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Journal

Proceedings of the Annual Meeting of the Cognitive Science Society, 42(0)

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Publication Date

2020

Peer reviewed

Auricular Transcutaneous Vagus Nerve Stimulation (tVNS) Affects Mood and Anxiety during Second Language Learning

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Abstract

Vagus nerve stimulation (VNS) has been used to address the symptoms of treatment-resistant depression (Rush et al., 2000) and is proposed to also alleviate anxiety effects (George et al., 2008). Transcutaneous VNS (tVNS) offers a less invasive treatment mechanism for clinical populations; however, little is known about tVNS effects on mood and anxiety in a non-clinical adult population. Using auricular tVNS, the present study showed that 10 minutes of tVNS immediately preceding second-language learning across three consecutive days reduced state negative affect, somatic anxiety, and cognitive anxiety, dependent on task performance and/or trait mood/anxiety.

Keywords: tVNS; mood; anxiety; second language learning

Introduction

Mood and anxiety effects on well-being and cognitive outcomes have often been studied within clinical populations suffering from affective disorders, such as generalized anxiety disorder and major depressive disorder, which includes abnormal mood symptoms. Anxiety can be separated into 1) somatic anxiety, the physical manifestation of anxiety (e.g., trembling); and 2) cognitive anxiety, the mental aspect of anxiety (e.g., worrying; Ree et al., 2008). Mood can be separated into 1) positive affect, associated with enthusiasm and alertness; and 2) negative affect, associated with distress and depression (Watson et al., 1988). A meta-analysis of antidepressant treatment for individuals with generalized anxiety or major depressive disorder suggests that these disorders are characterized by dysfunction in the limbic system and reduced activity in the dorsolateral prefrontal cortex (dlPFC), which is necessary for emotion regulation (Ma, 2015).

Although it is important to investigate the symptomology and outcomes of individuals who suffer from affective disorders, it is also beneficial to investigate how negative mood and anxiety affect non-clinical populations in typical learning environments, such as second language learning. Second language learning is of particular interest, because this environment often induces anxiety (Price, 1991), which can affect learning and task performance (e.g., MacIntyre & Gardner, 1989). For example, foreign language classroom anxiety is negatively correlated with test performance (Dewaele & Alfawzan, 2018). The present study investigates whether a non-invasive form of vagus nerve simulation (VNS) influences mood and anxiety during a stress-inducing activity, second language learning.

VNS involves applying a low electrical current to the vagus, a cranial nerve that transmits parasympathetic signals to many organs, including the heart, lungs, and digestive tract, and terminates in several brain regions (Yuan & Silberstein, 2015). In a clinical trial with patients who had treatment-resistant major depressive disorder, VNS resulted in a 40% response rate within the first three months of treatment (Rush et al., 2000). In assessing whether VNS affected anxiety, George et al. (2008) found evidence that VNS decreased anxiety in patients with treatment-resistant generalized anxiety disorder.

One proposed mechanism of the effect of VNS on the brain is through the afferent fibers of the vagus nerve that terminate in the nucleus of the solitary tract (NTS) within the medulla region of the brainstem. The NTS projects to the pedonucleopontine nucleus, raphe nucleus (RN), and locus coeruleus (LC; George et al., 2004; Yuan & Silberstein, 2015). The LC is the brain's major source of norepinephrine (NE) and has been implicated as one of the causes of reduced seizures (Krahl et al., 1998). The RN, the brain's primary source of serotonin, is involved in emotion regulation, which may enable cognitive functioning (Zhao et al., 2015). Furthermore, the NTS has direct and indirect connections to brain regions within the limbic system such as the amygdala, thalamus, and hippocampus, which are often associated with decreased activation after VNS (Yuan & Silberstein, 2015), but are sometimes associated with increased activation (Dietrich et al., 2008).

