

Brain effect of transcutaneous vagal nerve stimulation: A meta-analysis of neuroimaging evidence

Rebekah Rajiah¹  | Kazuya Takahashi¹ | Qasim Aziz¹ | James K. Ruffle^{1,2} 

¹Centre for Neuroscience and Trauma, Blizard Institute, Wingate Institute of Neurogastroenterology, Barts and the London School of Medicine & Dentistry, Queen Mary University of London, London, UK

²UCL Queen Square Institute of Neurology, London, UK

Correspondence

James K Ruffle, High-Dimensional Neurology, UCL Queen Square Institute of Neurology, London WC1N 3BG, UK.
Email: j.ruffle@ucl.ac.uk

Qasim Aziz, Centre for Neuroscience and Trauma, Blizard Institute, Wingate Institute of Neurogastroenterology, Barts and the London School of Medicine & Dentistry, Queen Mary University of London, London, UK.
Email: q.aziz@qmul.ac.uk

Abstract

Background: Dysfunction in the autonomic nervous system is common throughout many functional gastrointestinal diseases (FGIDs) that have been historically difficult to treat. In recent years, transcutaneous vagal nerve stimulation (tVNS) has shown promise for improving FGID symptoms. However, the brain effects of tVNS remain unclear, which we investigated by neuroimaging meta-analysis.

Methods: A total of 157 studies were identified, 4 of which were appropriate for inclusion, encompassing 60 healthy human participants. Using activation likelihood analysis estimation, we statistically quantified functional brain activity changes across three domains: (1) tVNS vs. null stimulation, (2) tVNS vs. sham stimulation, and (3) sham stimulation vs. null stimulation.

Key Results: tVNS significantly increased activity in the insula, anterior cingulate, inferior and superior frontal gyri, caudate and putamen, and reduced activity in the hippocampi, occipital fusiform gyri, temporal pole, and middle temporal gyri, when compared to null stimulation (all corrected $p < 0.005$). tVNS increased activity in the anterior cingulate gyrus, left thalamus, caudate, and paracingulate gyrus and reduced activity in right thalamus, posterior cingulate cortex, and temporal fusiform cortex, when compared to sham stimulation (all corrected $p < 0.005$). Sham stimulation significantly increased activity in the insula and reduced activity in the posterior cingulate and paracingulate gyrus (all corrected $p < 0.001$), when contrasted to null stimulation.

Conclusions: Brain effects of tVNS localize to regions associated with both physiological autonomic regulation and regions whose activity is modulated across numerous FGIDs, which may provide a neural basis for efficacy of this treatment. Functional activity differences between sham and null stimulation illustrate the importance of robust control procedures for future trials.

KEYWORDS

autonomic nervous system, brain activation, meta-analysis, neuroimaging, transcutaneous vagal nerve stimulation, tVNS

Qasim Aziz, James K Ruffle joint senior authors.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Neurogastroenterology & Motility* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

The autonomic nervous system (ANS) is a bidirectional brain-body interface that assimilates information from the external environment with the internal milieu, to maintain homeostasis.¹ This role in physiological regulation is extensive, spanning from mediating metabolism, inflammation, and gastrointestinal function, to modulating nausea or pain perception.¹⁻³ Considering the central role of the ANS in regulation of the brain-gut axis, dysfunction of the ANS is placed at the center of disease pathophysiology for an array of functional gastrointestinal disorders (FGIDs) – including reflux hypersensitivity, irritable bowel syndrome (IBS), and chronic constipation (CC).^{2,4-6} These disorders are notoriously difficult to treat with current clinical means, posing a significant yet unmet health-care need.

Transcutaneous vagal nerve stimulation (tVNS) has shown promise as a therapy that may restore the disturbance to the ANS associated with the FGIDs.⁷ The clinical benefits of tVNS reported to date span multiple gastrointestinal pathologies, including evidence of tVNS-induced improvements in abdominal pain and constipation in IBS⁸; reduced symptomatic profiles in functional dyspepsia (FD),⁹ and the prevention and reversal of esophageal hypersensitivity in healthy patients with a validated model of acid-induced esophageal pain.¹⁰ Moreover, there is evidence to support a clinical role for tVNS in pediatric gastroenterology with improvements in abdominal pain observed in adolescent patients with abdominal pain-related FGIDs,¹¹ and in addition tVNS studies which imply an anti-inflammatory effect, illustrating therapeutic potential across a range of diseases.¹²⁻¹⁶

The principal mechanism of tVNS-related gastrointestinal effects is thought to be broadly based on the anatomical distribution and functional connectivity of the tenth cranial nerve (CNX), the vagus.⁷ Structurally, CNX carries fibers from the abdominal and thoracic viscera which ascend firstly to key neural substrates in the brainstem and subsequently to a cluster of cortical and subcortical regions comprising the central autonomic network (CAN).^{12,17-20} Through modulating the activity of CNX, tVNS is thought to exert an effect in relevant CAN structures, such as the insula, amygdala, and anterior cingulate cortex (ACC), in addition to numerous brainstem nuclei.^{21,22} While functional neuroimaging has provided some evidence in support of these central mechanisms, discrepancies remain, perhaps attributable to the small numbers of participants investigated in a single study, variation between stimulation parameters and/or the control used.²¹⁻²⁶ A lack of agreement between studies exploring tVNS brain activation patterns renders it difficult to infer with any certainty the true neural correlates of this intervention, a significant rate-limiting step in its prospective use. To overcome these limitations, we conducted a neuroimaging meta-analysis coalescing all previous tVNS imaging studies in healthy humans to establish a more robust estimate of its central mechanism.

Key points

- We present a neuroimaging meta-analysis that – by coalescing all previous studies – renders a more statistically robust representation detailing the brain effects of tVNS.
- This provides a neural basis for the numerous pre-existing research studies demonstrating efficacy of tVNS in FGIDs.
- Control paradigms in tVNS studies cannot be considered physiologically inert; a robustly controlled sham stimulation procedure is essential.

2 | MATERIALS & METHODS

2.1 | Study eligibility

Eligibility criteria were determined a priori. Studies were required to use a neuroimaging technique in conjunction with tVNS in healthy individuals aged between 18 and 65 years. We specifically focused on the inclusion of healthy individuals only, so as to ensure results ascertained were not confounded by an imaging signature of pathology. We only considered studies where there was an appropriate control arm, where tVNS would be statistically contrasted against a sham or null stimulation. Only trials presenting primary findings and published coordinate data were used. The language or date of publication was not considered; amidst concerns, this would narrow the pooling of relevant studies. Meta-analytic PICO criteria are provided in Table S1.

2.2 | Study selection

A literature search was performed with PubMed via MEDLINE and Web of Science databases on 31 May 2022 using a conjunction of two search terms: (1) synonyms pertaining to brain activation including 'brain activity', 'neuroimaging', 'fMRI', 'functional magnetic resonance imaging', 'neural activity' and 'functional brain network' and (2) synonyms for transcutaneous vagal nerve stimulation, including 'tVNS', 'transcutaneous vagus nerve stimulation', 'non-invasive vagal nerve stimulation', 'non invasive vagal nerve stimulation', 'non-invasive vagus nerve stimulation' and 'nVNS' (Figure S1).

For Web of Science, the search strategy generated 76 studies where 65 articles necessitated exclusion, with 11 remaining eligible (Figure 1). The PubMed search through MEDLINE generated a total of 337 results (Figure 1). After filtering by our inclusion criteria, this yielded a total collection of 81 studies, of which 76 were deemed unsuitable. Eligible articles were subsequently screened for repeats and then further assessed for suitability. Where coordinate or demographic data were missing from an article, we contacted the article's corresponding author to seek such data. This yielded a total of 12

