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Review

# Optimizing non-invasive vagus nerve stimulation for treatment in stroke

**Sheharyar S. Baig[1,*], Samantha Dorney[1], Mudasar Aziz[2], Simon M. Bell[1], Ali N. Ali[1], Li Su[1], Jessica N. Redgrave[1], Arshad Majid[1,*]**

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***From the Contents***



## Abstract

Stroke remains a leading cause of long-term disability worldwide. There is an unmet need for neuromodulatory therapies that can mitigate against neurovascular injury and potentially promote neurological recovery. Transcutaneous vagus nerve stimulation has been demonstrated to show potential therapeutic effects in both acute and chronic stroke. However, previously published research has only investigated a narrow range of stimulation settings and indications. In this review, we detail the ongoing studies of transcutaneous vagus nerve stimulation in stroke through systematic searches of registered clinical trials. We summarize the upcoming clinical trials of transcutaneous vagus nerve stimulation in stroke, highlighting their indications, parameter settings, scope, and limitations. We further explore the challenges and barriers associated with the implementation of transcutaneous vagus nerve stimulation in acute stroke and stroke rehabilitation, focusing on critical aspects such as stimulation settings, target groups, biomarkers, and integration with rehabilitation interventions.

**Key Words:** neuromodulation; neuroplasticity; rehabilitation; stroke; vagal nerve stimulation; vagus nerve stimulation

## Introduction

Stroke is a leading cause of adult-onset disability (GBD 2019 Stroke Collaborators, 2021). The estimated cost of stroke in the UK is £26 billion a year with a large proportion of this cost going towards unpaid care (Patel et al., 2020).

While there have been sizeable advances in acute stroke care with the wider availability of advanced neuroimaging and mechanical thrombectomy, most people with acute stroke are either ineligible or unable to access revascularization therapies (Jadhav et al., 2021). While current rehabilitation programs can promote recovery after stroke, around 50% of stroke survivors have persistent arm weakness (Wafa et al., 2020). In the chronic phases of stroke (defined here at > 6 months post-onset), the spontaneous recovery in limb function is limited, and therapy-mediated improvements require intensive programs that are inaccessible to many by way of availability, cost, time commitments or functional ability (Ward et al., 2019).

Invasive vagus nerve stimulation (VNS) paired with rehabilitation has been demonstrated to significantly improve upper limb motor function in people with chronic stroke (Dawson et al., 2016, 2021). Non-invasive vagus nerve stimulation can be delivered via transcutaneous stimulation (tVNS) of either the auricular branch in the outer ear or the cervical branch in the neck (Redgrave et al., 2018; Baig et al., 2019). **Figure 1** outlines the stimulation sites for invasive VNS and auricular tVNS. Invasive VNS requires an operation to implant an electrode cuff and an electrical stimulator; VNS devices are commonly used. Auricular tVNS (taVNS) activates an afferent branch of the vagus nerve which terminates in the nucleus tractus solitarii with ongoing projections to noradrenergic and cholinergic pathways within the brain (Baig et al., 2023); this can be stimulated using commercially available wearable devices. Cervical tVNS (tcVNS) activates afferent and efferent vagus nerve fibers and is typically delivered by handheld stimulators on the skin of the neck; these are frequently used in clinical practice e.g. for headache disorders (Baig et al., 2023).

tVNS has been shown to be safe, tolerable and to activate brainstem nuclei associated with vagus nerve activation (Kraus et al., 2007; Baig et al., 2022). Small, pilot randomized controlled trials of tVNS have been promising (Capone et al., 2017; Redgrave et al., 2018; Baig et al., 2019). In acute stroke in humans, tVNS can be delivered safely with some early

[1]Sheffield Institute for Translational Neuroscience, Department of Neuroscience, University of Sheffield, Sheffield, UK; [2]Doncaster and Bassetlaw Teaching Hospitals NHS Foundation Trust, Doncaster, UK

***Correspondence to:** Sheharyar S. Baig, sbaig1@sheffield.ac.uk; Arshad Majid, MD, Arshad.majid@sheffield.ac.uk.
https://orcid.org/0000-0003-1415-8784 (Sheharyar S. Baig); https://orcid.org/0000-0002-1144-039X (Arshad Majid)

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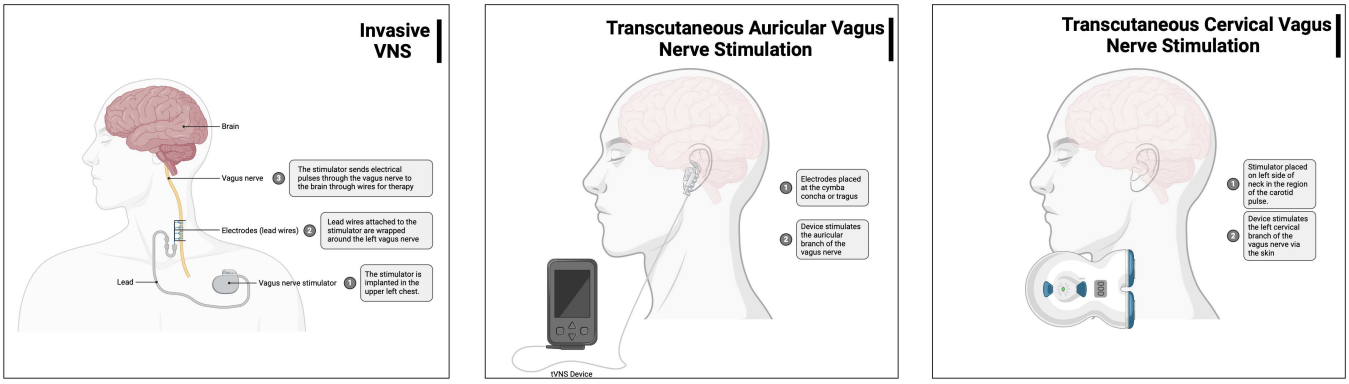


**Figure 1** | **Invasive vagus nerve stimulation (VNS) compared with transcutaneous vagus nerve stimulation.**
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suggestion of a trend towards reduction in relative ischemic lesion growth on diffusion weighted imaging MRI (Arsava et al., 2022). In chronic stroke, there is early evidence that tVNS paired with rehabilitation can promote motor recovery in chronic stroke akin to invasive VNS with studies showing significant improvements in the upper limb Fugl-Meyer score after 6 weeks of in-clinic therapy (Redgrave et al., 2018).

