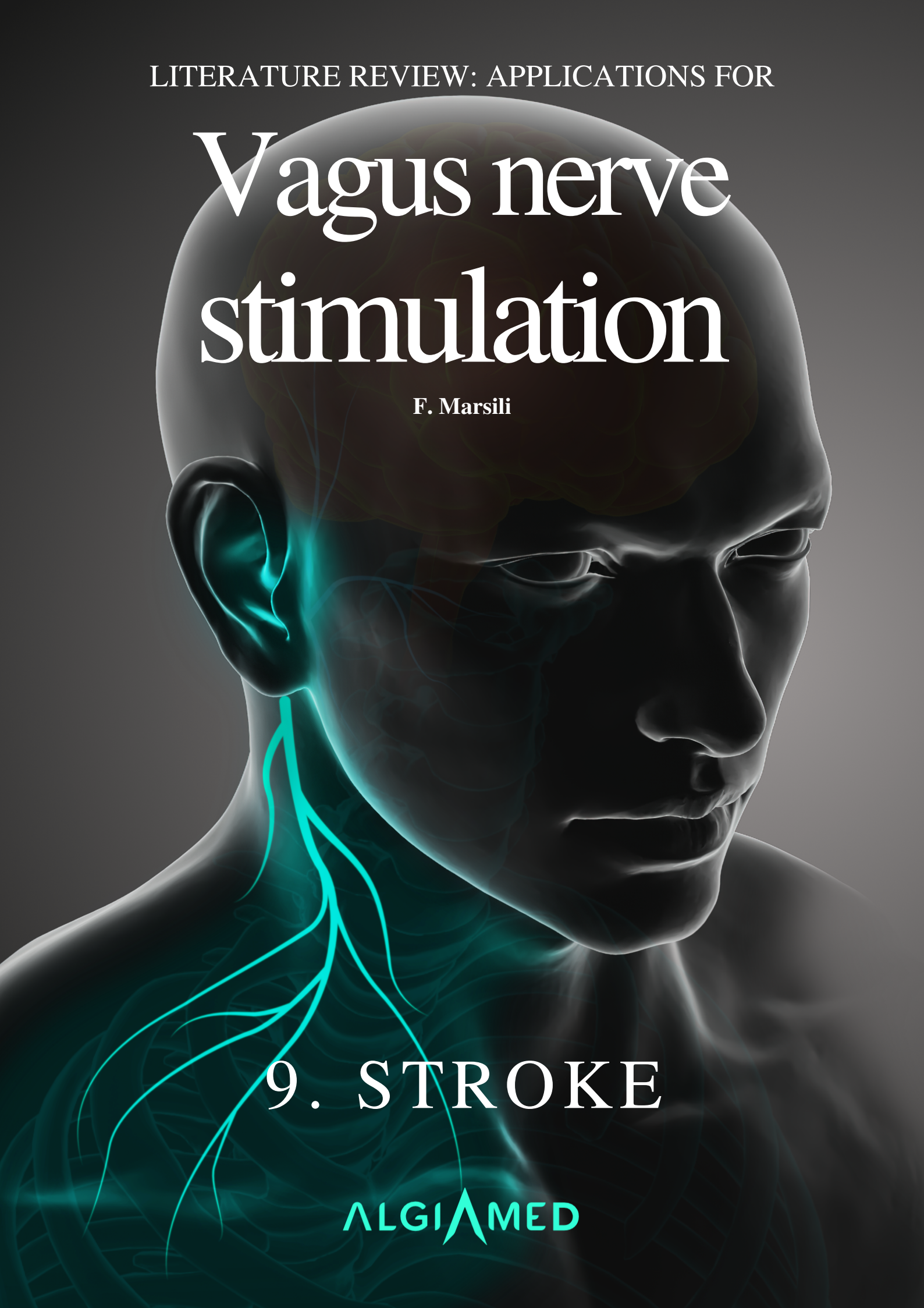
LITERATURE REVIEW: APPLICATIONS FOR

Vagus nerve stimulation

F. Marsili



9. STROKE

ALGIAMED

Author's choice

The papers in this collection focus on the application of Vagus Nerve Stimulation (VNS) as established therapeutic solution for difficult-to-treat conditions.

The vagus nerve is the longest cranial nerve and is widely distributed throughout the body, traversing the neck, thorax and abdomen. It is composed by motor fibres and sensory fibres from sympathetic and parasympathetic branches. [1], [2]. Afferent branches of the vagus nerve innervate brain behavioural areas involved in depressive states, and it desynchronises cortical activity with anti epileptic effects [3], [4]. Efferent branches of the vagus nerve regulate gastrointestinal secretory and motor function [5]. Recent advances in the field, have unraveled an anti-inflammatory role of the efferent vagus nerve via the Cholinergic Anti-inflammatory Pathway (CAP), a known mechanism for neural inhibition of inflammation linked to the activation of the autonomic nervous system (ANS) [6], [7].

Electrical stimulation of the VN modulates the nervous system at central, peripheral, and autonomic levels without the need for pharmacological interventions. For decades, invasive techniques of VNS have demonstrated their clinical efficacy in VN-related diseases and, to these days, efforts have been made to create a more safe, effective, and non-invasive solution to VNS.

The auricular branch is the only peripheral branch of the VN on the human body, it is part of the afferent portion of the VN that directly connects to the brainstem. Thus, auricular VN has become the most favourable access point for non-invasive VNS. Neuroimaging studies on animal models and humans have confirmed the modulatory efficacy of auricular VNS (aVNS). For examples, fMRI studies show identical activation patterns in the brain between invasive and aVNS, with significant inhibitory and anti-inflammatory effects. Due to the existence of different control systems, the anti-inflammatory effects of aVNS (i.e., release of norepinephrine and noradrenaline, and neurotrophic factors) seem to occur immediately after intervention, while neuroplastic changes only occur as a consequence of sustained regenerative efforts [7].

Colleciton 1 and collection 2 are the most extensive selections, since VNS has been standard-of-care for epilepsy and depression for decades. Collection 3 explores the possibility of using VNS for the treatment of posttraumatic stress disorders. Collection 4 focuses on fibromyalgia and collection 5 on multiple sclerosis. Collection 6 and 7 corroborates the hypothesis that VNS can be used to activate the cholinergic anti-inflammatory pathway to treat inflammatory diseases, such as inflammatory bowel disease or rheumatoid arthritis. Collection 8 and 9 focus on the use of VNS for ameliorating pain sensitivity in chronic pain conditions and for rehabilitating upper limb motor fibres after ischemic strokes, respectively. In conclusion, collection 10 opens up other possibilities for clinical applications of VNS, ranging from cardiovascular diseases, through ADHD disorders, to tinnitus.

To summarise, VNS is a novel technology and its non-invasive configuration is still under investigation. Further clinical examinations are mandatory in order to understand the underlying mechanism of VNS and to open the door to new possible therapeutic applications. However, being a non-invasive, safe, and efficient therapeutic solution, VNS is an attractive tool for further implementation and new creative clinical applications.

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9. VNS and stroke

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Vagus Nerve Stimulation Paired With Upper Limb Rehabilitation After Chronic Stroke

A Blinded Randomized Pilot Study

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Background and Purpose—We assessed safety, feasibility, and potential effects of vagus nerve stimulation (VNS) paired with rehabilitation for improving arm function after chronic stroke.

Methods—We performed a randomized, multisite, double-blinded, sham-controlled pilot study. All participants were implanted with a VNS device and received 6-week in-clinic rehabilitation followed by a home exercise program. Randomization was to active VNS (n=8) or control VNS (n=9) paired with rehabilitation. Outcomes were assessed at days 1, 30, and 90 post-completion of in-clinic therapy.

Results—All participants completed the course of therapy. There were 3 serious adverse events related to surgery. Average FMA-UE scores increased 7.6 with active VNS and 5.3 points with control at day 1 post–in-clinic therapy (difference, 2.3 points; CI, −1.8 to 6.4; P=0.20). At day 90, mean scores increased 9.5 points from baseline with active VNS, and the control scores improved by 3.8 (difference, 5.7 points; CI, −1.4 to 11.5; P=0.055). The clinically meaningful response rate of FMA-UE at day 90 was 88% with active VNS and 33% with control VNS (P<0.05).

Conclusions—VNS paired with rehabilitation was acceptably safe and feasible in participants with upper limb motor deficit after chronic ischemic stroke. A pivotal study of this therapy is justified.

Clinical Trial Registration—URL: https://www.clinicaltrials.gov. Unique identifier: NCT02243020. **(*Stroke*. 2018;49:2789-2792. DOI: 10.1161/STROKEAHA.118.022279.)**

Key Words: motor cortex ■ neuromodulation ■ plasticity ■ rehabilitation ■ stroke ■ upper extremity ■ vagus nerve

Impaired use of the upper limb is one of the most common symptoms after stroke, and improving upper limb function is a priority for many patients.[1] Clinical trials of increased dose of upper extremity task-specific training have been disappointing.[2] This suggests new interventions are needed to maximize poststroke motor recovery.[3]

Vagus nerve stimulation (VNS) paired with movement has been shown to drive task-specific plasticity in the motor cortex in rodent models and improve forelimb function after experimental stroke.[4] In our first-in-human, randomized, controlled, open clinical trial, VNS paired with upper limb rehabilitation was safe and feasible in people with upper limb deficit at least 6 months after ischemic stroke.[5]

The purpose of this pilot study was to further assess safety, feasibility, and efficacy of VNS paired with upper limb rehabilitation in chronic ischemic stroke, with blinded, sham VNS control.

Methods

This article adheres to the American Heart Association Journals' implementation of the Transparency and Openness Promotion Guidelines. Requests for data will be considered by the corresponding author after Food and Drug Administration postmarket approval.

This was a randomized, sham stimulation controlled, and fully blinded study of VNS paired with rehabilitation in people with arm weakness after ischemic stroke. Participants in both groups were implanted with the VNS device. Participants, therapists, and outcome assessors were blinded to group allocation.

The study was approved by an institutional review board at each institution and subject to appropriate regulatory approvals (Food and Drug Administration investigational device exemption No. 130287 and UK Medicines and Healthcare Products Regulatory Agency [MHRA]

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No. CI/2015/0011). It was registered on http://www.clinicaltrials.gov (NCT02243020). Written informed consent was obtained in compliance with the requirements set forth in US Food and Drug Administration, Code of Federal Regulations Title 21. The study was conducted according to the Declaration of Helsinki.

Participants

Enrollment at the 4 sites is shown in Table I in the online-only Data Supplement. People with a history of unilateral supratentorial ischemic stroke that occurred between 4 months to 5 years before randomization, aged ≥30 and ≤80 years, and with an FMA-UE between 20 to 50 were eligible for inclusion (Table II in the online-only Data Supplement).

Protocol Summary

A presurgery assessment was performed. After VNS implantation and ≈1 week of recovery, participants were randomized to either active VNS (0.8 mA) or control VNS (0.0 mA), and baseline assessments were repeated. In-clinic rehabilitation therapy began on the next day and was delivered ≈3× a week for 6 weeks (18 visits; Figure I in the online-only Data Supplement). Outcomes assessments were performed on days 1, 7, 30, and 90 after completion of in-clinic therapy.

After 6 weeks of in-clinic therapy, all participants began daily, therapist-prescribed home exercises. For the first 30 days of at-home therapy, all participants received 0 mA VNS. Thereafter, participants received VNS according to their randomized allocation. After the day-90 assessment, the control VNS group crossed over to receive 6 weeks of in-clinic rehabilitation paired with active VNS (0.8 mA) followed by outcome assessments at days 1, 7, 30, and 90 thereafter.

Further details on methodology are given in Appendix in the online-only Data Supplement.

Main Study Outcome Measures

The main safety outcome measure was the number of serious adverse events related to the device or therapy. The main feasibility measure was the number of participants who completed the minimum number of visits during the randomized portion of the study (at least 12 therapy visits).

Efficacy outcomes included the FMA-UE,[6] Wolf Motor Function Test (WMFT; time and functional), Box and Block Test, Nine-Hole Peg Test, Stroke Impact Scale, and Motor Activity Log. Because this was a pilot study, no primary or secondary efficacy measures were designated.

Sample Size and Statistical Analysis

No formal sample size calculation was performed for this pilot study. Efficacy analyses were performed on the intention-to-treat population and included all randomized participants. Missing data were not imputed. The change in outcome measures at each time point was compared between groups using 2-tailed, unpaired *t* tests. Fisher exact test was used to calculate the significance for response rates. For all comparisons, α was set at 0.05.

