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SPECIAL ISSUE: EFFECTS OF TVNS ON BRAIN AND COGNITION

Transcutaneous auricular vagus nerve stimulation modifies cortical excitability in middle-aged and older adults

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Abstract

There is a growing interest in the clinical application of transcutaneous auricular vagus nerve stimulation (taVNS). However, its effect on cortical excitability, and whether this is modulated by stimulation duration, remains unclear. We evaluated whether taVNS can modify excitability in the primary motor cortex (M1) in middle-aged and older adults and whether the stimulation duration moderates this effect. In addition, we evaluated the blinding efficacy of a commonly reported sham method. In a double-blinded randomized cross-over sham-controlled study, 23 healthy adults (mean age 59.91 ± 6.87 years) received three conditions: active taVNS for 30 and 60 min and sham for 30 min. Single and paired-pulse transcranial magnetic stimulation was delivered over the right M1 to evaluate motor-evoked potentials. Adverse events, heart rate and blood pressure measures were evaluated. Participant blinding effectiveness was assessed via guesses about group allocation. There was an increase in short-interval intracortical inhibition ($F = 7.006$, $p = .002$) and a decrease in short-interval intracortical facilitation ($F = 4.602$, $p = .014$) after 60 min of taVNS, but not 30 min, compared to sham. taVNS was tolerable and safe. Heart rate and blood pressure were not modified by taVNS ($p > .05$). Overall, 96% of participants detected active stimulation and 22% detected sham stimulation. taVNS modifies cortical excitability in M1 and its effect depends on stimulation duration in middle-aged and older

adults. taVNS increased $GABA_A$ergic inhibition and decreased glutamatergic activity. Sham taVNS protocol is credible but there is an imbalance in beliefs about group allocation.

KEYWORDS

aging, auricular vagus nerve stimulation, cortical excitability, neuroplasticity, TMS, vagus nerve

1 | INTRODUCTION

Vagus nerve stimulation (VNS) appears to modify neurochemistry and promote adaptive changes within the brain (Badran et al., 2017; Kaniusas et al., 2019). Preclinical evidence has shown that VNS influences the release of neurotransmitters, including norepinephrine, acetylcholine, serotonin, and gamma-aminobutyric acid (GABA) (Badran et al., 2017; Cheyuo et al., 2011; Frazer et al., 2014; Groves & Brown, 2005; Hulsey et al., 2019; Nichols et al., 2011). These neurotransmitters play critical roles in synaptic transmission, neuronal excitability, and neuroplasticity (Brunoni et al., 2008). By modulating these neurotransmitters, VNS can potentially enhance or suppress specific neural pathways, facilitating neuroplastic changes within the brain (Badran et al., 2017; Frazer et al., 2014; Groves & Brown, 2005; Hulsey et al., 2019). For example, VNS has been shown to increase synaptic connectivity six-fold in the lesioned hemisphere of animal stroke models when paired with rehabilitation, leading to enduring neuroplastic changes for up to two months after cessation of treatment (Meyers et al., 2018).

There is growing interest in clinical applications of noninvasive VNS due to ease of application, affordability (Bremner et al., 2020; Wang, Li, et al., 2021), and avoiding risks associated with invasive VNS, such as localized infection and voice alteration (Ben-Menachem et al., 2015). In humans, emerging evidence from noninvasive forms of VNS, such as transcutaneous auricular vagus nerve stimulation (taVNS), indicates that taVNS might improve behavior and mood. For example, taVNS was shown to reduce the frequency of seizures in epilepsy (Bauer et al., 2016; Liu et al., 2018; Stefan et al., 2012), improve mood in major depression (Guerriero et al., 2021), and further enhance upper-limb recovery after stroke when combined with physiotherapy (Capone et al., 2017; Li, Xie, et al., 2022; Redgrave, Moore, et al., 2018; Wu et al., 2018). Although promising, taVNS research is in its infancy, and it is important to understand the neural mechanisms that may underpin any effects. This might help refine the delivery of taVNS by identifying optimal stimulation parameters.

One approach to measure neural mechanisms in humans is to use transcranial magnetic stimulation (TMS). Applying single TMS pulses to the primary motor cortex (M1) elicits a response in a peripheral muscle known as a motor-evoked potential (MEP) that can be used as an estimate of corticospinal excitability (Opie & Semmler, 2021). Measures of cortical excitability can be explored using paired-pulse TMS techniques (Hordacre et al., 2021). These measures include short-interval intracortical inhibition (SICI), a measure of $GABA_A$ergic inhibition (Di Lazzaro et al., 2000; Ilić et al., 2002; Mrachacz-Kersting et al., 2021; Ziemann et al., 1996), long-interval intracortical inhibition (LICI), a measure of $GABA_B$ergic inhibition, intracortical facilitation (ICF), a measure of glutamate excitability and short-interval intracortical facilitation (SICF) (Opie & Semmler, 2021). SICF is thought to index activities within the I-waves generating circuits (Opie & Semmler, 2021; Wagle-Shukla et al., 2009). These I-waves are believed to result from indirect activation of corticospinal neurons by presynaptic intracortical circuits (Opie & Semmler, 2021; Wagle-Shukla et al., 2009).