There are several mechanisms through which tVNS may counteract post-stroke inflammatory cascades and improve long-term outcomes in stroke. In acute stroke, these include reducing cortical spreading depression, reducing expression of inflammatory cytokines and matrix metalloproteinases, stabilization of the blood-brain barrier, and reducing apoptosis and pyroptosis (Baig et al., 2023). Several of these pathways are mediated through cholinergic activation of the alpha-7 nicotinic acetylcholine receptor (α7nAChR) (Baig et al., 2023). In subacute and chronic stroke, tVNS increases expression of brain-derived neurotrophic factor and growth differentiation factor 11, potent regulators of neurogenesis and angiogenesis after stroke (Baig et al., 2023).

With fewer risks and associated costs than invasive VNS, there is clear potential for tVNS in the care of stroke survivors. We have previously extensively reviewed the limited pre-clinical and clinical evidence base for tVNS in acute and chronic stroke (Baig et al., 2023). These studies largely confirm the safety and feasibility of tVNS in stroke. We and others have previously concluded that the current evidence base is insufficient to recommend the adoption of tVNS in clinical practice with several unanswered questions regarding efficacy and several challenges to optimal implementation (Andalib et al., 2023; Baig et al., 2023). However, there is a dynamic evolution in the field of neuromodulation after stroke with numerous ongoing clinical studies addressing the efficacy and mechanism of tVNS in stroke. Understanding this landscape is imperative to effectively plan for a future where tVNS may be a part of standard care and to prospectively identify the limitations of the evolving evidence base to optimally guide future research in this area. In this review, we outline the ongoing studies of tVNS in stroke, critically evaluate the outstanding challenges, and make evidence-based suggestions for implementation of tVNS into clinical practice.

## Methods

### Search strategy

We searched the following registries/journals (17/07/2024) for upcoming, active, and completed studies of transcutaneous vagus nerve stimulation in acute and chronic stroke:
(1) ClinicalTrials.gov (https://clinicaltrials.gov);
(2) The International Clinical Trials Registry Platform (https://trialsearch.who.int/);
(3) The Chinese Clinical Trial Registry (https://www.chictr.org.cn/);
(4) The ISRCTN Registry (https://www.isrctn.com/);
(5) The EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/);
(6) Clinical Trials Registry – India (https://ctri.nic.in);
(7) BioMedCentral Trials Journal (https://trialsjournal.biomedcentral.com/).
Search terms included "vagal nerve stimulation," "vagus nerve stimulation," "tVNS," "VNS," "auricular," "stroke," "cerebrovascular," "ischemic," "ischaemic," "haemorrhage," and "hemorrhage." Further studies were identified from citation searches of previously published articles.

### Study selection

Studies were selected if they met the following criteria:
(1) Population: included adults with stroke (ischemic and/or hemorrhagic).
(2) Intervention: non-invasive VNS delivered by transcutaneous stimulation auricular or cervical branches of the vagus nerve.
(3) Comparator: compared against a control group of either sham tVNS or other active treatment group.
(4) Outcome: included either clinical outcome measures and/or mechanistic outcome measures into the biological effects of VNS.

### Data collection

Study information was independently collected on a review proforma by two authors (SSB, SD). This included a systematic collection of the study population, type of stroke included, time point post-stroke, nature of tVNS intervention including parameter settings and duration, primary and secondary outcome measures, study duration, estimated completion date, and study status.

Studies were labeled as having an "unknown" status if: (1) the study status was not available on the relevant clinical trials registry or (2) more than 2 years elapsed since the estimated completion date, without publication of results or trial registry update.

## Results

### Summary of study characteristics

The current review identified 48 registered RCTs using tVNS in stroke.

#### *Stroke subtype*

Twenty-four studies include ischemic stroke and fifteen include ischemic or hemorrhagic stroke. Nine studies do not define stroke type. No studies using tVNS exclusively in hemorrhagic stroke were identified.

#### *Time point post-stroke*

Of the studies reviewed, 16 studies plan to include participants with chronic stroke and 17 in those with subacute stroke (defined here as 1 week – 6 months). Two studies are investigating acute stroke whereas one study is exploring the acute/subacute phase (0.5 hours – 14 days) (ChiCTR2400082197). There are 3 registered studies that include participants from the subacute and chronic phase. Nine studies did not adequately specify the time since stroke in the inclusion criteria.

#### *Site of stimulation*

Transcutaneous auricular vagus nerve stimulation (taVNS) is the most common stimulation method, with 35 of the reviewed studies using this method. Of the few taVNS studies that have reported stimulation location, the cymba concha/concha and tragus have been reported for active stimulation whereas the limbus and lobe have been reported for sham stimulation. One study (NOVIS NCT04050501) plans to use cervical stimulation. The remaining studies did not define the type of stimulation used.

#### *Laterality of stimulation*

Approximately one-quarter of studies report the side of stimulation. Eleven of these studies plan to use left-sided stimulation and one study in acute post-stroke dysphagia plans to use bilateral stimulation.

#### *Stimulation settings*

Just over one-quarter of studies report stimulation parameters. The most common frequency was 20–25 Hz. Pulse width varied during 0.1–0.3 ms and stimulation intensity varied during 0.5–6 mA. One study in motor recovery (ChiCTR2300068033) planed to utilize taVNS at 30, 300 and 3000 Hz, however, other stimulation parameters such as pulse width, intensity, and stimulation location were not detailed. The type of stimulation device is rarely prospectively reported. Use of the gammaCore Sapphire was reported for the single study of tcVNS.

There is variability in the planned duration and intensity of sessions, with the average stimulation per session being 30 minutes (range 15–60 minutes). The duration of therapy varied from 10 days to 3 months however one study planned a single tVNS session.

#### *Timing of stimulation*

Most of the registered rehabilitation studies pair tVNS with the rehabilitation activity however the timing of stimulation and activity is not specified in all studies. Four studies employ a priming approach with tVNS being delivered prior to rehabilitation however one study plans to deliver tVNS after physiotherapy. One study has included two active tVNS groups, where tVNS is paired or unpaired with motor training (NCT05943431).

#### *Transcutaneous vagus nerve stimulation and other stimulation*

Some studies plan to combine tVNS with other neuromodulation techniques such as transcranial direct current stimulation (NCT06244914), neuromuscular electrical stimulation (NCT05779293), trigeminal nerve stimulation (NCT06288217), or repetitive transcranial magnetic stimulation (ChiCTR2100054543).

#### *Sham techniques*

Specific parameters used for sham stimulation are sparsely reported. Of the 48 studies identified, 18 studies do not specify the sham procedure. Twenty-one studies include no stimulation, either with the tVNS device switched off (6 studies) or by comparing with conventional treatment or therapy alone (15 studies). Six studies include sham stimulation at different locations, namely the earlobe (5 studies) or limbus (1 study). Additionally, 3 studies use low or subthreshold stimulation as a sham technique.