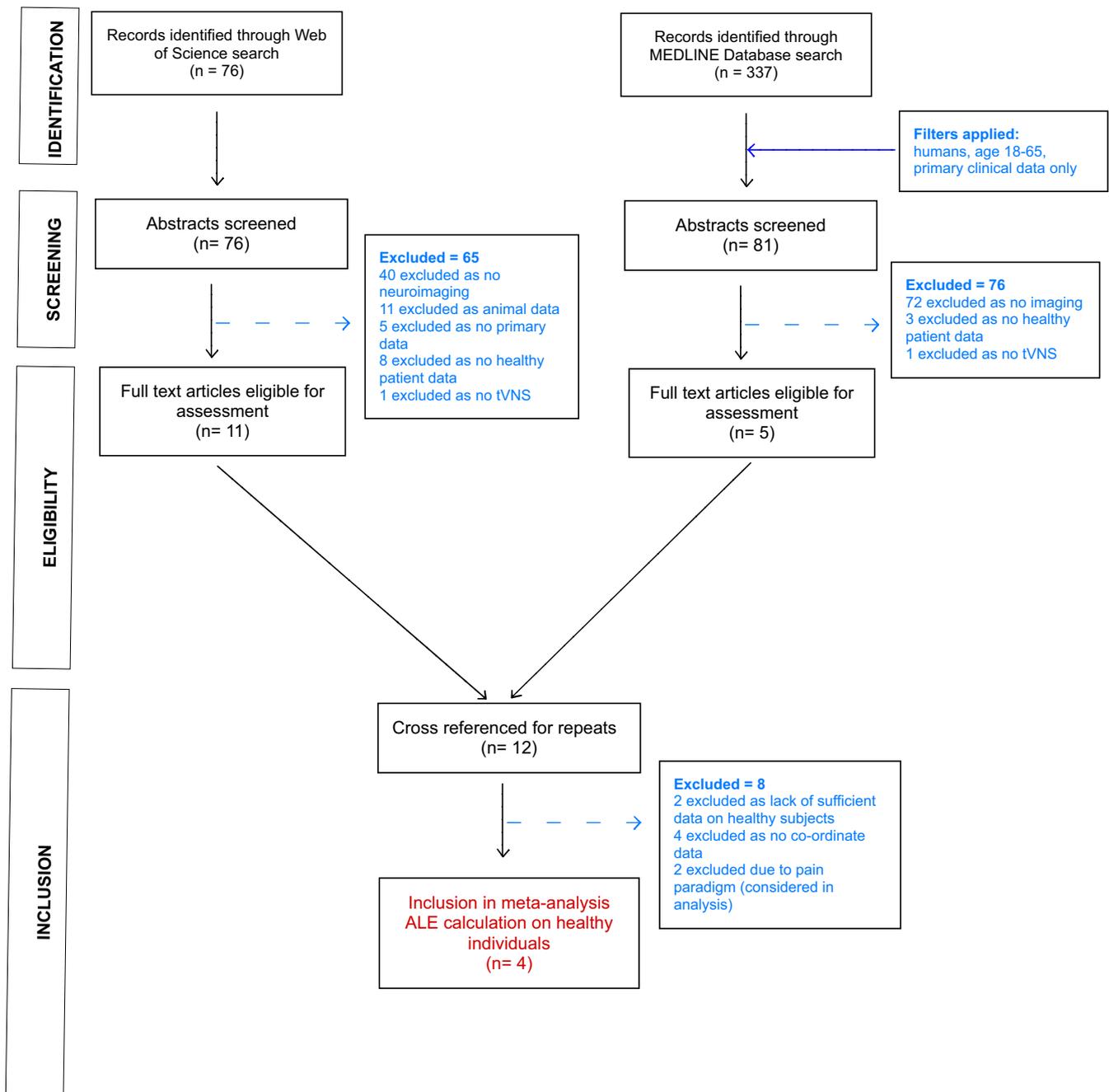


FIGURE 1 Initial search strategy for the meta-analysis. Diagram to show stages of the initial meta-analysis search strategy based on the four-phase Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart. ALE, activation of likelihood estimation; n, number of participants; tvNS, transcutaneous vagal nerve stimulation

studies, where 8 were excluded under further interrogation for either a lack of sufficient coordinate data, a lack of data available on healthy subjects or were excluded due to use of a pain paradigm in the study protocol which would be considered a confounding factor, leaving 4 remaining as suitable. Therefore, our total sample size encompassed 60 healthy human participants (17 males, 27 females, and 16 where gender was not provided), aged between 18 and 65, and 139 brain imaging foci to be considered in the meta-analytic statistical analysis.²²⁻²⁵ Recognizing the existence of relevant allied literature that was unsuitable in the neuroimaging statistical analysis,

we separately undertook a qualitative literature review of all prior relevant studies and the brain imaging foci associated with change in activity secondary to tvNS (Tables S2 and S3).

2.3 | Statistical analysis of meta-analytic brain imaging data by activation likelihood estimation

We utilized activation likelihood estimation (ALE) for meta-analysis of brain imaging data, a well-validated statistical method of spatial

concordance, including within autonomic neuroscience.²⁷⁻²⁹ This study was further justified as presently no meta-analytic map of tVNS exists in popular online fMRI meta-analytic frameworks.^{30,31} Given that eligible studies provided data during both tVNS stimulation and sham stimulation, three domains of comparison were determined: (1) tVNS vs. no stimulation, (2) tVNS vs. sham stimulation, and (3) sham stimulation vs. no stimulation (Table 1). Between studies, the site of active stimulation differed and included the application of tVNS to left tragus,²³ left external acoustic meatus,^{22,25} and the right anterolateral surface of the neck.²⁴ The site of sham stimulation also varied and included either somatic non-noxious stimulation of the left ear lobe^{22,23,25} or the right posterolateral aspect of the neck.²⁴

All ALE analyses calculations were conducted using GingerALE 3.02 (<http://www.brainmap.org/ale/>; Research Imaging Centre, University of Texas). As a widely used tool in coordinate-based meta-analyses, GingerALE enables inferences of commonly activated or deactivated regions across studies to be deduced where coordinates are available.²⁸ The coordinate space in the available studies was mixed, with two studies in Talairach space and two in Montreal Neurological Institute (MNI) space; therefore, all foci reported in Talairach space were transformed to MNI using *icbm2tal* transform prior to the ALE analysis.³²

To undertake ALE, a series of calculations are performed to produce both an ALE score, functioning as an indicator of spatial agreement likelihood among foci, and an ALE cluster map, providing a visual representation of the numerical information generated in the ALE analysis output. Briefly, the calculations that ensue can be outlined in several steps: (1) the creation of a modeled activation (MA) map to denote a 3D image for each experiment group using the foci, the mask selected, and the full width half maximum

(FWHM) of the subject size; (2) a union of each MA map to create an ALE image; (3) the translation of MA maps into values that are used for histograms where the likelihood of activation at each voxel can be estimated; (4) thresholding of the ALE map to detect the presence of significant clusters; and (5) correction for multiple comparisons through permutation-based thresholding.^{29,33} Considering that in the present study, data were available for both increased and decreased activity within each category of comparison, six separate ALE analyses were conducted by Ginger ALE to assess for spatial concordance for the three comparisons aforementioned (Table 1). All analyses used a cluster forming threshold of corrected $p < 0.05$, a cluster level inference threshold of corrected $p < 0.05$ and a permutation threshold of 5000 with utilization of the conservative mask size. All p values reported within the manuscript are corrected for multiple comparisons by the aforementioned methodology.

3 | RESULTS

3.1 | Comparison of tVNS vs. no stimulation

The analysis of brain activity during tVNS, compared to resting state without stimulation (4 studies, 46 participants, 53 foci), revealed widespread increased activity with significant results observed in the bilateral insula ($p < 0.0003$), bilateral anterior cingulate cortex, right caudate, right putamen, and left superior and bilateral inferior frontal gyri (all $p < 0.004$) (Table 2). Additional regions that showed increased activity associated with tVNS included the bilateral frontal pole, bilateral central opercular cortex, left frontal operculum cortex, left postcentral gyri, left paracingulate gyrus, and right orbitofrontal cortex ($p < 0.004$) (Figure 2A). In terms of reduced brain activity secondary to tVNS, contrasted to the no stimulation group (3 studies, 29 participants, 27 foci), clusters of decreased activity were identified in the bilateral hippocampi, bilateral parahippocampal gyri, bilateral temporal occipital fusiform gyri, right temporal pole, right middle temporal gyrus, and the brainstem (all $p < 0.0007$) (Table 2 and Figure 2B). We provide these meta-analytic result maps as supplementary data (S2 and S3).

3.2 | Comparison for tVNS vs. sham stimulation

Comparison of tVNS brain activation against sham stimulation in the ALE analysis (3 studies, 38 participants, 50 foci) revealed significant clusters of activation in the bilateral anterior cingulate cortex, the left thalamus, left caudate, left paracingulate gyrus (all $p < 0.0005$), right frontal pole ($p < 0.003$), and brainstem ($p < 0.05$) (Table 3 and Figure 3A). Regarding decreased activity in the brain for tVNS vs. sham stimulation (2 studies, 21 participants, 9 foci), ALE revealed a pattern of decreased activity in the brainstem, right thalamus, left posterior cingulate cortex, and left temporal

TABLE 1 Data available for the 6 activated likelihood estimation analyses conducted

| | No. of Participants | No. of experimental groups | No. of foci |
|---------------------------------|---------------------|----------------------------|-------------|
| Increased Activity: tVNS vs. NS | 46 | 4 | 53 |
| Decreased Activity: tVNS vs. NS | 29 | 3 | 27 |
| Increased Activity: tVNS vs. SS | 38 | 3 | 50 |
| Decreased Activity: tVNS vs. SS | 21 | 2 | 9 |
| Increased Activity: SS vs. NS | 46 | 4 | 25 |
| Decreased Activity: SS vs NS | 29 | 3 | 9 |

Note: Table shows the number of participants, number of experimental groups, and the number of foci for each of the 6 analyses conducted. Abbreviations: SS, sham stimulation; NS, no stimulation; tVNS, transcutaneous vagal nerve stimulation.