Using TMS to measure the neural mechanisms of taVNS appears warranted. First, the balance between excitation and inhibition is linked to neuroplasticity (Grigoras & Stagg, 2021), so would provide valuable insight to understand how taVNS modifies cortical excitability. To date, studies have only evaluated inhibitory mechanisms (Capone et al., 2015; Mertens et al., 2022; van Midden et al., 2023), suggesting a need to further explore facilitatory mechanisms. Second, studies that have investigated the taVNS effect on cortical excitability have included younger adults and are often limited to a single stimulation intensity or duration (Capone et al., 2015; Mertens et al., 2022; van Midden et al., 2023). It is therefore unclear whether the effects of taVNS are similar for older adults, who can be more similar in age to patients evaluated in clinical trials, or whether different stimulation parameters influence cortical changes. For example, 60 min of taVNS led to increased SICI, i.e., increased $GABA_A$ergic inhibition, in the contralateral primary motor cortex (M1) compared to sham, in 10 adults (average age 30.2 years) (Capone et al., 2015). A

more recent study found that 30 min of taVNS increased SICI compared to sham when measured online, but the effect was less prominent when measured offline (van Midden et al., 2023). Another study found no significant effect of 60 min of taVNS on SICI in 13 males (average age 27.5 years) except in a subgroup of participants who tolerated taVNS intensity greater than 2.5 mA (Mertens et al., 2022). It is unclear why these individual results differed. One hypothesis is that there is a dose effect of stimulation, given that different stimulation parameters were used. Indeed, preclinical evidence shows a nonlinear, U-shape relationship between VNS dose and both behavioral and physiological effects (Clark et al., 1998; Hays et al., 2014a), but this relationship has not been elucidated in humans.

To robustly evaluate effects of taVNS on cortical excitability requires a reliable sham stimulation protocol as a control. Several sham methods have been reported in clinical trials but the most common was attaching the electrode to the same anatomical site that was stimulated in active taVNS while delivering no stimulation (Gerges et al., 2024). A previous study showed that this sham stimulation method did not affect cortical excitability (van Midden et al., 2023), potentially making it a suitable control for physiological studies. However, the effectiveness of this sham method in blinding participants has been rarely evaluated (Gerges et al., 2024). One study showed that 60–70% of participants who received a sham treatment (with zero current delivered to the same location as active taVNS) believed they had undergone active stimulation, whereas all participants who received active taVNS correctly perceived the treatment as active (Hein et al., 2013). Evaluation of sham effectiveness is important to increase confidence in the results of taVNS effects.

The primary purpose of this study was to evaluate whether taVNS can alter cortical excitability in M1 in middle-aged and older adults and test whether this effect is influenced by the duration of stimulation. The secondary aim was to evaluate the effectiveness of a commonly reported sham method. We hypothesized that taVNS would increase intracortical inhibition and decrease intracortical facilitation and that the effect would depend on the duration of stimulation.

2 | METHODOLOGY

2.1 | Participants

Participants were included if they were healthy adults aged 40 to 75 years and were naive to VNS. Participants were excluded if they had a history of neurological, psychological or cardiac conditions, were on neuroactive medications, had active upper limb musculoskeletal conditions, were contraindicated for TMS, or had a history of vagus nerve surgery, i.e., vagotomy. Before starting the first experimental session, all participants provided written informed consent and were screened for brain stimulation safety (Rossi et al., 2010). This study was approved by the University of South Australia Human Research Ethics Committee (HREC), and the study protocol was pre-registered on Open Science Framework on September 12, 2022: https://osf.io/7jbyc. Participants were recruited via social media and from the UniSA City East campus.

2.2 | Sample and power calculation

Although previous work reported a large effect size of 0.91 (Capone et al., 2015) for change in short interval intracortical inhibition (SICI) following taVNS, we conservatively adjusted this to a moderate effect size (0.7) for a power calculation. Power calculations were conducted using G*power (Faul et al., 2007), and it was determined that a minimum of 19 participants were required, given an alpha level of 0.05 and a power of 80%.

2.3 | Study design

We conducted a double-blind, randomized cross-over sham-controlled trial where participants received, in random order: active taVNS for 60 min, active taVNS for 30 min, and sham taVNS for 30 min. The sessions were separated by at least one week. The order of the experimental sessions was randomized using randomization software, with codes held by a researcher not involved in data collection or analysis. Each session took approximately 2.5 h and was conducted in the same room by the same researcher and, where possible, at the same time of the day. During the intervention, participants did not engage in any cognitive or physical activities. A procedural schematic is provided in Figure 1.

2.4 | Transcutaneous auricular vagus nerve stimulation

A transcutaneous auricular vagus nerve stimulation was performed using tVNS-R device (tVNS Technologies, Bayern, Germany). Participants were provided with alcohol wipes to clean the inside of their left auricle. A conductive cream, supplied by the device manufacturer, was applied to the electrodes before use. An earpiece carrying two hemispheric electrodes was fitted to the participant's left ear. The electrodes were made of titanium/

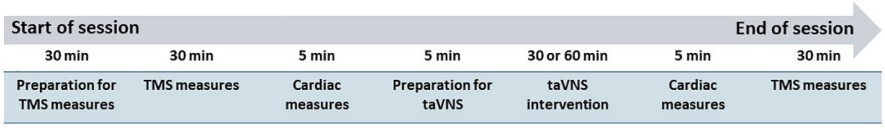

FIGURE 1 A schematic representation of a study session. Preparation for TMS measures included (1) attaching EMG electrodes, (2) identifying 'hot spot' for evoking motor evoked potentials, and (3) measuring resting motor threshold. Preparation for taVNS included, (1) connecting the electrode to the left ear, and (2) measuring sensory threshold, pain threshold and maximal tolerable stimulus. Each participant completed three sessions, one with each experimental condition: active taVNS for 30 or 60 min and sham for 30 min.

