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ORIGINAL ARTICLE

The Effects of Transcutaneous Vagus Nerve Stimulation on Functional Connectivity Within Semantic and Hippocampal Networks in Mild Cognitive Impairment

Aidan J. Murphy[1,3,6] · Alexandria G. O'Neal[1,2,3,5] · Ronald A. Cohen[2,3,5] · Damon G. Lamb[1,2,3] · Eric C. Porges[2,3,5] · Sarah A. Bottari[1,3,5] · Brian Ho[2,5] · Erin Trifilio[1,3,5] · Steven T. DeKosky[2,4] · Kenneth M. Heilman[3,4] · John B. Williamson[1,2,3,4,5]

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Abstract

Better treatments are needed to improve cognition and brain health in people with mild cognitive impairment (MCI) and Alzheimer's disease (AD). Transcutaneous vagus nerve stimulation (tVNS) may impact brain networks relevant to AD through multiple mechanisms including, but not limited to, projection to the locus coeruleus, the brain's primary source of norepinephrine, and reduction in inflammation. Neuropathological data suggest that the locus coeruleus may be an early site of tau pathology in AD. Thus, tVNS may modify the activity of networks that are impaired and progressively deteriorate in patients with MCI and AD. Fifty patients with MCI (28 women) confirmed via diagnostic consensus conference prior to MRI (sources of info: Montreal Cognitive Assessment Test (MOCA), Clinical Dementia Rating scale (CDR), Functional Activities Questionnaire (FAQ), Hopkins Verbal Learning Test — Revised (HVLT-R) and medical record review) underwent resting state functional magnetic resonance imaging (fMRI) on a Siemens 3 T scanner during tVNS (left tragus, $n=25$) or sham control conditions (left ear lobe, $n=25$). During unilateral left tVNS, compared with ear lobe stimulation, patients with MCI showed alterations in functional connectivity between regions of the brain that are important in semantic and salience functions including regions of the temporal and parietal lobes. Furthermore, connectivity from hippocampi to several cortical and subcortical clusters of ROIs also demonstrated change with tVNS compared with ear lobe stimulation. In conclusion, tVNS modified the activity of brain networks in which disruption correlates with deterioration in AD. These findings suggest afferent target engagement of tVNS, which carries implications for the development of noninvasive therapeutic intervention in the MCI population.

Keywords Transcutaneous vagus nerve stimulation (tVNS) · Alzheimers disease · Mild cognitive impairment (MCI) · Resting state fMRI · Functional connectivity · Semantic network

Aidan J. Murphy and Alexandria G. O'Neal contributed equally to the composition of this manuscript.

✉ John B. Williamson
john.williamson@ufl.edu

[1] Center for OCD and Anxiety Related Disorders, Department of Psychiatry, College of Medicine, McKnight Brain Institute, University of Florida, Gainesville, FL, USA

[2] Center for Cognitive Aging and Memory, McKnight Brain Institute, University of Florida, Gainesville, FL, USA

[3] Brain Rehabilitation Research Center, Malcom Randall VAMC, Gainesville, FL, USA

[4] Department of Neurology, College of Medicine, University of Florida, Gainesville, FL, USA

[5] Department of Clinical and Health Psychology, College of Public Health and Health Professions, University of Florida, Gainesville, FL, USA

[6] Department of Human Evolutionary Biology, Harvard University, Cambridge, MA, USA

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Introduction

Alzheimer's disease (AD) is the most common cause of dementia in people aged 65 years and older. The Alzheimer's Association predicts that there will be more than12 million Americans with AD by the year 2050 [1]. Because of our aging population, if adequate treatments and preventive measures are not identified, AD will reach epidemic levels and cause enormous human suffering and economic burden. Interrupting brain deterioration early in the degenerative process, i.e., during the mild cognitive impairment (MCI) stage, is a major target of intervention to reduce future disability. Transcutaneous vagus nerve stimulation (tVNS) is a promising potential treatment with minimal side effects and multiple vectors of potential impact on systems that affect cognition, including primary areas of deterioration in patients with MCI and AD.

Postmortem studies of patients with AD have provided evidence that the locus coeruleus (LC) is one of the earliest sites of AD pathology [2]. Disease process may be influenced by involvement of the LC early in the progression of AD. Activation of neurons in the LC modulates several processes that are altered in brains of patients with AD, including synaptic plasticity, inflammation, metabolism, and blood–brain-barrier permeability [2]. Furthermore, norepinephrine (NE) deficiency resulting from LC degeneration increases beta-amyloid deposition in the brain [3]. Thus, the LC may be a good target for intervention in MCI to modify disease progression in AD [4]. Another important structure in the path of the afferent vagus is the nucleus of the solitary tract (NTS). The NTS, LC, and hippocampal pathway are important in memory function [5]. Furthermore, the NTS projects to the basal forebrain (a cholinergic structure) [6], a critical structure in semantic functions and memory, with dense interconnectivity to temporal cortex and hippocampus [7]. Vagal pathways offer a potential conduit to affect multiple structures and projections relevant to disease progression and cognitive sequalae of AD. Vagal afferents may offer a potential vector for improved function of connected networks and cognitive process.

