis that the true effect size is smaller than the effect size obtained by Neuser et al.

4 | DISCUSSION

Replicating scientific findings is crucial for scientific progress as it provides more robust knowledge, in our case about possible applications of tVNS in fundamental research and clinical practice. In the current study, we replicated the study of Neuser et al. (2020), in which participants were required to exert effort by repeatedly pressing a button with their right index finger on a controller joystick to obtain either monetary or food rewards. Although, in line with Neuser et al., we found strong effects of task variables on effort invigoration and maintenance, we were not able to replicate their key finding: taVNS did not increase the strength of invigoration, regardless of reward type, reward magnitude, and trial difficulty. The data were five times more likely ($BF_{10} = 0.19$) under the null hypothesis than under the alternative hypothesis that taVNS modulates invigoration. Similar to Neuser et al., we found no effect of taVNS on effort maintenance, although the relative strength of evidence for the null hypothesis was stronger in our data ($BF_{10} = 0.20$ compared to 0.51). Together, these findings cast doubt on the idea that active taVNS, compared to sham stimulation, can alter the effort or drive to work for rewards.

Importantly, for the most part, we were able to replicate the key task-related effects reported in Neuser et al. (2020). Notably, we found a robust effect of reward magnitude on invigoration and effort maintenance, suggesting that participants were more prone to invigorate their effort, and maintain this effort, when larger rewards were at stake. Furthermore, we replicated the effect of task difficulty on effort maintenance, showing that participants reduced the frequency of button presses when the trial was more difficult. We also replicated the null associations between subjective

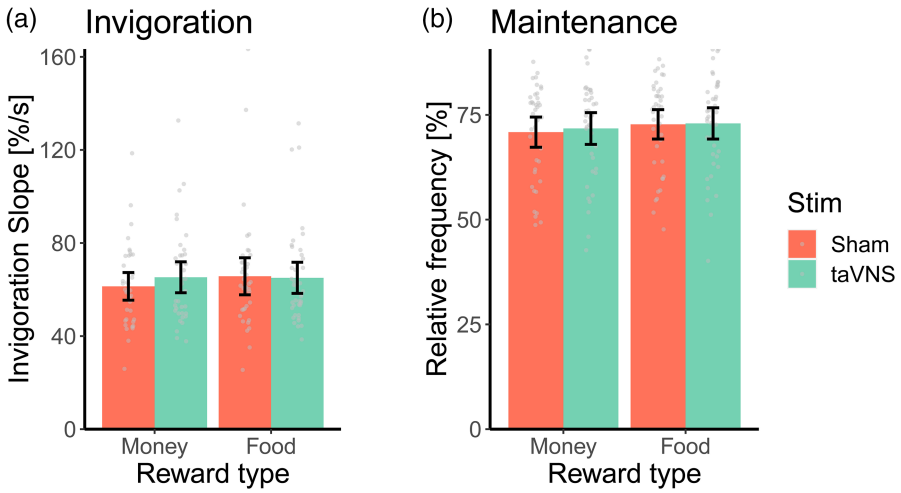

FIGURE 3 Effects of taVNS on the invigoration (a) and maintenance (b) of effort. Gray dots indicate participant means per condition; error bars refer to 95% confidence intervals at the trial level. %/s, button press rate.

ratings of wanting and exertion and effort maintenance and the positive association between wanting and invigoration.

There are essentially three possible explanations for our non-replication of an effect of tVNS on invigoration. First, it is possible that we did not manage to (sufficiently) increase vagus nerve activity. We cannot properly address this possibility, because we did not collect (and nor did Neuser and colleagues) a non-invasive biomarker to demonstrate that our tVNS methods managed to increase vagus nerve activity. It is important that tVNS researchers continue the search for, and validation of, valid non-invasive biomarkers, and that follow-up studies on the role of the (human) vagus nerve on effort management collect a biomarker to verify the effect of taVNS on vagus nerve activity (i.e., do a manipulation check). However, given that we used the same tVNS parameters as Neuser and colleagues, it seems unlikely that this explanation can account for the discrepant results. Second, it is possible that we managed to stimulate the vagus nerve, but that increased vagus nerve activity does not affect effort invigoration. This explanation would imply that Neuser and colleagues reported a false positive. A third possible explanation is that increased vagus nerve activity affects effort invigoration, but does so only in a limited set of circumstances. Below we discuss a number of potential moderator variables, based on procedural differences between the two studies (stimulation side and taVNS initiation period) and differences between the participant samples (sex and baseline motivation levels). Future studies should actively explore potential moderator variables that may affect the impact of taVNS on cognitive and physiological measures.