Germane to the present study, non-invasive VNS studies show that transcutaneous VNS (tVNS) results in similar outcomes as invasive VNS. For example, auricular tVNS, which stimulates the auricular branch of the vagus nerve, appears to activate similar brain regions as invasive VNS (e.g., Dietrich et al., 2008; Kraus et al., 2007). When investigating tVNS effects on patients with mild or moderate major depressive disorder, Fang et al., (2016) found that patients who received 30 minutes of tVNS twice a day for four weeks showed reductions in depression and anxiety

measures compared to a sham tVNS group. Furthermore, increased functional connectivity between the default mode network and rostral anterior cortex and medial prefrontal cortex (mPFC) was correlated with a decrease in anxiety and depression. When specifically examining changes within the emotion regulation neural network, Liu et al. (2016) showed that a tVNS group of patients with mild or moderate symptoms of depression showed increased functional connectivity between the right amygdala and left dlPFC, indicating an increase in emotion regulation, whereas a sham group showed the opposite effect.

The effects of tVNS on behavioral outcomes and neural connectivity in clinical populations suggest that it is a promising treatment. A study conducted with healthy adults showed that auricular tVNS administered at low (perception) and high (just below pain threshold) levels resulted in increased mood and well-being (Kraus et al., 2007). When the same participants received a placebo on a different day, their mood and self-reported well-being worsened. No effects of tVNS were found on anxiety. tVNS has also been used with healthy adults in fear conditioning paradigms with mixed success; for example, tVNS increased fear extinction rates, but did not affect fear retention (Burger et al., 2017).

To more closely examine tVNS effects during typical learning activities, the present study examined the effect of tVNS on mood and anxiety in a non-clinical adult sample learning a foreign language. To investigate whether the type of learning interacts with tVNS effects on mood and anxiety, we included two separate learning environments, 1) vocabulary (i.e., paired associates) learning, and 2) grammar learning. Overall, based on findings with clinical (Liu et al., 2016) and non-clinical populations (Kraus et al., 2007), we hypothesize that tVNS: 1) will increase positive affect and decrease negative affect and 2) will reduce cognitive and somatic anxiety. Further, tVNS effects may differ between vocabulary and grammar learning tasks, which require different cognitive processes.

Study Overview

The study included five sessions completed over five consecutive days. Session 1 was a pre-training session consisting of a variety of individual difference measures; only trait mood and anxiety measures are reported here. Sessions 2-4 included tVNS training sessions for vocabulary or grammar along with pre- and post- measures of state mood and anxiety. Session 5 consisted of a post-training session that did not include any tVNS. To ease scheduling burdens, participants were not required to complete sessions at the same time of day across the five sessions. Although NE levels fluctuate throughout the day, any effects related to time of day should occur across the majority of participants, and thus should be largely unrelated to stimulation condition.

Method

Participants

A total of 234 participants were recruited from a United States university and the surrounding community as part of a larger study examining the effects of tVNS on second language learning. The research protocol was reviewed and approved by the university's Institutional Review Board. Eligibility requirements included being between the ages of 18 and 35 and having normal or corrected-to-normal vision, normal hearing including no blockage of the ear canal, and no learning disabilities. Importantly, participants had no history of neurological, psychiatric, or neuropsychiatric disorders and were not taking psychoactive medications. All participants gave informed consent. Thirty participants were excluded from analyses for failing to comply with task instructions or having missing data. Of the remaining 204 participants 63 were male, 140 were female, and one identified as other (age: $M = 20.97$, $SD = 3.15$). Of these 204 participants, 83 were assigned to sham or priming conditions (described under *Procedure*) of the grammar or vocabulary tasks. The remaining 121 participants were assigned to one of three other conditions in which tVNS was administered during, rather than before, learning or testing tasks. Only the priming and sham conditions are reported here, because they most closely align with previous research investigating the effects of tVNS and VNS on mood/anxiety in clinical populations in which participants did not receive tVNS during tasks (Fang et al., 2016; Liu et al., 2016; Rush et al., 2000).

Measures

Participants completed self-report measures of mood and anxiety, vocabulary or grammar training in Indonesian, and individual difference measures. Individuals were assigned pseudorandomly to stimulation condition and task (vocabulary or grammar) based on working memory updating and visuospatial working memory tasks; these measures are not presented here because they are unrelated to the present study.

Positive Affect and Negative Affect Schedule (PANAS)
Trait and State versions of PANAS were administered. PANAS measures positive and negative affect (Watson et al., 1988). The measure has 20 items; half indicate positive affect (e.g., enthusiastic, strong), and half indicate negative affect (e.g., scared, depressed). Participants indicated how strongly they currently feel (state) or felt over the past week (trait) on a five-point Likert-type scale (ranging from "Very slightly or not at all" to "Extremely"). PANAS generates two scores: 1) a positive affect score, and 2) a negative affect score.

State-Trait Inventory for Cognitive and Somatic Anxiety
Trait and State versions of STICSA served as anxiety measures (Ree et al., 2000). STICSA consists of 21 statements (e.g., "I can't concentrate without irrelevant

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thoughts intruding."). Using a four-point Likert-type item scale (ranging from "Not at all" to "Very much so"), participants indicated the degree to which the statement describes how they feel in the present moment (state) or how the statements described their general anxiety level (trait). STICSA generates two scores: 1) cognitive anxiety, and 2) somatic anxiety.

Vocabulary and Grammar Learning Tasks Both the vocabulary and grammar tasks involved Indonesian learning, recall, and recognition tasks. Indonesian was chosen as the target language because it has a similar orthography to English but differs in some grammatical rules and should be unfamiliar to most native English speakers from the selected population. The vocabulary learning task involved paired associates learning of 80 Indonesian nouns. In the recall task, participants typed the English translation of the Indonesian word. In the recognition task, participants indicated which of two presented Indonesian words was the correct translation of an English word.

The grammar learning task involved explicit inductive learning of grammatical rules for Indonesian noun phrases (two to seven words long). During the learning task, both English and Indonesian phrases were presented on a screen. Translation pairs were highlighted in the same color. Indonesian words that did not have an English translation, such as classifiers, were not highlighted. In the recall task, participants constructed the Indonesian translation of the English phrase. In the recognition task, an Indonesian phrase was presented below an English phase, and participants indicated whether the Indonesian phrase was grammatical.

Item-level feedback was not provided, but a percentage score was provided at the end of the recognition task, prior to the post-tVNS questionnaire with state versions of PANAS and STICSA.

Procedure

The pre- and post-training sessions (Sessions 1 and 5, respectively) were two hours each, and the three training sessions with the tVNS manipulation (Sessions 2-4) were one hour and 15 minutes each. Training sessions occurred on consecutive days. Participants were paid at a rate of $20 per hour. Because the post-training session did not include tVNS or trait mood/anxiety measures, analyses of tasks at that session are not presented here.

Pre-training Session In the pre-training session, participants completed questionnaires and cognitive tasks, including the trait versions of PANAS and STICSA.

Training Sessions During training sessions, participants wore earbuds designed to administer tVNS to the outer ear canal of the left ear only. Training sessions consisted of three parts: 1) Pre-tVNS questionnaire with the State version of PANAS and STICSA, 2) vocabulary or grammar training with tVNS, and 3) Post-tVNS questionnaires with the State

versions of PANAS and STICSA, and a post-stimulation comfort questionnaire.

tVNS Parameters and Calibration Participants in the priming condition received continuous tVNS for 10 minutes while watching an animated video prior to any learning tasks. Participants in the sham condition did not receive any tVNS, other than during calibration, described below. Because the study was double blind, all participants heard static-like pink noise during the video and throughout the tasks to mask sound that occurred during tVNS administration.

The tVNS signal originated from a Digitimer DS8R Biphasic Constant Current Stimulator (DS8R; Digitimer North America, LLC, Fort Lauderdale, FL), set with a 50 ms pulse width, using a biphasic mode with alternating polarity, 350 ms interphase dwell interval, and a 100% recovery phase ratio. Participants wore a pair of modified Nervana headphones (Nervana, LLC, Deerfield Beach, FL), connected to the DS8R. A custom left earbud was created for each participant, altering the existing Nervana left ear-cap by using Axelgaard AG735 and AG2550 hydrogel (Axelgaard Manufacturing Co., Ltd.) as the transmission medium.

At the start of each session, participants underwent tVNS calibration. Calibration started at 2.0 mA, sending a 2000 ms sample of tVNS stimulation, pausing randomly between 1000-3000 ms, and increasing in amplitude by 0.5 mA. Once a participant indicated feeling stimulation, the amplitude was reduced by 2.0 mA, and the process restarted using 0.1 mA intervals until the participant again indicated feeling stimulation. The tVNS amplitude for the session was then set to 0.2 mA below the participant's threshold level, to remain below perceptual threshold. To maintain double blindness, all participants underwent calibration and a ramping procedure in which stimulation began at zero, increased to perceptual threshold, and then decreased back to zero. During the priming video, participants in the priming condition received stimulation below perceptual threshold, whereas those in the sham condition did not receive stimulation. Proctors and researchers who performed statistical analyses were blind to participants' stimulation condition.

Model Building Procedures

Linear mixed effects models run in R (version 3.4.3) were used to estimate effects of tVNS on changes in mood/anxiety. The Satterwaithe approximation method was used to calculate degrees of freedom for model coefficients, using the package lmerTest (Kuznetsova et al., 2017). The outcome variable was the difference score of state mood/anxiety scores for each training session (post minus pre). Difference scores reflect changes in mood/anxiety due to experimental conditions, in which more negative scores indicate a decrease in state mood/anxiety from pre- to post-tVNS. The initial models included participant as a random intercept, fixed effects of trait mood/anxiety, training session (session 2, 3, and 4), task (vocabulary vs. grammar), and condition (priming vs. sham) with all possible interactions. The interaction of recognition score, trait mood/anxiety, and

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condition was also included to determine if task performance influenced condition effects on changes in mood/anxiety across a session when accounting for trait scores. All continuous predictor variables were centered in all models; trait scores were grand mean centered, and recognition scores were centered by session and task. The intercept for all models was the grammar task, sham condition, average recognition score, Session 2 (i.e., the first training session), and average trait mood/anxiety score.

Next, models were built in a forward stepwise manner to test the addition of a random effect of session on slope. If a likelihood ratio test revealed that the next model explained significantly more variance than the previous model ($p < .05$), and the correlation between the random slope and intercept was $< .95$, the random slope for that model was retained. Finally, fixed effects were eliminated in a backwards stepwise fashion using likelihood ratio tests. If the next model did not significantly differ from the last ($p > .05$), then the more parsimonious model was retained. If the next model significantly differed from the last ($p < .05$), the previous model was retained, and the building process stopped. All results presented are from the final model of each building process. As we are primarily interested in the effects of tVNS on mood and anxiety, the results below focus on main effects of Condition and interactions with Condition.

Results

Of the 83 participants, 24 were male, 58 were female, and one identified as other (age: $M = 21.20$, $SD = 3.59$). Of these participants, 21 were assigned to the grammar priming condition (16 female; age: $M = 20.43$, $SD = 2.40$), 19 to the grammar sham condition (13 female; age: $M = 22.53$, $SD = 4.98$), 22 to the vocabulary priming condition (14 female, one other; age: $M = 20.73$, $SD = 3.09$), and 21 to the vocabulary sham condition (15 female; age: $M = 21.29$, $SD = 3.48$).

Prior to testing effects of tVNS, 2 (Condition) x 2 (Task) ANOVAs tested any potential differences in participants' trait mood/anxiety. There were no significant main effects or interactions for trait Negative Affect (ps $> .683$), trait Positive Affect (ps $> .573$), trait Cognitive Anxiety (ps $> .292$), or trait Somatic Anxiety scores (ps $> .505$).

Mood Results

Results for the state positive affect model revealed a Condition × Task interaction (Table 1), such that there was a significant main effect of Condition only for the grammar task; the priming group displayed an overall decrease in positive affect from pre- to post-tVNS, but this was not true for the vocabulary task ($\beta = 1.10$, $SE = 1.20$, $t = 0.91$; Figure 1).