titanium-iridium and were placed in contact with the cymba concha. The stimulation device which delivers a bi-phasic waveform electric current, was set to its lowest intensity level, 0.1 mA, and activated. The participant was then asked to describe any sensations they felt at the electrode site, such as tingling, warmth, pricking/stinging, or vibration. Stimulation intensity was gradually increased in 0.1 mA increments. After each adjustment, participants were asked if they experienced a sensation at the electrode site. Once the participants reported a noticeable sensation, the intensity level at which this sensation occurred was noted as the sensation threshold. Then, the researcher continued increasing the intensity until the participant reported non-tolerable discomfort or pain at the electrode site, this intensity was recorded as the pain threshold. Intensity was then lowered down by 0.1 mA increment and the participant was asked if the sensation became tolerable. Once the participant reported that the sensation was tolerable, this intensity level was noted as a maximal tolerable intensity. Stimulation intensity was set at the individual's maximal tolerable level for the active taVNS sessions (Redgrave, Moore, et al., 2018; Suk et al., 2018). The pulse width was 0.25 ms, the pulse frequency was 25 Hz, and the pulse–pause ratio was 30 sec on and 30 sec off. For sham taVNS, the same setup procedure was followed as in active taVNS sessions including connecting electrodes to the cymba concha of the left ear and measuring sensory and pain thresholds, as well as maximal tolerable intensity. The device was then deactivated immediately after recording these measures to not deliver any stimulation. Participants were asked to report the sensation they felt in the ear immediately after the intervention started and to inform the researcher if this sensation changed throughout the intervention. In all three sessions, participants were informed that any sensation experienced after the intervention started would be intermittent, lasting no longer than a minute, and with pauses of no longer than a minute. During active taVNS stimulation, if a participant reported feeling nothing, the researcher verified the device setup to ensure connectivity and electrode contact with the skin until the participant perceived the stimulus again. In the sham intervention, if a participant reported no sensation, the researcher checked the device and its connection, reassured the participant of proper device connectivity, and reminded them that sensation could vary depending on stimulation parameters. The researcher monitored the taVNS device activity during all sessions to ensure the device was functioning during active taVNS sessions; the device's screen and controlling application were kept out of sight of participants in all sessions.

2.5 | Electromyography

Surface electromyography (EMG) was used to record motor evoked potentials (MEP) from the first dorsal interosseous (FDI) muscle of the left hand using Ag/AgCl electrodes (3 M™ Red Dot™ ECG Monitoring Electrode) in a belly tendon montage. Skin overlying the FDI was cleaned with alcohol and prepared by lightly abrading with NuPrep paste. A ground strap was placed on the wrist. Signals were sampled at 5 kHz (Cambridge Electronic Design 1401, Cambridge, UK), amplified 1000× (Cambridge Electronic Design 1902, Cambridge, UK), band-pass filtered (20–1000 Hz) and stored for offline analysis using Signal Software Version 5.08 (Cambridge Electronic Design, Cambridge, UK).

2.6 | TMS measures

Transcranial magnetic stimulation (TMS) was performed with a Magstim 200 stimulator (Magstim, Whitland, UK) and a BiStim module for paired pulse TMS that delivered magnetic pulses via a figure-of-eight coil (internal wing diameter 70 mm). The 'hot spot' for evoking MEPs of the highest peak-to-peak amplitude in the left FDI was identified by initially placing the coil over the right hemisphere with the handle oriented posterolaterally at approximately 45 degrees from the sagittal plane to induce a posterior–anterior current across the right primary motor cortex (M1) and the center of the coil applied tangentially to the scalp. Then the coil was moved forward, backward, and sideways in small increments until the optimal spot for evoking MEPs was found. This location was

then marked on the scalp using a colored water-soluble marker. TMS pulses were delivered at 5 seconds intervals ±10%. The resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a MEP (at least 50 μV) in at least 50% of ten trials at rest (Wang, Li, et al., 2021). RMT was measured twice, once for single pulse (RMT1) and once for paired-pulse TMS measures (using single pulses TMS but with the BiStim connected) (RMT2). The TMS intensity that elicited MEPs of approximately 1 mV in peak-to-peak amplitude (SI1mV) was determined.

Corticospinal excitability was determined by recording MEPs following stimuli delivered at an intensity of 120% RMT1 with the muscle at rest (Wang, Li, et al., 2021). For paired-pulse TMS measures, two stimuli were provided through the same magnetic coil over the right M1 (Kloosterboer & Funke, 2019). For SICI, the conditioning stimulus (CS) was 70% of RMT2, the test stimulus (TS) was SI1mV, and the inter-stimulus interval (ISI) was 2 ms (Kujirai et al., 1993). For measuring long-interval intracortical inhibition (LICI), CS was 110% of RMT2, TS was SI1mV, and ISI was 100 ms (McDonnell et al., 2006). For short interval intracortical facilitation (SICF), CS was 100% of RMT2, TS was SI1mV, and ISIs were 1.5 ms ($SICF_{1.5ms}$), 2.5 ms ($SICF_{2.5ms}$), and 4.7 ms ($SICF_{4.7ms}$). For intracortical facilitation (ICF), CS was 80% of RMT2, TS was SI1mV, and ISI was 10 ms (Talelli et al., 2011). TMS measures were recorded in three blocks: the 120% RMT1 in the first block; SICI and LICI in the second block, and $SICF_{1.5ms}$, $SICF_{2.5ms}$, $SICF_{4.7ms}$, and ICF in the third block. Eighteen MEPs were recorded for 120% RMT1, SICI, LICI, $SICF_{1.5ms}$, $SICF_{2.5ms}$, $SICF_{4.7ms}$, and ICF. Eighteen single-pulse MEPs were recorded for TS alone in the SICI/LICI and SICF/ICF blocks. For paired-pulse TMS, the sequence of pulses was semi-randomized within each block. All TMS measures were recorded before and after taVNS, in all sessions.

2.7 | Blinding

Both participants and the researcher who analyzed the neurophysiological measures were blinded to the intervention. To facilitate participant blinding, a script was developed that was read to each participant before delivering taVNS. This script stated: "*In this study, we will use different stimulation parameters in each session, and the stimulation may feel different to you between sessions. So it may be that you feel nothing at all.*" The researcher who analyzed all TMS measures was blind to the study protocol and the conditions delivered in each session. To evaluate blinding effectiveness, participants were asked to guess whether they had received active or sham stimulation, or if they were unsure ('Don't Know'). Participants who were unsure were then asked to provide their best guess (forced response of active sham). Participants were also asked to provide reasons for their guesses. See supplementary file 1 for the full blinding questionnaire including follow-up questions (adapted from Bang et al. [2010]). All participants completed the blinding questionnaire immediately after each taVNS session (a total of three guesses per participant).