The vagus is the primary visceral nerve (heart, respiration, digestive functions) and conveys both motor and sensory information. It is a compelling clinical target due to its multi-system involvement. Implanted vagus nerve stimulators were initially used to treat patients with poorly controlled seizures. Vagus nerve stimulation (VNS) has been an FDA approved treatment for epilepsy since 1997 [8]. VNS and tVNS have shown treatment promise for other disorders as well, including Alzheimer's-related cognitive decline [9–11]. A pilot study investigated the effect of VNS on cognition in ten patients with AD [12] and showed promise for the potential impact of VNS longitudinally, with stability or improvement in cognitive performance noted over 6 months of stimulation. Subsequently, 17 patients who met the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association criteria for probable AD were studied [9, 13]. These patients were assessed with the Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) and Mini Mental State Examination (MMSE) before and after one year of VNS (30 s on, 5 min off, 20 Hz, 500 microsecond pulse width). After 1 year, 12 out of 17 patients either improved or did not decline from baseline.

While some studies utilizing implanted VNS showed promising effects in AD, no studies have been published examining the impact of VNS or tVNS on brain function in MCI. If participants with MCI show tVNS-induced modulation of the activity of brain regions involved in AD-associated decline, this would be further evidence of target engagement and the potential for long term upregulation of systems underlying the dysfunction experienced in this population. tVNS may delay or help to reverse the decline associated with brain network disruption caused by AD. We hypothesized that tVNS would enhance neural function of cortical and subcortical brain areas that receive projections from the nucleus tractus solitarius (NTS) and LC, including the hippocampus and other regions modulated by NE, and basal forebrain, the source neurons for hippocampal and cortical cholinergic innervation (see Fig. 1). Semantic networks are critical to the clinical features of AD-related decline, thus, we assessed tVNS-related effects on semantic network functional

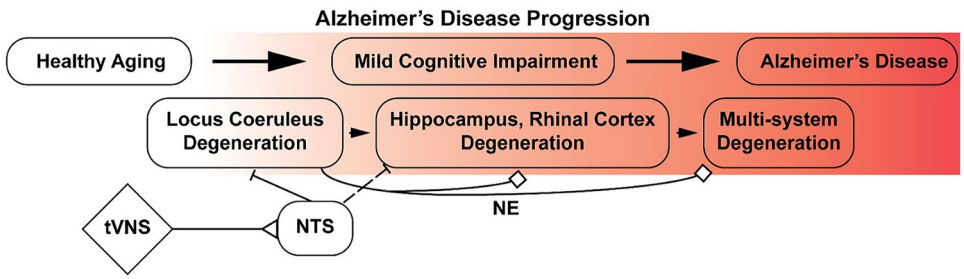

Fig. 1 A proposed pathway of Alzheimer's disease progression. Given its projections, neurodegeneration in the LC leads to hippocampal and compounding multi-system degeneration. Because of its potential impact on hippocampal and prefrontal systems, tVNS may impact memory encoding, retrieval, and working memory in the progression of AD

connectivity (e.g., anterior temporal poles, temporal and parietal structures). Furthermore, due to the role of hippocampal dysfunction in AD, we assessed functional connectivity in response to tVNS from the hippocampus to other areas of the brain. In patients with MCI and AD, prior research has demonstrated changes in resting state networks (e.g., the default mode network) that are associated with further cognitive decline [14–16]. We therefore assessed the effects of tVNS on resting state networks as well. tVNS offers a multisystemic approach to AD-relevant neuromodulation through potential impact on cholinergic, noradrenergic, and serotonergic networks [17]. However, it should be noted that a precise biological pathway was not the focus of this paper.

Methods

Experimental Design

Participants completed an intake session consisting of informed consent, cognitive testing, and medical history review. After a diagnostic consensus conference in which amnestic MCI criteria were confirmed, eligible participants were scheduled for an MRI including structural and functional imaging sequences. A mixed design was used, with random assignment to stimulus conditions: tragus (experimental condition) or ear lobe stimulation (control condition).

Participants

The protocol was approved by the University of Florida human subjects review board and all participants signed an informed consent prior to participation. Premorbid intelligence was estimated with the word list from the Wechsler Test of Adult Reading. Dementia and MCI diagnoses were verified by a variety of measures, including instrumental activities of daily living (IADLs) using the Functional Activities Questionnaire [18], the Clinical Dementia Rating Scale (CDR) [19], and the Montreal Cognitive Assessment (MoCA) [20]. The MoCA is a brief test that has high sensitivity (90%) and specificity (87%) for detecting individuals with MCI and dementia and distinguishing them from individuals with normal cognition. In 114 participants with MCI that progressed to dementia and 51 who did not, 90.5% of participants with MCI with a MoCA score of less than 20/30 at baseline converted to AD within the average follow-up period of 18 months, compared with 52.7% of participants with MCI above the cutoff [21]. To verify memory impairment, in addition to a review of recent neuropsychological data performed at memory disorder clinics (the primary referral source for participants in this study), we administered the CDR, the MoCA, and Form 5 of the Hopkins Verbal Learning Test-Revised (HVLT-R) [22]. Form 5 was used to minimize any interference between our measures and clinical cognitive assessment occurring before or after our study.

The diagnosis of MCI was arrived upon via diagnostic consensus conference with the study neuropsychologist (Williamson) and neurologist (DeKosky) based on the above information and a review of medical records. Participants who scored < 27 on the MoCA and had a CDR sum of boxes (CDRsb) score of 0.5 along with verified memory deficits in performance on memory testing (HVLT-R) were enrolled in the study [23]. Clinician judgment was used in order to identify amnestic forms of MCI, determined primarily on memory scores and the absence of strong support for alternative etiologies, including any acute onset events. Furthermore, individuals with other neurological diseases or medical conditions that may be associated with impaired cognition, such as large vessel stroke, epilepsy, or Parkinson's disease, were excluded from the study.