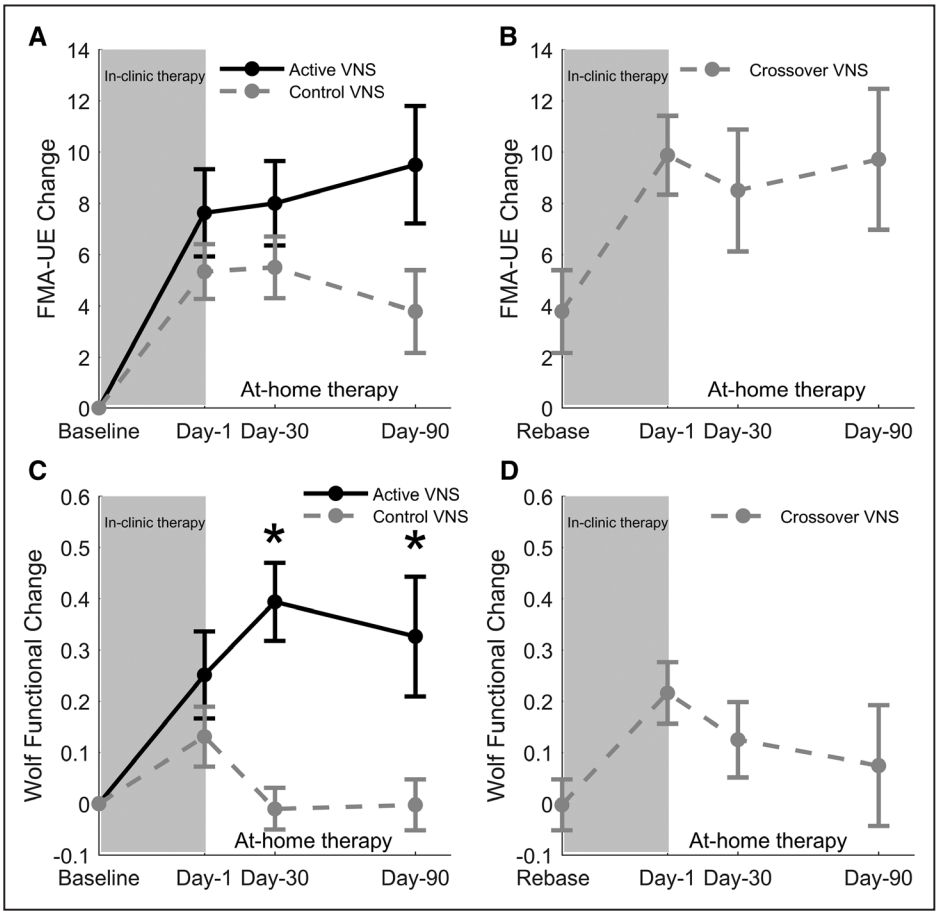

Figure 1. Fugl-Meyer assessment–upper extremity (FMA-UE; mean±SEM) and Wolf Motor Function Test (WMFT) scores (mean±SEM). **A**, Change in FMA-UE score during blinded follow-up for active vagus nerve stimulation (VNS) and controls from baseline and 3 posttreatment assessments. **B**, Change in FMA-UE score following crossover to active VNS. **C**, Change in WMFT functional score during blinded follow-up for active VNS and controls. **D**, Change in WMFT score following crossover to active VNS. Shaded area indicates the 6 wk of in-clinic therapy. Rebase, baseline in controls before starting active VNS. Days 1 to 30 (after in-clinic therapy) consisted of at-home therapy with no VNS for both groups. From days 30 to 90, active VNS group received VNS (0.8 mA) and controls received control VNS (0 mA) with at-home therapy. *P=0.029 at post-90 d and P<0.001 at post-30 d.

Table. Change in Outcome Measures (Intention-to-Treat Analysis, n=17 [Active VNS, 8; Control, 9])

Measure	Day-1 Difference Post–In-Clinic Therapy*		Day-90 Difference Post–In-Clinic Therapy*	
	95% CI	*P* Value	95% CI	*P* Value
FMA-UE	2.29 (–1.9 to 6.47)	0.2604	5.72 (–0.15 to 11.6)	0.055
WMFT functional	0.12 (–0.10 to 0.33)	0.2625	0.33 (0.04 to 0.61)	0.029
WMFT time, s	–3.02 (–11 to 5.24)	0.4215	–4.04 (–14 to 5.64)	0.362
Stroke Impact Scale (hand)	5.66 (–11 to 22.7)	0.4889	2.71 (–14 to 19.9)	0.741
Box and Block Test	–2.93 (–6.3 to 0.44)	0.0835	–0.23 (–4.1 to 3.66)	0.903
Nine-Hole Peg Test	–2.25 (–58 to 53.5)	0.9245	–9.18 (–48 to 29.2)	0.580
Motor Activity Log	NA	NA	17.93 (–0.37 to 36.2)	0.054

FMA-UE indicates Fugl-Meyer assessment–upper extremity; NA, not applicable; VNS, vagus nerve stimulation; and WMFT, Wolf Motor Function Test.

*Difference between groups: active VNS - control VNS.

Results

Twenty-two people consented to participate in the study. Of these, 17 participants were implanted and randomized (8 to active VNS and 9 to control; Figure II in the online-only Data Supplement). All participants completed the randomized portion of the study. Baseline characteristics of participants are shown in Table III in the online-only Data Supplement. Details on protocol adherence, feasibility, and blinding are provided in the online-only Data Supplement.

Safety

There were 3 serious adverse events related to implantation surgery, including 1 implantation wound infection requiring treatment with intravenous antibiotics but resolved; 1 case of shortness of breath and dysphagia, likely because of intubation, which recovered; and 1 case of hoarseness because of vocal cord palsy. There were no serious adverse events reported as associated with stimulation. Full details of adverse events are shown in Appendix in the online-only Data Supplement.

Efficacy

Between-group differences in FMA-UE are shown in Figure 1 and the Table. At day 90, the response rate (defined as FMA-UE change ≥6 points[7]) was 88% in the active group and 33% in control (P=0.03; Figure 2). Between-group differences in Wolf Motor Function Test are shown in Figure 1 and the Table.

After crossover to active VNS in controls, FMA-UE scores increased to 9.8 points above baseline at day 1 after in-clinic therapy (P<0.001) and by 9.7 points at day 90 (P=0.01; Figure 1). Response rates were 88% and 57% at these time points, respectively (Figure 2). Wolf Motor Function Test data are shown in Figure 1. Full details on all outcome measures are shown in Tables V and VI in the online-only Data Supplement.

Discussion

The primary objective of this pilot study was to assess the safety and feasibility of using paired VNS to improve arm function after chronic ischemic stroke. We found this technique to be feasible, including the use of home-based VNS, and demonstrated safety in-line with that expected for VNS devices. The study was not powered to assess efficacy, although there were significant differences between groups in some measures at day 90.

There are several important differences between this and our previous clinical study.[5] This study was fully blinded, all participants were implanted with a VNS device, control participants crossed over to receive the active VNS therapy, and participants continued rehabilitation exercises at home for several months.

There were no significant differences between groups immediately after in-clinic therapy completion, but there was a significant difference by 90 days because of maintained benefit by the VNS group with corresponding decline in the control group and a higher percentage of responders who

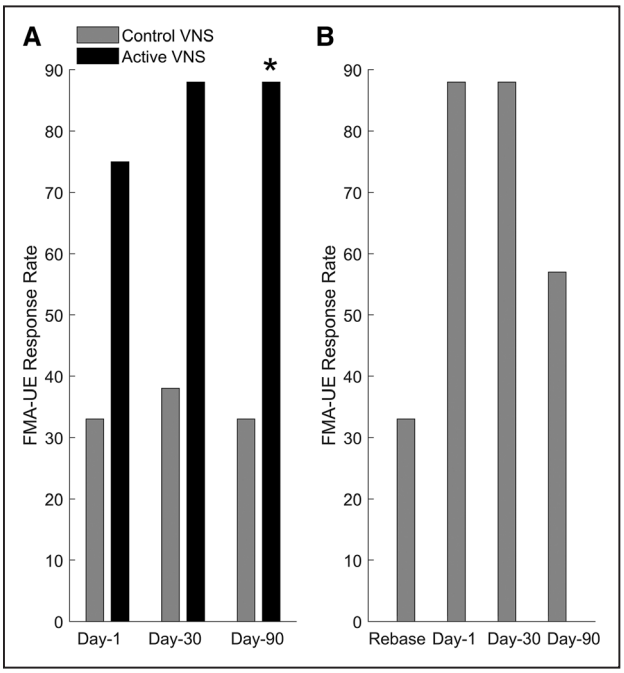

Figure 2. Average Fugl-Meyer assessment–upper extremity (FMA-UE) response rate. **A**, Responder rate (defined as FMA-UE change ≥6 from baseline) for the first 90 d in paired vagus nerve stimulation (VNS; black) and controls (gray). **B**, Responder rates after control group crossed over to receive active VNS therapy. Rebase, baseline in controls before starting active VNS therapy. *P<0.05, Fisher exact test.

achieved a clinically meaningful change for the FMA-UE (change, ≥6 points) with active VNS treatment.[7] Although we cannot definitively conclude these differences are because of paired active VNS treatment, our findings are consistent with the effect of a neuroplastic treatment where time may be needed for benefit to accrue. It is of note that control participants experienced a benefit similar to the initial VNS participants when they crossed over to active VNS treatment.

This pilot study showed that rehabilitation paired with VNS is an acceptably safe and feasible intervention for the treatment of upper limb weakness after ischemic stroke. The study demonstrated sufficient safety, feasibility, and potential efficacy to support a larger pivotal trial.

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Disclosures

Drs Dawson, Kimberley, and Prudente have received reimbursement for conference attendance where results of the study were presented from MicroTransponder, Inc. Dr Cramer has served as a consultant for MicroTransponder, Inc, as well as Roche, and Dart Neuroscience. D. Pierce, Dr Engineer, Dr Prudente, and B. Tarver are employees of MicroTransponder, Inc. The other authors report no conflicts.

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frontiers in Neuroscience

REVIEW
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Targeted Vagus Nerve Stimulation for Rehabilitation After Stroke

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Stroke is a leading cause of disability worldwide, and in approximately 60% of individuals, upper limb deficits persist 6 months after stroke. These deficits adversely affect the functional use of the upper limb and restrict participation in day to day activities. An important goal of stroke rehabilitation is to improve the quality of life by enhancing functional independence and participation in activities. Since upper limb deficits are one of the best predictors of quality of life after stroke, effective interventions targeting these deficits may represent a means to improve quality of life. An increased understanding of the neurobiological processes underlying stroke recovery has led to the development of targeted approaches to improve motor deficits. One such targeted strategy uses brief bursts of Vagus Nerve Stimulation (VNS) paired with rehabilitation to enhance plasticity and support recovery of upper limb function after chronic stroke. Stimulation of the vagus nerve triggers release of plasticity promoting neuromodulators, such as acetylcholine and norepinephrine, throughout the cortex. Timed engagement of neuromodulators concurrent with motor training drives task-specific plasticity in the motor cortex to improve function and provides the basis for paired VNS therapy. A number of studies in preclinical models of ischemic stroke demonstrated that VNS paired with rehabilitative training significantly improved the recovery of forelimb motor function compared to rehabilitative training without VNS. The improvements were associated with synaptic reorganization of cortical motor networks and recruitment of residual motor neurons controlling the impaired forelimb, demonstrating the putative neurobiological mechanisms underlying recovery of motor function. These preclinical studies provided the basis for conducting two multi-site, randomized controlled pilot trials in individuals with moderate to severe upper limb weakness after chronic ischemic stroke. In both studies, VNS paired with rehabilitation improved motor deficits compared to rehabilitation alone. The trials provided support for a 120-patient pivotal study designed to evaluate the efficacy of paired VNS therapy in individuals with chronic ischemic stroke. This manuscript will discuss the neurobiological rationale for VNS therapy, provide an in-depth discussion of both animal and human studies of VNS therapy for stroke, and outline the challenges and opportunities for the future use of VNS therapy.