2.8 | Adverse events

Participants were asked to complete an adverse events questionnaire that was developed following current literature reporting on taVNS safety (Redgrave, Day, et al., 2018) (supplementary file 2). Both heart rate (HR) and blood pressure (BP) were measured immediately before, at 15-min intervals during taVNS and following taVNS using a Rossmax Pulse Oximeter, Fingertip SB220 (Rossmax Swiss GmbH, Heerbrugg, Switzerland) and an automated BP measuring device, OMRON HEM-7320 (OMRON, Kyoto, Japan). The pulse oximeter was placed on the right index finger, and the BP measures were recorded from the participants' left arm.

2.9 | Data processing of TMS measures

MEP amplitudes were obtained in Signal Software Version 5.08 (Cambridge Electronic Design, Cambridge, UK). Contaminated trials were excluded, which were defined as those with a background EMG activity during 100 ms or less before the TMS pulse. An average of one trial was excluded for each physiological measure per participant (range 0 to 12). The mean peak-to-peak amplitudes of MEP were extracted for each stimulus, subject and time point. The ratio of inhibition or facilitation for paired-pulse TMS measures was determined as the mean conditioned MEP amplitude divided by the mean test pulse MEP amplitude. Therefore, values greater than 1 indicate facilitation and values less than 1 indicate inhibition. For analysis, delta values were calculated as pre-taVNS measures subtracted from post-taVNS measures. Therefore, a delta value of greater than 0 indicates decreased inhibition/increased facilitation, and a delta value of less than 0 indicates increased inhibition/decreased facilitation.

2.10 | Data analysis

Measures of SI1mV, RMT1, RMT2, test stimulus amplitudes, baseline measures of peak-to-peak amplitudes

of nonconditioned (TS-alone) and maximal tolerable taVNS intensity between sessions were analyzed using linear mixed models. The dependent variables were SI1mV, RMT1, RMT2, non-conditioned (TS-alone) amplitudes, and maximal tolerable taVNS intensity with fixed effect of condition (active taVNS 30 min; active taVNS 60 min; sham 30 min) and a random effect of the participant. For single- and paired-pulse TMS measures of cortical excitability, several linear mixed-model analyses were performed. The dependent variables were the delta measure (difference from pre to post-taVNS intervention) and there was a fixed factor of condition (active taVNS 30 min; active taVNS 60 min; sham 30 min) and random effect of the participant. Covariates for physiological measures were the baseline (pre-taVNS) physiological measure, RMT1 (for single pulse MEP), RMT2 (for paired-pulse measures), age, sex, session order and maximal tolerable intensity of taVNS. For HR and systolic and diastolic BP measures, linear mixed model analysis was performed with a fixed factor of condition (active taVNS 30 min; active taVNS 60 min; sham 30 min) and a random effect of the participant. Covariate for cardiac measures were the baseline (pre-taVNS) measures. All analyses were performed in IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp, 2021), with the level of significance set at $p < .05$ and 95% confidence interval (CI). Adverse events and blinding data were analyzed descriptively. Blinding was considered successful when approximately equal numbers of participants believed they received the active intervention in each condition (i.e., balanced beliefs about allocation) (Bang et al., 2010). Exploratory analyses of blinding patterns were computed using Bang's blinding index (BI) (Bang et al., 2010). Bang's BI was calculated for each condition separately (active and sham) and estimated the proportion of unblinding beyond random chance. Bang's BI values range from −1.00 to +1.00 (Bang et al., 2010). If all guesses were correct BI equals 1.00 (complete unblinding). If all guesses were incorrect, BI equals −1.00 (opposite guessing). A BI close to zero indicates random guessing. Unblinding may be claimed if the lower bound of the 95% CI is greater than zero (Bang et al., 2010).

3 | RESULTS

3.1 | Participants

Thirty-four participants were screened for eligibility. Twenty-five of those were eligible, and nine were excluded: four were on neuroactive medication, three had cardiac conditions, one had a pacemaker, and one had a neurological condition. Of the eligible participants, 23 completed the study (15 females, mean age 59.91 ± 6.87 years, range 42–71 years), and two chose not to participate in the study.

3.2 | Effect of taVNS on cortical excitability

There was no significant difference between sessions for SI1mV ($F = 0.84$, $p = .44$), RMT1 ($F = 0.46$, $p = .64$), RMT2 ($F = 0.9$, $p = .42$), and maximal tolerable taVNS intensity ($F = 2.27$, $p = .13$). A summary of the mean and standard deviation of these measures is provided in Table 1. The average non-conditioned MEP amplitudes per participant ranged from 0.72 mV to 0.95 mV. There were no significant differences between nonconditioned MEP amplitude across conditions at baseline (all $p > .08$).

There was a significant effect of condition on SICI ($F = 7.01$, $p = .01$). Sixty minutes of active taVNS led to an increase in SICI compared to sham (adjusted mean difference = − 0. 17, $t = -2.98$, $p = .004$, 95% CI [−0.29, −0.57]; Figure 2), but 30 min of active taVNS did not differ from sham (adjusted mean difference = 0.03, $t = 0.49$, $p = .623$, 95% CI [−0.09, 0.15]; Figure 2). There was a significant effect for two covariates on SICI; (1) age, showing an increase in SICI with increasing age (adjusted mean difference = −0.01; $t = -2.23$, $p = .03$, 95% CI [−0.02, −0.01]), and (2) sex, showing that females had greater SICI compared to

TABLE 1 TMS and taVNS intensities.

Condition	RMT1 %MSO (SD)	RMT2 %MSO (SD)	SI1mV %MSO (SD)	taVNS maximal tolerable intensity mA (SD)
Active taVNS-60 min	49.39 (9.20)	54.13 (8.76)	64.57 (11.13)	2.68 (2.68)
Active taVNS-30 min	50.04 (10.23)	53.48 (10.60)	63.87 (12.75)	3.06 (1.50)
Sham taVNS-30 min	49.57 (9.53)	54.43 (10.33)	65.26 (14.33)	2.70 (1.61)

Abbreviations: %MSO, per cent of maximal stimulator output; RMT1, resting motor threshold of single-pulse TMS measures; RMT2, resting motor threshold of paired-pulse TMS measures; S1mV, TMS intensity that elicited MEPs of approximately 1 mV in peak-to-peak amplitude; taVNS, transcutaneous auricular vagus nerve stimulation; TMS, transcranial magnetic stimulation.