Table 1: Mood models

	β	SE	df	t	
Positive Affect					
(Intercept)	-1.73	0.98	104.76	-1.77	
Sess	2.53	0.36	164.91	6.95	*
Cond	-4.08	1.25	78.65	-3.27	*
Task	-1.19	1.25	78.72	-0.95	
Recog	14.78	2.40	184.57	6.15	*
Cond × Task	5.18	1.74	78.73	2.98	*
Negative Affect					
(Intercept)	-1.36	0.61	208.25	-2.24	*
Sess	0.83	0.44	159.53	1.88	
Cond	0.86	0.84	208.63	1.02	
Task	1.78	0.84	208.17	2.12	*
Trait	-0.16	0.09	209.29	-1.73	
Recognition	1.15	1.72	122.53	0.67	
Sess × Cond	-1.19	0.62	161.53	-1.93	
Sess × Task	-1.39	0.62	158.74	-2.26	*
Cond × Task	-1.98	1.16	208.90	-1.71	
Sess × Trait	0.11	0.07	161.36	1.65	
Cond × Trait	0.21	0.13	209.18	1.63	
Task × Trait	0.05	0.14	209.26	0.34	
Cond × Recog	4.87	2.45	133.49	1.99	*
Trait × Recog	0.90	0.21	163.07	4.28	*
Sess × Cond × Task	1.48	0.86	159.42	1.73	
Sess × Cond ×Trait	-0.26	0.10	172.73	-2.59	*
Sess × Task × Trait	-0.09	0.10	159.06	-0.94	
Cond × Task ×Trait	-0.33	0.18	209.04	-1.82	
Sess × Cond × Task ×Trait	0.31	0.14	167.62	2.24	*

Note. * $p < .05$; Task = Vocabulary (Grammar as reference); Cond = Priming (Sham as reference); Sess = Session; Recog = Recognition score; Trait = Positive or Negative Affect for a given model.
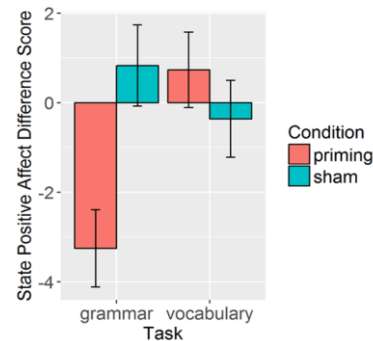

Figure 1: State positive affect Condition x Task interaction.

Results for the state negative affect model revealed a significant Condition × Recognition interaction, showing that

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for the priming group, lower recognition scores were associated with greater reductions in state negative affect from pre- to post-tVNS (Figure 2).
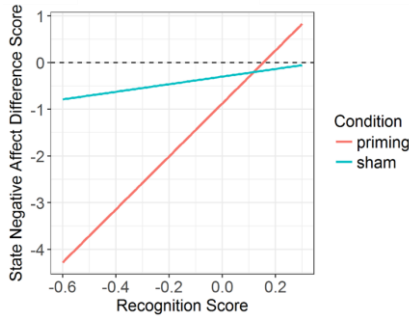

Figure 2: State negative affect Condition × Recognition interaction.

There was also a Session × Condition × Task × Trait interaction, which revealed that, for the grammar learning group, initially the sham group showed a larger reduction in negative affect than the priming group at higher levels of negative trait affect; this pattern reversed by the final training session (Figure 3a). For the vocabulary learning group, the priming condition showed a larger decrease in negative affect than the sham condition, and this effect was larger at higher levels of trait negative affect (Figure 3b). For the vocabulary learning group, the effect of condition and trait negative affect appeared to reduce as training sessions progressed.
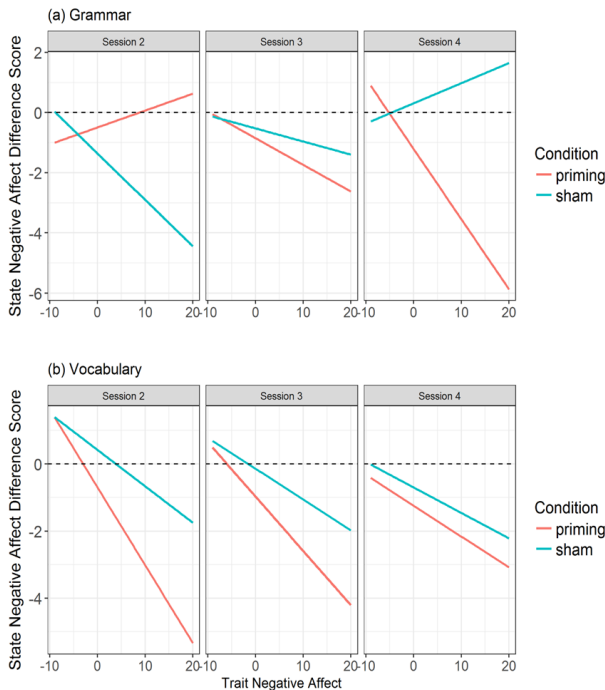