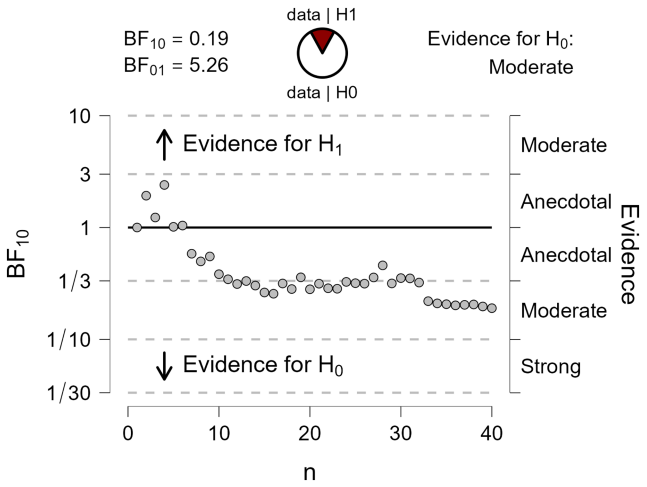

FIGURE 4 Evolution of the Bayes factor reflecting the evidence for the null hypothesis.

Our experimental procedure differed in two ways from that of Neuser et al. (2020). First, as discussed above, we were allowed to use only left-ear stimulation in our participants. We did not consider this a problem because Neuser and colleagues found the effect of taVNS on invigoration to be essentially identical for left- and right-ear stimulation (interaction between taVNS/sham and side of stimulation: $p = .95$). Nevertheless, future research is necessary to determine if the reported positive effect of right-ear taVNS on invigoration is replicable. This question is especially important in the light of recent evidence from rodents that the right vagus nerve promotes appetitive behavioral responses and strongly activates dopaminergic midbrain nuclei (Brougher et al., 2021), which regulate the motivation to obtain rewards.

Second, after stimulation was started, the participants in Neuser et al. (2020) first carried out a 20-min food-cue reactivity task (Müller et al., 2022) before they started the effort allocation task. This raises the possibility that we might also have found a positive effect of taVNS on invigoration if our participants had started the task after

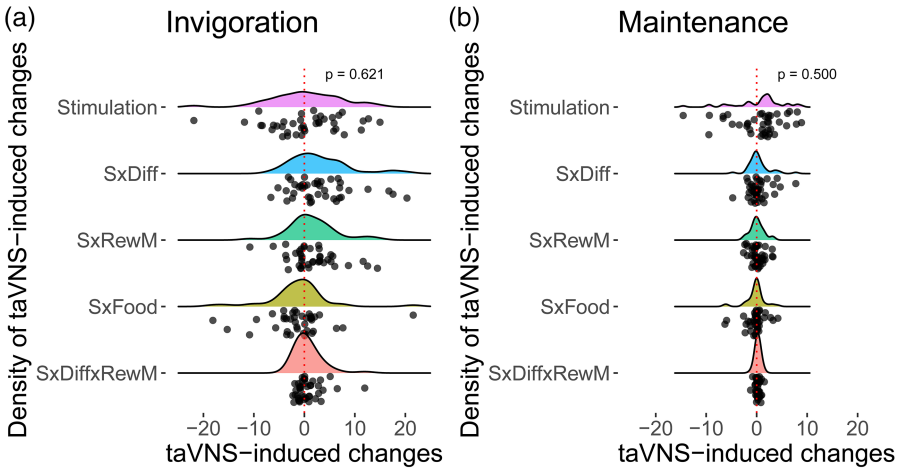

FIGURE 5 Stimulation effects induced by taVNS on invigoration (a) and effort maintenance (b). *p* values refer to two-sided *t* contrasts of the mixed-effects models. Black points refer to the empirical Bayes estimates from the mixed-effects models. Diff, difficulty; RewM, reward magnitude; S, stimulation.

a 20-min initiation period. To address this possibility, we compared the effects of taVNS on invigoration during the first and the second 20 min of the effort allocation task by repeating the mixed-effects model analyses with an additional predictor (first vs. second half). These analyses yielded nonsignificant interactions between stimulation (taVNS/sham) and session half (first/second) for invigoration, $b = 0.59$, $t(38) = 0.36$, $p = .79$, and maintenance, $b = 0.42$, $t(38) = 1.15$, $p = .26$. These results suggest that 20 min of additional stimulation did not have substantial effects on the dependent variables.