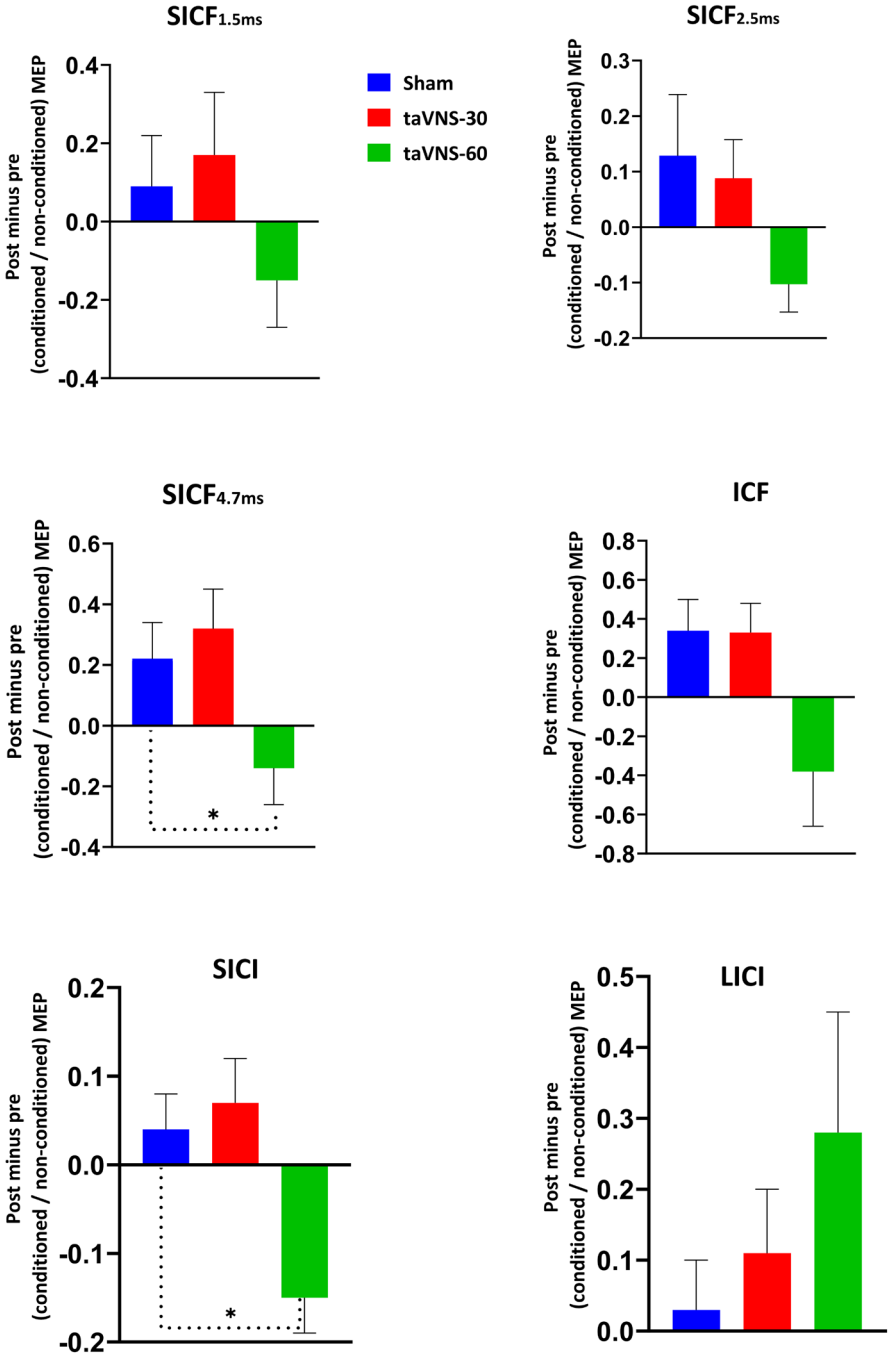

FIGURE 2 Transcutaneous auricular vagus nerve stimulation (TaVNS) effect on paired-pulse transcranial magnetic stimulation measures of the contralateral primary motor cortex in humans. For analysis, delta values were calculated as pre-taVNS measures subtracted from post-taVNS measures. Therefore, a delta value of greater than 0 indicates decreased inhibition/increased facilitation, and a delta value of less than 0 indicates increased inhibition/decreased facilitation. SICI: Short interval intracortical inhibition, LICI: Long interval intracortical inhibition, SICF: Short interval intracortical facilitation (the dominator of this acronym represents the interstimulus intervals: 1.5, 2.5, and 4.7 ms), and ICF: Intracortical Facilitation. The ratio of inhibition or facilitation for paired-pulse TMS measures was determined as the mean conditioned motor evoked potential (MEP) amplitude divided by the mean test pulse MEP amplitude. Sham: 30 min of sham stimulation, Active-30: 30 min of active stimulation, Active-60: 60 min of active stimulation and *: indicate p-value <.05.

males (adjusted mean difference = −0.18, $t = -3.22$, $p = .01$, 95% CI [−0.29, −0.07]). Figure 3 shows that both male and female participants had increased SICI but on average female participants showed a greater increase in SICI.

There was a significant effect of condition on $SICF_{4.7ms}$ ($F = 4.602$, $p = .014$). Sixty minutes of taVNS led to a decrease in $SICF_{4.7ms}$ compared to sham taVNS (adjusted mean difference = −0.36, $t = -2.10$, $p = .04$, 95% CI [−0.70, −0.02]; Figure 2), but 30 min of active taVNS did not differ from sham taVNS (adjusted mean difference = 0.15, $t = 0.89$, $p = .38$, 95% CI [−0.19, 0.49]). There was a significant effect for one covariate, RMT2 indicating an increase in $SICF_{4.7ms}$ with increasing RMT2 (adjusted mean difference = 0.06, $t = 2.13$, $p = .04$, 95% CI [0.01, 0.11]).