TABLE 2 Brain activation for tVNS compared to null stimulation in healthy participants according to ALE

| Brain Region | Hemisphere(s) | x | y | z | Max. ALE Score (4.d.p.) | p value (4.d.p.) |
|--|---------------|----------|-----------|-----------|-------------------------|-------------------|
| Increased Brain Activity (<i>n</i> = 46, studies = 4) | | | | | | |
| Insula (posterior) | BL | -40 36 | -6 -12 | 4 16 | 0.0111 0.0076 | <0.0001 0.0002 |
| Frontal pole | BL | -36 26 | 50 52 | -16 -6 | 0.0080 0.0057 | 0.0001 0.0034 |
| Insula (middle) | L | -38 | 2 | -10 | 0.007 | 0.0002 |
| Central opercular cortex | BL | -58 48 | -18 -6 | 16 8 | 0.0074 0.0079 | 0.0004 0.0001 |
| Postcentral gyrus | L | -60 | -20 | 30 | 0.0074 | 0.0004 |
| Frontal operculum cortex | L | -40 | 20 | 2 | 0.0060 | 0.0019 |
| Inferior frontal gyri | BL | -48 56 | 32 10 | 6 4 | 0.0059 0.0058 | 0.0019 0.0031 |
| Superior frontal gyrus | L | -8 | 38 | 46 | 0.0075 | 0.0003 |
| Perigenual anterior cingulate cortex | R | 10 | 34 | 12 | 0.0074 | 0.0003 |
| Dorsal anterior cingulate cortex | BL | -2 8 | 22 22 | 34 20 | 0.0074 0.0058 | 0.0003 0.0031 |
| Caudate | R | 16 | 24 | 6 | 0.0074 | 0.0003 |
| Paracingulate gyrus | L | -8 | 22 | 46 | 0.0073 | 0.0004 |
| Subgenual anterior cingulate cortex | R | 16 | 42 | 0 | 0.0057 | 0.0034 |
| Insula (anterior) | R | 38 | 20 | 0 | 0.0074 | 0.0004 |
| Frontal orbital cortex | R | 40 | 30 | -2 | 0.0074 | 0.0004 |
| Putamen | R | 24 | 12 | -4 | 0.0074 | 0.0004 |
| Decreased Brain Activity (<i>n</i> = 29, studies = 3) | | | | | | |
| Temporal occipital fusiform gyrus | BL | -30 27 | -46 -46 | -9 -12 | 0.0112 0.0073 | <0.0001 <0.0001 |
| Parahippocampal gyrus | BL | -26 29 | -28 -27 | -21 -20 | 0.0060 0.0102 | 0.0004 <0.0001 |
| Brainstem (pons) | - | -5 | -30 | -28 | 0.0060 | 0.0004 |
| Hippocampus | BL | -28 33 | -21 -16 | -13 -16 | 0.0060 0.0074 | 0.0004 <0.0001 |
| Temporal pole | R | 54 | 12 | -25 | 0.0058 | 0.0005 |
| Middle temporal gyrus | R | 53 | 3 | -24 | 0.0057 | 0.0006 |

Note: Brain region provides a location for the altered neural activity detected, the ALE score represents a measure of spatial concordance among studies, coordinates (x, y, z) provide the center of mass of the cluster of activation, and p represents the p value for cluster significance. For regions bilaterally related to tVNS, the left hemispheric region coordinate is reported first followed by the right hemisphere, separated by a vertical bar. Abbreviations: ALE, activated likelihood estimation; BL, bilateral; L, left; R, right.

fusiform cortex (all $p < 0.0009$) (Table 3 and Figure 3B). We provide the meta-analytic result NIFTI as supplementary data (S4 and S5).

3.3 | Comparison for sham stimulation vs. null stimulation

Sham stimulation – contrasted to no stimulation – was associated with increased activity in the right insula, right parietal operculum, right central opercular cortex, and right postcentral gyrus (Table 4 and Figure 4A). Similarly, reduced activity was observed in the left frontal operculum cortex, left posterior cingulate, left frontal pole,

left paracingulate cortex, left subcallosal cortex, right lateral occipital cortex, right cerebellum, and the bilateral precentral gyri (all corrected $p < 0.001$) (Table 4 and Figure 4B).

4 | DISCUSSION

In this meta-analysis, we reveal the brain regions whose functional activity is most plausibly affected by tVNS, statistically contrasted against both sham and null stimulation. By coalescing all previous relevant literature in a statistically formalized way, using a validated meta-analytic framework, we posit these meta-analytic maps provide to date the best approximation of the ground truth as to

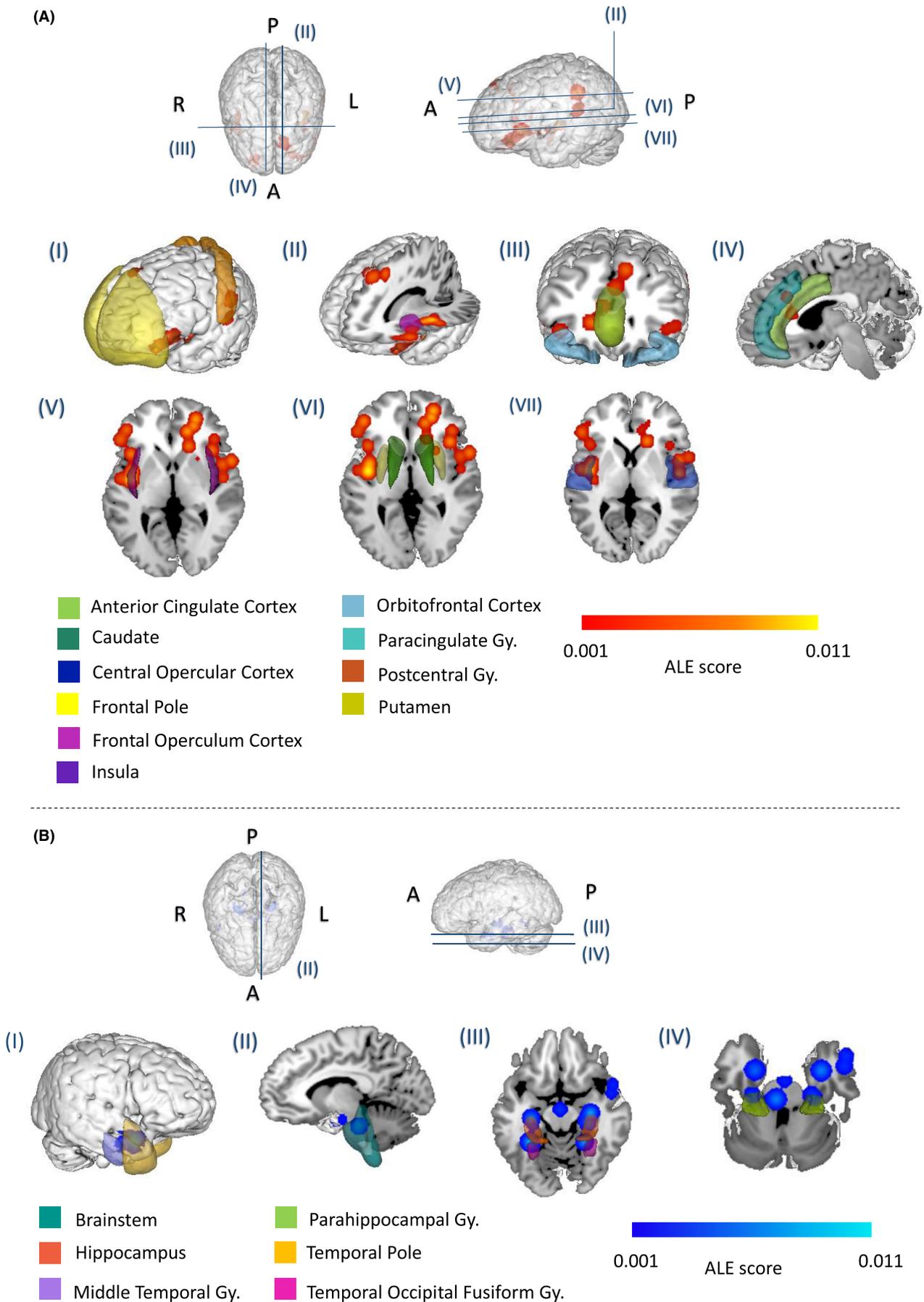


FIGURE 2 Results of the ALE analysis showing brain regions associated with (A) increased activity tVNS vs. null stimulation and (B) decreased activity tVNS vs. null stimulation. , anterior; ALE, activated likelihood estimation; L, left; P, posterior; R, right