#### *Primary outcomes*

Many of the identified studies are exploring the feasibility of using tVNS alongside stroke rehabilitation. Of the studies identified, 26 focus on motor recovery. Thirteen of these studies focus on the chronic phase of recovery. Eighteen studies include the Fugl-Meyer Assessment-Upper Extremity (FMA-UE) as a primary outcome measure, predominantly assessing upper limb function. Other motor studies have included balance, gait, dexterity, and functional motor tasks. The timing of tVNS delivery relative to rehabilitation activity varies across studies. Regarding therapy provision, two studies utilize robot-assisted physiotherapy whereas most studies involve therapy with a physiotherapist.

Five studies focus on cognitive performance. One pilot study plans to explore the use of tVNS in relation to language recovery in post stroke aphasia, however, this is a secondary outcome measure, with the primary aim to explore feasibility. Five studies focus on the use of tVNS for post stroke dysphagia, utilizing common assessment methods such as videofluoroscopy. Other studies focus on pain, consciousness, and depression.

#### *Mechanistic measures*

Ten studies have reported using functional near-infrared spectroscopy (fNIRS) in comparison to only three studies using fMRI. Other techniques such as electroencephalogram (EEG),

electromyography (EMG), and motor-evoked potentials have been reported. Several studies are analyzing blood samples to assess inflammatory markers and brain-derived neurotrophic factor (plasma/serum). Other potential tVNS biomarkers such as heart rate variability have been included in outcome measures of several studies. Only one study has reported using pupillary dilation and no studies have reported using salivary alpha-amylase.

**Summary of major upcoming studies**

There are promising, large studies in acute, subacute, and chronic stroke that will determine the efficacy of tVNS at different time points in stroke. Below we highlight the largest ongoing studies of tVNS at each of these time points.

In acute stroke, the NOVIS trial is randomizing 150 participants with acute ischemic stroke within 12 hours of symptom onset to active or sham cervical tVNS (van der Meij et al., 2020). In addition to assessing MRI infarct volumes at day 5, the authors will assess change in the NIHSS score, penumbral salvage on MRI, blood–brain barrier leakage on CT perfusion imaging, and day 90 mRS scores. This will establish the clinical effects of tVNS as an adjunct to acute stroke management and will help whether a potential mechanism is through blood-brain barrier stabilization.

In subacute stroke, NCT05943431 will investigate the effects of 2 weeks of daily auricular tVNS with physiotherapy on upper limb motor recovery (Xiao et al., 2024). The novel aspect of this study is the inclusion of three treatment arms; in the two active groups, auricular tVNS will be delivered either paired with arm movement (detected by limb EMG) or at regular 5–7-second intervals unpaired with physiotherapy. This trial will help establish whether tVNS can enhance the effects of early rehabilitation and spontaneous recovery in subacute stroke. Furthermore, it will help delineate whether the pairing of tVNS with therapy needs to be precisely timed. The relevance of this is discussed in detail below.

In chronic stroke, the TRICEPS trial (ISCRTN 20221867) will investigate movement-activated tVNS paired with rehabilitation on upper limb motor function in over 240 participants with an ischemic stroke between 6 months and 10 years prior. In this study, tVNS will be delivered via a wrist accelerometer-triggered system that participants can use independently at home over a 12-week period, eliminating the need for in-clinic therapy sessions. The primary outcome measure is the FMA-UE score at 3 months with additional mechanistic sub-studies using multimodal neuroimaging to examine the effects of tVNS on cortical plasticity.

## Discussion

The current review details the scope of tVNS-related research in clinical stroke and outlines the expected medium-term outputs in the field. In acute stroke, ongoing studies will signal the potential benefit of tVNS in improving neurological outcomes in acute stroke and may provide sufficient experience and data to warrant an adequately powered multicenter study to establish its efficacy. In subacute and chronic stroke, only two studies are including more than 200 participants; these larger studies are likely to be adequately powered to determine the efficacy of tVNS as an adjunct to post-stroke arm rehabilitation. Our study highlights the redundancy in clinical cohorts and stimulation paradigms being used globally. While some of the ongoing studies of tVNS detailed above will provide key insights into the efficacy and mechanism of VNS in acute stroke management and stroke recovery, there are missed opportunities to use these resources to answer mechanistic and pragmatic questions about tVNS in stroke. The following sections will highlight some of the major barriers to implementation of tVNS in clinical practice including knowledge gaps that need addressing and make recommendations for future clinical trial consideration. The major recommendations are summarized in **Table 1**.

**Transcutaneous vagus nerve stimulation in hyperacute stroke**

Neuroprotective strategies in hyperacute stroke beyond revascularization have been largely unsuccessful (Pérez-Mato et al., 2024). Despite promising evidence of many preclinical drug and neurostimulation therapies, none have been successful in clinical practice. There may be several potential reasons for this including the diversity of stroke-related presentations and significant differences in the physiology of young rodent models compared to adult human populations with comorbidities and polypharmacy (Fisher et al., 2009). Nevertheless, the preclinical evidence for tVNS is encouraging with animal models showing consistent reductions in infarct size following tVNS in the hyperacute and subacute phases after stroke (Baig et al., 2023).

Translating hyperacute stroke therapies into clinical practice presents several challenges. First, undifferentiated stroke populations are heterogeneous in terms of stroke location, time from onset to presentation, mechanism (ischemic *vs.* hemorrhagic, cardioembolic *vs*. intracranial), and study population (age of participants and comorbidities) which may potentially dilute the effects if certain subcategories are responders *vs*. non-responders. Second, balancing the timing of intervention is key – although early intervention with tVNS e.g. in prehospital settings may allow treatment at the first medical contact, a large proportion of stroke mimics may be treated which would require an inflated sample size. Third, there is a logistical problem of treating acute stroke patients with an additional therapy that may require monitoring while several other therapeutic and diagnostic interventions are taking place. Fourth, some animal models show a benefit of sustained treatment with tVNS including up to 28 days (Li et al., 2020); this may present a challenge to deliver clinically as many stroke survivors are discharged earlier. Clinical trials in the hyperacute phase should aim to deliver tVNS in pre-hospital settings and assess the dose-response relationship with some treatment arms having higher frequency or longer duration of tVNS.

**Optimizing transcutaneous vagus nerve stimulation delivery**

Several tVNS-related parameters can be varied (**Figure 2**). Modification of these variables at an individual or group level is desirable to maximize the potential utility. Unfortunately, the pre-existing literature assesses a narrow range of

**Table 1** | **Key challenges in the implementation of tVNS in clinical practice and recommendations for future research**

| Domains | Unanswered questions | Recommendation(s) for future studies |
|---|---|---|
| Hyperacute stroke | Is tVNS more effective when delivered in a pre-hospital setting? | Pre-hospital clinical trials of tVNS delivered in pre-hospital settings (e.g. during ambulance transfer) and comparison with studies delivering tVNS on hospital arrival. |
| | Is intermittent or continuous tVNS more effective? | Establishing a dose-response relationship of tVNS with studies of single session, multiple session, and continuous tVNS in hyperacute stroke. |
| | Does tVNS reduce blood-brain barrier permeability and excitotoxicity in clinical stroke populations? | Establishing mechanistic outcomes of tVNS in hyperacute stroke based on preliminary pre-clinical evidence e.g. assessing cortical spreading depression using EEG and blood-brain barrier integrity using contrast MRI. |
| tVNS parameter settings | What is the optimal current amplitude and frequency of tVNS? | Mechanistic studies of cortical activation patterns (e.g. fMRI or fNIRS) using various current amplitudes and frequencies. |
| | Are the effects of tVNS lateralized within the brain? | Subgroup analysis of responsiveness to tVNS in individuals with left versus right sided stroke. |
| | What is the optimal duration of tVNS? | Extension studies to randomized clinical trials where a subset of participants continue to use tVNS over a longer timeframe (3 months – 1 year) |
| | Is there a difference between tcVNS and taVNS? | Head-to-head studies of taVNS and tcVNS. |
| Rehabilitation | Which forms of rehabilitation synergize with tVNS? | Clinical trials combining tVNS with alternative forms of rehabilitation beyond repetitive task practice e.g. strength training, constraint-induced movement therapy and passive stretching. |
| | What is the optimal timing of tVNS with respect to rehabilitation? | Crossover studies comparing tVNS delivered prior to rehabilitation (priming), at the onset of movement (paired), and after movement (reinforcement). |
| Upper limb outcomes | Which aspects of arm function respond best to tVNS? | Detailed assessment of multiple aspects of arm function including strength, spasticity, flexor synergy, kinematics and sensory testing within clinical trials. |
| Indications for tVNS | Which post-stroke deficits respond to tVNS? | Pilot studies of tVNS in clinical studies of leg weakness, balance disorders, visual field impairment, aphasia, and dysphagia. |
| Biomarkers | What are the ideal markers of autonomic activation from tVNS and can these be utilized to stratify potential responders *vs*. non-responders? | Multimodal assessment of acute biomarkers of autonomic activation at baseline assessment (e.g., pupillometry, heart rate variability) with subsequent assessment of overall treatment response rate to intervention according to change in acute biomarkers. |
| | What are some longer term biomarkers of tVNS that correlate with clinical outcome measures? | Mechanistic studies of tVNS including functional neuroimaging to assess dynamic changes in cerebral function before and after intervention. |
| Target populations | Do individuals with intracerebral hemorrhage respond similarly to ischemic stroke? | Dedicated pre-clinical models and clinical trials for individuals with hemorrhagic stroke or large clinical trials with adequate sample size to demonstrate response rate in ischemic *vs.* hemorrhagic stroke. |
| | Do comorbidities that affect autonomic function e.g., diabetes mellitus, affect tVNS response? | Subgroup analysis of larger clinical trials assessing response rate in individuals with/without diabetes mellitus. |
| | Do medications affecting the cholinergic, noradrenergic, and serotonergic systems affect tVNS response? | Collection of medication history in clinical trials of tVNS with subsequent reporting of response rate in groups taking centrally active medication. |
| Accessibility and affordability | Can tVNS stimulator technology be adapted to resource-poor environments? | Safety and feasibility testing of transcutaneous electrical nerve stimulation devices adapted to deliver tVNS. |

EEG: Electroencephalogram; fMRI: functional magnetic resonance imaging; fNIRS: functional near-infrared spectroscopy; MRI: magnetic resonance imaging; taVNS: transcutaneous auricular vagus nerve stimulation; tcVNS: transcutaneous cervical vagus nerve stimulation; tVNS: transcutaneous vagus nerve stimulation.

stimulation amplitudes and frequencies; most currently active trials do not investigate the effect of varying stimulation parameters (**Additional Table 1**). It is unlikely that individual clinical trials can be set up to modify each of these variable parameters. As such, mechanistic studies that assess the effect of individual treatment parameters are required.

***Stimulator settings***

Several device-related tVNS stimulation parameters can be varied including amplitude, pulse width, duration of pulse, and frequency. In many of the commercially available devices, such as the TVNS Technologies and Parasym devices, the pulse width and frequency are fixed but the intensity and duration of the pulse can be set by the clinician/user.

The effect of stimulation current amplitude and frequency is not a linear dose-response relationship. In invasive VNS and rehabilitation, there appears to be an inverted U-shaped relationship with higher amplitudes and frequencies associated with reduced cortical plasticity (Buell et al., 2018; Pruitt et al., 2021). The optimal settings for tVNS amplitude, frequency, and pulse width are unknown. In an fMRI study in migraine, Sacca et al. (2022) showed different activation patterns in low-frequency (1 Hz) *vs*. high-frequency (20 Hz) taVNS. One Hz taVNS was associated with increased NTS/LC-occipital cortex static functional connectivity and a decrease in NTS-thalamus static functional connectivity whereas 20 Hz taVNS was associated with an increase in LC-anterior cingulate cortex static functional connectivity. In the case of current amplitude, this may be beneficial in clinical populations as the autonomic nerve fibers are activated at a lower threshold than the unmyelinated C fibers conveying pain sensation (Bolz and Bolz, 2022). There is considerable inter-individual variation in the perception of stimulation hence devices used in clinical practice will need to offer some flexibility to accommodate this.

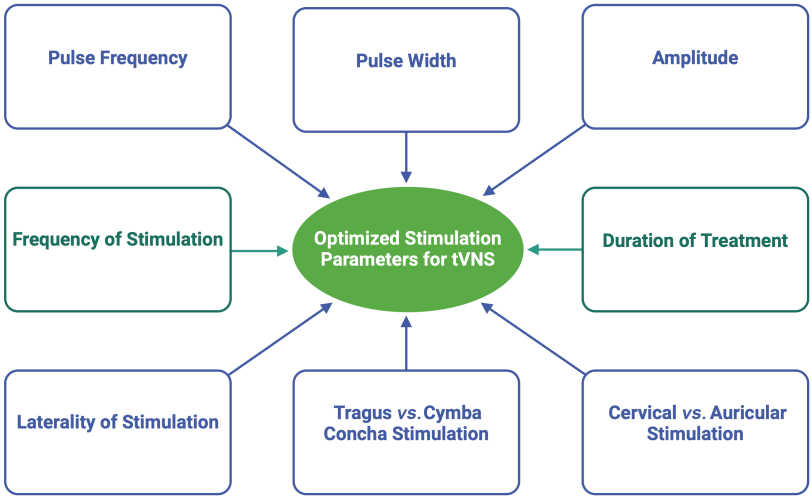


**Figure 2 | Transcutaneous vagus nerve stimulation (tVNS) parameters that can be modulated in tVNS therapy.**
Created with BioRender.com.