There was no significant effect of condition for single pulse MEP ($F = 0.473$, $p = .626$; Figure 4), $SICF_{1.5ms}$ ($F = 1.848$, $p = .167$), $SICF_{2.5ms}$ ($F = 2.593$, $p = .084$), ICF ($F = 2.339$, $p = .106$), and LICI ($F = 1.457$, $p = .241$) (Figure 3).

3.3 | Adverse events

All participants tolerated taVNS well, with no serious adverse events (AEs) reported. Nineteen AEs were reported

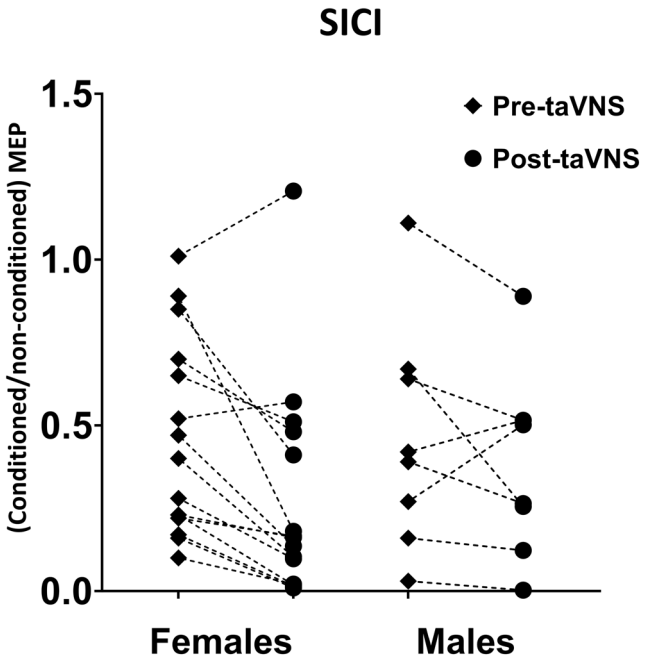

FIGURE 3 Transcutaneous auricular vagus nerve stimulation (taVNS) effect on short interval intracortical inhibition (SICI) in both male and female participants. MEP: Motor evoked potential.

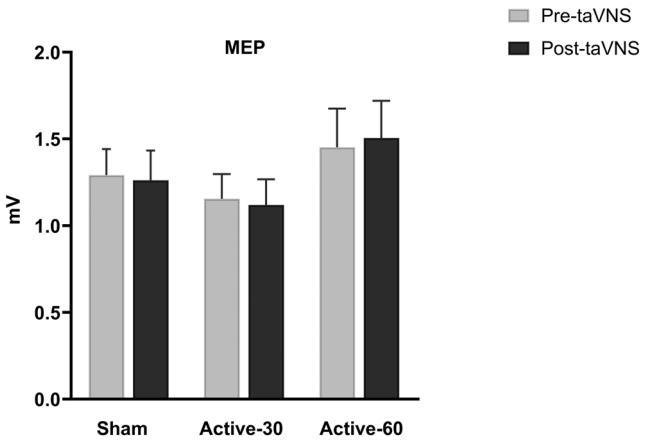

FIGURE 4 Transcutaneous auricular vagus nerve stimulation (TaVNS) effect on motor evoked potential (MEP) of contralateral primary motor cortex in humans. Sham: 30 min of sham stimulation, Active-30: 30 min of active stimulation, Active-60: 60 min of active stimulation.

in total, but they were all mild and localized to the stimulation site (Table 2). All AEs were transient, occurred during stimulation, and ceased immediately when stimulation stopped, except one (redness), which persisted for 10 min after stimulation and recovered spontaneously. All AEs except one occurred during active stimulation, 11 during 60 min of stimulation, and seven during 30 min of stimulation. During the sham, the AE occurred while measuring the participant's maximal tolerable intensity and not during the intervention. Instantaneous HR, systolic BP, and diastolic BP were not modified by taVNS, as there was no significant effect of condition on any of these measures (all p values >.05: supplementary file 3).

TABLE 2 Summary of reported adverse events related to taVNS.

Symptom	Number of participants reporting AEs	Number of AEs	% participants
Irritation	5	5	21.7
Itchiness	5	8	21.7
Pain	3	5	13
Redness	1	1	0.4

Abbreviation: AE; adverse event.

3.4 | Blinding

Table 3 presents the blinding data (participant guesses about allocation). All participants correctly identified active taVNS 60 min, (BI = 1.00 [95% CI: 1.00–1.00]), 91% correctly identified active taVNS 30 min (BI = 0.91 [95% CI: 0.80–1.03]), and 21% correctly identified the sham 30 min (BI = 0.26 [95% CI: −0.58–0.06]). 100% of participants who correctly identified active stimulation reported that the reason for their guess was the sensation felt at electrode site during the intervention while 100% of participants who correctly identified sham reported that lack of sensation at electrode site was the reason for their guess (refer to supplementary file 4). The reasons for incorrect guesses of the sham intervention varied including (1) sensation felt during the pre-intervention preparation process where the researcher measured sensory threshold, (2) trust in the researcher, and (3) discomfort at the electrode site. Four of the participants who identified sham correctly (two in the primary guesses and two in the forced guesses) reported that the sensation felt at the electrode site was different during the sham session compared to their previous active taVNS sessions.

4 | DISCUSSION

This study aimed to evaluate if taVNS can alter cortical excitability in middle-aged and older adults and whether the duration of taVNS moderates the change in cortical excitability. To our knowledge, this is the first study to investigate the effect of taVNS on cortical excitability in this age group. Our results support the hypotheses that taVNS increased $GABA_A$ergic inhibition and decreased intracortical facilitation and the effect depends on stimulation

TABLE 3 Blinding data.