TABLE 3 Brain activation for tVNS compared to sham stimulation in healthy participants according to ALE

| Brain Region | Hemisphere | x | y | z | Max ALE Score (4.d.p.) | p Value (4.d.p.) |
|--|------------|-----|-----|-------|------------------------|------------------|
| Increased Brain Activity (n = 38, studies = 3) | | | | | | |
| Perigenual anterior cingulate cortex | R | 6 | 36 | 12 | 0.0102 | <0.0001 |
| Paracingulate gyri | L | -2 | 42 | 30 | 0.0075 | 0.0002 |
| Dorsal anterior cingulate cortex | L | -2 | 16 | 34 | 0.0074 | 0.0003 |
| Caudate | L | -12 | -2 | 16 | 0.0074 | 0.0004 |
| Thalamus | L | -10 | -16 | 10 | 0.0074 | 0.0004 |
| Frontal pole | R | 24 | 46 | -6 | 0.0058 | 0.0021 |
| Brainstem | - | 2.6 | -28 | -16.6 | 0.0025 | 0.0232 |
| Decreased Brain Activity (n = 21, studies = 2) | | | | | | |
| Brainstem (Pons) | - | -6 | -28 | -30 | 0.0105 | <0.0001 |
| Posterior cingulate | L | -4 | -52 | 24 | 0.0059 | 0.0001 |
| Thalamus | R | 20 | -26 | 12 | 0.0058 | 0.0003 |
| Temporal occipital fusiform cortex | L | -32 | -46 | -10 | 0.0057 | 0.0003 |
| Brainstem (Medulla) | - | -2 | -45 | -60 | 0.0046 | 0.0008 |

Note: Brain region provides a location for the altered neural activity detected, the ALE score represents a measure of spatial concordance among studies, coordinates (x, y, z) provide the center of mass of the cluster of activation, and p represents the p value for cluster significance.

Abbreviations: ALE, activated likelihood estimation; BL, bilateral; L, left; R, right.

how tVNS acutely modifies brain function in health. We illustrate a change in activity of numerous brain regions sequential to tVNS, ranging across the cortex, including frontal, temporal and parietal lobes; subcortex, including basal ganglia and thalamus; and brainstem level. Importantly, brain regions identified align with established regions implicated in both the physiological regulation of the autonomic nervous system and regions whose activity is disrupted across a range of FGIDs.^{19,27} As such, a neural basis for the purported efficacy of tVNS shown in FGID clinical trials may be identified.

4.1 | The brain signature of tVNS

The meta-analysis found evidence for significantly decreased activity in the parahippocampal gyri and hippocampi during the active tVNS state. Reduced activity in these regions is consistently reported in the broader literature related to tVNS.^{22,24-26} For example, Kraus et al. demonstrated reduced activity in limbic areas of the brain including the hippocampi and parahippocampal gyri, alongside reports of significantly improved wellbeing scores following tVNS but not in the sham stimulation group which indicates that tVNS could assert its mood-enhancing effects through modulation of the limbic system.²⁵ Moreover, the tVNS-induced improvements in diagnostic scales for depression are associated with neurophysiological changes in limbic circuitry.³⁴ For example, Fang et al. have shown that statistically significant reductions in depression scores observed following a treatment regime of tVNS in patients with depression are associated with reduced functional connectivity between the default mode network and anterior insula and parahippocampal gyri.³⁵ Considering the well-established evidence to support a bidirectional

link between the brain and the gut, it is reasonable to speculate that these mood-altering effects could instigate changes in gastrointestinal function.⁵

Furthermore, the meta-analysis also demonstrated increased activity in the cingulate cortex, a key component of both limbic circuitry and the CAN,³⁶ during active tVNS compared to sham stimulation. Neuroanatomically, tVNS-associated altered cingulate cortex activity likely occurs as a consequence of activation of the mono-synaptic projections ascending from the nucleus tractus solitarius to higher cortical structures.³⁷⁻³⁹ As an area of significance for emotional regulation, behavioral flexibility, and the affective component of pain, modulation of the cingulate cortex may assert favorable behavioral effects.^{40,41} Indeed, studies of tVNS in patients with depression have demonstrated that the strength of functional connectivity of the rostral ACC is directly associated with improvements in depression scores.³⁴ Given our findings of reproducibly altered brain activity induced by tVNS in key pain and emotion processing nodes, tVNS could utilize these neuroanatomical relay stations to assert its gastrointestinal therapeutic effects.

Our data reveal increased activity in the frontal and temporal lobes – in regions comprising the left superior, bilateral inferior frontal gyri, bilateral frontal pole, and bilateral central opercular cortex – sequentially associated with tVNS. Activations within the frontal lobe likely arise as a consequence of vagal afferents stimulating higher-order brain centers such as the orbitofrontal and prefrontal cortices. tVNS-induced activity in these regions has been suggested to play a role in mediating analgesic and antidepressant qualities.^{22,35,40,42-44} Of note, our finding of tVNS-induced decreased temporal lobe activity in regions comprising the right temporal pole, right middle temporal gyrus, and bilateral temporal occipital fusiform gyri is contrary to what has

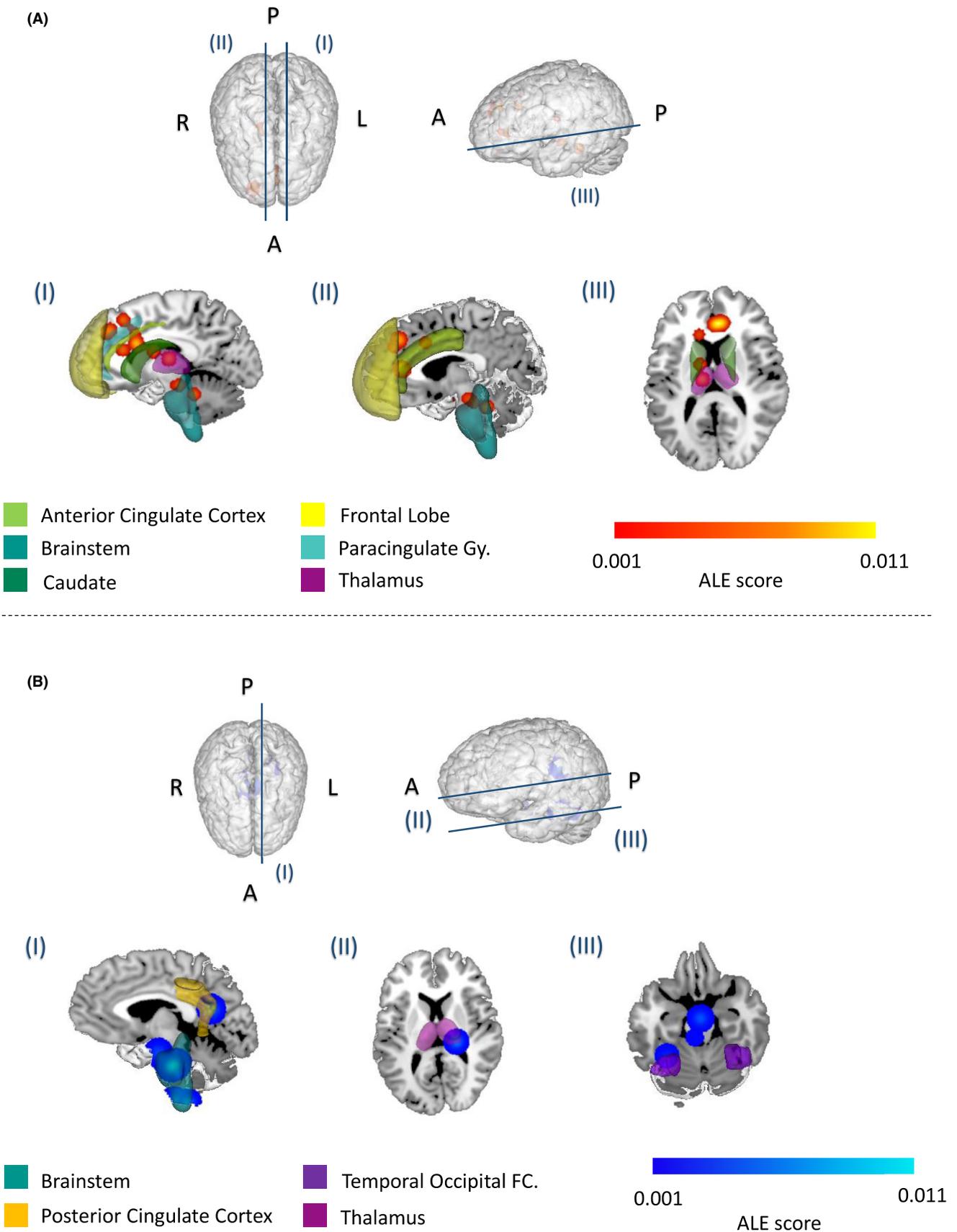


FIGURE 3 Results of the ALE analysis showing brain regions associated with (A) increased activity tVNS vs. sham stimulation and (B) decreased activity tVNS vs. sham stimulation. A, anterior; ALE, activated likelihood estimation; FC, fusiform cortex; L, left, P, posterior; R, right

TABLE 4 Brain activation for sham stimulation compared to null stimulation in healthy participants according to ALE

| Brain Region | Hemisphere(s) | x | y | z | Max. ALE Score (4.d.p.) | p value (4.d.p.) |
|---|---------------|-----|---------|-------|-------------------------|------------------|
| Increased Brain Activity (<i>n</i> = 52, studies = 4, foci = 25) | | | | | | |
| Insula (middle) | R | 36 | -14 | 18 | 0.0089 | <0.0001 |
| Parietal operculum | R | 40 | -20 | 20 | 0.0089 | <0.0001 |
| Central opercular cortex | R | 48 | -4 | 8 | 0.0086 | <0.0001 |
| Insula (posterior) | R | 44 | -6 | 0 | 0.0079 | <0.0001 |
| Postcentral gyrus | R | 62 | -16 | 22 | 0.0075 | 0.0001 |
| Decreased Brain Activity (<i>n</i> = 35, studies = 3, foci = 9) | | | | | | |
| Frontal operculum cortex | L | -40 | 20 | 2 | 0.0079 | <0.0001 |
| Posterior cingulate cortex | L | -4 | -52 | 24 | 0.0078 | <0.0001 |
| Frontal pole | L | -26 | 60 | 10 | 0.0076 | <0.0001 |
| Paracingulate cortex | L | -6 | 38 | -12 | 0.0076 | <0.0001 |
| Subcallosal cortex | L | -4 | 11 | -10 | 0.0075 | <0.0001 |
| Lateral occipital cortex | R | 38 | -84 | -8 | 0.0074 | <0.0001 |
| Precentral gy | BL | 0 4 | -26 -26 | 60 60 | 0.0050 0.0049 | 0.0008 0.0008 |
| Cerebellum | R | 36 | -40 | -32 | 0.0049 | 0.0008 |

Note: Brain region provides a location for the altered neural activity detected, the ALE score represents a measure of spatial concordance among studies, coordinates (x, y, z) provide the center of mass of the cluster of activation, and p represents the p-value for cluster significance. For regions bilaterally related to sham stimulation, the left hemispheric region coordinate is reported first followed by the right hemisphere, separated by a vertical bar.

Abbreviations: ALE, activated likelihood estimation; BL, bilateral; L, left; R, right.

been observed following invasive vagal nerve stimulation (iVNS), which has been associated with increased temporal lobe activity.⁴⁵ To explain this finding, reference has been made to needle and electrical acupuncture neuroimaging studies which have frequently reported reduced activity of the temporal lobes, and therefore, it could be inferred that decreased temporal lobe activation may serve as a neural correlate for the transcutaneous technique itself, rather than a direct consequence of CNX modulation.^{25,45-47}

Another finding of note was the presence of increased activity in the caudate and putamen, regions of the brain comprising the basal ganglia, all structures implicated in normal ANS physiological regulation.^{2,19} This concept is strengthened by evidence that perturbations in the basal ganglia have been associated with conditions involving dysautonomia, such as Parkinson's disease.^{2,20,48,49} Hence, it is reasonable to speculate that increased tVNS-induced activity in these brain regions could be associated with vagally mediated alterations in autonomic control, lending further support to the proposed mechanism of action.

Our study also found evidence of increased activity in the bilateral insula, well implicated in the CAN.¹⁷ Considering the functional importance of the insula in sensory processing and emotional cognition, it can be speculated that altered activity in this region may be a mediator of tVNS-induced analgesic and antidepressive qualities.^{23,50} This concept is supported by prior neuroimaging research that has shown that lower pre-treatment levels of metabolic activity detected in the anterior insula are predictive of a therapeutic response to VNS in treatment-resistant major depression, as validated through symptom severity scores.⁵¹

Noteworthy, our finding of tVNS-induced changes in brain activity in key neural substrates comprising the thalami, hypothalamus, orbitofrontal cortex, anterior cingulate cortex, and temporal pole closely mimics prior reports of neural activity sequential to invasive vagal nerve stimulation (iVNS).⁵²⁻⁵⁴ Importantly, these changes have been noted across a variety of neuroimaging techniques including positron emission tomography (PET), single photon emission computed tomography (SPECT), and fMRI, lending further support to our proposed tVNS brain mechanism.⁴⁵ It should however be noticed that the mainstay of iVNS neuroimaging literature focuses on elucidating a functional brain network in disease states, as opposed to identifying VNS neural correlates in the healthy individual, as was the aim of our study.

4.2 | Sham stimulation mimics some tVNS brain effects

Though we present evidence that tVNS induces increased insula and decreased posterior cingulate cortex activity, it is important to note that we observed a similar pattern of activity for sham stimulation when statistically contrasted to null stimulation in these brain regions. Considering three of the four studies included in our meta-analysis incorporated participant blinding in study design, it is possible that these findings of altered brain activity in the absence of active treatment are in part attributable to some form of placebo effect. As the insula and the posterior cingulate cortex are areas of the brain that are important in emotional regulation^{50,55} and

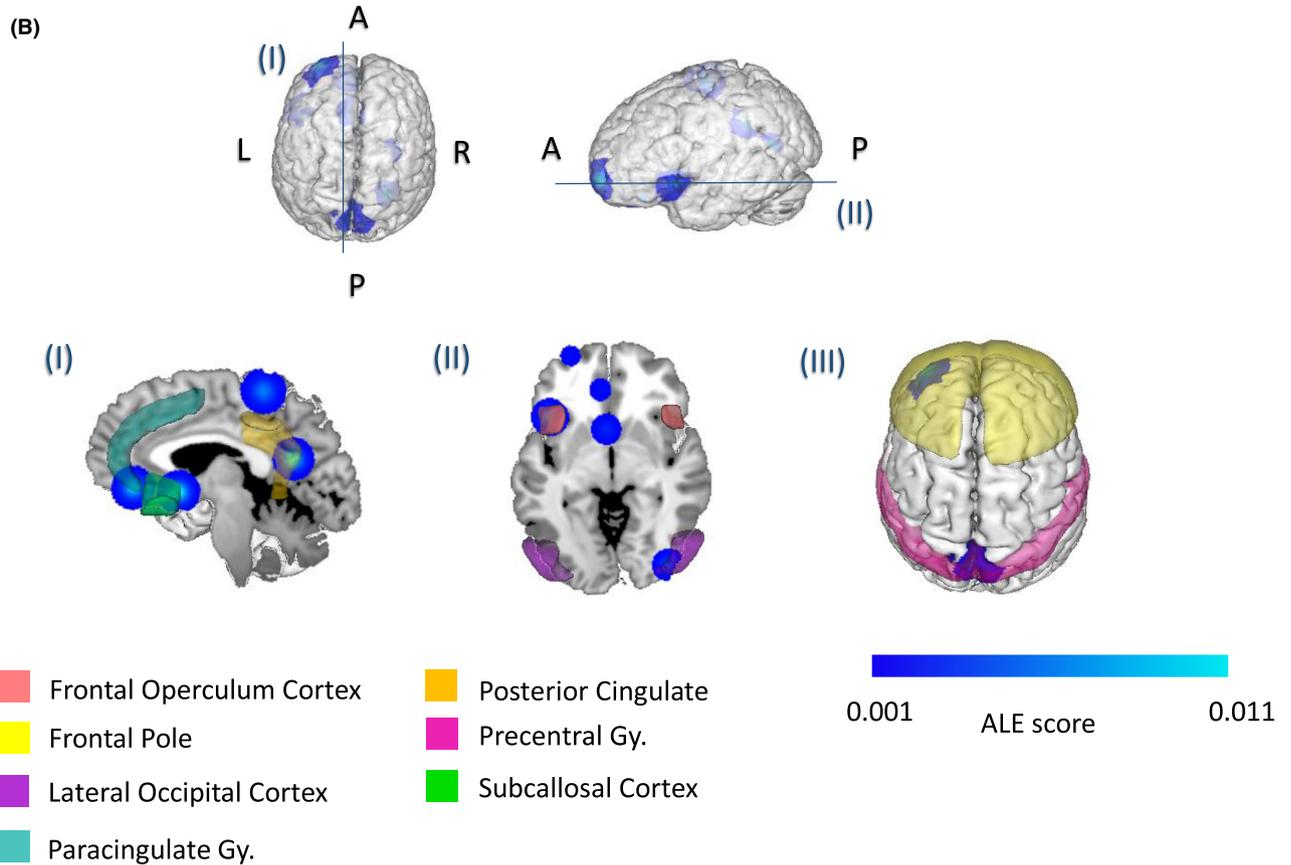
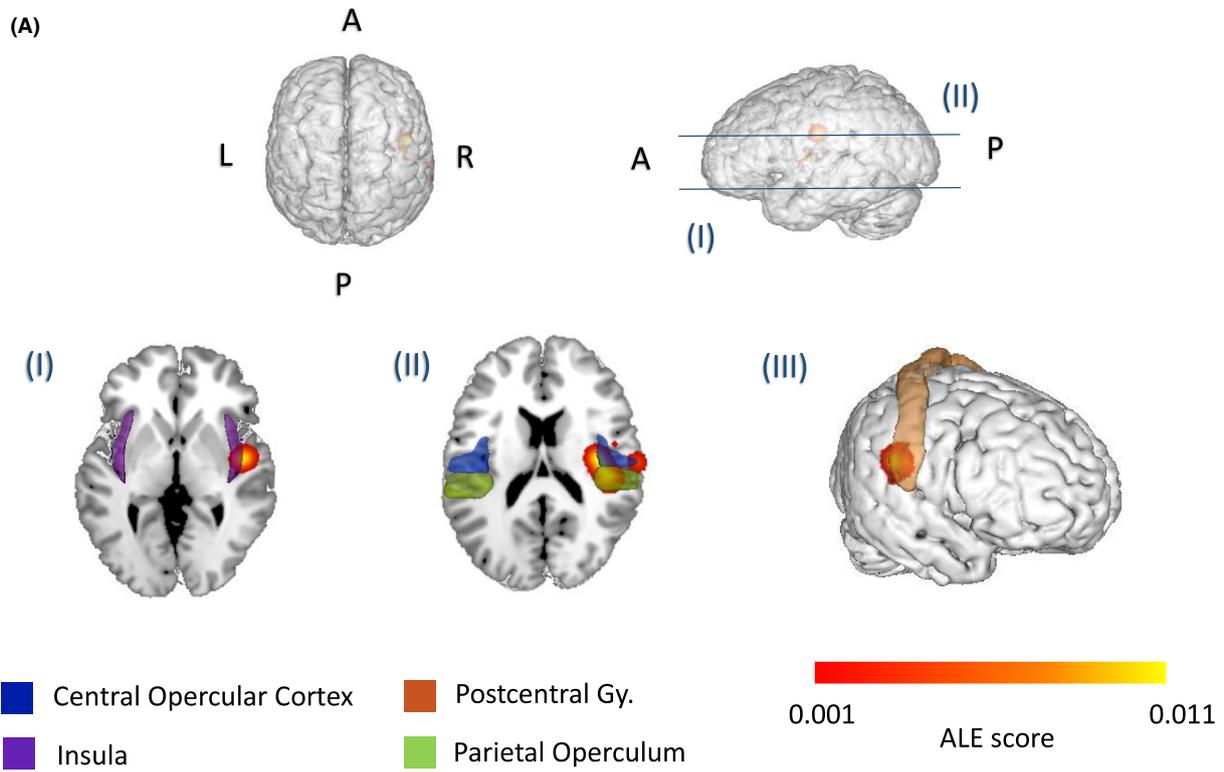


FIGURE 4 Results of the ALE analysis showing brain regions associated with (A) increased activity sham stimulation vs. null stimulation and (B) decreased activity sham stimulation vs. null stimulation. A, anterior; ALE, activated likelihood estimation; Gy, gyrus; L, left; P, posterior; R, right

self-reflection^{56–58} – both mechanisms which interface with the perception of pain – it is reasonable to propose that to eliminate any confounding effects observed in this brain region, it is essential for tVNS neuroimaging research to consistently utilize robust control stimulation procedures and to help elucidate the neural correlates of genuine tVNS effects.

4.3 | Rigorous statistical control is essential

Through systematic assessment of the risk of bias within tVNS neuroimaging literature, a number of issues have become apparent. Firstly, in view of the fact that the present analysis found evidence of a similar pattern of brain activation across both tVNS and control stimulation states, there seems reason to doubt the adequacy of current control locations. What is more, the only tVNS fMRI study to use a marker of parasympathetic tone (high-frequency heart rate variability) has shown this parameter is increased by both control and active intervention states, confirming that the control regions used in the current literature cannot be considered 'physiologically inert'.^{59–61} By improving the validity of future research, it is essential that adequate understanding of the effects driven by mere control stimulation is known, so as to not confound the results presented by the active site.

A second issue of contention refers to the inadequate utilization of explicitly stated and robustly conducted randomization and blinding procedures, implying the risk of bias in trials to date cannot be considered low. Currently, most trials do not use a randomized trial design; they simply conduct both sham and active stimulation on the same individual, with either minutes or days between interventions, if any control stimulation is even used. Though this carries the benefit of assessing changes in neural activation patterns to different interventions within the same individual, it introduces the possibility that participants may be expecting the intervention with an inadequate wash-out period, thereby confounding the results.

4.4 | Heterogeneity of tVNS parameters

A further limitation presented by the current literature lies in the substantial differences in experimental protocols utilized. Regretfully, it has become commonplace across studies investigating tVNS to utilize an immense variety of stimulus parameters, which significantly hinders the ability to draw clear inference as to the efficacy of this device. It is likely that this significant heterogeneity among trials contributes to the inconsistent results across the wider literature. Testimony to this argument are articles identifying similar brain regions associated with tVNS when comparable stimulation parameters were used: for example, both Badran et al. and Yakunina et al. demonstrate similarity in the pulse width and stimulation frequency used and consequently observed comparable effects in the angular gyrus, caudate, cerebellum, cingulate, and frontal cortex.^{23,26}

Another important limitation in the literature is the large number of interventional studies exploring the effects of tVNS on clinical populations, seemingly despite lack of an established evidence base of the physiological mechanism on those healthy. Considering this medical device in the same remit as a medicinal product, then pre-clinical testing and phase I clinical trials should necessitate its safety, efficacy, and dosimetry (for tVNS, its stimulation parameters). Yet, to date there exists a wealth of investigatory studies using tVNS across a vast array of pathology, with far smaller numbers aiming to derive expected function on normal physiology. Specific to brain imaging, our meta-analysis demonstrates only four neuroimaging studies with published coordinates providing information about specific central effects induced by tVNS in healthy populations. Yet, the clinical effects of tVNS have been broadly assessed in different disease contexts including depression, migraine, epilepsy, and functional pain syndromes.^{11,35,37,62–64}

4.5 | Strengths and limitations

Though the present analysis has provided some strong points of commonality regarding brain activation in tVNS research, there are some limitations to consider. Firstly, although every effort was made to acquire maximal data, only four trials were suitable to be considered in the ALE meta-analyses. Additionally, within these four studies, there were constraints on the data, leading to some ALE calculations only considering 2 experimental groups and 9 foci in their estimations. Due to these limitations in available data, our study reports some evidence of arguably inconsistent alterations in brain activity. For example, relating to the comparison of tVNS vs. sham stimulation, we identified a pattern of increased activity in the left thalamus, concomitant with decreased activity in the right thalamus. Moreover, we also identify no significant findings of altered brain activity to some limbic regions such as the amygdala, contrary to our expectation for this neural substrate which has been suggested to play an integral role in the CAN. It is anticipated that with further – and larger scale – research, an effect of tVNS on additional brain regions would be more suitably characterized. Further, the limited consideration of autonomic parameters in existing tVNS neuroimaging studies to date restricts one's ability to draw inference between alterations in functional activity to changes in physiological monitoring of the autonomic state, such as with cardiovascular or respiratory-based monitoring, which would be an important area for future study. Further, considerations could not be made for temporal factors pertaining to the initiation of stimulation and the commencement of the imaging sequence. To our strength, by capturing this heterogeneous stimulation data and its attached central brain signature, it seems plausible that we offer a best approximation of the brain effects of tVNS across the wide variety of stimulation parameters that groups instigate, quantified with a robust and well-validated meta-analytic neuroimaging statistical methodology.

The present study was unable to assert conclusive changes in the brainstem due to the discord between the findings presented

across all studies. While a pattern of reduced activity was observed in brainstem of our ALE analysis, we also note the converse has been reported in some (but not all) literature (we provide a full literature review of all relevant data as Table S2).^{21,22,24,61,65} It seems plausible that the disparity in these findings is threefold: (i) due to the limited availability of studies for the ALE analysis detailing brainstem findings in healthy participants (only one study), (ii) due to the existing predominance of tVNS neuroimaging studies utilizing a whole brain approach with slice acquisitions too broad to reliably infer perturbations at the brainstem level, and (iii) the multi-faceted role and large number of nuclei that comprise the brainstem. The brainstem is a difficult area to image with conventional whole brain field of view imaging paradigms, now seemingly forming an entirely separate domain of neuroimaging with finer voxel resolutions, 7 T strength, and dedicated brainstem sequences. Given the caliber of brainstem nuclei implicated in autonomic regulation (such as the locus coeruleus, which is approximately 1–2 mm in its widest axial plane), for whole brain fMRI paradigms the volume of a single voxel would often exceed nuclei size. As such, we would recommend some caution in the ability to reliably characterize brainstem effects under these constraints and welcome dedicated brainstem imaging studies to better probe this area for further characterization of its influence in autonomic regulation.