Fifty-six participants with MCI were enrolled; six were excluded from analyses due to excessive movement during the acquisition of functional magnetic resonance imaging (fMRI). The participants had a mean age of 75 years, an average of 16 years of education, a WTAR score of 41.1, a MoCA score of 22.3, and an HVLT total recall T score of 36.9 and HVLT retention T score of 27.1. Table 1 presents a breakdown of demographic and key cognitive intake scores. A chi-square test of independence was used to compare groups (tragus versus ear lobe stimulation) on categorical variables (sex X^2 (1,50) = 0.33, $p = 0.569$; $p > 0.05$), and ANOVA was used to compare continuous variables. Groups did not differ statistically on these factors ($p > 0.05$) (see Table 1).

tVNS Approach

Self-adhesive 10 × 25 mm hydrogel stimulation electrodes were placed over the left auricular branch of the vagus nerve with one electrode placed anterior to the tragus oriented vertically and one placed on the posterior face of the tragus to the interface of the auditory canal and oriented horizontally to slightly oblique (see Fig. 2). This site was selected due to support within the literature for the largest, averaged vagus sensory evoked potentials (VESPs) when compared to other stimulation sites [24]. It is important to note that there are other potentially viable non-invasive access points to the auricular branch of the vagus including the cymba-conchae. Alternately, one may target the cervical branch in the neck, though this carries a risk of additional off-target effects [25]. There may be differences in effects associated with stimulation at these regions. For the control condition, electrodes were placed on opposite sides (mesial and lateral faces) of the earlobe. The return electrode for tVNS was placed anterior to the tragus to minimize off-target stimulation, and the sham

Table 1 Group demographic data

	Tragus stimulation ($n = 25$)	Lobe stimulation ($n = 25$)
Age	74.52 (7.0)	75.88 (6.6)
Female	15	13
Education	16.24 (2.8)	15.96 (2.8)
MOCA	22.40 (2.6)	22.20 (2.4)
WTAR	41.68 (7.4)	40.60 (7.4)
FAQ	2.20 (2.3)	3.80 (3.1)
HVLT total recall T-score	37.75 (7.5)	35.96 (8.6)
HVLT retention T-score	26.21 (10.6)	28.04 (12.5)

Demographic and key cognitive intake data divided by tragus and ear lobe stimulation groups. Groups did not differ statistically on any of the above factors ($p > 0.05$)

return electrode was placed on the mesial face of the earlobe (Fig. 2). Stimulation was provided by a transcutaneous electrical nerve stimulation (TENS) device with an rf filter for use in an MRI environment (Biopac models STM100C + STMISOC; Biopac,Galeta, CA). Positive pulses were delivered at a 20 Hz, 50 µs pulse width within the range of published stimulus parameters (frequency range: 8–30 Hz, pulse width range: 20–300 µs) [26–35]. Stimulation was delivered continuously during one fMRI resting state condition. Stimulus intensity for sham and tVNS was progressively increased from 0 to the threshold of discomfort, then reduced to 80% of threshold, as per prior investigations [27–29, 33, 34, 36, 37]. Due to device limitations, stimulation intensity was capped at 10 mA, which most subjects reached without discomfort.

Average stimulation intensity did not significantly differ between the active tVNS and control group, as determined through independent samples *t*-test conducted in R-Studio (average stim: 7.3, average sham: 7.7; t[48] = 0.435, $p > 0.05$). Discomfort level was assessed with a questionnaire after each visit on a 10 cm line scale bracketed by 1 and 10. Participants were instructed to put a mark on the line to rate their discomfort with 1 being no discomfort and 10 being high discomfort. The line was measured using a ruler and that number was used as the indicator of discomfort level. The stimulation and control groups both reported low levels of discomfort (not surprising given that we set intensity below the threshold of discomfort) and did not report significantly different levels of discomfort while receiving tVNS (average stim: 1.6/10, average sham:1.5/10; $W = 290$, $p = 0.669$). In a post-MRI questionnaire, participants (who were blinded as to the consequence of their stimulation condition and not explicitly told which electrode condition they were receiving) were asked whether they felt the stimulation had an effect on their thinking or memory. Six subjects out of twenty-five in the lobe condition stated "yes," and nine out of twenty-five in the tragus condition stated, "yes" (X^2 (1,50) = 0.857, $p = 0.355$). This suggests that subjective experience was not altered by the simulation condition.

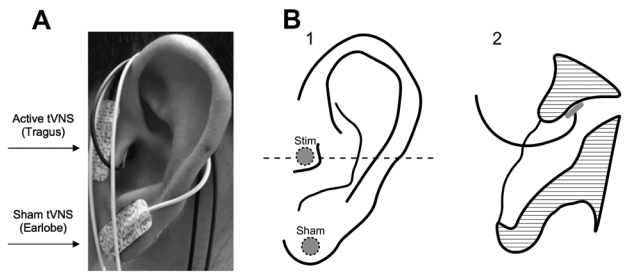

Fig. 2 Stimulus location. **A** Electrode placement from pilots. tVNS and sham electrodes are simultaneously placed. **B** Diagram of stimulus sites

Magnetic Resonance Imaging

Participants underwent structural and functional imaging at the Advanced Magnetic Resonance Imaging and Spectroscopy Facility (AMRIS) at the University of Florida on a 3 T Siemens Prisma (Siemens USA, Washington, DC). This MRI system has a 64-channel head coil. Participants were screened for MRI safety prior to scanning. Sequences acquired were (1) structural: MPRAGE T1-weighted, sagittal $FOV = 256$ mm, 256×256 matrix, slice thickness = 1.00 mm, $TR/TE = 1230/2.26$ ms; (2) functional: echo-planar BOLD imaging (EPI) methods, with a TR of 3000 ms, TE 30 ms, number of volumes = 120, field of view = 240 mm, 80×80 matrix, and voxel size of 3 mm^3. fMRI acquisitions were made at an axial-oblique angle.