Keywords: stroke, vagus nerve stimulation, plasticity, rehabilitation, neuromodulation

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INTRODUCTION

Stroke is a leading cause of disability and a significant health burden in the United States and worldwide (Murray et al., 2013; Feigin et al., 2016). Upper limb deficits persist in approximately 60% of individuals after stroke (Wade et al., 1983), limiting their use in day to day activities and impacting quality of life of the individual (Franceschini et al., 2010; Morris et al., 2013). An important goal of stroke rehabilitation research is to develop effective, evidence-based therapies to reduce impairment, facilitate functional upper limb use and improve participation in activities without resorting to compensatory strategies after chronic stroke.

Neurophysiological and neuroimaging studies have provided an improved understanding of the neurobiological processes underlying the brain's ability to restore function by capitalizing on residual networks after stroke (Krakauer, 2004; Ward, 2004; Nudo, 2006; Murphy and Corbett, 2009; Dimyan and Cohen, 2011; Boyd et al., 2017; Sampaio-Baptista et al., 2018). One approach for improving chronic upper limb deficits is to augment this capacity to reorganize, referred to as plasticity. Rehabilitation by itself drives some reorganization of motor networks, but these changes occur within a framework of architectural and anatomical constraints which are believed to limit substantial improvements (Kleim and Jones, 2008). As a result, strategies that can enhance reorganization in conjunction with rehabilitation may support greater recovery. Here, we will describe the neurophysiological basis and implementation of VNS during rehabilitation as a means to enhance plasticity and improve post-stroke recovery.

CHOLINERGIC AND NORADRENERGIC MODULATION OF CORTICAL PLASTICITY

Activation of neuromodulatory networks is strongly linked to plasticity (Gu, 2002), thus engaging these mechanisms provides a potential strategy to enhance plasticity for stroke recovery. Cholinergic neurons within the nucleus basalis (NB) and noradrenergic neurons in the locus coeruleus (LC) are part of the ascending neuromodulatory system that projects diffusely to wide areas of the cortex. Release of acetylcholine (ACh) from NB neurons and norepinephrine (NE) from LC neurons plays an important role in many behavioral and cognitive processes including arousal, memory consolidation and attentional modulation of goal-directed behavior (Gu, 2002; Aston-Jones and Cohen, 2005; Sarter et al., 2005; Hasselmo and Sarter, 2011). The vagus nerve sends projections to the nucleus tractus solitarius (NTS), which in turn projects to the neuromodulatory nuclei. Therefore, understanding the role of these neuromodulatory networks in cortical plasticity is instructive for defining the basis for delivering VNS paired with sensory or motor events to facilitate plasticity.

In a constantly changing world, the brain must extract behaviorally relevant information to drive useful goal-directed behaviors. Neuromodulatory networks, including the cholinergic and noradrenergic systems which provide diffuse neuromodulatory innervation throughout the cortex, are uniquely poised to serve that role. Cholinergic and noradrenergic neurons show phasic discharge during specific epochs of behavior that may signal cue detection, novelty or reinforcement feedback (Hasselmo, 1995; Arnold et al., 2002; Bouret and Sara, 2004; Sarter et al., 2005, 2006, 2009; Parikh et al., 2007; Hasselmo and Sarter, 2011). For example, transient cholinergic activity in cortical neurons signals behaviorally relevant cues while decreased activity is observed with missed cues (Parikh et al., 2007). Rapid cholinergic activation provides reinforcement feedback in response to both positive and negative events (Hangya et al., 2015). Similarly, phasic discharge from LC neurons predicts correct responses in a visual discrimination task with increased cross-correlation among LC neurons (Usher et al., 1999). These studies demonstrate that brief bursts of ACh or NE are likely involved in the attentional modulation of cortical neurons to encode the behavioral relevance of stimulus-specific features during task performance.

The neuromodulator-driven attentional modulation of cortical neurons must eventually be encoded into long-lasting changes in synaptic efficacy with successful task learning (Hess and Donoghue, 1994; Hess and Krawczyk, 1996; Kirkwood et al., 1999; Rioult-Pedotti et al., 2000; Ziemann et al., 2006; Seol et al., 2007; Cohen and Maunsell, 2009; Korchounov and Ziemann, 2011; Carcea and Froemke, 2013; Hasan et al., 2013). At a systems level, the changes in synaptic efficacy may underlie reorganization of cortical maps specific to the learned features of the task (Merzenich et al., 1988; Recanzone et al., 1992, 1993; Pascual-Leone and Torres, 1993; Elbert et al., 1995; Buonomano and Merzenich, 1998; Sterr et al., 1998; Feldman and Brecht, 2005; Feldman, 2009; Froemke, 2015). Furthermore, depletion of cortical ACh or NE resulting from lesions of their respective nuclei or pharmacologic modulation with cholinergic and noradrenergic antagonists blocks cortical plasticity and impairs learning (Sato et al., 1987; Juliano et al., 1991; Heron et al., 1996; Kilgard and Merzenich, 1998; Zhu and Waite, 1998; Conner et al., 2003, 2005; Ramanathan et al., 2009; Vitrac and Benoit-Marand, 2017). Together, these studies established a causal role for the neuromodulatory networks in task-specific learning and plasticity.

The vagus nerve projects to the NTS (Foley and DuBois, 1937; Prechtl and Powley, 1990) and consequently provides rapid activation of the cholinergic and noradrenergic systems (Roosevelt et al., 2006; Nichols et al., 2011; Porter et al., 2012; Hulsey et al., 2017). Therefore, the engagement of these neuromodulatory systems by VNS led to the prediction that brief bursts of VNS paired with sensory or motor experience could enhance cortical plasticity that was specific to the paired experience. Repeatedly pairing a tone with VNS reorganized the rat auditory cortex map, resulting in an expansion for the paired tone (Engineer et al., 2011). A tone paired with trigeminal nerve stimulation did not result in specific auditory cortex plasticity, demonstrating that the enhancement of plasticity was unique to stimulation of the vagus nerve.

Neuromodulatory networks share some features in mediating plasticity in motor and auditory cortices (Gu,

2002; Ramanathan et al., 2009). Since VNS paired with sensory experience drives robust, specific plasticity in the primary sensory cortex, this raised the possibility that pairing VNS with motor training could also facilitate plasticity in naïve rat motor cortex. Indeed, repeatedly pairing VNS with a forelimb movement during motor training increased the corresponding map representation of that movement in motor cortex compared to equivalent training in rats that did not receive VNS (Porter et al., 2012). These studies laid the groundwork for using VNS paired with motor training for improving upper limb deficits after stroke.

VNS IMPROVES MOTOR FUNCTION IN ANIMAL MODELS OF STROKE

Post-stroke recovery is associated with plasticity in motor networks (Murphy and Corbett, 2009). The development of strategies to enhance this plasticity and subsequently generate greater recovery has been the focus of intense research. Based on its ability to drive training-dependent neuroplasticity in uninjured motor networks, a number of animal studies have evaluated VNS paired with rehabilitative training to support the recovery of motor function after stroke.

A study performed in an animal model of ischemic stroke tested the hypothesis that VNS paired with rehabilitative training could enhance post-stroke recovery (Khodaparast et al., 2013). This study sought to evaluate the ability of VNS delivered during motor training to improve recovery of forelimb strength, a main contributor to disability after stroke (Canning et al., 2004; Harris and Eng, 2007). Rats were trained to proficiency on a strength-based forelimb task, and then underwent ischemic lesion of the motor cortex. Rats that received brief bursts of VNS paired with forelimb movement during motor training demonstrated significantly greater recovery of volitional forelimb strength compared to rats that received equivalent training without VNS. Recovery persisted when assessed 1 week after the cessation of stimulation, consistent with the notion that VNS drives stable plasticity and providing an initial indication that the benefits of VNS therapy may be lasting.

A second study built upon these initial findings and assessed the ability of VNS to improve forelimb movement speed after stroke. Rats were pre-trained on a skilled task that measured rapid movement of the forelimb and underwent an ischemic lesion of the motor cortex (Khodaparast et al., 2014). Corroborating findings from the initial study, VNS paired with forelimb movement during rehabilitative training resulted in significant enhancement of functional recovery compared to equivalent rehabilitation training without VNS (**Figure 1A**).

Rehabilitation can become less effective with increasing time after stroke. To evaluate whether a long delay in therapy delivery would impact the efficacy of VNS, a study evaluated whether VNS paired with rehabilitative training could improve recovery in a rat model of chronic ischemic stroke (Khodaparast et al., 2016). VNS and rehabilitative training were initiated on the 7th week post-stroke in rats with chronic, stable forelimb impairment. Despite the delay in starting therapy, VNS delivered with rehabilitative training produced significantly greater forelimb recovery compared to equivalent training without stimulation (**Figure 1B**). The degree of forelimb recovery after chronic stroke was comparable to that observed in previous studies of subacute stroke (Khodaparast et al., 2013, 2014). These findings provide an initial demonstration that the efficacy of VNS paired with rehabilitative training is not dependent on time to begin the intervention after stroke. Additionally, the observation that VNS therapy improves recovery when initiated long after stroke suggests that VNS does not act by augmenting the action of pro-plasticity factors upregulated in response to stroke (Khodaparast et al., 2016). Alternatively, VNS likely acts to enhance recovery by generating repeated, temporally precise, consistent engagement of pro-plasticity neuromodulatory circuits to reinforce rehabilitation-related neural activity (Hays et al., 2013; Hays, 2016). The independence from stroke-related plasticity is consistent with the ability of VNS paired with training to drive cortical plasticity in uninjured animals (Porter et al., 2012; Hulsey et al., 2016).

Advanced age is a major risk factor for stroke and is associated with reduced plasticity, which could in turn influence the effectiveness of VNS therapy. Thus, a study sought to determine whether VNS delivered during rehabilitative training could improve post-stroke recovery in aged rats (Hays et al., 2016). Rats aged at least 18 months were pretrained on a skilled forelimb task and subsequently underwent ischemic lesions of the motor cortex. Pairing VNS with rehabilitative training generated robust improvements in recovery of forelimb strength compared to equivalent training without VNS in aged rats. The magnitude of recovery observed in aged rats that received VNS therapy was comparable to that reported in previous studies using young rats receiving the same intervention (Khodaparast et al., 2013). The similar effectiveness in aged and young rats receiving VNS is consistent with studies suggesting that age alone is not a determinant in the benefits of rehabilitation and provides initial evidence that advanced age does not preclude VNS-dependent enhancement of post-stroke recovery (Bagg et al., 2002).

Generalization of improved functional recovery to tasks that are not explicitly trained during rehabilitation is an important consideration in the translation of therapies for clinical use, as it has practical implications for administration of the therapy. Given a fixed duration for a session of rehabilitation, a therapist would need to determine whether a patient should receive a greater number of stimulation pairings during a more constrained set of rehabilitative exercises or whether to deliver fewer stimulation pairings distributed across a greater breadth of rehabilitative exercises. To provide data to guide this determination, a recent study tested whether the VNS-dependent recovery after stroke would generalize to a similar, untrained task (Meyers E.C. et al., 2018). Rats were pre-trained on a task that measured skilled forelimb rotation, then underwent an ischemic lesion to motor cortex followed by training on the same rotational task with or without VNS. Delivery of VNS paired with rehabilitative training significantly enhanced recovery of forelimb rotation compared to equivalent training without VNS. After the completion of 6 weeks of motor training on the rotation task, all rats were tested on a similar, but distinct task that

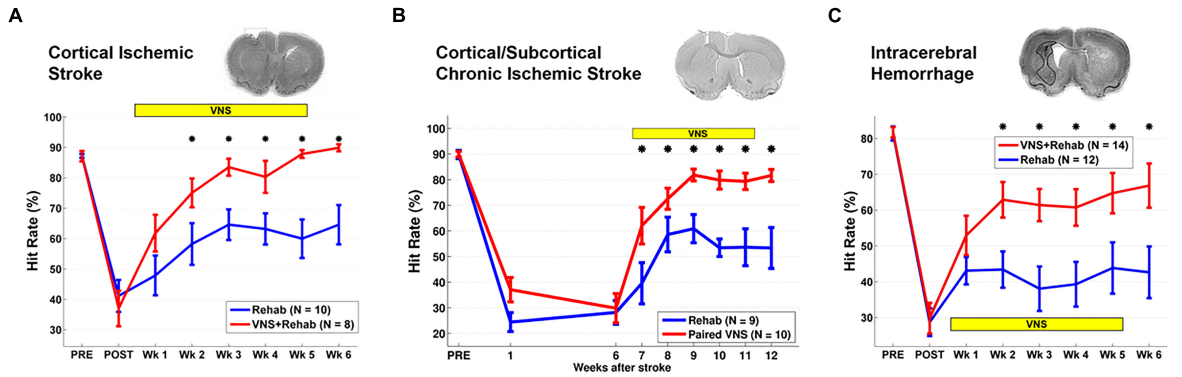

FIGURE 1 | Vagus Nerve Stimulation-dependent recovery of motor function in rat models of stroke. **(A)** VNS paired with rehabilitative training significantly improves recovery of forelimb motor function compared to equivalent training without VNS in a model of cortical ischemic stroke. The top panel shows a coronal brain section with a representative ischemic lesion. Similarly, VNS paired with rehabilitative training enhances recovery of forelimb function after **(B)** chronic combined cortical and subcortical ischemic and **(C)** intracerebral hemorrhage. The symbol "*" indicates $p < 0.05$ across groups at each time point (Adapted from Hays et al., 2014a,b; Khodaparast et al., 2016).

measured volitional forelimb strength. Rats that had previously received VNS paired with rehabilitative training on the rotation task exhibited significantly improved recovery on the volitional strength task compared to rats that had previously received rotation training without VNS, suggesting that VNS-dependent recovery may generalize to similar untrained movements. The magnitude of recovery observed on the untrained task was similar to that observed when VNS was paired with training on the primary task, providing evidence of generalization. Moreover, in this study, VNS-dependent recovery persisted at least 7 weeks following cessation of stimulation, providing additional corroborating evidence that the benefits of VNS are long-lasting.

Other studies provide insight into the implementations of VNS therapy that may be most beneficial. To determine the stimulation paradigm that yields the greatest enhancement in recovery, a study evaluated a range of distinct VNS parameters on recovery of forelimb strength after stroke (Hays et al., 2014b). Delivery of an equivalent amount of VNS that is temporally dissociated from rehabilitative training is less effective at promoting recovery than VNS that is paired with forelimb movement during rehabilitative training, suggesting that non-specific effects of stimulation that do not require precise timing, such as reduction of inflammation or neurogenesis, do not contribute to VNS-dependent enhancement of recovery. Additionally, a paradigm that delivered sixfold more stimulation in rapid succession generated significantly less recovery than VNS explicitly paired with forelimb movement rehabilitation. Together, the results from this study emphasize the need to optimize both the dose and timing of stimulation paradigms for VNS therapy.

Additional studies support the use of VNS therapy for mechanistically distinct forms of cerebrovascular injury. Intracerebral hemorrhage (ICH) is a common and devastating subtype of stroke with few post-injury treatment options. Evidence from preclinical studies indicates that reorganization of spared circuits supports recovery after ICH, similar to ischemic stroke (Auriat et al., 2010; Liang et al., 2013; Santos et al., 2013). Based on the premise that VNS enhances plasticity, a study evaluated whether VNS paired with rehabilitative training may lead to improved recovery in a model of ICH (Hays et al., 2014a). Rats were trained to proficiency on a skilled forelimb task and then received an injection of collagenase into the dorsolateral striatum to induce hemorrhage. Delivery of VNS paired with rehabilitative training significantly enhanced recovery compared to equivalent training without VNS, providing a preliminary demonstration that VNS therapy can improve motor function after ICH (**Figure 1C**). Emerging evidence extends these findings to other distinct forms of neurological damage, indicating VNS can improve recovery in models of traumatic brain injury (Pruitt et al., 2016), spinal cord injury (SCI; Ganzer et al., 2018), and peripheral nerve damage (Meyers E. et al., 2018; **Figure 2**).

Cognitive deficits are not uncommon in patients following ischemic stroke (Tatemichi et al., 1994). Preclinical studies document improvements in memory retention with VNS (Clark et al., 1995, 1998). While a small number of clinical studies provide corroborating evidence for the role of VNS in improving memory function, placebo-controlled studies in larger clinical populations are needed to determine whether VNS facilitates long-term improvement in cognitive function in humans after stroke (Hoppe et al., 2001; Boon et al., 2006; Ghacibeh et al., 2006; Sun et al., 2017). It is possible that short bursts of VNS combined with a cognitive rehabilitative training paradigm may promote plasticity and improve cognitive impairments after stroke. While considerably more development is needed, these findings raise the prospect that pairing VNS with cognitive rehabilitation may represent a potential intervention for post-stroke cognitive impairment.

Despite the evidence demonstrating VNS-dependent enhancement of recovery across a range of preclinical models of neurological injury, the mechanisms that underlie recovery are not thoroughly characterized. In the following section, we will discuss the putative mechanisms by which VNS modulates neural plasticity to support recovery of function.

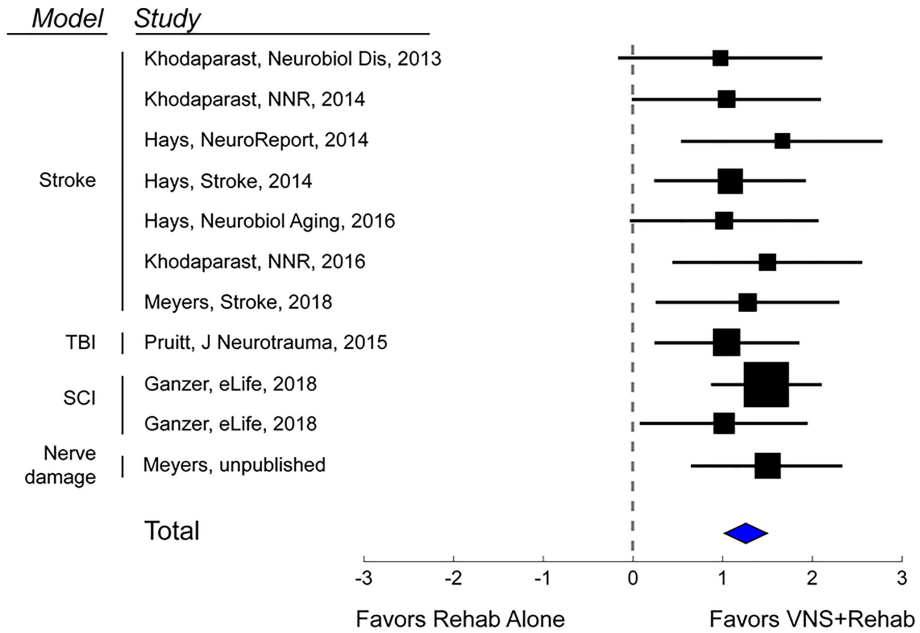

FIGURE 2 | Vagus Nerve Stimulation therapy improves recovery in a variety of models of neurological injury. A meta-analysis of recovery across a range of rat models of neurological damage demonstrates that VNS paired with rehabilitative training (VNS+Rehab) consistently improves recovery of forelimb motor function compared to equivalent rehabilitative training without VNS (Rehab Alone). The data are presented as a forest plot. Markers denote standardized mean difference for VNS+Rehab compared to Rehab Alone for each study, and horizontal lines indicate 95% confidence interval. The size of the indicator represents the number of subjects. The blue diamond represents the summary effect.

NEUROBIOLOGICAL MECHANISMS OF MOTOR RECOVERY AFTER PAIRED VNS

Structural plasticity in descending cortical spinal circuits has been associated with recovery after stroke. A recent study evaluated whether VNS paired with rehabilitative training influenced reorganization of corticospinal tract (CST) connectivity (Meyers E.C. et al., 2018). A retrograde transsynaptic tracing study in rats revealed that VNS paired with rehabilitation tripled synaptic connectivity in CST networks controlling the impaired forelimb compared to equivalent rehabilitation without VNS, providing a direct quantification of VNS-dependent plasticity in motor networks after stroke. This reorganization of CST connectivity was observed 2 months after the cessation of VNS, suggesting that this plasticity is robust and enduring, and consistent with the notion that this plasticity subserves long-term restoration of motor function (**Figure 3**).

Vagus Nerve Stimulation engages a variety of molecular and neuronal mechanisms via the ascending neuromodulatory systems that may underlie the observed reorganization of motor networks. After a stroke, treatment with brain-derived neurotrophic factor (BDNF) increases functional recovery, whereas reduction of BDNF levels prevented the benefits of rehabilitative training (Schabitz et al., 2004; Ploughman et al., 2009). In rodents, both acute and chronic VNS increased levels of BDNF in the hippocampus but the elevated BDNF levels were not associated with improvements in the forced swim or elevated plus-maze tests (Follesa et al., 2007). It remains to be determined whether elevated BDNF levels contribute to motor reorganization and stroke recovery.

Engagement of neuromodulatory networks that regulate synaptic plasticity also represents a means by which VNS likely supports recovery. VNS drives activation of multiple neuromodulatory networks, including the noradrenergic, cholinergic, and serotonergic systems (Nichols et al., 2011; Hulsey et al., 2017). These neuromodulators, in turn, act synergistically to alter spike-timing dependent plasticity (STDP) properties in active networks (Dan and Poo, 2004; Seol et al., 2007). These neuromodulators are known to act within a short window of approximately 5–10 s after neural activity, referred to as the synaptic eligibility trace, to allow STDP (He et al., 2015). Two studies provide initial evidence that VNS generates temporally precise neuromodulatory feedback within the synaptic eligibility trace to drive synaptic plasticity. First, in a study examining plasticity in auditory cortex, only tones presented concurrently with VNS were reinforced (Engineer et al., 2011). Tones delayed 15 s after VNS, which falls outside the time window for synaptic eligibility, failed to generate plasticity. Second, a study examined the requirement for a temporal association between VNS and optimal trials during rehabilitative exercises after SCI (Ganzer et al., 2018). VNS delivery immediately after or within 2 s of the optimal trials significantly enhanced recovery of motor function, while a delay of approximately 25 s from the optimal trials failed to yield any benefits compared to equivalent rehabilitation without VNS.

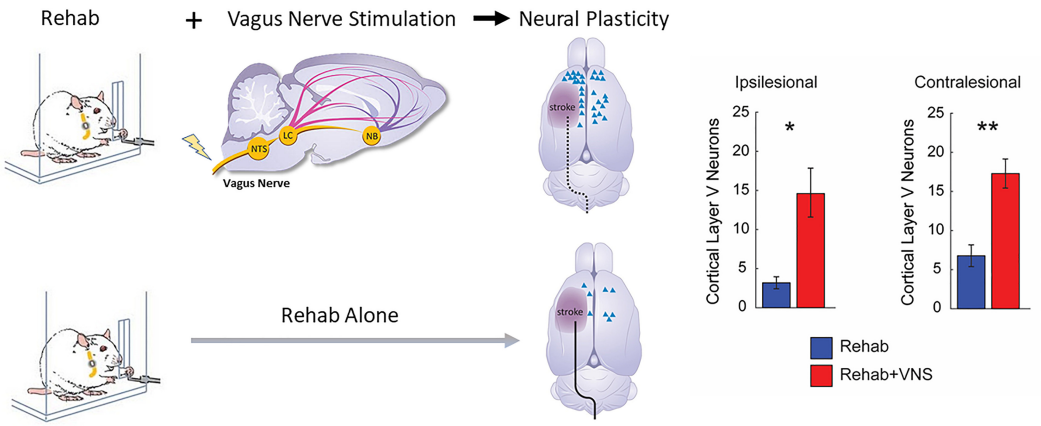

FIGURE 3 | Vagus Nerve Stimulation paired with motor training enhances synaptic reorganization after stroke. Rats that receive VNS paired with motor training (red bar) after stroke demonstrated a significantly greater increase in corticospinal tract (CST) connectivity to rehabilitated muscles compared to equivalent training without VNS (blue bar). CST connectivity originating in both the ipsilesional and contralesional hemispheres was increased. These findings indicate that VNS drives large-scale reorganization in motor networks after stroke which may underlie recovery of function (Adapted from Meyers E.C. et al., 2018). * indicates $p < 0.05$; ** indicates $p < 0.01$.

These studies align well with the time scale of the synaptic eligibility trace and provide a means by which VNS may drive temporally precise neuromodulatory release to reinforce ongoing neural activity related to the paired event.

RANDOMIZED CLINICAL TRIALS TO ASSESS SAFETY AND EFFICACY OF PAIRED VNS AFTER CHRONIC ISCHEMIC STROKE

Transitioning from basic science investigation to clinical studies moves the field closer to determining if these promising findings can translate into improvements in clinical care. Studies are now attempting to translate these preclinical VNS experiments into clinical practice through feasibility, safety, and more recently, pivotal clinical trials in individuals with chronic stroke (Dawson et al., 2016; Kimberley et al., 2018).

A single-blinded, randomized feasibility study evaluating VNS paired with motor rehabilitation was performed by Dawson et al. (2016) in 20 participants with chronic ischemic stroke who had moderate to severe upper limb weakness. Subjects were randomized to VNS paired with rehabilitation ($n = 9$; implanted) or rehabilitation alone ($n = 11$; not implanted). VNS was triggered by a therapist pushing a button during task-specific movements,

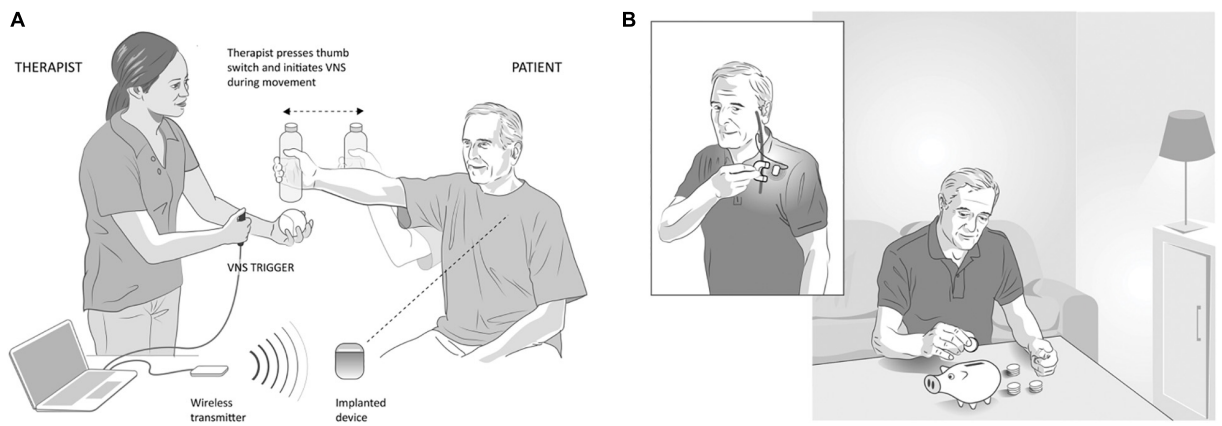

FIGURE 4 | Vagus Nerve Stimulation paired with rehabilitation in the clinic and at home, **(A)** In-clinic rehabilitation with VNS: VNS is delivered by a therapist using a push button timed with a task-specific movement. Pressing the button delivers a brief burst of VNS (0.5 s) during an active goal-directed movement. The VNS system includes an implantable pulse generator (implanted device) that is implanted under the individual's chest wall, an implantable lead, wireless transmitter (for communication between the device and computer) and custom programming software. **(B)** Home-based VNS therapy: Participants are provided a magnet (inset) to swipe over the device once before the start of each rehabilitation session to self-initiate 30 min of VNS (0.5 s burst of VNS every 10 s for 30 min). During the 30 min, participants performed at-home exercises prescribed by the therapist and adapted to their functional level and goals.

based on the notion that VNS provides timed engagement of neuromodulatory networks to support rehabilitation-dependent plasticity (**Figure 4A**). Stimulation parameters were selected based on earlier preclinical studies (Engineer et al., 2011; Porter et al., 2012; Khodaparast et al., 2013, 2014, 2016; Hays et al., 2016; Hulsey et al., 2016). The main outcome measures were a change in upper extremity Fugl-Meyer Assessment (FMA-UE) score and response rate (FMA-UE change ≥6 points was considered clinically meaningful, discussed below). After 6 weeks of in-clinic rehabilitation, participants in the paired VNS group showed a 9.6-point improvement from baseline while the control group improved by 3 points in the per-protocol analysis (between group difference = 6.5 points, CI: 0.4 to 12.6, $p = 0.038$). The response rates were 66 and 36.4% in VNS and control groups, respectively. No serious adverse device effects were reported. These results demonstrated the feasibility of using paired VNS and did not raise safety concerns. Two limitations of this study were the absence of an implanted control VNS group and the lack of assessment of long-lasting effects of paired VNS. These limitations were addressed in a second pilot study (Kimberley et al., 2018).

This second study was a multicenter, fully blinded and randomized study (Kimberley et al., 2018). All participants were implanted with the VNS device, which allowed the control group to crossover to receive paired VNS therapy after completion of blinded follow-up and permitted within-subject comparison of gains. To evaluate the lasting effects of paired VNS, home-based therapy was included as part of the study (**Figure 4B**). Differences between the two studies are highlighted in **Table 1**.

Seventeen participants with chronic ischemic stroke who had moderate to severe upper extremity impairment were enrolled at four sites, with similar surgical procedure and randomization (**Figure 5A**) to the first study. The study design is shown in **Figure 5B**. Participants performed 6 weeks of in-clinic therapy followed by home-based therapy. After 6-weeks of in-clinic therapy, participants in both groups had 1 month of at-home exercises with no VNS followed by 2 months of home-based therapy. During home therapy, participants in both groups activated the VNS device at the start of each 30-min session via a magnet swipe over the implanted pulse generator to deliver either Active or Paired VNS (0.8 mA) or Control VNS (0 mA), respectively (**Figure 4B**).

After 2 months of home-based therapy, the Paired VNS group continued the VNS therapy while the Control Group switched over to receive paired VNS (**Figure 5B**). After 6 weeks of in-clinic therapy, the FMA-UE score increased by 7.6 points for the VNS group and 5.3 points for controls. Three months after the end of in-clinic therapy (post-90), the FMA-UE increased by 9.5 in the paired VNS group and 3.8 points in controls. At post-90, response rate (FMA-UE change ≥6 points) was 88% in the VNS group and 33% in controls ($p = 0.03$) (**Figures 6A,B**).

After controls crossed-over to receive in-clinic Active VNS, FMA-UE improved by 9.8 points from baseline ($p < 0.001$) after 6 weeks. After an additional 2 months of home-based VNS, FMA-UE improvement was maintained at 9.7 points ($p = 0.01$). Therefore, the improvements in upper limb impairment more than doubled after rehabilitation paired VNS compared to rehabilitation alone, an effect of approximately the same magnitude observed in the preclinical studies of VNS for ischemic stroke (Khodaparast et al., 2013).

It is of note that controls received similar intensity of in-clinic and home rehabilitation (without VNS) and showed

TABLE 1 | Comparison of the two pilot VNS studies (Dawson et al., 2016; Kimberley et al., 2018).

	Dawson et al., 2016	Kimberley et al., 2018
Number of sites	2 United Kingdom	4 United States and United Kingdom
Study design	Randomized, single-blind (Assessor)	Randomized, blinded (Assessor, Therapist, Participant), sham-controlled, cross-over
Number of participants	20 (VNS: $n = 9$; Control: $n = 11$)	17 (VNS: $n = 8$; Control: $n = 9$)
VNS implantation	Only VNS group implanted	Both VNS and Control group implanted
Long-term home therapy	No	Yes
Inclusion criteria	ARAT (Action Research Arm Test)	FMA-UE (Fugl-Meyer Assessment – Upper Extremity)
Outcome measure end-points	End of in-clinic (6 weeks) assessment followed by a 30-day assessment	End of in-clinic (6 weeks) assessment followed by 30-day and 3-month assessment
Imaging (Structural MRI)	Yes	Yes
Safety	(One) Transient vocal cord palsy and dysphagia after implant, (Five) minor events including nausea, metallic taste in the mouth. No serious adverse device effects.	(Three) Serious adverse events related to implantation surgery including wound infection, shortness of breath with dysphagia and hoarseness. No serious adverse device effects.
Efficacy (FMA change from baseline at 6 weeks)	9.6 vs. 3 (between group difference = 6.5 points, CI: 0.4 to 12.6, $p = 0.038$). *Response rate: 66% vs. 36.4%	7.6 vs. 5.3 (between group difference = 2.3 points, CI: −1.8 to 6.4, $p = 0.2$). *Response rate: 75% vs. 33%. At 3 months, post-therapy, 9.5 vs. 3.8 (between group difference = 5.7 points, CI: −1.4 to 11.5, $p = 0.055$). *Response rate: 88% vs. 33% ($p = 0.03$).

*FMA change ≥6 points.

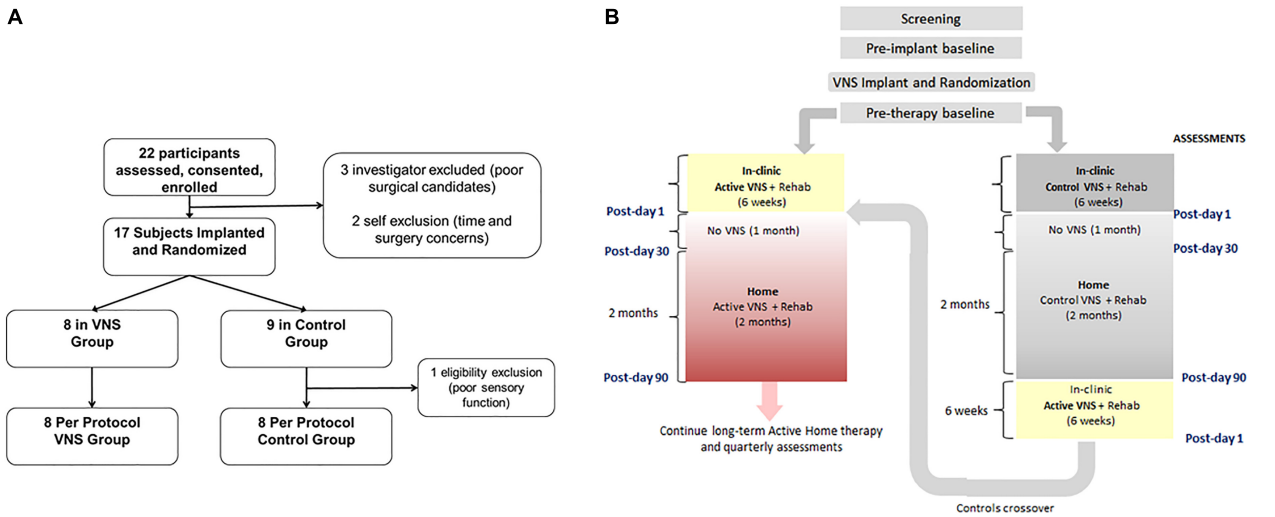

FIGURE 5 | (A) Consort Diagram for the pilot study (from Kimberley et al., 2018). Twenty-two participants were enrolled in the study of which 17 were implanted. Eight participants were randomized to the VNS group and 9 to rehabilitation only **(B)**. Clinical study flowchart. After screening and baseline evaluations, all participants were implanted with a VNS device and randomized to receive either Active (0.8 mA) or Control VNS (0.0 mA) paired with upper limb rehabilitation. Participants received 18 sessions of in-clinic therapy for 6 weeks, followed by a home-based therapy for 3 months (no VNS was delivered to either group during the 1st month of home therapy). The 3-month time point is referred to as Post-day 90. After Post-day 90, the Active VNS group continued with home-based Active VNS, and the Control group crossed over to receive 6-weeks of in-clinic therapy with Active VNS followed by home-based Active VNS, similar to the Active VNS+Rehab group. Outcome measures were evaluated at baseline, Post-day 1, Post-day 30, and Post-day 90.

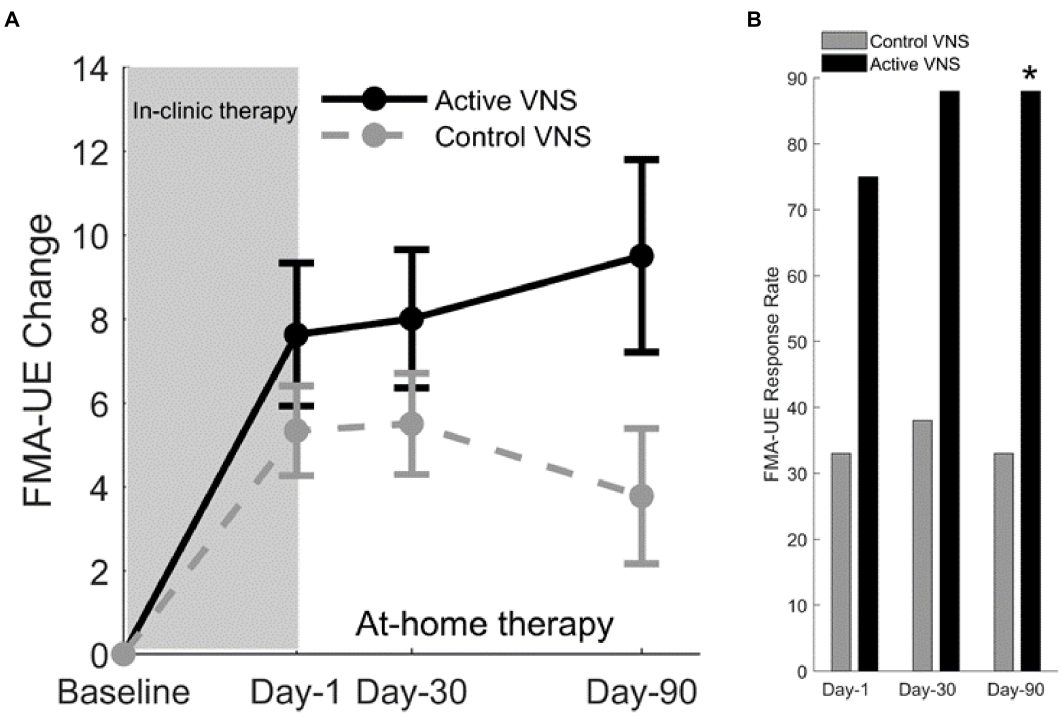

FIGURE 6 | (A) Fugl-Meyer assessment–upper extremity (Kimberley et al., 2018). Change in FMA-UE score at three posttreatment assessments from baseline for Active VNS (solid line) and Control VNS + Rehab (dashed line). Shaded area indicates 6 weeks of in-clinic therapy. Error bars indicated standard error of the mean (s.e.m). **(B)** FMA-UE responder rate (defined as FMA-UE change ≥6 points from baseline) for Active VNS (black) and Control VNS + Rehab (gray). *$p < 0.05$, Fisher exact test.

minimal improvement in the randomized portion of the study, especially as more time elapsed following the in-clinic rehabilitation. After crossover to Active VNS, the control participants showed a clinically meaningful outcome that was similar to the initial Active VNS group. This is consistent with studies suggesting that intense rehabilitation or standard of care rehabilitation for individuals with chronic stroke may be insufficient to significantly improve motor outcomes (van der Lee et al., 1999; Langhorne et al., 2009; Teasell et al., 2014).

In addition to improving motor impairment, Active VNS therapy also improved upper limb functional performance. At post-90 (3 months after the end of in-clinic therapy), the Wolf Motor Function Test (WMFT-Functional) difference between the Paired VNS and Control groups was 0.33 points (CI, 0.04 to 0.61; $p = 0.029$). Thus, participants showed significant improvements on both impairment (FMA-UE) as well as functional scales (WMFT-Functional) after Paired VNS therapy. These results suggest that improvement reflects true motor recovery rather than improved movement compensation. The study showed that rehabilitation paired with VNS was an acceptably safe and feasible intervention for patients with chronic stroke and demonstrated sufficient safety and feasibility to support a larger pivotal trial.

The benefits of Paired VNS require time to emerge and may suggest that progressive neural reorganization is facilitated by paired VNS (Porter et al., 2012; Meyers E.C. et al., 2018). VNS responders had greater cortico-spinal tract (CST) injury compared to control responders, which suggests that VNS-induced neuroplastic mechanisms could facilitate improvements in the VNS responders who would otherwise not have responded to rehabilitation alone (Dawson et al., 2016). These findings also mirror the reorganization of the CST observed with VNS therapy in preclinical models in which VNS paired with rehabilitation significantly increased synaptic connectivity in both ipsilesional and contralesional CST networks controlling the impaired forelimb (Meyers E.C. et al., 2018; **Figure 3**). Assessment of plasticity in multiple brain regions that accompanies improvements in recovery would strengthen future clinical studies by providing a more detailed description of the mechanisms that support VNS-dependent benefits.

A change in FMA-UE score of $\geq$6 points was used to indicate a clinically meaningful improvement. Previous studies have assessed FMA-UE scores using anchor-based methods to determine the clinically important change in FMA-UE from baseline. The FMA-UE change ranged from 4.24 to 7.25 points (Page et al., 2012). A $>$50% improvement in the overall arm and hand function, which was considered an excellent improvement, corresponded to FMA-UE change of 5.25 points. If the 9.5-point increase in FMA-UE score observed at day-90 following Paired VNS and the 9.8-point change from baseline after crossover to Paired VNS in Controls is a true effect of VNS, the therapy enhances the modest improvements seen with rehabilitation alone, up to more clinically meaningful levels.

CLINICAL AND NEUROPHYSIOLOGICAL CONSIDERATIONS FOR FUTURE CLINICAL STUDIES

Although the studies described above present initial evidence that VNS paired with rehabilitation may support recovery after stroke, there are several important considerations for continued translation of the VNS therapy.

Clinical and Neurophysiological Biomarkers

Clinical and neurophysiological biomarkers are important for predicting response to interventions, especially in a heterogeneous chronic stroke population (Milot and Cramer, 2008; Burke and Cramer, 2013; Wu et al., 2015; Boyd et al., 2017). It would be valuable to identify biomarkers in patient subpopulations that are non-responsive to the VNS therapy. Biomarker evaluation across a range of stimulation parameters, including intensity, frequency, and pulse width, would be useful to guide the selection of paradigms to maximize plasticity and recovery after stroke. Future studies with larger sample sizes may determine whether clinical and neurophysiological markers will help identify participants more or less responsive to VNS therapy.

A number of characteristics, including age, type of stroke (e.g., ischemic or hemorrhagic), stroke location (e.g., supratentorial or infratentorial), stroke severity, amount of spasticity, associated contractures that may limit movement, time since stroke onset, associated sensory loss, comorbidities (e.g., diabetes), are known to affect outcomes. Moreover, factors that directly impact neuromodulatory function, including Parkinson's disease, Alzheimer's disease and concomitant use of pharmacotherapeutic agents, may specifically impact the efficacy of VNS. These factors will be discussed below.

Supratentorial and Infratentorial Strokes

The clinical VNS studies described above included participants with supratentorial, ischemic stroke and excluded infratentorial strokes. Infratentorial or posterior strokes such as those involving the cerebellum, pons or medulla, were excluded because the behavioral benefits of paired VNS have not yet been demonstrated in preclinical models. Furthermore, individuals with posterior strokes presenting with upper limb weakness likely have other symptoms including dizziness, double vision, visual field deficits, dysphagia, clumsiness of the hand and ataxia that may impact upper limb motor training and therefore would likely require a different rehabilitation protocol. Previous studies have demonstrated that brainstem infarcts can result in the activation or reorganization of motor cortex (Kwon and Jang, 2010). It is possible that Paired VNS therapy could recruit upstream spared CSTs to regain lost function. Furthermore, studies in rat models of SCI showed that VNS paired with motor training drives plasticity in upstream motor neurons, suggesting that VNS-dependent plasticity in residual cortical or subcortical motor circuits could mediate recovery (Ganzer et al., 2018).

Hemorrhagic Stroke

In rat models of hemorrhagic stroke, rehabilitation improves motor outcomes along with changes in dendrite morphology suggesting that plasticity within residual neurons supported recovery (Auriat et al., 2010). Furthermore, studies in a rat model of ICH provide direct evidence that VNS paired with motor training significantly improves forelimb function compared to equivalent training alone (Hays et al., 2014a). However, the clinical VNS studies excluded individuals with hemorrhagic stroke to maximize the ability to detect effects in ischemic stroke patients. Considering the flexibility of VNS to enhance recovery in a wide range of neurological injury animal models including hemorrhagic stroke, future studies evaluating VNS in patients with these types of stroke is warranted.

Age

Age is an important non-modifiable risk factor for ischemic stroke (Bagg et al., 2002; Kelly-Hayes et al., 2003; Saposnik et al., 2008; Hays et al., 2016; Lui and Nguyen, 2018). Advanced age is associated with a reduction in neuroplasticity, which raises the prospect that advanced age may reduce the efficacy of VNS therapy (Kelly-Hayes et al., 2003; Burke and Barnes, 2006; Freitas et al., 2011). However, preclinical studies provide an initial demonstration that age does not limit VNS-dependent enhancement of recovery after stroke, as aged rats benefited from the therapy as much as young rats (Hays et al., 2016). The pilot clinical study (Kimberley et al., 2018) included a wide age-range of participants (37–73 years), and after 3 months of paired VNS therapy, 50% of participants over 65 years of age showed significant improvement in FMA-UE scores (≥6-point change). Therefore, age by itself did not preclude VNS-dependent benefit in responders; and less improvement in non-responders suggests that other factors are involved in determining response to therapy.

Chronic Stroke

The clinical studies included individuals with chronic stroke for the following reasons: First, highlighting the need for interventions that are effective long after the acute stroke episode, an estimated 7.2 million Americans live with chronic post-stroke disability (Benjamin et al., 2018). Second, evidence from preclinical studies supports the efficacy of VNS paired with rehabilitative training when initiated several weeks after stroke (Khodaparast et al., 2016). Thus, VNS likely acted by engaging plasticity-enhancing neuromodulatory circuits during training rather than pro-plasticity factors upregulated by stroke (Meyers E.C. et al., 2018). Third, since spontaneous recovery of upper limb motor deficits is often observed during the first 6 months after stroke, any improvements in upper limb deficits obtained from interventions carried out during this acute phase would be difficult to dissociate from this spontaneous recovery. Indeed, participants with sub-chronic stroke often show greater improvements on the FMA-UE compared to participants with chronic stroke (Shelton et al., 2001; Masiero et al., 2007; Narayan Arya et al., 2011). Finally, acute stroke is a life-changing event for the majority of individuals, and it is likely that most individuals, physicians, and other healthcare professionals would be somewhat reluctant to undergo a non-emergency surgical procedure. The chronic population was therefore selected as a starting point for investigation.

Severity of Upper Limb Deficits

The VNS clinical studies excluded individuals with very severe upper limb deficits who had minimal to no movement in their upper extremity (typically FMA-UE < 15). VNS could be combined with other interventions to initiate movements in this severe population. Since paired VNS in rats facilitated recruitment of residual neurons and increased synaptic connectivity in cortico-spinal networks controlling the impaired forelimb (Meyers E.C. et al., 2018), it is possible that the severity of CST injury may not preclude recovery of the impaired limb function in humans. This would be an interesting area for study once proven effective in a moderately severe population.

Centrally Acting Drugs May Interfere With the Effects of VNS

Since VNS acts via the activation of neuromodulatory pathways, it is possible that certain medications could interfere with the effects of VNS therapy. For example, lipophilic muscarinic antagonists (e.g., scopolamine) or adrenergic antagonists (e.g., metoprolol) easily cross the blood-brain barrier and are known to have central adverse effects which could interfere with the effects of VNS. Animal studies provide supporting evidence that interfering with neuromodulatory networks prevents the plasticity enhancing effects of VNS (Hulsey et al., 2016; Hulsey, 2018). Unlike pharmacological blockade, animal studies utilized methods that resulted in a permanent, virtually complete reduction of neuromodulators. Therefore, pharmacological antagonism may differentially influence the effects of VNS. Nevertheless, given the well-documented literature regarding the central effects of some cholinergic and noradrenergic antagonists on mood, cognitive processing, behavioral performance and neurophysiological indicators of plasticity, some drug exclusions need to be considered in clinical studies.

Sensory Loss

Impaired tactile sensation, stereognosis, and proprioception are common after stroke. Sensory disruption can affect motor function and recovery, since sensorimotor integration is important for successful goal-directed movements (Xerri et al., 1998; Bolognini et al., 2016). With severe sensory loss, the motor deficits can appear to be worse, even in the absence of significant muscle weakness. The motor cortex receives significant input from somatosensory areas, and peripheral nerve lesions or lesions in the somatosensory cortex can significantly alter movement representations in motor cortex and impact motor skill learning (Donoghue and Sanes, 1987; Xerri et al., 1998). Furthermore, lesions of motor cortex can also disrupt sensory function (Nudo et al., 2000).

It is possible that repeatedly pairing VNS with tactile rehabilitation may improve sensory deficits in individuals with significant sensory loss. In a case report study involving a

72-year-old male with sensory deficits, VNS paired with tactile rehabilitation showed clinically meaningful improvements in sensory threshold, proprioception and stereognosis that were long-lasting (Kilgard et al., 2018). It is possible that the pairing narrowed receptive fields from the hand to individual fingers, which may have contributed to the improved tactile perception. Thus, individuals with motor deficits and significant sensory deficits may benefit from VNS combined with tactile training and could show improvements in both sensory as well as motor function.

Comorbid Conditions

Neurodegenerative diseases (e.g., Alzheimer's disease and Parkinson's disease) can deplete neuromodulator reserves in basal forebrain cholinergic neurons and LC neurons (Whitehouse et al., 1981; Coyle et al., 1983; Gesi et al., 2000; Zarow et al., 2003). Since cholinergic and noradrenergic modulation is essential for the effects of VNS, it is possible that decreased neuromodulator reserves may impact VNS-induced plasticity. In such individuals, it is possible that different stimulation parameters may be needed to generate appropriate activation of remaining neuromodulatory networks. Future studies evaluating VNS in both animal models and patients with neurodegenerative diseases is warranted.

Future preclinical and clinical studies in larger populations along with neurophysiological biomarkers as predictors of improvement will help adapt the VNS therapy to different patient subgroups.

OPTIMIZATION OF VNS PARAMETERS

Identification of stimulation parameters and paradigms that yield maximal recovery is an important step in the translation of VNS-based targeted plasticity therapy for stroke. Both the preclinical and clinical studies evaluating motor recovery described above utilized identical stimulation settings of 0.8 mA, 100 μs pulse width, 30 Hz frequency and a pulse train of 0.5 s (Engineer et al., 2011; Porter et al., 2012; Dawson et al., 2016; Kimberley et al., 2018).

Given that VNS-directed plasticity is believed to underlie recovery, a number of studies have characterized stimulation paradigms aimed at increasing the magnitude of VNS-dependent plasticity. The parameter that has been most thoroughly investigated is stimulation intensity. Higher intensity stimulation recruits a larger proportion of vagal fibers and triggers stronger activation of neuromodulatory nuclei, which may improve stroke recovery (Roosevelt et al., 2006; Castoro et al., 2011; Mollet et al., 2013; Hulsey et al., 2017). Paradoxically, a number of studies examining the effects of VNS on neural plasticity and memory indicate that moderate intensity stimulation generates the greatest effects compared to lower and higher intensity stimulation (Clark et al., 1995, 1998, 1999), suggesting that non-linear interactions in upstream targets may be responsible for these effects and VNS operates across a specific range of stimulation parameters.

Increasing the pulse width can compensate for a reduction in stimulation amplitude, indicating that total charge delivered to the nerve is the main predictor of VNS-dependent engagement of neuromodulatory networks and VNS-dependent plasticity (Hulsey et al., 2017; Loerwald et al., 2017). Several studies have examined the influence of varying other stimulation parameters on VNS-dependent plasticity. Increasing the interval between stimulation trains increases the magnitude of VNS-dependent plasticity, an effect ascribed to desensitization of neuromodulatory receptors (Borland et al., 2018). Additionally, similar to the effect of stimulation intensity, the pulse frequency during a VNS train also demonstrates an inverted-U relationship with plasticity. Trains consisting of pulses delivered at moderate frequency rates enhanced cortical plasticity, while slower and faster pulse rates both fail to significantly enhance plasticity (Buell et al., 2018). Taken together, the studies illustrate the influence of both the timing and intensity of stimulation parameters on the magnitude of VNS-dependent plasticity, suggesting manipulation of either or both parameters may be required to optimize efficacy for clinical implementation.

The precise mechanisms that underlie the observed inverted-U relationship between plasticity and several VNS parameters are not fully understood. However, several possibilities could explain this response, the most apparent of which is the effect of stimulation intensity. First, lower stimulation intensities could recruit pro-plasticity neuromodulatory circuits, while higher intensities recruit overriding anti-plasticity networks. As a result, moderate stimulation intensities would produce the greatest enhancement of plasticity by maximally recruiting the low threshold system while suppressing activation of the high threshold system. Other possible explanations relate to receptor activation. Noradrenergic receptors are required for VNS effects and are known to display considerable adaptation (Gainetdinov et al., 2004). Low intensity stimulation may avoid desensitization and allow repeated effective signaling and thus drive plasticity, while high intensity stimulation may produce desensitization that prohibits repeated activation and limits plasticity. Alternatively, activation of different receptor types at differing stimulation intensities could produce an inverted-U effect. Low and moderate intensities of VNS may result in appropriate norepinephrine release to engage higher-affinity α2-adrenergic receptors and promote potentiation, whereas high intensity stimulation may increase norepinephrine levels further to activate lower-affinity β-adrenergic receptors to oppose potentiation. Indeed, this concentration-dependent dichotomy in control of the polarity of plasticity by adrenergic receptors has been described previously (Salgado et al., 2012). A recent study demonstrated that stimulation frequency also imposes an inverted-U effect on the degree of plasticity, consistent with postsynaptic receptor activation as the primary mediator of the response (Buell et al., 2018). It is important to note that both the desensitizing and opposing activation models, as well as many others, may contribute to the inverted-U, as they are not mutually exclusive.

It is not known whether the inverted-U response results from a common underlying principle of cellular and network activity across all brain regions or whether differences in network

architecture across different systems would produce different outcomes. It is possible that non-responders to the standard VNS therapy may benefit from a different set of stimulation parameters that operate within this range or circumvent the conditions that perturb neuromodulatory pathways, such as alterations in vagal tone or neuromodulatory function. Furthermore, given the heterogeneity of patient characteristics as well as stroke manifestations described above, it is possible that some subgroups may be more responsive to one set of stimulation settings than others. Clinical studies described above utilized a standard, non-individualized set of stimulation parameters and observed significant improvement in motor deficits in most patients, supporting the notion that a relatively wide effective therapeutic range exists and individual variability is unlikely to preclude benefits (Dawson et al., 2016; Kimberley et al., 2018). Regardless of the underlying mechanism, the differential responses to stimulation parameters highlight the utility of optimizing stimulation parameters to yield the greatest response.

NON-INVASIVE VAGUS NERVE STIMULATION

In recent years, non-invasive transcutaneous methods of stimulating the vagus nerve have emerged as a potential alternative strategy to generate VNS without necessitating a surgical implant. There are two primary ways of delivering non-invasive VNS. The first method, commonly termed tVNS or aVNS, targets the auricular branch of the vagus nerve (ABVN) and consists of the application of stimulation to the skin of the external ear on the tragus and cymba. The second is transcutaneous stimulation of the skin in the neck region over the cervical vagus nerve, commonly referred to as nVNS and targets the underlying cervical vagus.

The two main sites for auricular VNS include the tragus and cymba concha. Recent reports suggest that the extent to which vagal branches innervate the tragus is unclear (Badran et al., 2018a; Burger and Verkuil, 2018) due to inconsistencies in a human cadaver study that described the innervation of the human auricle (Peuker and Filler, 2002). Furthermore, inconsistencies in electrode placement and skin contact coupled with the effects of varying tissue impedance on nerve activation from individual to individual may be impediments to reliable stimulation with tVNS. For example, the electrode is placed over the auricular skin in a relatively small area with dense innervation and it is possible that the spread of current could activate nearby nerves such as the auriculotemporal branch of the mandibular nerve. This combined recruitment complicates the assessment and interpretation of the effects of stimulation of the vagus nerve.

Stimulation parameters using implanted cervical VNS have been well characterized and strongly influence the plasticity effects of VNS. The challenge of identifying and consistently delivering stimulation within a particular range of parameters is magnified by non-invasive stimulation strategies. While tVNS may be able to stimulate the auricular branches of the vagus, the inability to provide consistent, reliable activation may hamper the ability to observe robust effects. Furthermore, the ABVN has five times less A-β fibers compared to the cervical vagus nerve (Safi et al., 2016), which may contribute to its weaker activation of central targets (Ay et al., 2015).

Therefore, while avoiding surgical implantation has advantages, the preponderance of evidence in well-controlled studies points to the failure of these devices to sufficiently and reliably activate key brain structures. For example, in rat models of acute ischemic stroke, cervical VNS resulted in a greater reduction of infarct volume compared to non-invasive VNS (Ay et al., 2009, 2015). Non-invasive VNS also generated less intense c-fos staining in NTS neurons compared to cervical VNS, suggesting less robust activation (Ay et al., 2015). Available data from human studies describing regional brain activation in response to non-invasive VNS varies substantially from study to study (Kraus et al., 2007; Frangos et al., 2015; Yakunina et al., 2017; Badran et al., 2018a). Moreover, human studies using tVNS at the tragus failed to demonstrate significant activation of the locus coeruleus, a key brainstem nucleus in the actions of VNS, compared to sham stimulation (Yakunina et al., 2017; Badran et al., 2018b). These studies may explain the reduced efficacy of human studies with non-invasive VNS compared to cervical VNS (Bauer et al., 2016; Barbella et al., 2018).

A second non-invasive approach is stimulation delivered to the neck region above the cervical vagus nerve (nVNS). This method of non-invasive stimulation has shown efficacy for the treatment of acute episodes of cluster headaches and migraine (Silberstein et al., 2016; Goadsby et al., 2018; Grazzi et al., 2018; Tassorelli et al., 2018). The mechanism of action is thought to arise from VNS-driven activation of NTS, which in turn modulates the activity of the trigeminal cervical complex (TCC) (Moeller et al., 2018) and suppresses the transmission of nociceptive signals to higher pain processing centers (Bohotin et al., 2003). However, NTS also receives direct inputs from the trigeminal and cervical nerves. Since these nerves lie near the vagus nerve, it is possible that these nerves can also activate NTS via the spread of current. Indeed, trigeminal nerve stimulation or peripheral nerve stimulation can modulate nociceptive signals in the TCC via activation of NTS (Contreras et al., 1982; Lewis et al., 1987; Du and Zhou, 1990; Zerari-Mailly et al., 2005; Liu et al., 2014; Mercante et al., 2017) and have therefore been used for the treatment of headaches (Magis et al., 2007, 2013; Saper et al., 2011). Activation of these nerves during nVNS could contribute to headache relief (Henssen et al., 2019). Therefore, both VNS and TNS can modulate nociceptive input via NTS activation and may represent a generalized anti-nociceptive response to stimulation. In contrast, the induction of cortical plasticity is unique to VNS inputs. Repeatedly pairing a tone with cervical VNS, but not TNS, resulted in tone-specific plasticity in the auditory cortex (Engineer et al., 2011).

In addition to NTS, which receives 95% of the vagal input (Magdaleno-Madrigal et al., 2010), key brain regions activated by cervical VNS are also activated by non-invasive VNS including locus coeruleus, amygdala, hippocampus, cingulate and insula (Chae et al., 2003). This implied that the actions of non-invasive VNS were similar to cervical VNS since both methods activate similar upstream targets, and could, therefore, be used as an alternative to cervical VNS. However, many studies have

demonstrated these key brain regions are also activated by peripheral nerve stimulation, trigeminal nerve stimulation, and cutaneous stimulation (Kwon et al., 2000; Rouzade-Dominguez et al., 2001; Scherder et al., 2003; Frangos and Komisaruk, 2017; De Cicco et al., 2018). Furthermore, LC neurons can be activated by both aversive stimulation (e.g., tail pinch) as well as cervical VNS (Hulsey et al., 2017). In other words, brain regions activated by VNS are also activated by tactile, arousing or aversive sensory stimuli, suggesting that the activation of these regions is not specific to the vagus nerve. Therefore, nVNS activation of common brain regions does not entail equivalence to cervical VNS.

Furthermore, cervical VNS stimulation parameters have been well characterized and have been shown to modulate plasticity effects across a twofold range of intensities and suggest the existence of a potentially useful therapeutic range of activity (Borland et al., 2016). With non-invasive VNS, the ability to deliver consistent and reliable stimulation within a particular range of parameters to induce plasticity for therapeutic use has not yet been demonstrated. Taken together, these results demonstrate that brain activation of common targets by cervical VNS and non-invasive VNS does not entail similar plasticity or behavioral outcomes. More studies are needed to determine the extent to which the vagus nerve is activated using non-invasive approaches along with a parametric characterization of stimulation parameters.

Recently, two clinical studies were conducted using non-invasive VNS combined with upper limb rehabilitation in individuals with chronic upper extremity weakness after stroke. In a study by Capone et al., (Capone et al., 2017) individuals with chronic ischemic or hemorrhagic stroke were randomized to either tVNS combined with robotic rehabilitation (n = 7) or auricular-sham VNS (ear lobe) combined with robotic rehabilitation (n = 5). The therapy was delivered for 10 days over 2 weeks. After 2 weeks, no significant differences between the tVNS and sham group were observed on the FMA-UE score (5.4 vs. 2.8 points, p = 0.16). While the results are interesting, the sample size precludes drawing distinct conclusions about tVNS efficacy.

In the second single-arm feasibility study (Redgrave et al., 2018), 13 participants more than 3 months post-stroke underwent rehabilitation combined with tVNS for 6 weeks. After tVNS rehabilitation training, the FMA-UE score increased by 17.1 $\pm$ 7.8 points with a >10-point change in 83% of patients. It should be noted that the FMA-UE scores used in this study combined motor, sensory, and joint components (0–126 points score) instead of the 0–66 points score that is typically used in many upper-limb stroke studies. Therefore the results are not directly comparable with the cervical VNS studies (Dawson et al., 2016; Kimberley et al., 2018). Several limitations of this study are worth considering. First, the study did not include a sham stimulation control group. Since stimulation was delivered at the maximally tolerable intensity and was thus perceptible, a placebo effect of stimulation cannot be ruled out. Second, some participants were less than 6 months post-stroke, and it is possible that spontaneous recovery could contribute to some of the improvement (Narayan Arya et al., 2011). A future randomized, blinded, placebo-controlled study in chronic stroke patients would be required to determine the efficacy of non-invasive VNS as applied to upper limb rehabilitation.

Further studies are needed to explore the effectiveness of non-invasive VNS, with a specific focus on parametric characterization. Ideally, any non-invasive VNS effects would be benchmarked against implanted VNS to determine the magnitude. As non-invasive stimulation would have demonstrable advantages for patients over implanted VNS, a thorough evaluation in robust, well-designed studies is needed to guide future clinical implementation.

CONCLUSION AND FUTURE DIRECTIONS

The studies reviewed provide a compelling demonstration that VNS-based rehabilitation is a potentially useful strategy to target plasticity and improve motor function for chronic stroke. VNS-dependent rapid engagement of neuromodulatory networks provides a signal to facilitate plasticity in pathways activated by rehabilitative exercises. While the effects of cholinergic and noradrenergic modulation on cortical plasticity have been well documented, other neuromodulators could also play a role in VNS-induced cortical plasticity. Emerging evidence highlights a similar role of serotoninergic systems in the VNS-dependent enhancement of plasticity, paralleling studies demonstrating that VNS activates these neuromodulatory systems (Manta et al., 2009, 2012; Hulsey, 2018). The neurophysiological mechanisms underlying VNS-driven cortical plasticity are complex and likely involve top-down control of neuromodulatory inputs involved in the planning of movements, reward, and decision making (Zmarowski et al., 2005; Convento et al., 2014).

The effects of VNS paired with rehabilitation have been tested across several different animal models of stroke and other neurological injuries and consistently demonstrate significantly greater recovery and enhancement of plasticity when rehabilitation is paired with VNS compared to equivalent rehabilitation without VNS. The flexibility to improve recovery across several injury models demonstrates that VNS engages a generalized mechanism to potentiate benefits specific to rehabilitation. The improved behavioral outcomes across different models along with objective evidence of plasticity after paired VNS informed clinical studies for the inclusion of appropriate patient populations who are likely to benefit from the therapy.

The encouraging findings from the two pilot clinical studies supported the design of a phase III pivotal, multi-site, double-blind, randomized trial (VNS-REHAB) of this intervention with 120 implanted participants and approximately 20 study sites. This study is powered to detect the difference seen in the FMA-UE score at the end of 6-weeks of in-clinic therapy with 80% power. The VNS-REHAB study is

approximately 75% enrolled, with enrollment expected to complete in Spring 2019.

Despite the observed improvements across a range of conditions, it is possible that additional factors, including comorbid conditions, stroke etiology, individual variations in anatomy, and drugs or diseases that influence neuromodulatory function, could influence the efficacy of VNS therapy. Evaluation of the clinical effectiveness of paired VNS therapy in heterogeneous stroke populations along with continued development of stimulation parameters and rehabilitative paradigms to individualize and optimize the therapy for specific patient subgroups will improve the potential of this therapy to improve human function and well-being.

AUTHOR CONTRIBUTIONS

NE and SH wrote the manuscript. All authors participated in the discussion of the manuscript and provided significant revisions. All authors approved the final version of the manuscript for submission.

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Transcutaneous Auricular Vagus Nerve Stimulation (tAVNS) Delivered During Upper Limb Interactive Robotic Training Demonstrates Novel Antagonist Control for Reaching Movements Following Stroke

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Implanted vagus nerve stimulation (VNS) delivered concurrently with upper limb rehabilitation has been shown to improve arm function after stroke. Transcutaneous auricular VNS (taVNS) offers a non-invasive alternative to implanted VNS and may provide similar therapeutic benefit. There is much discussion about the optimal approach for combining VNS and physical therapy, as such we sought to determine whether taVNS administered during robotic training, specifically delivered during the premotor planning stage for arm extension movements, would confer additional motor improvement in patients with chronic stroke. Thirty-six patients with chronic, moderate-severe upper limb hemiparesis (>6 months; mean Upper Extremity Fugl-Meyer score = 25 ± 2, range 13–48), were randomized to receive 9 sessions (1 h in length, 3x/week for 3 weeks) of active (N = 18) or sham (N = 18) taVNS (500 ms bursts, frequency 30 Hz, pulse width 0.3 ms, max intensity 5 mA, ∼250 stimulated movements per session) delivered during robotic training. taVNS was triggered by the onset of a visual cue prior to center-out arm extension movements. Clinical assessments and surface electromyography (sEMG) measures of the biceps and triceps brachii were collected during separate test sessions. Significant motor improvements were measured for both the active and sham taVNS groups, and these improvements were robust at 3 month follow-up. Compared to the sham group, the active taVNS group showed a significant reduction in spasticity of the wrist and hand at discharge (Modified Tardieu Scale; taVNS = –8.94% vs. sham = + 2.97%, $p < 0.05$). The EMG results also demonstrated significantly increased variance for the bicep peak sEMG amplitude during extension for the active taVNS group compared to the sham group at discharge (active = 26.29% MVC ± 3.89, sham = 10.63% MVC ± 3.10, mean absolute change admission to discharge, $p < 0.01$), and at 3-month follow-up, the bicep peak sEMG

amplitude was significantly reduced in the active taVNS group ($P < 0.05$). Thus, robot training improved the motor capacity of both groups, and taVNS, decreased spasticity. taVNS administered during premotor planning of movement may play a role in improving coordinated activation of the agonist-antagonist upper arm muscle groups by mitigating spasticity and increasing motor control following stroke.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier (NCT03592745).

Keywords: stroke, vagus nerve stimulation (VNS), transcutaneous auricular vagus nerve stimulation (taVNS), hemiparesis, rehabilitation, robotic therapy

INTRODUCTION

Stroke is the leading cause of long-term disability in the United States (American Heart Association [AHA], 2021). Even with aggressive standard rehabilitation, more than 40% of patients experience chronic upper limb hemiparesis (Cramer et al., 1997). Recently, the combination of upper limb rehabilitation with vagus nerve stimulation (VNS) was demonstrated to improve motor outcomes in individuals with chronic post-stroke hemiparesis (Dawson et al., 2016, 2021; Kimberley et al., 2018). VNS has been shown to activate cholinergic basal forebrain, noradrenergic locus coeruleus networks important for plasticity and learning, and to enhance the release of GABA (Capone et al., 2015; Hays, 2016; Colzato and Beste, 2020), thereby potentially facilitating improvement when it is combined with motor rehabilitation.

Animal models of motor recovery following stroke have indicated specificity of recovery for only those tasks or stimuli paired with VNS. Motor behaviors paired with implanted VNS following stroke demonstrated selective increases in the size of their motor representations within the motor cortex, while motor representations for untrained tasks or tasks performed without VNS remained relatively unchanged (Porter et al., 2011; Khodaparast et al., 2014). A similar specificity of recovery for VNS-stimulated tasks has been documented in studies that focused on tinnitus reduction, in which selective pairing of VNS with tones outside of the tinnitus white noise perceptual range resulted in significant reductions in the perception of tinnitus for up to three months (Engineer et al., 2011; De Ridder et al., 2014). Taken together, these results suggest that timing, frequency, total dose delivered and characteristics of the electrical stimulation with respect to the stimulated task may all be important factors for treatment effectiveness.

In the rehabilitation of patients with chronic stroke, a notable obstacle to motor recovery is the persistence of maladaptive upper and lower limb flexor synergy patterns that impair independent control of individual joints (Zackowski et al., 2004). Some argue this aspect of the upper motor syndrome after stroke is a more significant obstacle to recovery than the traditionally defined passively elicited velocity dependent hyperactive stretch reflex (Ellis et al., 2017), commonly termed spasticity (Lance, 1980). Upper extremity flexor synergy is characterized by a fixed pattern of scapular retraction, shoulder abduction/external rotation, elbow/wrist/finger flexion, and wrist supination, resulting a "curling in" of the arm toward the body with a rigid, closed hand. It is caused by damage to the corticospinal tract and subsequent upregulation of interneuron spinal networks, and ultimately results in movement limitations, particularly for extension (McMorland et al., 2015). We have previously shown that robotic therapy provides clinically significant benefits to upper limb motor recovery after stroke (Volpe et al., 2009; Lo et al., 2010; Chang et al., 2017; Edwards et al., 2019), and can specifically reduce upper limb flexor synergy patterns through shoulder/elbow robotic training (Dipietro et al., 2007). We have also demonstrated that treatment aimed at passively elicited spasticity reduction can unmask latent motor potential (Paget-Blanc et al., 2019). In this study, we tested whether maximal and optimized current delivered to the auricular branch of the vagus nerve during pre-motor activity for robot-trained extensor movements would reduce spasticity and generate additional motor recovery of arm function after stroke.

Although many previous studies of VNS depend on invasive stimulation paired with motor training, transcutaneous auricular vagus nerve stimulation (taVNS) has emerged as a viable, efficacious, and non-invasive alternative that likely activates similar cortical networks as implanted VNS (Kraus et al., 2007; Badran et al., 2018). Here, we performed a double-blinded study using taVNS or sham stimulation paired with 3 weeks of shoulder/elbow robotic therapy. We selected a 3-week course of robotic training for study efficiency, as it has been shown to induce a reliably detectable improvement on clinical scales (Volpe et al., 2009). This study investigates whether specifically timed taVNS augments a robot trained clinical benefit and additionally produces an objective surface electromyography (sEMG) biomarker of clinical improvement in the trained muscle groups. taVNS stimulation was selectively delivered during the onset of a visual cue for extension movements to alter flexor synergy patterns and to target improved planning and execution of extension.

MATERIALS AND METHODS

Participants

Thirty-six patients with a diagnosis of stroke and chronic (>6 months) upper limb hemiparesis were recruited by treating clinicians in the Departments of Neurology and Physical Medicine and Rehabilitation at Northwell Health (18 males, 18 females; 59.02 years of age ± 1.98, range 27.9–81.1; 2.16 years post stroke ± 0.39; **Table 1**). This trial was approved

TABLE 1 | Patient demographics.

Parameters (*n* = 36)	Mean (SEM)	Range
Sex, F/M, n	18/18	N/A
Age	59.02 (1.98)	27.9–81.1
Time after stroke, years	2.16 (0.39)	0.5–12.8
Type of stroke (Ischemic/Hemorrhagic)	27/9	N/A
Affected side (Dominant/Non-dominant)	17/19	N/A
Baseline Fugl-Meyer	25.27 (2.14)	13–48
Baseline MTS total upper extremity	23.5 (0.73)	12–31
Baseline MTS shoulder	7.1 (0.39)	4–12
Baseline MTS elbow	8.1 (0.27)	5–11
Baseline MTS wrist	8.6 (0.32)	3–14

by the Institutional Review Board at Northwell Health, and all subjects provided written informed consent. Inclusion criteria were: (a) $\geq$ 18 years and $\leq$ 85 years of age; (b) First single focal unilateral supratentorial stroke with diagnosis verified by brain imaging (CT or MRI) that occurred at least 6 months prior; (c) Cognitive function sufficient to understand the experiments and follow instructions; (d) Upper Extremity Fugl-Meyer (UE-FM) assessment score of 12–48 points (neither hemiplegic nor fully recovered motor function in the muscles of the shoulder, elbow, and wrist). Exclusion criteria were: (a) