TITLE

Brain Effect of Transcutaneous Vagal Nerve Stimulation: A Meta-Analysis of Neuroimaging Evidence

RUNNING TITLE

Brain Activity in Transcutaneous Vagal Nerve Stimulation

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All data used are publicly available and fully referenced in the manuscript.

KEY POINTS

- We present a neuroimaging meta-analysis that – by coalescing all previous studies - render 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.

KEY WORDS

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

ABSTRACT

BACKGROUND: Dysfunction to 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: 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.

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 healthcare 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 IBS [8]; 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]. Whilst 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 render 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.

MATERIALS & METHODS

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 Supplementary Table 1.

Study selection

A literature search was performed with PubMed via MEDLINE and Web of Science databases on 31st 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' (Supplementary Figure 1).

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 was missing from an article, we contacted the article's corresponding author to seek such data. This yielded a total of 12 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 male, 27 female and 16 where gender were 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 (Supplementary Tables 2 and 3).

[Insert Figure 1]

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, San Antonio, TX). 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 modelled 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 was 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.

[Insert Table 1]

RESULTS

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 post central 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.

[Insert Table 2]

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

[Insert Table 3]

[Insert Figure 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).

[Insert Table 4]

[Insert Figure 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 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.

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 is 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 monosynaptic 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 reveals 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 been observed following invasive vagal nerve stimulation (iVNS), which been associated with increased temporal lobe activation [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 anti-depressive 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].

Noteworthily, 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 mimic 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.

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 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 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, to help elucidate the neural correlates of genuine tVNS effects.

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]. In improving the validity of future research, it is essential that adequate understanding of the effects driven by mere control stimulation are 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.

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 to 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 preclinical 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].

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 restrict 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 heterogenous 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. Whilst 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 Supplementary Table 2) [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 notoriously 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, 7T strength, and dedicated brainstem sequences. Given the calibre of brainstem nuclei implicated in autonomic regulation (such the locus coeruleus) is approximately 1-2mm in its widest axial plane, often across whole brain fMRI paradigms the volume of a single voxel would exceed this. As such, we would recommend some caution in the ability to reliably characterise

brainstem effects under these constraints, and welcome dedicated brainstem imaging studies to better probe this area for further characterisation of its influence in autonomic regulation, including recent efforts in quantifying tVNS-induced brainstem effects in meta-analysis combining both healthy and clinical participants[66], which interestingly report – akin to the findings of our study – a mixed direction of activity within brainstem nuclei [66].

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 [67].

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.

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Declarations of interest: none

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TABLES

	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

Table 1: Data available for the 6 activated likelihood estimation analyses conducted.

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

Brain Region	*Hemisphere(s)*	*x*	*y*	*z*	*Max. ALE Score (4.d.p.)*	*P value (4.d.p.)*
Increased Brain Activity (n=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 (n=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

Table 2: Brain activation for tVNS compared to null stimulation in healthy participants according to ALE.

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 centre 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.

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

Table 3: Brain activation for tVNS compared to sham stimulation in healthy participants according to ALE.

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 centre 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.

Brain Region	***Hemisphere(s)***	***x***	***y***	***z***	***Max. ALE Score (4.d.p.)***	***P value (4.d.p.)***
Increased Brain Activity (n=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 (n=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

Table 4: Brain activation for sham stimulation compared to null stimulation in healthy participants according to ALE.

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 centre 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.

FIGURE LEGENDS

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. Abbreviations: ALE, activation of likelihood estimation; n, number of participants; tVNS, transcutaneous vagal nerve stimulation

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.

Abbreviations: A, anterior; ALE, activated likelihood estimation; L, left; P, posterior; R, right.

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.

Abbreviations: A, anterior; ALE, activated likelihood estimation; FC, fusiform cortex; L, left, P, posterior; R: right.

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.

Abbreviations: A, anterior; ALE, activated likelihood estimation; Gy, gyrus; L, left; P, posterior; R, right.