### *Timing*

The optimal timing of tVNS in motor learning is contentious. VNS could be delivered before movement (priming) where it may increase arousal, during movement or after movement (reinforcement). While the initial pre-clinical studies of invasive VNS during motor action are optimal for promoting plasticity (Khodaparast et al., 2014), there is recent evidence that reinforcement of only the successful performance of a motor task with stimulation may optimize motor training (Bowles et al., 2022). In studies of acute stroke where the pairing of stimulation with motor activity is not essential, it is unclear whether tonic or phasic stimulation is optimal.

Some groups have tried to enhance tVNS by gating stimulation to the exhalation phase of the respiratory cycle as this is when there is facilitatory influence on the NTS. Ultrahigh field 7T MRI of the brainstem in migraine shows that exhalation-gated tVNS enhances the fMRI response in a region corresponding to the NTS greater than inhalation-gated tVNS (Sacca et al., 2022). While there was a clinical trial registered to investigate respiratory gated tVNS in stroke (NCT03292159), this was discontinued due to termination of funding. Although there is some evidence that tVNS may differentially affect EEG-derived functional brain networks dependent on the time of the day (von Wrede et al., 2022), the potential diurnal effects of tVNS in stroke have not been systematically investigated.

### *Cervical versus auricular transcutaneous vagus nerve stimulation*

The auricular vagus nerve is purely afferent while the cervical vagus nerve contains both afferent and efferent nerve fibers. There have not been any head-to-head comparisons in tVNS in stroke. It is difficult to compare the effect size between studies of auricular and cervical tVNS as the stimulation parameters for optimal activation of the vagus nerve will vary according to the greater degree of subcutaneous tissue in the neck versus the ear. While one could make a theoretical argument that efferent nerve fibers are not directly activated by taVNS, there is evidence that auricular nerve stimulation can have similar effects to efferent nerve stimulation, e.g., on serum cytokines and heart rate variability – this is likely to be via the regulation of sympatho-vagal balance centrally. Furthermore, the afferent nerve fibers in the cervical region are activated at lower amplitudes than efferent nerve fibers, therefore it may not necessarily be the case that cervical tVNS is activating efferent fibers in all studies (Ahmed et al., 2022). One of the benefits of auricular stimulation is that there is the potential for bilateral stimulation which may counteract some of the suggestions that some of the taVNS effects are lateralized (Colzato and Beste, 2020). Furthermore, as noted by Ay and colleagues, there is a theoretical risk that acute administration of cervical tVNS could precipitate carotid artery embolization in at-risk individuals (Arsava et al., 2022).

### *Rehabilitation protocols*

In chronic stroke, tVNS alone is unlikely to be sufficient to drive task-specific neural plasticity and motor recovery. It likely requires combining with rehabilitative therapy. The optimal rehabilitation protocol to combine with tVNS is unclear. Currently, the mainstay of rehabilitative therapies combined with tVNS is repetitive task practice or robotic rehabilitation. Future studies with tVNS alongside other techniques, e.g., virtual reality interventions or constraint-induced movement therapy would add a range of options and novelty in stroke rehabilitation in clinical practice. It is not clear which aspects of arm function respond greatest to tVNS; it is important to differentiate effects on strength, mobility, spasticity, and sensory function. A wider range of outcome measures specific to each of these domains is required. While most registered trials of tVNS in rehabilitation are using the FMA-UE assessment, the sensory domains in this assessment are not detailed. The use of an additional sensory-focused outcome measure such as the Nottingham Sensory Assessment could illuminate the interplay between sensory and motor recovery in tVNS-mediated restitution.

Currently, the published studies of tVNS paired with rehabilitation require in-hospital rehabilitation. This is a big challenge to upscaling tVNS for widespread use and developing clinical trials for home-based tVNS is essential. This has previously been shown to be feasible in studies of tVNS in a study of COVID-19 (Badran et al., 2022), however, the disability associated with stroke presents unique challenges to this.

***Transcutaneous vagus nerve stimulation systems***

If tVNS is shown to be effective in large multi-center trials then there will be great interest in incorporating advanced technology to optimize and deliver therapy. Movement-activated tVNS systems are one such approach, this enables tVNS to be paired to limb movement and deliver the paradigm of paired stimulation which currently requires in-hospital therapy. The TRICEPS trial adopts this approach. With the development of biomarkers of vagus nerve activation (e.g., EEG or heart rate based), a feedback system may be able to automatically change the stimulation parameters during a given therapy session to maximize vagus stimulation (Yu et al., 2022). An alternative approach of movement-paired tVNS delivery is being employed in NCT05943431 where muscle activation detected by EMG triggers tVNS. On a longer-term scale, devices that measure the speed and quality of limb movements could track improvements with different stimulation protocols; such closed-loop systems could recommend and implement the most effective stimulation paradigms on an individual basis. For other post-stroke deficits, the pairing of stimulation to repetitive tasks will require novel ideas e.g. voice-activated tVNS.

***Target populations***

Given the relatively small size of clinical studies of tVNS in stroke, there is limited information on the effect of age, sex, comorbidities, and medication on the response to tVNS. For instance, in the elderly, degenerative processes may affect autonomic nerve fibers and/or central cholinergic receptor density and distribution (Schliebs and Arendt, 2011). Similarly, the presence of diabetes and autonomic neuropathy may theoretically affect vagus nerve activation. The sexual dimorphism in post-stroke inflammation necessitates more female animal models in pre-clinical research and targeting similar proportions of male and female participants in clinical trials (Baig et al., 2023).

VNS has been shown to exert effects through cholinergic, noradrenergic, and serotonergic pathways (Morrison et al., 2022). This naturally raises the question as to whether centrally acting medications e.g., muscarinic antagonists, beta blockers, and selective serotonin reuptake inhibitors will influence the effects of tVNS in clinical practice. One study found that daily injections of oxybutynin, prazosin, or duloxetine did not block invasive VNS-dependent reorganization of the motor cortex after stroke in rats (Morrison et al., 2022). Similar studies have not been performed in tVNS. In the future, collection and reporting of the use of these medications in clinical trials will be an effective tool to determine whether these are independent predictors of treatment response. Similarly, given that nicotine can activate the α7nAChR, reporting of smoking status and nicotine use is important.

The subgroup analysis of the VNS-REHAB study revealed no significant differences in upper limb motor recovery scores among subgroups categorized by age (< 62 years old *vs*. ≥ 62 years old), sex, stroke severity (FMA-UE score ≤ 34 *vs*. > 34), time since stroke (≤ 2 years *vs*. > 2 years), side of paresis, or presence/absence of cortical involvement (Dawson et al., 2022). It is unclear if tVNS will be similarly effective across a range of patient profiles. Additionally, the VNS-REHAB study excluded individuals with very severe strokes (FMA-UE < 20), infratentorial stroke, intracerebral hemorrhage, or multiple infarcts. Further research is needed to determine whether VNS is effective in different stroke subtypes and whether likely responders *versus* non-responders can be differentiated at baseline. This is best achieved through subgroup analysis of large clinical trials.