Figure 3: Four-way interactions of state negative affect model of Session × Condition × Task × Trait (a) Session × Condition × Trait for the Grammar condition; (b) Session × Condition × Trait for the Vocabulary condition.

Anxiety Results

The results from the state somatic anxiety model revealed only a Condition × Recognition interaction (Table 2). For the priming condition, participants with lower recognition scores showed a larger decrease in somatic anxiety from pre- to post-tVNS than those with higher recognition scores. The sham group's recognition scores did not seem to affect changes in state somatic anxiety (Figure 4).

Table 2: Anxiety models

	β	SE	df	t	
Somatic Anxiety					
(Intercept)	0.63	0.25	79.18	2.53	*
Cond	-0.44	0.35	79.18	-1.27	
Recog	-0.88	1.35	176.82	-0.65	
Cond × Recog	5.06	1.90	193.00	2.66	*
Cognitive Anxiety					
(Intercept)	-1.26	0.41	92.62	-3.11	*
Session	0.35	0.29	79.49	1.20	
Condition	0.24	0.53	77.96	0.46	
Task	1.11	0.28	77.56	4.01	*
Trait	-0.08	0.05	101.94	-1.74	
Sess × Cond	-0.71	0.41	79.61	-1.74	
Sess × Trait	0.02	0.04	106.56	0.50	
Task × Trait	0.10	0.04	77.58	2.20	*
Sess × Cond × Trait	-0.11	0.03	76.67	-3.30	*

Note. * $p < .05$; Task = Vocabulary (Grammar as reference); Cond = Priming (Sham as reference); Trait = Somatic or Cognitive Anxiety for a given model.
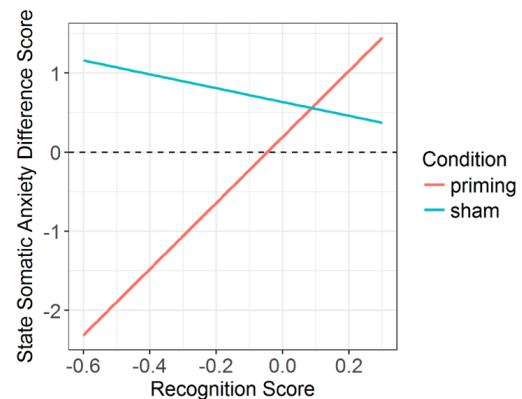

Figure 4: State somatic anxiety model Recognition x Condition interaction.

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The cognitive anxiety model revealed a significant Session × Condition × Trait interaction, in which the priming condition showed a larger decrease in state cognitive anxiety for participants with higher trait cognitive anxiety, and this effect grew across sessions (Figure 5).
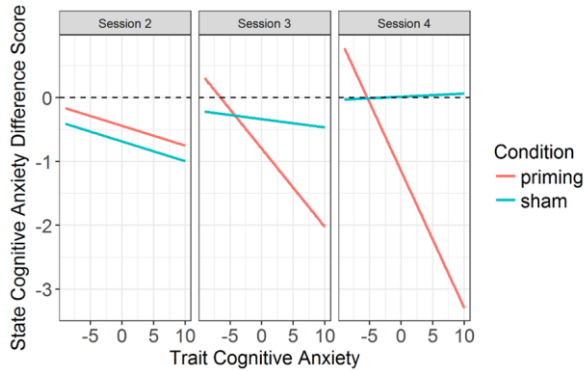

Figure 5: State cognitive anxiety model Session × Condition × Trait interaction.