Intervention	Number of primary guesses				Number of forced guesses	
	Real	Sham	"Don't know"	Total	Real	Sham
Active taVNS- 60 min	23	0	0	23	NA	NA
Active taVNS- 30 min	21	0	2	23	2	0
Sham taVNS- 30 min	11	5	7	23	4	3

duration. Sixty minutes of taVNS increased SICI and decreased $SICF_{4.7ms}$ in the hemisphere contralateral to the stimulated ear, while 30 min of taVNS showed no effect compared to sham. taVNS was safe and well tolerated by all study participants. All reported AEs were mild, transient, and localized to electrode location. Participants were better at correctly identifying active taVNS compared to sham taVNS.

Our results suggest an increase in SICI after 60 min of taVNS compared to sham. Our findings, in middle-aged and older adults, are consistent with previous studies that investigated the taVNS effect on cortical excitability using TMS measures in young adults. For example, 60 min of taVNS performed at the cymba concha (of left ear) in 10 healthy adults (mean age 30.2 ± 3.6 (SD) years) led to greater $GABA_A$ergic inhibition in the right motor cortex compared to sham taVNS (Capone et al., 2015). Another found that 30 min of continuous stimulation to cymba concha of 30 adults (mean age 27.5 years), increased $GABA_A$ergic inhibition (van Midden et al., 2023). Similarly, invasive VNS led to greater $GABA_A$ergic inhibition compared to no stimulation in five people with epilepsy (age range: 24–51) (Di Lazzaro et al., 2004). Invasive VNS was also shown to increase free GABA levels in the cerebrospinal fluid, measured 3-months after initiation of VNS in people with epilepsy compared to baseline, and the change in cerebrospinal fluid levels was more prominent in responders compared to non-responders (Ben-Menachem et al., 1995). Together, this indicates that both invasive and noninvasive VNS have similar effects on cortical excitability. On the other hand, our results were not consistent with the findings of Mertens et al., (2022). This study recorded TMS-evoked potentials (TEP) using electroencephalography and (single and paired-pulse TMS) MEPs measures from 15 healthy males (age range: 22 to 32 years) immediately after 60 min of active or sham taVNS. They found no significant differences in SICI between active and sham taVNS (Mertens et al., 2022). There are two key differences between our study and Mertens et al., which might have contributed to these divergent results. First, we included both females (n = 13) and males (n = 8) while Mertens et al. included males only. This is noteworthy as we observed a significant effect of sex on SICI. There is supporting evidence for sex differences in SICI, with healthy females having greater SICI compared to males (Shibuya et al., 2016). However, the changes that we observed in SICI were not solely driven by female participants (Figure 3). Second, we delivered no stimulation during sham sessions, while Mertens et al. delivered a maximal tolerable stimulus to the earlobe. Although the earlobe is not innervated by the vagus nerve, stimulation of the earlobe can modulate cortical excitability via different mechanisms, which might confound the active taVNS effect (Rangon, 2018). Indeed, the authors reported modulation of SICI after sham taVNS as measured by TEP. It is, therefore, unclear whether this sham taVNS protocol is a robust control.

That our results are similar to younger adults is promising, but an important advance of our work is that we evaluated middle-aged and older adults. This is important because taVNS has been used therapeutically in several pathologies that are more common in these age groups including stroke (Capone et al., 2017; Li, Xie, et al., 2022; Wu et al., 2020), tinnitus (Kreuzer et al., 2014; Raj-Koziak et al., 2023; Shim et al., 2015; Suk et al., 2018; Ylikoski et al., 2020), and Parkinson's disease (Marano et al., 2022) to improve function or reduce symptoms through driving cortical plasticity. For example, in people with stroke aged between 53.7 and 64.5 years, taVNS was shown to enhance upper-limb function when combined with physical rehabilitation, compared to sham plus physical rehabilitation (Capone et al., 2017; Li, Xie, et al., 2022; Wu et al., 2020). In tinnitus, taVNS has been shown to reduce symptoms when used alone (Kreuzer et al., 2014; Suk et al., 2018), or combined with sound therapy (Shim et al., 2015) for people aged between 44 to 62.3 years. In Parkinson's disease, taVNS reduced symptoms (Marano et al., 2022) for people who were 67.64 (on average). Therefore, our study results add to this literature by providing evidence of neural mechanisms associated with taVNS.

This study's results add to this literature by providing evidence of neural mechanisms associated with taVNS. In

addition to increasing SICI, we also observed a reduction in $SICF_{4.7ms}$. The underlying physiology of SICF is not fully understood, but it is thought to index glutamatergic activity within the I-wave generating circuits (Van den Bos et al., 2018; Ziemann, 2020). Pharmacological studies have shown that SICF is suppressed by $GABA_A$ agonists (Ziemann et al., 1998, 2015). Therefore, the decrease in SICF might be partly driven by the increased $GABA_A$ergic inhibition. It is noteworthy that there was no facilitation in $SICF_{2.5ms}$ across conditions. This might be due to the proximity of the ISI (2.5 ms) of that measure to that of SICI (2 ms) which might have resulted in inhibition rather than facilitation. On the other hand, there was facilitation in $SICF_{1.5ms}$ and ICF across all conditions, but the 60 min of taVNS showed a trend toward reduced facilitation in both measures and this was not evident in the 30 min of active stimulation or sham (Figure 2).

The ratio between glutamatergic excitation and GABAergic inhibition in the motor cortex is often referred to as excitation/inhibition balance (E/I) and has been linked to neuroplasticity (Chen et al., 2022). Brain stimulation modalities such as repetitive TMS and transcranial direct current stimulation that are known to drive neuroplasticity have been shown to modulate E/I balance (Cywiak et al., 2020; Kloosterboer & Funke, 2019; Krause et al., 2013; Sousa et al., 2022). Our results suggest that taVNS might bias E/I balance towards greater inhibition in the contralateral M1 through increasing inhibition and decreasing facilitation. This might inform the potential role of VNS in some pathological groups. For example, there is evidence that E/I imbalance is linked to the pathophysiology of epilepsy, where evidence indicates a decreased GABAergic inhibition, i.e. the balance is skewed towards increased facilitation (van van Hugte et al., 2023). Invasive VNS was associated with improvement in this E/I imbalance in the amygdala and hippocampus in people with epilepsy (Ooi et al., 2023). Modifying E/I balance might be one of the physiological mechanisms underlying VNS therapeutic effects.