**Broadening the application of transcutaneous vagus nerve stimulation in chronic stroke**

The majority of studies in subacute to chronic stroke investigate the pairing of tVNS with upper limb physiotherapy on upper limb motor recovery. While this is a natural extension of the current evidence base in invasive VNS, there is a mismatch in the number of small studies addressing this compared to other pressing post-stroke impairments including aphasia, leg weakness, balance impairment, and visual field impairment. If the postulated mechanisms of tVNS are correct – that it promotes task-specific plasticity through the combination with training – then the same effects on cortical and subcortical adaptations may be of significant value across a range of stroke sequelae.

Some of the outstanding research gaps where tVNS is not being utilized include post-stroke sensory impairment, visuospatial deficits, and fatigue. These indications are best assessed through specifically designed pilot studies that address the unique factors for each condition.

***Aphasia***

Aphasia after stroke is present in up to one-third of stroke cases with the majority having sustained deficits in the longer term (Williams et al., 2024). Recovery from aphasia shares some similar processes of neuroplasticity with upper limb recovery (Morrison et al., 2021). Other forms of non-invasive brain stimulation such as repetitive transcranial magnetic stimulation and transcranial direct current stimulation have been trialed in smaller studies of language recovery (Williams et al., 2024). There have not been any published reports of tVNS in language recovery after stroke. As previously summarized by Morrison et al. (2021), VNS may reorganize networks through several mechanisms including influencing auditory networks and corticobulbar pathways mediating jaw movement.

The TRANSLATE study (NCT06403475) is a single-center pilot study of tVNS in post-stroke aphasia. This is, to our knowledge, the only study of VNS in aphasia. Participants with chronic stroke will have a home-based program consisting of tVNS paired with an established computer-based speech therapy intervention. While the primary outcome measures are feasibility and tolerability, secondary outcome measures will include performance on language tasks and mechanistic outcome measures including brain-derived neurotrophic factor levels.

Assessing tVNS in aphasia after stroke has potential challenges. Language function may be distributed differently between individuals; the variation in lesion site in people with aphasia

is an additional variable that may affect responsiveness (Kyeong et al., 2019). Further, the timing of tVNS pairing with language tasks is more complex than simple motor tasks; while short tasks such as object naming could be paired with a tVNS stimulus, more complex tasks of comprehension and complex speech production occur over several seconds. It is unclear whether continuous or pulsed stimulation would be more effective to promote tVNS-mediated plasticity.

***Balance***

Mobility and balance impairments after stroke are complex with contributory factors including impairments in strength, proprioception, tone, praxis, coordination, cardiovascular fitness and confidence. While repetitive transcranial magnetic stimulation and transcranial direct current stimulation have been trialed in studies of post-stroke balance and gait disorders (Veldema and Gharabaghi, 2022), there are no published studies of tVNS. Similar to language, the processes that regulate balance and gait are broadly distributed throughout the brain (Moon et al., 2016). As such, tVNS that provides more diffuse rather than targeted neuromodulatory effects may be a potentially useful adjunct in a range of post-stroke balance deficits.

In Australia, a study of tVNS paired with physiotherapy is assessing the effect of tVNS on balance (ACTRN12623000376640). This study of 40 participants will pair tVNS with during task-specific training (e.g., walking tasks or balance training), aerobic exercise, core and limb strengthening. Participants will have 2–3 sessions per week, each lasting 1 hour, for 6 weeks. This varied, holistic exercise program provides an evidence-based framework to characterize the additive effect of tVNS.

Future studies of tVNS in stroke should aim to assess the effects of training these individual components of balance rehabilitation alongside tVNS and to increase participants with cerebellar stroke where balance difficulties are more common.

**Biomarkers**

The characterization and development of biomarkers of tVNS in stroke will have numerous clinical and research applications. First, the use of clinical scoring systems to track and monitor recovery has some challenges including ceiling effects. Tools to track the biological effect of tVNS will allow clinicians to determine whether beneficial improvements tVNS are occurring or if they have plateaued in individual patients. Second, integrating both clinical and biomarker-based assessments could enable the development of resilient and effective protocols for future trials, incorporating diverse tVNS parameters and treatment durations. The early identification of biomarker-based outcomes may be effectively utilized in adaptive trial designs (Pallmann et al., 2018). For example, parameters can be varied as treatment combinations and investigated simultaneously allowing futile treatment combinations to be dropped or new combinations to be added to an ongoing trial. Third, biomarkers could be used on an individual level to develop integrated tVNS systems where tVNS stimulation parameters are automatically adjusted to achieve optimal personalised therapy (this is discussed in further detail below). Fourth, research on biomarkers may further delineate the underlying mechanism of tVNS in stroke and enable the development of targeted neurostimulation and non-neurostimulation treatments in the future.

When considering biomarkers of tVNS in stroke, different perspectives can be considered (**Figure 3**). For instance, there are potential biomarkers of autonomic nervous system activation versus biomarkers potentially associated with an effective treatment response. With the former, these could be used as a feedback tool to ensure the safety of tVNS and establishing effective stimulation parameters at treatment onset. With the latter, these could be used as outcome measures in clinical trials and in clinical practice to guide treatment parameters and duration. An alternative classification is to consider biomarkers of acute vagus nerve activation versus biomarkers of the sustained downstream pathways triggered by VNS. It is important to consider that not all the pathways activated by tVNS are necessarily related to the mechanism of effect in acute or chronic stroke. Furthermore, experimental markers of tVNS in healthy volunteers may not necessarily translate into the stroke population where acute stroke can perturb autonomic function and chronic stroke is associated with multimorbidity (Xiong et al., 2018).

***Markers of autonomic activation***

One of the pitfalls of research in electrical stimulation is the confirmation of whether the target is being stimulated, whether it is being stimulated at the right time, and whether the parameters need to be adjusted to optimize the degree of stimulation in an individual. As such, there has been great interest in identifying potential markers of acute autonomic activation from tVNS. The principal modalities investigated include measures of heart rate variability, pupillometry, and salivary alpha-amylase (Burger et al., 2020a).

Increased heart rate variability (HRV) is a marker of parasympathetic innervation to the heart (Burger et al., 2020a). By convention, a study of tVNS has avoided using the right cervical vagus nerve as the sinoatrial node is predominantly innervated by this nerve (Burger et al., 2020a). There have been mixed results when investigating whether tVNS can alter any parameters of HRV in healthy volunteers with some showing increased HRV and others showing no effect (Wolf et al., 2021). Some of this variability may relate to different stimulation parameters being used between studies and the use of different metrics used to measure HRV. There are also no clear differences between right or left taVNS (Burger et al., 2020a). It is important to note that in taVNS, there may be activation of vagal afferent fibers which occur and exert a central effect without necessarily affecting efferent vagus transmission to the heart.