We considered two other potential explanations for our null results. First, in our study only 8 out of 40 (20%) participants were male, whereas in Neuser et al. (2020) 34 out of 81 (42%) participants were male. This difference could potentially account for the discrepancy between the two studies, because men and women tend to differ in heart rate variability, an index of cardiac vagal tone (Koenig & Thayer, 2016), and can also differ in the motivation to work for rewards (Lewis et al., 2023). To directly address the question whether our non-replication can be due to a difference in sex distribution, we examined in both data sets whether men and women differed in the effect of taVNS on effort invigoration and effort maintenance. However, this was not the case (Supplementary Results). Second, we examined whether the groups of participants in the two studies differed in their motivation to perform well on the task. If participants in the current study were more motivated than those in the Neuser et al. (2020) study, there might have been less room for a tVNS-evoked increase in invigoration, suggesting an explanation for the obtained null effect. However, a close examination of the data from the two studies suggested that, if anything, our participants were somewhat *less* motivated than the participants in the original study (Supplementary Results).

"Invigoration" and "vigor" are well-developed concepts that generally refer to the (inverse) latency to initiate and/or complete a response and have long been known to depend on dopaminergic activity (Niv et al., 2007). It remains to be determined to what extent the invigoration slope in the effort allocation task used here, which does not reflect the latency of a discrete response, corresponds with these concepts. The moderate correlation with effort maintenance scores ($r = .28$), the high test–retest reliability ($r = .85$), and the significant effects of task variables suggest that the invigoration slope is a meaningful motivational measure. Nonetheless, future research using more common measures of invigoration is needed to further assess the effects of invasive and non-invasive VNS on the motivation to work for monetary and food rewards.

Several recent taVNS studies have not managed to replicate the effects of invasive VNS on psychophysiological measures such as pupil size and P300 amplitude, reporting null effects instead (Burger et al., 2020; D'Agostini et al., 2022; Gadeyne et al., 2022; Keute et al., 2019; Warren et al., 2019). In the present work and another replication attempt (Lloyd et al., 2023), we find that even the results of high-profile taVNS studies can be difficult to replicate. Parametric exploration of tVNS stimulation parameters (D'Agostini et al., 2023; Urbin et al., 2021) and pre-registration, to combat *p*-hacking and publication bias (Munafò et al., 2017), may help this nascent field of inquiry to overcome these challenges.

AUTHOR CONTRIBUTIONS

Federica Lucchi: Data curation; project administration; writing – original draft. **Beth Lloyd:** Formal analysis; project administration; supervision; visualization; writing – review and editing. **Sander Nieuwenhuis:** Conceptualization; funding acquisition; supervision; writing – review and editing.

ACKNOWLEDGMENTS

We would like to thank Anisha Koeldiep for her help with setting up the study. We would also like to thank the authors of the original paper (Neuser et al.) for their support in the replication of the study. Nils kindly provided us task and preprocessing scripts and gave us helpful advice on our analyses.

FUNDING INFORMATION

This work was supported by the Netherlands Organization for Scientific Research (grant no. VI.C.181.032).

CONFLICT OF INTEREST STATEMENT

Authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Analysis code can be found here: https://github.com/bethlloyd/neuser_replication. Raw and processed behavioral data can be found here: https://doi.org/10.6084/m9.figshare.24427312.v1.

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REFERENCES

Bauer, S., Baier, H., Baumgartner, C., Bohlmann, K., Fauser, S., Graf, W., Hillenbrand, B., Hirsch, M., Last, C., Lerche, H., Mayer, T., Schulze-Bonhage, A., Steinhoff, B. J., Weber, Y., Hartlep, A., Rosenow, F., & Hamer, H. M. (2016). Transcutaneous vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: A randomized, double-blind clinical trial (cMPsE02). *Brain Stimulation*, *9*(3), 356–363. https://doi.org/10.1016/j.brs.2015.11.003

Borgmann, D., Rigoux, L., Kuzmanovic, B., Edwin Thanarajah, S., Münte, T. F., Fenselau, H., & Tittgemeyer, M. (2021). Technical note: Modulation of fMRI brainstem responses by

transcutaneous vagus nerve stimulation. *Neuroimage*, *244*, 118566. https://doi.org/10.1016/j.neuroimage.2021.118566

Brainard, D. H. (1997). The psychophysics toolbox. *Spatial Vision*, *10*(4), 433–436. https://doi.org/10.1163/156856897X00357

Brougher, J., Aziz, U., Adari, N., Chaturvedi, M., Jules, A., Shah, I., Syed, S., & Thorn, C. A. (2021). Self-administration of right vagus nerve stimulation activates midbrain dopaminergic nuclei. *Frontiers in Neuroscience*, *15*, 782786. https://doi.org/10.3389/fnins.2021.782786

Burger, A. M., D'Agostini, M., Verkuil, B., & Van Diest, I. (2020). Moving beyond belief: A narrative review of potential biomarkers for transcutaneous vagus nerve stimulation. *Psychophysiology*, *57*(6), e13571. https://doi.org/10.1111/psyp.13571

Busch, V., Zeman, F., Heckel, A., Menne, F., Ellrich, J., & Eichhammer, P. (2013). The effect of transcutaneous vagus nerve stimulation on pain perception—An experimental study. *Brain Stimulation*, *6*(2), 202–209. https://doi.org/10.1016/j.brs.2012.04.006

Chen, M., Yu, L., Ouyang, F., Liu, Q., Wang, Z., Wang, S., Zhou, L., Jiang, H., & Zhou, S. (2015). The right side or left side of noninvasive transcutaneous vagus nerve stimulation: Based on conventional wisdom or scientific evidence? *International Journal of Cardiology*, *187*(1), 44–45. https://doi.org/10.1016/j.ijcard.2015.03.351

D'Agostini, M., Burger, A. M., Franssen, M., Perkovic, A., Claes, S., von Leupoldt, A., Murphy, P. R., & Van Diest, I. (2023). Short bursts of transcutaneous auricular vagus nerve stimulation enhance evoked pupil dilation as a function of stimulation parameters. *Cortex*, *159*, 233–253. https://doi.org/10.1016/j.cortex.2022.11.012

D'Agostini, M., Burger, A. M., Villca Ponce, G., Claes, S., von Leupoldt, A., & Van Diest, I. (2022). No evidence for a modulating effect of continuous transcutaneous auricular vagus nerve stimulation on markers of noradrenergic activity. *Psychophysiology*, *59*(4), e13984. https://doi.org/10.1111/psyp.13984

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

Ellrich, J. (2011). Transcutaneous vagus nerve stimulation. *European Neurological Review*, *6*(4), 254–256. https://doi.org/10.17925/ENR.2011.06.04.254

Farmer, A. D., Strzelczyk, A., Finisguerra, A., Gourine, A. V., Gharabaghi, A., Hasan, A., Burger, A. M., Jaramillo, A. M., Mertens, A., Majid, A., Verkuil, B., Badran, B. W., Ventura-Bort, C., Gaul, C., Beste, C., Warren, C. M., Quintana, D. S., Hämmerer, D., Freri, E., … Koenig, J. (2021). International consensus based review and recommendations for minimum reporting standards in research on transcutaneous vagus nerve stimulation (version 2020). *Frontiers in Human Neuroscience*, *14*, 568051. https://doi.org/10.3389/fnhum.2020.568051

Ferstl, M., Teckentrup, V., Lin, W. M., Kräutlein, F., Kühnel, A., Klaus, J., Walter, M., & Kroemer, N. B. (2020). Non-invasive vagus nerve stimulation boosts mood recovery after effort exertion. *Psychological Medicine*, *52*, 3029–3039. https://doi.org/10.1017/S0033291720005073

Fischer, R., Ventura-Bort, C., Hamm, A., & Weymar, M. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances conflict-triggered adjustment of cognitive control. *Cognitive, Affective, & Behavioral Neuroscience*, *18*(4), 680–693. https://doi.org/10.3758/s13415-018-0596-2

Frangos, E., Ellrich, J., & Komisaruk, B. R. (2015). 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. https://doi.org/10.1016/j.brs.2014.11.018

Gadeyne, S., Mertens, A., Carrette, E., Van den Bossche, F., Boon, P., Raedt, R., & Vonck, K. (2022). Transcutaneous auricular vagus nerve stimulation cannot modulate the P3b event-related potential in healthy volunteers. *Clinical Neurophysiology*, *135*, 22–29. https://doi.org/10.1016/j.clinph.2021.11.079

Giraudier, M., Ventura-Bort, C., Burger, A. M., Claes, N., D'Agostini, M., Fischer, R., Franssen, M., Kaess, M., Koenig, J., Liepelt, R., Nieuwenhuis, S., Sommer, A., Usichenko, T., Van Diest, I., Von Leupoldt, A., Warren, C. M., & Weymar, M. (2022). Evidence for a modulating effect of transcutaneous auricular vagus nerve stimulation (taVNS) on salivary alpha-amylase as indirect noradrenergic marker: A pooled mega-analysis. *Brain stimulation*, *15*(6), 1378–1388.