Discussion

Overall, the results generally supported our hypotheses; tVNS had an effect on state mood and anxiety outcomes, and these results mostly align with prior research (e.g., Fang et al., 2016; Kraus et al., 2007; Liu et al., 2016). In our second language learning environment, tVNS effects on state mood/anxiety depended on task, trait mood/anxiety, and recognition performance. The effect of tVNS on negative affect was larger for participants who performed poorly on recognition tasks and for participants with higher trait negative affect. Although we did not collect neural data, this effect could be driven by a dampening of the limbic system's response to negative emotion (Ma, 2015), due to tVNS enhancing activation of the LC-NE and/or RN-serotonin systems. tVNS effects on negative affect across sessions differed between tasks, with a larger effect at the end of training for the grammar learning group, and smaller effect at the end of training for the vocabulary learning group. The task differences suggest that more tVNS training may be necessary to show effects for a novel, grammar learning task.

Although we predicted that tVNS would increase positive affect, our findings showed that positive affect decreased for participants in the grammar learning group receiving tVNS, whereas the vocabulary learning group showed no differences in positive affect between sham and tVNS priming conditions. One potential explanation is that task demands affected the effect of tVNS on mood. In a review of mood effects on executive function, Mitchell and Phillips (2007) showed that positive mood is more associated with heuristic processing. Our findings suggest that tVNS priming interferes with heuristic processing, perhaps by inducing attentional states more conducive to full interpretation of goal-related information. Given that heuristics could be more useful in the grammar task, which involves discovering hierarchical syntactic relationships, it is not surprising to see the effect of tVNS on mood in this task as opposed to the paired-associates vocabulary learning task.

Our findings on anxiety in neurotypical adults generally align with findings of tVNS effects on anxiety in clinical populations (Fang et al., 2016; Liu et al., 2016), in that tVNS reduced anxiety. Our results, nevertheless, add nuance in that in clinical research cognitive and somatic anxiety are not typically assessed separately (e.g., George et al., 2008).

Regarding effects on state cognitive anxiety, tVNS had a larger effect in reducing state cognitive anxiety for participants with higher trait cognitive anxiety. Furthermore, the effect increased as participants progressed through the training sessions. This finding provides evidence that tVNS can attenuate anxiety in high trait anxiety individuals, who typically have poorer attention control (e.g., Bishop, 2009). A reduction in cognitive anxiety also aligns with a tVNS study with high worriers that found tVNS reduced spontaneous negative thoughts associated with worrying (Burger et al., 2019). Decreased cognitive anxiety could be due to a similar emotion regulation mechanism that resulted in reduced negative affect in the present study. Alternatively, as Burger et al. (2019) suggest, increased activity in the default mode network and its connectivity to the mPFC (Fang et al., 2016) could enhance inhibition of worrisome thoughts. Our findings also indicate that tVNS can lead to incremental changes over time, rather than isolated effects that do not persist, that appear to vary by task demands.

With regard to somatic anxiety, we found that participants in the tVNS condition who had lower recognition scores showed larger reductions in somatic anxiety. This contrasts with previous findings that poorer cognitive performance is associated with greater somatic anxiety (e.g., Jones & Cale, 1989). Interestingly, one study found that tVNS led to increased confidence about performance (on an interoceptive detection task), but not performance enhancement (Villani, Tsakiris, & Azevedo, 2019). Perhaps the lower performers are susceptible to this effect, and, believing they are performing well, become more relaxed through the session.

In conclusion, the present study provides additional evidence that tVNS influences mood and anxiety levels in a non-clinical adult population. Furthermore, we report that contextual factors (traits and performance) influence the effects of tVNS. One limitation of the current study is that vocabulary and grammar are usually taught concurrently, which could potentially lead to different mood and anxiety effects. Furthermore, language learning environments often require learners to recite passages or engage in spoken dialogue, which may increase anxiety and negative affect. Thus, future work could assess second language learning in classroom settings and how other situation factors (e.g., task difficulty, evaluation type) across different domains influence how tVNS affects mood and anxiety.

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