Vagal pathways to the NTS project to the locus coeruleus which, in turn, mediates noradrenergic signalling to the cortex (Ludwig et al., 2021; Komisaruk and Frangos, 2022). Invasive VNS has been shown to phasically activate the locus coeruleus (Hulsey et al., 2017) and alpha-2 adrenoreceptor antagonism in the motor cortex appears to prevent invasive VNS-mediated motor plasticity (Tseng et al., 2021). This has led to interest

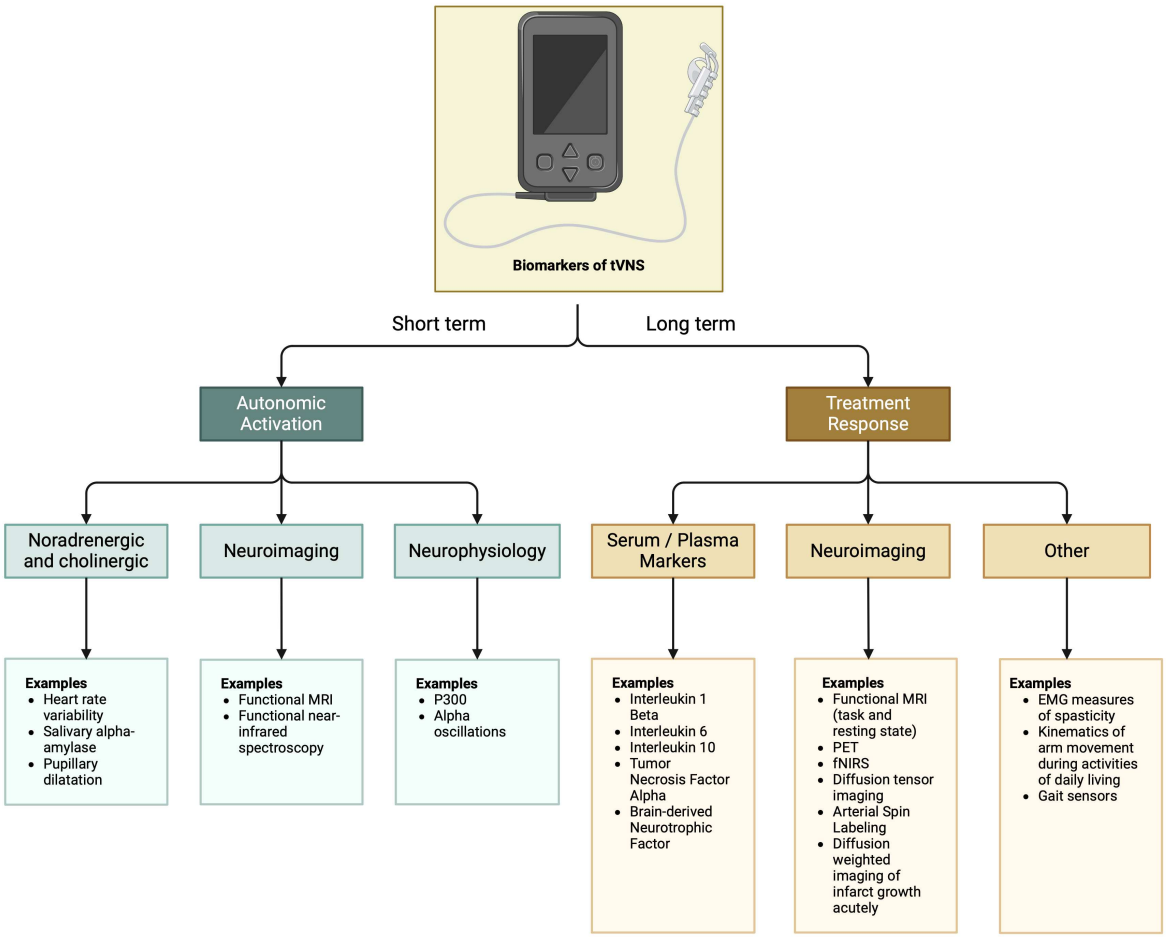


**Figure 3** | **Potential biomarkers of tVNS.**
Created with BioRender.com. EMG: Electromyography; fNIRS: functional near-infrared spectroscopy; MRI: magnetic resonance imaging; PET: positron emission tomography; tVNS: transcutaneous vagus nerve stimulation.

in the use of markers of noradrenergic activity as biomarkers of tVNS. Measures of pupillary dilatation following tVNS have shown varied and inconsistent findings (Burger et al., 2020b; Capone et al., 2021; Sharon et al., 2021). The secretion of salivary alpha-amylase is another putative biomarker of tVNS activation (Giraudier et al., 2022).

### *Electroencephalography*

The P300 event related potential may potentially be a marker of noradrenergic activity but studies assessing it as a biomarker of tVNS have not found a reliable response. Other candidate neurophysiological biomarkers of tVNS have recently been extensively reviewed (Gianlorenco et al., 2022). The StrokeVNS (NCT06226493) study will record resting state EEG during taVNS. Spectral analysis will be performed on the signal with the primary outcome measure assessing the power spectral density ratio of alpha to delta frequencies. This study may potentially identify a novel biomarker of taVNS responsiveness on an individual basis.

### *Neuroimaging*

Although there are studies investigating the effects of invasive VNS on cerebral perfusion and cortical activation in epilepsy (Liu et al., 2003) and depression (Nahas et al., 2007), there is a need to develop an evidence base for the effects of tVNS in stroke. When considering neuroimaging as a biomarker, this can once more be divided into acute effects of tVNS on brain regions or longer-term changes in structure and function that result from a tVNS-based intervention. In a meta-analysis of fMRI performed in 60 healthy adult volunteers, tVNS acutely modifies several brain regions including the frontal, temporal and parietal lobes, basal ganglia, thalamus, and brainstem (Rajiah et al., 2022).