Han, W., Tellez, L. A., Perkins, M. H., Perez, I. O., Qu, T., Ferreira, J., Ferreira, T. L., Quinn, D., Liu, Z. W., Gao, X. B., Kaelberer, M. M., Bohórquez, D. V., Shammah-Lagnado, S. J., de Lartigue, G., & de Araujo, I. E. (2018). A neural circuit for gut-induced reward. *Cell*, *175*(3), 665–678.e23. https://doi.org/10.1016/j.cell.2018.08.049

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. https://doi.org/10.1037/0735-7044.118.1.79

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. https://doi.org/10.1016/j.neurobiolaging.2015.02.023

Jeffreys, H. (1998). *The theory of probability*. Oxford University Press.

Keute, M., Demirezen, M., Graf, A., Mueller, N. G., & Zaehle, T. (2019). No modulation of pupil size and event-related pupil response by transcutaneous auricular vagus nerve stimulation (taVNS). *Scientific Reports*, *9*(1), 11452. https://doi.org/10.1038/s41598-019-47961-4

Kleiner, M., Brainard, D., Pelli, D., Ingling, A., Murray, R., & Broussard, C. (2007). What's new in psychtoolbox-3. *Perception*, *36*(14), 1–16.

Koenig, J., & Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: A meta-analysis. *Neuroscience & Biobehavioral Reviews*, *64*, 288–310. https://doi.org/10.1016/j.neubiorev.2016.03.007

Kreuzer, P. M., Landgrebe, M., Husser, O., Resch, M., Schecklmann, M., Geisreiter, F., Poeppl, T. B., Prasser, S. J., Hajak, G., & Langguth, B. (2012). Transcutaneous vagus nerve stimulation: Retrospective assessment of cardiac safety in a pilot study. *Frontiers in Psychiatry*, *3*, 70. https://doi.org/10.3389/fpsyt.2012.00070

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/JSS.V082.I13

Lakens, D. (2022). Sample size justification. *Collabra: Psychology*, *8*(1), 33267. https://doi.org/10.1525/collabra.33267

Lewis, C. A., Grahlow, M., Kühnel, A., Derntl, B., & Kroemer, N. B. (2023). Women compared with men work harder for small rewards. *Scientific reports*. https://doi.org/10.1038/s41598-023-32391-0

Liu, C. H., Yang, M. H., Zhang, G. Z., Wang, X. X., Li, B., Li, M., Woelfer, M., Walter, M., & Wang, L. (2020). Neural networks and the anti-inflammatory effect of transcutaneous auricular vagus nerve stimulation in depression. *Journal of Neuroinflammation*, *17*(1), 54. https://doi.org/10.1186/s12974-020-01732-5

Lloyd, B., Wurm, F., Lucchi, F., de Kleijn, R., & Nieuwenhuis, S. (2023). The neuromodulatory effects of transcutaneous vagus nerve stimulation: A replication. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, *16*(1), 165–166. https://doi.org/10.1016/j.brs.2023.01.154

Müller, F. K., Teckentrup, V., Kühnel, A., Ferstl, M., & Kroemer, N. B. (2022). Acute vagus nerve stimulation does not affect liking or wanting ratings of food in healthy participants. *Appetite*, *169*, 105813. https://doi.org/10.1016/j.appet.2021.105813

Munafò, M. R., Nosek, B. A., Bishop, D. V., Button, K. S., Chambers, C. D., Percie du Sert, N., Simonsohn, U., Wagenmakers, E. J., Ware, J. J., & Ioannidis, J. (2017). A manifesto for reproducible science. *Nature Human Behaviour*, *1*(1), 1–9.