In this study, taVNS duration appears to moderate its effect on cortical excitability. This is consistent with preclinical physiological and behavioral data. For example, in animal models of stroke, there was an inverted U relationship between the invasive VNS-driven plasticity and stimulation intensity where the optimum stimulation intensity ranged from 0.6 to 1.0 mA and lower or higher intensities failed to enhance plasticity or improve functional recovery (Hays et al., 2014b). Similarly, an intensity-dependent, inverted-U-shaped pattern of (memory) retention performance was shown in rats, with the greatest effect observed for 0.4 mA (Clark et al., 1998). Five trains of VNS for 30 sec achieved a greater and more robust anxiolytic effect in rats compared to one train of stimulation (Mathew et al., 2020). This is consistent with preliminary behavioral data that suggests low daily doses of taVNS might be less effective than higher doses. For example, 15 to 30 min of taVNS failed to show therapeutic effectiveness in depression (Hein et al., 2013) and epilepsy (Bauer et al., 2016), while a stimulation duration of 60 min or more per day showed more consistent effects (Fang et al., 2017; Li, Rong, et al., 2022; Liu et al., 2018; Stefan et al., 2012). It is important to further explore the effect of taVNS duration on clinical outcomes across different clinical populations to help refine therapeutic delivery.

Our blinding effectiveness data revealed an imbalance in participant beliefs about active versus sham conditions, which may have influenced our results. Participants were unblinded in both active conditions (lower bound of 95% CI greater than zero) but not in the sham condition. That the majority of participants in the sham condition believed the sham was active (incorrect guessing), or were unsure, suggests that the sham was credible. However, overall fewer participants in the sham group believed they received the active treatment than in the two active conditions. As such, our results may have been influenced by placebo or bias in favor of the active condition. This blinding scenario is common in clinical trials of physical interventions that are difficult to blind (e.g., needling therapy) (Braithwaite et al., 2019). Possible explanations for the imbalance are the distinct sensory experience of active electrical stimulation at the electrode site (e.g., pricking, tingling, vibration, stinging, warmth) compared to no sensation provided by the sham. Further, due to the crossover design, participants were afforded a direct comparison of active versus sham that likely informed their second or third guess about allocation.

Further work is needed to balance allocation beliefs between active and sham stimulation conditions. Nonetheless, the blinding method used in this study has been reported in previous work (Aranow et al., 2021; Boezaart & Botha, 2021; Hasan et al., 2015; Hein et al., 2013; Li, Xie, et al., 2022; Manning et al., 2019; Shiffer et al., 2019; Wang, Xu, et al., 2021; Wu et al., 2020), and it has been shown to have no significant effect on cortical excitability (van Midden et al., 2023). Another commonly reported sham method is to deliver stimulation to an area that is not innervated by the vagus nerve, e.g. earlobe (Fang et al., 2017; Li, Rong, et al., 2022; Liu et al., 2018; Stefan et al., 2012). We did not stimulate the earlobe in this study as the validity of this method has been questioned, with evidence suggesting that stimulation of the earlobe this method might modulate cortical physiology via the superficial cervical plexus earlobe (Rangon, 2018). Another limitation of this study is that we selected one sham condition a-priori that was 30 min in duration, but the effect of taVNS was evident at 60 min. A previous study

investigated the effect of 60 min of active and sham taVNS on cortical excitability using TMS, and their findings were consistent with ours (Capone et al., 2015). That study used a different sham method where they stimulated the earlobe, whereas we delivered no current at the cymba concha. Future studies should investigate the effects of 60 min of active taVNS compared to 60 min of sham (delivering zero current at cymba concha).

To further advance this work, future studies should: (1) explore the physiological and behavioral effects of different doses of taVNS to understand the nature between taVNS physiological effects and behavioral effects, (2) investigate taVNS effect on the excitability of other brain regions, (3) evaluate VNS effect on E/I balance in both healthy and clinical population to understand potential links with therapeutic effects, and (4) evaluate methods for blinding participants in taVNS studies.

5 | CONCLUSION

taVNS appears to modulate cortical excitability in the hemisphere contralateral to the stimulated ear in healthy middle-aged and older adults. The likely underlying mechanisms are an increase in $GABA_A$-ergic inhibition and a decrease in glutamatergic activity that mediates late-I wave generation. The duration of taVNS appeared important, with effects seen after 60 min of stimulation but not after 30 min. This physiological data might prove beneficial for future work exploring effect of taVNS duration on behavioral measures to identify optimal parameters and enhance therapeutic efficacy. While our sham was credible, further work is needed to optimize blinding for active versus sham taVNS.

AUTHOR CONTRIBUTIONS

Ashraf N. H. Gerges: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; writing – original draft. **Lynton Graetz:** Data curation; formal analysis; software; writing – review and editing. **Susan Hillier:** Conceptualization; methodology; project administration; supervision; writing – review and editing. **Jeric Uy:** Conceptualization; methodology; supervision; writing – review and editing. **Taya Hamilton:** Conceptualization; methodology; supervision; writing – review and editing. **George Opie:** Data curation; methodology; writing – review and editing. **Ann-Maree Vallence:** Methodology; writing – review and editing. **Felicity A. Braithwaite:** Formal analysis; methodology; writing – review and editing. **Saran Chamberlain:** Supervision; writing – review and editing. **Brenton Hordacre:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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

Supplementary file 1. Blinding assessment questionnaire.
Supplementary file 2. taVNS adverse effects questionnaire.
Supplementary file 3. The effect of taVNS on heart rate and blood pressure.
Supplementary file 4. Blinding data including reasons reported by participants for their guesses.

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