The activation of the frontal cholinergic pathways may mediate the biological effects of tVNS in stroke discussed above. Some of the challenges in interpreting fMRI changes after acute tVNS are the differences in control groups used in studies (e.g., low stimulation *vs*. no stimulation *vs*. earlobe stimulation), activation of vagal brainstem targets by non-auricular nerve fibers, activation of non-vagal brainstem targets by auricular stimulation and potential carry-over effects from alternating active and sham stimulation (Rajiah et al., 2022). Further, the fMRI response in healthy volunteers may not be similar in stroke survivors where there is structural damage and subsequent neural network reorganization. As discussed by Rajiah et al. (2022), the initial brain regions activated by tVNS reside in the brainstem, a region that is more difficult to image with conventional whole brain protocols where the voxel size may exceed the size of the brainstem nuclei being investigated.

fNIRS has been an underutilized tool in studies of tVNS. fNIRS may offer a cheap and effective way to monitor cerebral hemodynamics in real time in a non-invasive way and has previously been used to demonstrate adaptive changes following motor recovery in stroke (Yang et al., 2019). It can offer better temporal resolution and is less prone to movement artefacts than fMRI. In a study of invasive VNS in people with epilepsy, fNIRS performed during a verbal fluency task showed increases in cerebral blood flow with increases in VNS intensity (Höper et al., 2022). In consistency with this, taVNS has been shown to increase cerebral blood flow in the prefrontal cortex in adolescents (Höper et al., 2022). A recent fNIRS study of activation patterns pre/post auricular tVNS in participants with a subacute/chronic stroke indicated significant increases in the activation of the pre-motor cortex and supplementary motor cortex in the unaffected hemisphere (Wang et al., 2023).

With regards to the long-term effects of tVNS, there have not been studies of the dynamic changes in the brain that result from tVNS treatment after stroke rehabilitation. In longitudinal fMRI studies post-stroke, there is initially widespread activation of diffuse brain regions in the subacute phase post-infarction followed by a progressive focusing of brain activity; better spontaneous recovery is associated with this narrowing of brain activation while maladaptive plasticity may maintain these broad patterns of activation (Nair et al., 2007). It will be important to establish whether tVNS and/or other interventions in stroke rehabilitation are associated with patterns of activation that resemble spontaneous recovery.

In addition to fMRI, alternative neuroimaging modalities could add value. For instance, in the acute setting, CT perfusion or contrast MRI can be used to assess the integrity of the blood-brain barrier while arterial spin labeling could be used to assess collateral perfusion. In the chronic setting, PET imaging using $^{18}$F-FDG, including the advent of task-based functional PET, could be combined with fMRI to give a robust insight into the hemodynamic and metabolic interactions after tVNS treatment in stroke (Jamadar et al., 2020). The use of cholinergic tracers, including the potential use of ligands to the α7nAChR could be used to demonstrate whether the pre-clinical evidence for the importance of this pathway holds true in human disease.

Clinical neuroimaging in studies of tVNS in stroke may also serve as a potential predictor of treatment response. When predicting recovery from a stroke, initial stroke severity, infarct size, and location can all contribute towards broad predictions of anticipated spontaneous recovery (Heiss and Kidwell, 2014). However, there is still gross variability in the degree of spontaneous recovery between individuals with similar stroke lesions. Other more specific predictors of recovery include corticospinal tract thickness at the level of the pons (Lin et al., 2019). It is unclear whether these factors will also be predictors of the tVNS treatment response; as such, subgroup analysis of the baseline neuroimaging features of individuals in tVNS trials may help stratify likely responders versus non-responders so that tVNS can be targeted to the correct populations.

***Serum markers***

With activation of the cholinergic anti-inflammatory pathway, tVNS can affect levels of circulating cytokines. While not yet studied in stroke populations, there is emerging evidence in healthy volunteers and different disease states that tVNS can regulate the balance between M1 and M2 macrophages in humans. For instance, in healthy volunteers, 2 minutes of cervical tVNS (sequentially on the right then the left) was associated with a decrease in IL-1 and TNF levels at 24 hours compared to sham stimulation (Lerman et al., 2016). While the auricular vagus nerve, a purely afferent nerve, does not directly innervate the splenic cholinergic anti-inflammatory pathway, there is evidence that taVNS reduces serum IL-1, TNF and IL-6 in an animal model of lipopolysaccharide-induced inflammation (Zhao et al., 2012), reduces plasma IL-6 and CRP in individuals with COVID-19 (Corrêa et al., 2022), and reduces IL-6 in a study of delayed cognitive recovery after knee surgery (Zhou et al., 2022). One study suggests that this anti-inflammatory effect may be dependent on the stimulation frequency with 15 Hz taVNS being associated with greater reductions in inflammatory cytokines than 25 Hz taVNS in mice with an acute inflammatory state (Go et al., 2022). This suggests that taVNS causes a central effect that alters the sympatho-vagal balance in the periphery. To our knowledge, no studies have investigated to what extent auricular stimulation (an afferent nerve) can influence the cholinergic anti-inflammatory pathway relative to the cervical vagus nerve (a nerve with both afferent and efferent nerve fibers).

One study in healthy volunteers found that taVNS increased the inflammatory cytokines IL-1 and IL-6 (Veiz et al., 2022). This finding may relate to the fact that in healthy volunteers there is not a state of chronic inflammation or a rectifiable imbalance in sympathetic and parasympathetic activity. One of the caveats of blood-based biomarkers is that the integrity of the blood-brain barrier means that peripheral blood sampling may not capture the array of central changes that occur on a local level in the infarct and peri-infarct regions. For instance, if tVNS causes local adaptive changes in microglia and astrocytes then these will not easily be captured using standard blood sampling methods.

***Electromyography***

In the chronic phase of stroke, limitations in upper limb function are not purely restricted to deficiencies in muscle strength; spasticity, pain and sensory dysfunction can all contribute to functional impairment. Furthermore, the use of clinical rating scales such as the FMA-UE scale does not necessarily change proportionately with marginal improvements in function, particularly in the mid-scoring ranges. Chang et al. (2021) showed that just 3 weeks of taVNS paired with robotic training can significantly alter peak biceps surface EMG amplitude during extension movements. It is possible that combining clinical and EMG outcome measures could provide additional information about the effects of tVNS on spasticity.

**Accessibility and affordability**

In addition to safety considerations, non-invasive medical devices such as tVNS offer practical advantages over

invasive options in terms of affordability and re-use. While implanted medical devices cannot be re-purposed for use in other individuals in the event of non-responsiveness or ceiling effects, tVNS devices could be cleaned and used by other individuals. This is an appealing option for resource-poor settings where healthcare providers may not be able to source enough devices for each eligible individual. While advanced, closed-loop systems are likely to be more expensive than basic stimulators, there are alternative options that may make tVNS more affordable. For instance, pre-existing TENS machines could be re-purposed with electrodes that adhere to the tragus/cymba concha. This approach is being explored by an RCT in a trial of 100 people with stroke in India (CTRI/2023/11/059373).

## Summary

tVNS is a highly promising therapeutic intervention in acute stroke and post-stroke rehabilitation. The effective implementation of tVNS in clinical practice necessitates a coordinated approach at a pre-clinical and clinical level to identify solutions to the key barriers discussed in the present review.

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**Additional file:**
***Additional Table 1:*** *Ongoing clinical trials of tVNS in stroke as of July 17, 2024.*

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