Neuser, M. P., Teckentrup, V., Kühnel, A., Hallschmid, M., Walter, M., & Kroemer, N. B. (2020). Vagus nerve stimulation boosts the drive to work for rewards. *Nature Communications*, *11*(1), 3555. https://doi.org/10.1038/s41467-020-17344-9

Niv, Y., Daw, N. D., Joel, D., & Dayan, P. (2007). Tonic dopamine: Opportunity costs and the control of response vigor. *Psychopharmacology*, *191*(3), 507–520. https://doi.org/10.1007/s00213-006-0502-4

Oehrn, C. R., Molitor, L., Krause, K., Niehaus, H., Schmidt, L., Hakel, L., Timmermann, L., Menzler, K., Knake, S., & Weber, I. (2022). Non-invasive vagus nerve stimulation in epilepsy patients enhances cooperative behavior in the prisoner's dilemma task. *Scientific Reports*, *12*(1), 10255. https://doi.org/10.1038/s41598-022-14237-3

R Core Team. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.R-Project.Org/

Raedt, R., Clinckers, R., Mollet, L., Vonck, K., El Tahry, R., Wyckhuys, T., De Herdt, V., Carrette, E., Wadman, W., Michotte, Y., Smolders, I., Boon, P., & Meurs, A. (2011). Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *Journal of Neurochemistry*, *117*(3), 461–469. https://doi.org/10.1111/j.1471-4159.2011.07214.x

Raudenbush, S. W., Bryk, A. S., Cheong, Y. F., Richard, T., Congdon, J., & du Toit, M. (2011). *Hierarchical linear and nonlinear modeling*. Scientific Software International, Inc.

Sellaro, R., de Gelder, B., Finisguerra, A., & Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances recognition of emotions in faces but not bodies. *Cortex*, *99*, 213–223. https://doi.org/10.1016/j.cortex.2017.11.007

Sharon, O., Fahoum, F., & Nir, Y. (2021). Transcutaneous vagus nerve stimulation in humans induces pupil dilation and attenuates alpha oscillations. *Journal of Neuroscience*, *41*(2), 320–330. https://doi.org/10.1523/JNEUROSCI.1361-20.2020

Team, J. (2019). JASP (version 0.10.2). In *[Computer software]*.

Urbin, M. A., Lafe, C. W., Simpson, T. W., Wittenberg, G. F., Chandrasekaran, B., & Weber, D. J. (2021). Electrical stimulation of the external ear acutely activates noradrenergic mechanisms in humans. *Brain Stimulation*, *14*(4), 990–1001. https://doi.org/10.1016/j.brs.2021.06.002

Ventura-Bort, C., Wirkner, J., Genheimer, H., Wendt, J., Hamm, A. O., & Weymar, M. (2018). Effects of transcutaneous vagus nerve stimulation (tVNS) on the P300 and alpha-amylase level: A pilot study. *Frontiers in Human Neuroscience*, *12*, 202.

Verhagen, J., & Wagenmakers, E.-J. (2014). Bayesian tests to quantify the result of a replication attempt. *Journal of Experimental Psychology: General*, *143*(4), 1457–1475. https://doi.org/10.1037/a0036731

Warren, C. M., Tona, K. D., Ouwerkerk, L., van Paridon, J., Poletiek, F., van Steenbergen, H., Bosch, J. A., & Nieuwenhuis, S. (2019). The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the P3 event-related potential. *Brain Stimulation*, *12*(3), 635–642. https://doi.org/10.1016/j.brs.2018.12.224

Yakunina, N., Kim, S. S., & Nam, E. C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation*, *20*(3), 290–300. https://doi.org/10.1111/ner.12541

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

FIGURE S1 Distribution of VAS ratings from (a) Neuser et al. (2020) and (b) the current study.

FIGURE S2 Effort invigoration (top) and effort maintenance (bottom) as a function of stimulation (taVNS vs. sham) and sex. (a) Results based on the data from Neuser et al. (2020). (b) Results based on the current data. Colored bars indicate the mean. Grey lines indicate the results from individual participants.

FIGURE S3 Effect of taVNS on invigoration, separately for session 1 and 2. (a) Results based on the data from Neuser et al. (2020). (b) Results based on the current data.

FIGURE S4 Total money and calories won as a function of the maximum frequency of button presses recorded during the practice phase, separately for female and male participants for (a) Neuser et al. (2020) and (b) the current study.

FIGURE S5 The criterion value determined by the maximum frequency of button presses during the practice phase, separately for female and male participants for (a) Neuser et al. (2020) and (b) the current study.

TABLE S1 Model output predicting invigoration.

TABLE S2 Model output predicting effort maintenance.

How to cite this article: Lucchi, F., Lloyd, B., & Nieuwenhuis, S. (2024). Non-invasive vagus nerve stimulation and the motivation to work for rewards: A replication of Neuser et al. (2020, *Nature Communications*). *Psychophysiology*, *61*, e14484. https://doi.org/10.1111/psyp.14484