Resting State fMRI Characterization

Subjects underwent 6 min of pre-stimulation open-eye resting state acquisition. They were instructed to fixate on a cross in the center of a projected screen and to let their thoughts wander. After this pre-stimulation rest period, stimulation was turned on and calibrated. Subjects then underwent a second 6-min open-eye resting state period in which they were also instructed to fixate on a cross in the center of a projected screen and to let their thoughts wander. This approach allowed for assessment of tVNS versus sham induced within-subject change in functional connectivity.

Statistical Plan

CONN analysis: MRI data were processed in CONN toolbox v18b [38]. We applied the CONN default pre-processing pipeline which included slice timing correction, functional re-alignment and unwarping, normalization to the MNI152 space, segmentation to produce white-matter, gray-matter and CSF masks, and spatial smoothing with an 8 mm full width half maximum (FWHM) Gaussian kernel. All scans with frame-wise displacement of 0.9 mm or greater, or global blood-oxygen-level-dependent (BOLD) signal changes +/− 5 standard deviations, were excluded as potential outliers. During pre-processing, CONN also applies a default denoising pipeline that removes potential confounding effects in the BOLD signal using an ordinary least squares (OLS) regression. An anatomical, component-based noise correction procedure (aCompCor) extracts noise components from cerebral white matter and cerebrospinal area, minimizes estimated subject-motion parameters, and applies anomaly-outliers scrubbing [39–41]. Data was also de-noised with a 0.008–0.1 Hz band-pass filter.

To evaluate the effect of active stimulation on change in resting state functional connectivity, regions of interest (ROIs) were selected for ROI-ROI functional connectivity analyses within the CONN toolbox (see Table 2). These included the following anatomical ROIs that have been implicated in MCI and are critical in semantic and memory functions: bilateral temporal poles (TP), superior temporal gyrus (STG), middle temporal gyrus (MTG), inferior temporal gyrus (ITG), supramarginal gyrus (SMG), angular gyrus (AG), precuneus, anterior cingulate (AC), posterior cingulate (PC), bilateral hippocampi, parahippocampal gyrus, and amygdala. ROI-ROI analyses provide Fisher z-transformed bivariate correlations between brain regions' BOLD time-series to quantify connectivity while at rest. Conditions were set up to contrast the tVNS and sham-stimulation groups, as well as the period with stimulation versus the period prior to any stimulation, to evaluate condition × group effects. ROI-ROI connectivity results were false discovery rate (FDR)-corrected at the seed level (p-threshold < 0.05).

Table 2 Abbreviations for anatomical regions within ROI-to-ROI and seed-to-voxel analyses

Acronyms	
FP	Frontal pole
SFG	Superior frontal gyrus
FOrb	Frontal orbital cortex
TP	Temporal pole
PP	Planum polare
STG	Superior temporal gyrus
MTG	Middle temporal gyrus
ITG	Inferior temporal gyrus
HC	Heschl's gyrus
TOFusC	Temporal occipital fusiform cortex
TFusC	Temporal fusiform cortex
SMG	Supramarginal gyrus
AG	Angular gyrus
LG	Lingual gyrus
OFusG	Occipital fusiform gyrus
ACC	Anterior cingulate cortex
PCC	Posterior cingulate cortex
PaHC	Parahippocampal gyrus
PaCIG	Paracingulate gyrus
IC	Insular cortex
AMYG	Amygdala
HIP	Hippocampus
NA	Nucleus accumbens
PUT	Putamen

Furthermore, although connectivity to the hippocampus was not found to be significantly related to semantic networks in the ROI to ROI analysis, due to the importance of the hippocampus in AD, in an exploratory analysis, we examined whole-brain seed-to-voxel connectivity stemming from the left and right hippocampus in response to stimulation. In this analysis, Fisher-transformed bivariate temporal correlation coefficients were calculated between the hippocampi and all other individual voxels in the brain, to reveal interregional connectivity strength changes as a function of tVNS. All results surviving height (voxel) level threshold $p > 0.01$, uncorrected, and an extent (cluster)-level threshold $p < 0.05$, FDR corrected, were considered to be significant.

Results

During tVNS compared with earlobe stimulation, there were changes in connectivity within temporal and parietal regions associated with the semantic and salience networks (Table 3 and Fig. 3a). Significant contrasts with seed ROIs within the temporal lobe included the left temporal pole (TP) and the

Table 3 Functional connectivity relationships contrasting during tVNS or earlobe stimulation during resting state to resting state with no stimulation. ROI-to-ROI analysis results with brain regions implicated in semantic processing

Seed ROI	ROI	T statistic	Beta	p-FDR
Left TP	Left pMTG	T(48) = −3.74	−0.22	0.0165
Left TP	Right pSMG	T(48) = 3.15	0.21	0.0474
Left pITG	Left aITG	T(48) = −3.41	−0.25	0.0446
Left aSMG	Left pSTG	T(48) = 3.97	0.28	0.0082
Left aSMG	Right TP	T(48) = 3.13	0.22	0.0447
Left aSMG	Left aSTG	T(48) = 3.03	0.19	0.0447
Left aMTG	Right TP	T(48) = −3.45	−0.21	0.0406
Right TP	ACC	T(48) = 3.79	0.25	0.0135
Right TP	Left pMTG	T(48) = −3.47	−0.21	0.0135
Right TP	Right aSMG	T(48) = 3.23	0.20	0.0187

left posterior middle temporal gyrus (MTG) (t[48] = −3.74, $p < 0.05$) and right posterior supramarginal gyrus (SMG), (t[48] = 3.15, $p < 0.05$). There were also significant increases and decreases in connectivity from the right TP to the anterior cingulate cortex (AC) (t[48] = 3.79, $p < 0.05$), left posterior MTG (t[48] = −3.47, $p < 0.05$), and right anterior SMG (t[48] = 3.23, $p < 0.05$). In addition to connectivity changes between these regions and the temporal poles, there was decreased connectivity from the left posterior inferior temporal gyrus (ITG) to the left anterior ITG (t[48] = −3.41, $p < 0.05$), as well as from the left anterior MTG to the right TP (t[48] = −3.45, $p < 0.05$).

Significant contrasts containing seed ROIs within the parietal lobe demonstrated increased connectivity from the left anterior SMG to the left posterior superior temporal gyrus (STG) (t[48] = 3.97, $p < 0.01$), right TP (t[48] = 3.13, $p < 0.05$), and left anterior STG (t[48] = 3.03, $p < 0.05$).

We were also interested in evaluating connectivity from the hippocampus, given its relevance in MCI and AD and the connectivity of NTS and LC to hippocampus (apriori network prediction). Contrasting tVNS and sham stimulation, there were changes in connectivity from the left hippocampus to several cortical and subcortical regions bilaterally, with increased connectivity to prefrontal regions and cingulate, and decreased connectivity to anterior and medial temporal lobe, including temporal pole. A seed-to-voxel analysis from the right hippocampus yielded significant decrease in connectivity to a cluster of ROIs in the left anterior temporal lobe. These associations are displayed in detail within Tables 4 and 5, as well as in Fig. 3b, c.

Discussion

The purpose of the study was to determine effects of tVNS on functional connectivity during fMRI in brain networks relevant to AD. The primary results of this study of tVNS stimulation in a sample of older adults with MCI were changes in connectivity across critical cognitive networks. Changes in connectivity as a function of stimulation were evident between brain areas that typically deteriorate in

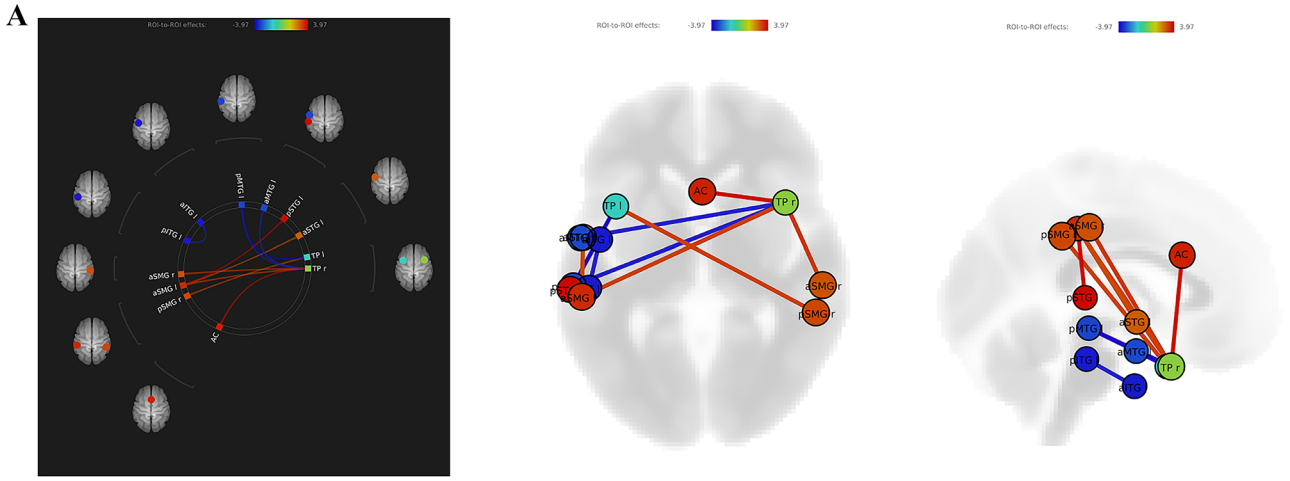

Fig. 3 Visual representation of significant functional connectivity contrasts following tVNS. **A** Change in connectivity was observed between semantically relevant regions including bilateral temporal poles, supramarginal gyrus, superior temporal gyrus, and anterior cingulate. **B** Changes in connectivity were observed from the left hippocampus to several diffuse, bilateral clusters of voxels covering multiple brain regions: A, left prefrontal cortex; B, left anterior temporal lobe; C, left anterior temporal lobe; D, posterior cingulate gyrus; E, bilateral superior frontal and cingulate cortex; F, right inferior temporal; G, right prefrontal cortex; H, anterior temporal lobe, parahippocampal gyrus. **C** Decreased connectivity was observed from the right hippocampus to a cluster including regions within the left anterior temporal lobe

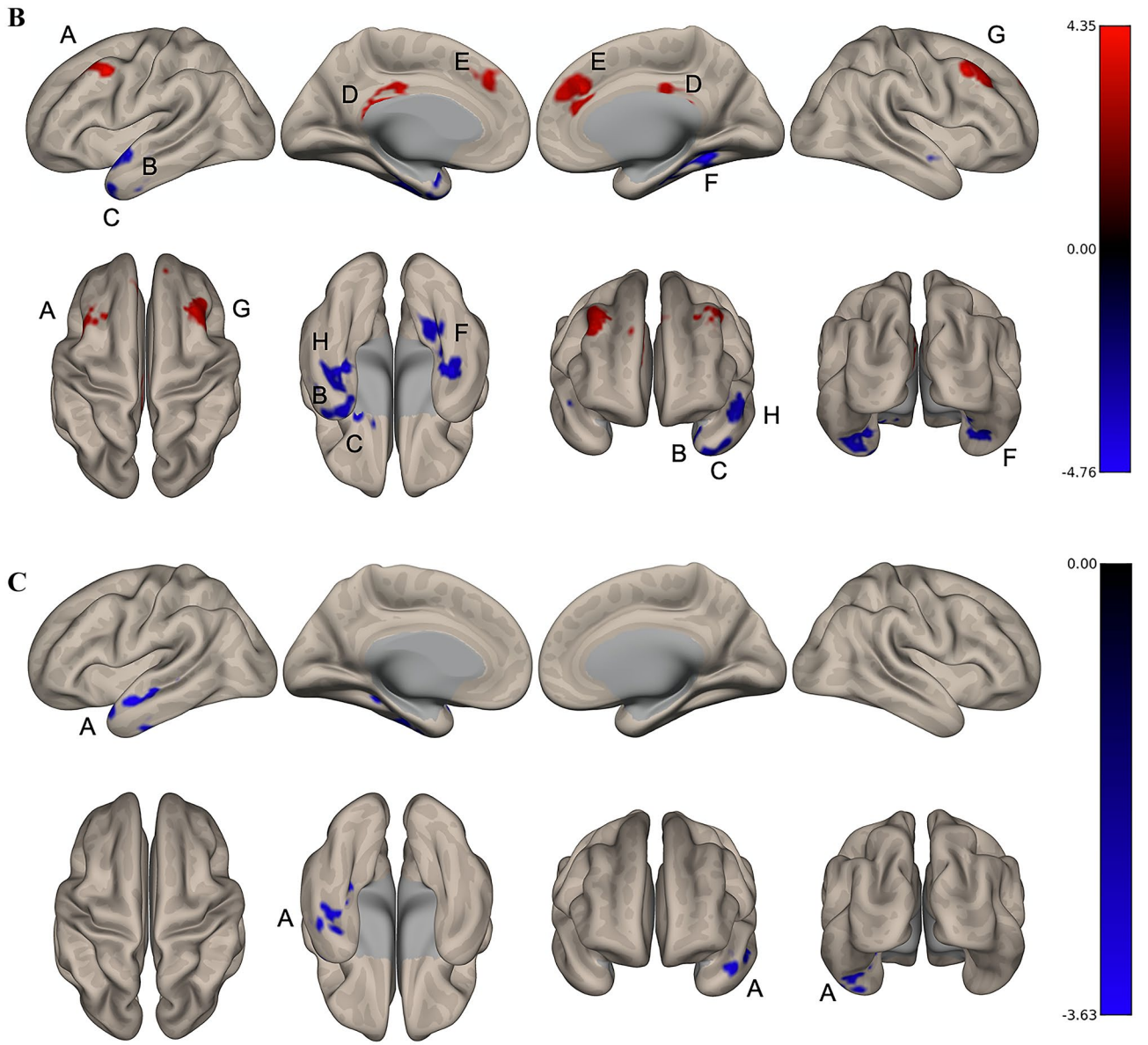

Fig. 3 (continued)

Table 4 Whole brain seed-to-voxel results seeding from the left hippocampus, listed in order of appearance in Fig. 3b. Prefixes: a, anterior division; p, posterior division; to, temporooccipital division

Hemisphere	Cluster regions	Peak MNI	Cluster size	T statistic	Beta	p-FDR
Left	MFG	24	289	4.38	0.18	0.032368
Left	TP, AMYG, PUT, FOrb, aPaHC, aTFusC, NA	−38	458	−6.24	−0.19	0.005747
Left	TP, aSTG, PP, aMTG	−64	284	−4.98	−0.19	0.032368
Bilateral	PCC	−42	297	4.63	0.16	0.032368
Bilateral	PaCIG, AC, SFG, FP	74	644	4.39	0.18	0.001409
Right	TOFusC, pTFusC, HIPP, LG, pITG, pPaHC, AMYG, aPaHC, aSTG, PP, pSTG, pMTG, OFusG	−6	717	−6.33	−0.2	0.001270
Right	MFG	104	447	4.12	0.18	0.005747
Left	pTFusC, pITG, aPaHC, PP, aTFusC, pPaHC, aITG, HG, IC	−80	583	−5.52	−0.2	0.001873

Table 5 Whole brain seed-to-voxel results seeding from the right hippocampus. Prefixes: a, anterior division; p, posterior division; to, temporooccipital division

Hemisphere	Cluster regions	Peak MNI	Cluster size	T statistic	Beta	p-FDR
Left	pITG, pTFusC, TP, pMTG, aSTG, aMTG, HIP, aTFusC, PP, aITG, toITG	92	615	−5.69	−0.19	0.003540

patients with AD. These changes included increased connectivity between the temporal poles bilaterally and the supramarginal gyrus, superior temporal gyrus, and anterior cingulate — critical structures in the semantic network [42]. Differences in connectivity among these regions are associated with progression to AD [42, 43]. These differences have been observed in patients with MCI, with decreased temporal and temporo-parietal functional connectivity found to be predictive of poorer outcomes in patients with MCI [44].

The temporal and parietal structures that show change in connectivity with tVNS are important to our ability to comprehend language, including the ability to derive meaning, form associations between concepts, and engage in symbolic reasoning [45, 46]. These lexical-semantic processes are among the first and most significantly impaired in those with AD. Semantic dysfunction can be detected in MCI, prior to an AD diagnosis, through deficits in categorical fluency and naming [43]. Relative decrements in memory (amnestic), naming (anomic), and semantic fluency performance are considered to be the cognitive triad indicative of a cognitive decline in patients with AD. These cognitive functions are supported largely by semantic networks, including the temporal and parietal lobes as well as limbic structures, i.e., those regions and networks affected by tVNS in our sample. Given the strengthened connectivity in these functional neuroanatomic systems in response to stimulation, tVNS may be useful as an intervention to modulate the progression of MCI to AD. Furthermore, there is potential to improve associative functions, particularly those dependent on parallel distributed cortical processing in the temporal and parietal regions.

In addition to strengthened connectivity among ROIs implicated in semantic processing, we found decreased connectivity from the bilateral temporal poles to the middle temporal gyrus, as well as within the inferior temporal gyrus. Recent studies have detected disruption of functional connectivity in early MCI, particularly between the medial temporal lobes (MTL) and posterior-medial parietal regions [47, 48]. Others have reported the presence of concurrent hyperexcitability within the MTL and neighboring limbic structures as early signs of AD pathology [49, 50]. Given the supporting literature for both hypo- and hyper- connectivity in MCI, the effect of AD progression on the functional connectivity within and between cortical and subcortical structures is complex and warrants continued study.

Functional connectivity changes were also observed from the left hippocampus to bilateral cortical and subcortical regions including the anterior temporal lobe, prefrontal cortex, cingulate gyri, and parahippocampal gyri in response to tVNS. Notably, there was decreased connectivity to temporal regions, consistent with findings of connectivity from the MTL to other semantic cortical areas, whereas connectivity from the left hippocampus to frontal and cingulate gyri increased. Multiple studies have shown altered connectivity from the hippocampus to these regions in prediction of progression of MCI and early stages of AD. Xue and colleagues used Granger causality analysis (GCA) based on voxels with rs-fMRI data and found significantly decreased functional connectivity from the right hippocampus to the left STG in patients with AD when compared to controls [51]. Another study also found decreased connectivity between the right hippocampus and the right STG in participants with AD [52]. Furthermore, Velayudhan and colleagues used 7-Tesla rsfMRI to conduct a seed-based ROI analysis from the bilateral hippocampus to 132 diffuse brain regions, and found significantly decreased functional connectivity of the left hippocampal seed to the left superior frontal gyrus (SFG) in healthy controls compared to individuals with AD [53]. Seed-based approaches have also been used to reveal reduction in intrinsic connectivity between the hippocampus and precuneus, as well as increased hippocampal glucose metabolism, in individuals with MCI and AD compared to normal controls [54]. While these reported findings demonstrate decreases in connectivity, Sohn and colleagues identified an initial increase in connectivity from the left hippocampus to the frontal and temporal lobes during aMCI and early AD stages, followed by a decrease in connectivity as AD progresses [55]. Our whole-brain seed-to-voxel analysis from the right hippocampus yielded a significant decrease in connectivity, confined to a single cluster of ROIs within the left anterior temporal lobe. Greater involvement of functional connectivity of the left hippocampus is logical in light of the findings regarding the effects of tVNS on semantic network, a language-based system. Changes in connectivity in response to tVNS in hippocampal connected regions implicate MCI/AD relevant system involvement. We cannot draw conclusions regarding the functional significance and therapeutic value of the directional change in connectivity at this point; however, future longitudinal studies using tVNS

relative to cognitive performance and neurophysiological responses are necessary to clinically corroborate findings.

The affected brain systems are critical to managing the interactions and accessibility of stored concepts and may have direct relevance to the enhancement of neuroplasticity within and between cortical association areas. Although our data do not address longitudinal changes in neuroplasticity with tVNS, vagus nerve stimulation does have the potential to modify neuroplasticity. One primary mechanism of neuroplasticity is through modulation of neurotransmitters such as acetylcholine and norepinephrine [56]. The left branch of the vagus nerve projects to the nucleus of the solitary tract, which synapses in the LC and the basal forebrain. These structures, LC and basal forebrain, contain the sources neurons for norepinephrine and acetylcholine distribution, respectively. Both of these neurotransmitters are neuromodulatory and are critical for neural plasticity. Though not yet demonstrated in humans, neuroplastic responses to VNS have been shown in the auditory cortex of rats when paired with specific auditory tones [57, 58] and have also been shown in the motor cortex of rats when a specific skilled motor task was paired with VNS [59]. These studies suggest alteration of these neurotransmitters as a potential mechanism for long-term effects of tVNS.

Support for the modulation of these neurotransmitters through tVNS in humans has been demonstrated through measurement of pupillary and EEG measures of arousal as potential biomarkers of LC-noradrenaline or norepinephrine network activity [60, 61]. However, others have reported alternative findings. A series of studies by Burger et al. found that tVNS did not increase pupil diameter or performance on an attentional blink task [62]. It is important to note that, in humans, response to tVNS has been mixed and it is unclear to what extent differences in populations (e.g., neurological disease versus healthy), parameter settings, timings, and context affect responses. Furthermore, the use of different biomarkers of vagus nerve activity (e.g., salivary alpha-amylase, P300) has demonstrated variable evidence [63].

While many conceptualize the potential brain effects of VNS based on its impact on the LC and norepinephrine, the afferent path of the vagus affects other structures and neurotransmitter systems that are germane to AD. Serotonergic pathways have also been implicated as a key mechanism of the positive effects of tVNS. A rodent study of invasive VNS found increased firing rate of serotonergic neurons after 14 days of stimulation and that this upregulation appeared to be mediated by norepinephrine release [64]. Furthermore, the vagus nerve also plays a key role in regulating peripheral cholinergic release, which modulates the release of several downstream inflammatory markers such as tumor-necrosis factor (TNF), interleukin-1 (IL-1), and other cytokines [65]. These inflammatory signaling molecules are elevated in AD and correlate with more severe cognitive impairment [66]. These factors may also be relevant to potential disease modifying features of longitudinally administered tVNS and will need to be evaluated in that context.

Despite the term MCI often being used to signify an intermediate state between healthy aging and dementia, MCI itself is not a homogenous category. Though all individuals within our sample demonstrated memory impairment (memory complaints, impairment on HVLT-R), some exhibited impairment in additional cognitive areas as well. Not all patients with MCI progress to AD, depending on the population from whom subjects are selected, the number of MCI cases that progress to AD vary from 5 to 40% (highest in populations like the population from which research clinics draw) [67]. Furthermore, despite excluding for significant medical comorbidities such as large vessel stroke, epilepsy, or other systemic factors that could explain the presentation of cognitive impairment, it is worth noting that our sample did include individuals with cardiovascular risk factors (i.e., high blood pressure). There is also the potential of unreported or undetected medical concerns that could be contributing to an amnestic deficit. A limitation of the present study is that we do not have any established biomarkers of Alzheimer's pathology (e.g., PET scans, CSF, genetic biomarkers). It is likely that MCI etiologies in our sample are mixed. However, as above, this is representative of the broader MCI population. In follow-up work, we hope to incorporate these methods for more precise identification of individuals on the AD trajectory. Sample size is another limitation of the present study and the influence of demographic factors (e.g., sex, age) and cognitive factors (e.g., premorbid ability, relative preservation of non-memory-based cognitive processes) was not analyzed. Furthermore, small sample sizes increase both type 1 and type 2 error risk. Sham approaches in stimulation studies are challenging, and the subjective experience of ear lobe versus tragus stimulation is different. Though ear lobe sham is commonly used in tVNS work, it is not clear that this is the best sham comparison [68]. Future research contrasting to other sham approaches and treatments may be helpful.

It should also be emphasized that this study investigates the acute effects of tVNS on brain function. What the long-term effects of tVNS on cognition and/or brain function would be in people with MCI is currently unknown. Acute alterations in connectivity may not result in consequent changes in long-term brain connectivity, although they may. It is also unknown if tVNS can provide tangible cognitive benefits in MCI populations and whether such effects would ameliorate the progression of MCI to AD. Others have reported various effects on cognitive function in other populations (e.g., depression, epilepsy, healthy people) both acutely and longitudinally [69–71]. However, the reasons for cognitive dysfunction (or lack thereof) in these populations are different. Such benefits might exist independently of the underlying deterioration of neural function and cause

no slowing of the neurodegenerative process itself. It may be that acute cognitive changes (during stimulation changes) are not induced or influenced by tVNS in patients with MCI, but that longitudinal stimulation may modify neuroplasticity such that advantages manifest in the longer term through improved or stabilized cognitive performance. This would be consistent with VNS and depression research showing increased effects over time [72]. The development of tVNS for treatment will require controlled longitudinal studies to investigate effects of stimulation parameters, stimulus locations, dosing length, and patient specific differences on brain function, health, and cognitive performance.

Conclusions

Our findings suggest that tVNS causes alterations during stimulation both within and between networks associated with cognitive impairment in AD and MCI. This suggests that tVNS engages targets within systems that deteriorate in MCI and AD populations. The relatively low efficacy of drugs and interventions on brain health in AD has led to an upsurge of interest in intervention on precursor stages of AD. Target engagement in systems that deteriorate in AD and MCI populations suggests that tVNS may be a potentially effective intervention. tVNS is a low-risk stimulation method that may provide brain health and cognitive benefits in subjects at high risk of converting to dementia, improving quality of life and potentially altering disease course. Further research is required on the long-term effects of tVNS on MCI, and investigations of its effects on specific cognitive tasks, in both neuropsychological testing and on in-scanner tasks.

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Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

Availability of Data and Materials A limited dataset will be provided upon request.

Declarations

Ethics Approval and Consent to Participate All participants consented to participate in the project via signed informed consent form. Procedures and study activities were reviewed and approved by the University of Florida Institutional Review Board.

Consent for Publication The co-authors have reviewed the manuscript and consent to its publication.

Competing Interests Drs. Williamson, Porges, Lamb, Cohen and DeKosky are on the scientific advisory board for Evren Technologies, a company developing a closed loop transcutaneous vagus nerve stimulation device based on a patent held by the University of Florida (Williamson, Lamb, Porges inventors). Drs. Porges, Lamb, and Williamson have an additional patent under development via the University of Florida related to an implementation technology associated with vagus nerve stimulation. No technology or resources from Evren were used in the current study. Furthermore, neither patented (or under review) technology was used in the current study. Mr. Aidan Murphy reported no biomedical financial interests or potential conflicts of interest. Ms. Alexandria O'Neal reported no biomedical financial interests or potential conflicts of interest. Ms. Sarah Bottari reported no biomedical financial interests or potential conflicts of interest. Mr. Brian Ho reported no biomedical financial interests or potential conflicts of interest. Ms. Erin Trifilio reported no biomedical financial interests or potential conflicts of interest. Dr. Kenneth Heilman reported no biomedical financial interests or potential conflicts of interest.

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