4.6 | Future directions

We reason three major future directions. *Firstly*, as the literature to date has shown that anatomical locations and temporal considerations can amplify the neural response to tVNS, optimization of tVNS parameters and stimulation sites is important for future research.^{22,26,61} *Secondly*, considering the reported efficacy of tVNS across numerous FGIDs, it would be clinically meaningful to ascertain changes in brain activity induced by tVNS in this patient group. *Thirdly*, in view of the proposed brain effects of tVNS on the CAN – yet the sparse availability of tVNS neuroimaging studies implementing robust use of autonomic parameters into study design – we pose further utilization of physiological correlates of autonomic activity such as heart rate variability (HRV) as an important adjunct for future research.⁶⁶

5 | CONCLUSION

This meta-analysis coalesces previous tVNS and brain imaging literature in healthy individuals to provide a best approximation of its effect on brain processing. In order to justify the usage of any new therapeutic device, understanding its precise mechanism is vital in assuring all physiological and clinical implications are well understood. We identify an array of brain regions with either increased or decreased activity, making this data directly available for further study. Notably, the majority of regions are implicated in both physiological autonomic regulation and similarly are areas with modulated

activity across the FGIDs. As such, these findings may provide a neural basis for the efficacy of tVNS shown across FGID clinical trials. Choice of control stimulation paradigm significantly influences findings. Future research should interrogate robust sham stimulation procedures and further characterize brain effects of tVNS in FGID populations.

AUTHOR CONTRIBUTION

R.R., J.K.R.: pioneered study concept design; acquisition of data; statistical analysis; manuscript preparation, study supervision and critical revision of manuscript for intellectual content. Q.A., K.T.: pioneered study concept and design, study supervision and critical revision of manuscript for intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

ACKNOWLEDGMENTS

This research has been previously presented at the United European Gastroenterology (UEG) Week Virtual 2020 and the European Congress of Radiology 2021.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

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

ORCID

Rebekah Rajiah  <https://orcid.org/0000-0001-9315-9157>

James K. Ruffle  <https://orcid.org/0000-0001-6248-7203>

REFERENCES

1. Jänig W. *Integrative Action of the Autonomic Nervous System: Neurobiology of Homeostasis*. Cambridge University Press; 2008.
2. Ruffle JK, Coen SJ, Giampietro V, et al. Morphology of subcortical brain nuclei is associated with autonomic function in healthy humans. *Hum Brain Mapp*. 2018;39(1):381-392. doi:10.1002/hbm.23850
3. Ruffle JK, Patel A, Giampietro V, et al. Functional brain networks and neuroanatomy underpinning nausea severity can predict nausea susceptibility using machine learning. *J Physiol*. 2019;597(6):1517-1529. doi:10.1113/JP277474
4. Drossman DA. Functional gastrointestinal disorders: history, pathophysiology, clinical features, and Rome IV. *Gastroenterology*. 2016;150(6):1262-1279. doi:10.1053/j.gastro.2016.02.032
5. Farmer AD, Aziz Q. Visceral pain hypersensitivity in functional gastrointestinal disorders. *Br Med Bull*. 2009;91(1):123-136. doi:10.1093/bmb/ldp026
6. Tsakiris M, Preester HD. *The Interoceptive Mind: from Homeostasis to Awareness*. Oxford University Press; 2018.
7. Butt MF, Albusoda A, Farmer AD, Aziz Q. The Anatomical Basis for Transcutaneous Auricular Vagus Nerve Stimulation. *J Anat*. 2019;236:588-611. doi:10.1111/joa.13122

8. Shi X, Hu Y, Zhang B, Li W, Chen JD, Liu F. Ameliorating effects and mechanisms of transcutaneous auricular vagal nerve stimulation on abdominal pain and constipation. *JCI Insight*. 2021;6(14):2-9. doi:10.1172/jci.insight.150052
9. Zhu Y, Xu F, Lu D, et al. Transcutaneous auricular vagal nerve stimulation improves functional dyspepsia by enhancing vagal efferent activity. *Am J Physiol Gastrointest Liver Phys Ther*. 2021;320(5):G700-G711. doi:10.1152/ajpgi.00426.2020
10. Farmer AD, Albusoda A, Amarasinghe G, et al. Transcutaneous vagus nerve stimulation prevents the development of, and reverses, established oesophageal pain hypersensitivity. *Aliment Pharmacol Ther*. 2020;52:988-996. doi:10.1111/apt.15869
11. Kovacic K, Hainsworth K, Sood M, et al. Neurostimulation for abdominal pain-related functional gastrointestinal disorders in adolescents: a randomised, double-blind, sham-controlled trial. *Lancet Gastroenterol Hepatol*. 2017;2(10):727-737. doi:10.1016/S2468-1253(17)30253-4
12. Bonaz B, Picq C, Sinniger V, Mayol JF, Clarençon D. Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. *Neurogastroenterol Motil*. 2013;25(3):208-221. doi:10.1111/nmo.12076
13. Bonaz B, Sinniger V, Pellissier S. Anti-inflammatory properties of the vagus nerve: potential therapeutic implications of vagus nerve stimulation. *J Physiol (Lond)*. 2016;594(20):5781-5790. doi:10.1113/JP271539
14. Busch V, Zeman F, Heckel A, Menne F, Ellrich J, Eichhammer P. The effect of transcutaneous vagus nerve stimulation on pain perception – an experimental study. *Brain Stimul*. 2013;6(2):202-209. doi:10.1016/j.brs.2012.04.006
15. Lerman I, Davis B, Huang M, et al. Noninvasive vagus nerve stimulation alters neural response and physiological autonomic tone to noxious thermal challenge. *PLoS ONE*. 2019;14(2):e0201212. doi:10.1371/journal.pone.0201212
16. Lerman I, Hauger R, Sorokin L, et al. Noninvasive transcutaneous Vagus nerve stimulation decreases whole blood culture-derived cytokines and chemokines: a randomized, blinded, healthy control pilot trial. *Neuromodulation*. 2016;19(3):283-290. doi:10.1111/ner.12398
17. Critchley HD, Harrison NA. Visceral influences on brain and behavior. *Neuron*. 2013;77(4):624-638. doi:10.1016/j.neuron.2013.02.008
18. Drake RL, Vogl AW, Mitchell AWM. Head and neck. In: *Gray's Anatomy for Students*. 4th ed. 2020:823-1121. Elsevier. <https://www.clinicalkey.com/#!/content/book/3-s2.0-B9780323393041000087?scrollTo=%23h0005384>. Accessed April 4, 2020.
19. Ruffle JK, Hyare H, Howard MA, et al. The autonomic brain: multi-dimensional generative hierarchical modelling of the autonomic connectome. *Cortex*. 2021;143:164-179. doi:10.1016/j.cortex.2021.06.012
20. Ruffle JK, Coen SJ, Giampietro V, Williams SCR, Aziz Q, Farmer AD. Preliminary report: parasympathetic tone links to functional brain networks during the anticipation and experience of visceral pain. *Sci Rep*. 2018;8(1):13410. doi:10.1038/s41598-018-31522-2
21. Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the Vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. *Brain Stimul*. 2015;8(3):624-636. doi:10.1016/j.brs.2014.11.018
22. Kraus T, Kiess O, Hösl K, Terekhin P, Kornhuber J, Forster C. CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal - a pilot study. *Brain Stimul*. 2013;6(5):798-804. doi:10.1016/j.brs.2013.01.011
23. Badran BW, Dowdle LT, Mithoefer OJ, et al. Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul*. 2018;11(3):492-500. doi:10.1016/j.brs.2017.12.009
24. Frangos E, Komisaruk BR. Access to vagal projections via cutaneous electrical stimulation of the neck: fMRI evidence in healthy humans. *Brain Stimul*. 2017;10(1):19-27. doi:10.1016/j.brs.2016.10.008
25. Kraus T, Hösl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *J Neural Transm (Vienna)*. 2007;114(11):1485-1493. doi:10.1007/s00702-007-0755-z
26. Yakunina N, Kim SS, Nam EC. Optimization of transcutaneous Vagus nerve stimulation using functional MRI. *Neuromodulation*. 2017;20(3):290-300. doi:10.1111/ner.12541
27. Beissner F, Meissner K, Bär KJ, Napadow V. The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function. *J Neurosci*. 2013;33(25):10503-10511. doi:10.1523/JNEUROSCI.1103-13.2013
28. Turkeltaub PE, Eickhoff SB, Laird AR, Fox M, Wiener M, Fox P. Minimizing within-experiment and within-group effects in activation likelihood estimation meta-analyses. *Hum Brain Mapp*. 2012;33(1):1-13. doi:10.1002/hbm.21186
29. Eickhoff SB, Bzdok D, Laird AR, Kurth F, Fox PT. Activation likelihood estimation meta-analysis revisited. *Neuroimage*. 2012;59(3):2349-2361. doi:10.1016/j.neuroimage.2011.09.017
30. Dockès J, Poldrack RA, Primet R, et al. In: Büchel C, Yeo T, Wager TD, eds. *NeuroQuery, Comprehensive Meta-Analysis of Human Brain Mapping*. Vol 9. eLife; 2020:e53385. doi:10.7554/eLife.53385
31. Yarkoni T, Poldrack RA, Nichols TE, Van Essen DC, Wager TD. Large-scale automated synthesis of human functional neuroimaging data. *Nat Methods*. 2011;8(8):665-670. doi:10.1038/nmeth.1635
32. Lancaster JL, Tordesillas-Gutiérrez D, Martinez M, et al. Bias between MNI and Talairach coordinates analyzed using the ICBM-152 brain template. *Hum Brain Mapp*. 2007;28(11):1194-1205. doi:10.1002/hbm.20345
33. Eickhoff SB, Laird AR, Grefkes C, Wang LE, Zilles K, Fox PT. Coordinate-based activation likelihood estimation meta-analysis of neuroimaging data: a random-effects approach based on empirical estimates of spatial uncertainty. *Hum Brain Mapp*. 2009;30(9):2907-2926. doi:10.1002/hbm.20718
34. Tu Y, Fang J, Cao J, et al. A distinct biomarker of continuous transcutaneous vagus nerve stimulation treatment in major depressive disorder. *Brain Stimul*. 2018;11(3):501-508. doi:10.1016/j.brs.2018.01.006
35. Fang J, Rong P, Hong Y, et al. Transcutaneous Vagus nerve stimulation modulates default mode network in major depressive disorder. *Biol Psychiatry*. 2016;79(4):266-273. doi:10.1016/j.biopsych.2015.03.025
36. Critchley HD, Mathias CJ, Josephs O, et al. Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain*. 2003;126(Pt 10):2139-2152. doi:10.1093/brain/awg216
37. Garcia RG, Lin RL, Lee J, et al. Modulation of brainstem activity and connectivity by respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) in migraine patients. *Pain*. 2017;158(8):1461-1472. doi:10.1097/j.pain.0000000000000930
38. Benarroch EE. The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin Proc*. 1993;68(10):988-1001. doi:10.1016/s0025-6196(12)62272-1
39. Saper CB. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annu Rev Neurosci*. 2002;25:433-469. doi:10.1146/annurev.neuro.25.032502.111311
40. Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci*. 2011;15(2):85-93. doi:10.1016/j.tics.2010.11.004
41. Sawamoto N, Honda M, Okada T, et al. Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula:

- an event-related functional magnetic resonance imaging study. *J Neurosci*. 2000;20(19):7438-7445.
42. Brighina F, De Tommaso M, Giglia F, et al. Modulation of pain perception by transcranial magnetic stimulation of left prefrontal cortex. *J Headache Pain*. 2011;12(2):185-191. doi:10.1007/s10194-011-0322-8
 43. Seminowicz DA, Wideman TH, Naso L, et al. Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function. *J Neurosci*. 2011;31(20):7540-7550. doi:10.1523/JNEUROSCI.5280-10.2011
 44. Aihara M, Ida I, Yuuki N, et al. HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. *Psychiatry Res*. 2007;155(3):245-256. doi:10.1016/j.psychres.2006.11.002
 45. Chae JH, Nahas Z, Lomarev M, et al. A review of functional neuroimaging studies of vagus nerve stimulation (VNS). *J Psychiatr Res*. 2003;37(6):443-455. doi:10.1016/s0022-3956(03)00074-8
 46. Hui KK, Liu J, Makris N, et al. Acupuncture modulates the limbic system and subcortical gray structures of the human brain: evidence from fMRI studies in normal subjects. *Hum Brain Mapp*. 2000;9(1):13-25. doi:10.1002/(sici)1097-0193(2000)9:1<13::aid-hbm2>3.0.co;2-f
 47. Kong J, Ma L, Gollub RL, et al. A pilot study of functional magnetic resonance imaging of the brain during manual and electroacupuncture stimulation of acupuncture point (LI-4 Hegu) in normal subjects reveals differential brain activation between methods. *J Altern Complement Med*. 2002;8(4):411-419. doi:10.1089/107555302760253603
 48. Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol*. 2015;21(37):10609-10620. doi:10.3748/wjg.v21.i37.10609
 49. Obeso JA, Rodriguez-Oroz MC, Stamelou M, Bhatia KP, Burn DJ. The expanding universe of disorders of the basal ganglia. *Lancet*. 2014;384(9942):523-531. doi:10.1016/S0140-6736(13)62418-6
 50. Uddin LQ, Nomi JS, Hebert-Seropian B, Ghaziri J, Boucher O. Structure and function of the human insula. *J Clin Neurophysiol*. 2017;34(4):300-306. doi:10.1097/WNP.0000000000000377
 51. Conway CR, Chibnall JT, Gangwani S, et al. Pretreatment cerebral metabolic activity correlates with antidepressant efficacy of vagus nerve stimulation in treatment-resistant major depression: a potential marker for response? *J Affect Disord*. 2012;139(3):283-290. doi:10.1016/j.jad.2012.02.007
 52. Bohning DE, Lomarev MP, Denslow S, Nahas Z, Shastri A, George MS. Feasibility of vagus nerve stimulation-synchronized blood oxygenation level-dependent functional MRI. *Invest Radiol*. 2001;36(8):470-479. doi:10.1097/00004424-200108000-00006
 53. Devous MD, Husain M, Harris TS, Rush AJ. Effects of VNS on regional cerebral blood flow in depressed subjects. *Eur Psychiatry*. 2002;17(S1):113s-114s. doi:10.1016/S0924-9338(02)80506-5
 54. Liu WC, Mosier K, Kalnin AJ, Marks D. BOLD fMRI activation induced by vagus nerve stimulation in seizure patients. *J Neurol Neurosurg Psychiatry*. 2003;74(6):811-813. doi:10.1136/jnnp.74.6.811
 55. Monkul ES, Silva LAP, Narayana S, et al. Abnormal resting state corticolimbic blood flow in depressed unmedicated patients with major depression: a (15)O-H(2)O PET study. *Hum Brain Mapp*. 2012;33(2):272-279. doi:10.1002/hbm.21212
 56. Craig ADB. How do you feel--now? the anterior insula and human awareness. *Nat Rev Neurosci* 2009;10(1):59-70. doi:10.1038/nrn2555
 57. Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ. Neural systems supporting interoceptive awareness. *Nat Neurosci*. 2004;7(2):189-195. doi:10.1038/nn1176
 58. Vogt BA, Laureys S. Posterior cingulate, precuneal & retrosplenial cortices: cytology & components of the neural network correlates of consciousness. *Prog Brain Res*. 2005;150:205-217. doi:10.1016/S0079-6123(05)50015-3
 59. Gilula MF, Kirsch DL. Cranial electrotherapy stimulation review: a safer alternative to psychopharmaceuticals in the treatment of depression. *J Neurother*. 2005;9(2):7-26. doi:10.1300/J184v09n02_02
 60. Kirsch DL, Nichols F. Cranial electrotherapy stimulation for treatment of anxiety, depression, and insomnia. *Psychiatr Clin North Am*. 2013;36(1):169-176. doi:10.1016/j.psc.2013.01.006
 61. Sclocco R, Garcia RG, Kettner NW, et al. The influence of respiration on brainstem and cardiovagal response to auricular vagus nerve stimulation: a multimodal ultrahigh-field (7T) fMRI study. *Brain Stimul*. 2019;12(4):911-921. doi:10.1016/j.brs.2019.02.003
 62. Bauer S, Baier H, Baumgartner C, et al. Transcutaneous Vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02). *Brain Stimul*. 2016;9(3):356-363. doi:10.1016/j.brs.2015.11.003
 63. Kiefe TM, Pinteá B, Muhammad S, et al. Cervical non-invasive vagus nerve stimulation (nVNS) for preventive and acute treatment of episodic and chronic migraine and migraine-associated sleep disturbance: a prospective observational cohort study. *J Headache Pain*. 2015;16:101. doi:10.1186/s10194-015-0582-9
 64. Tassorelli C, Grazi L, de Tommaso M, et al. Noninvasive vagus nerve stimulation as acute therapy for migraine: the randomized PRESTO study. *Neurology*. 2018;91(4):e364-e373. doi:10.1212/WNL.0000000000005857
 65. Yakunina N, Kim SS, Nam EC. BOLD fMRI effects of transcutaneous vagus nerve stimulation in patients with chronic tinnitus. *PLOS One*. 2018;13(11):e0207281. doi:10.1371/journal.pone.0207281
 66. Ruffle JK, Aziz Q, Farmer AD. Neuroimaging of vagal nerve stimulation: are we missing a trick? *Pain*. 2017;158(10):2053. doi:10.1097/j.pain.0000000000000987

SUPPORTING INFORMATION

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

How to cite this article: Rajiah R, Takahashi K, Aziz Q, Ruffle JK. Brain effect of transcutaneous vagal nerve stimulation: A meta-analysis of neuroimaging evidence. *Neurogastroenterology & Motility*. 2022;00:e14484. doi:10.1111/nmo.14484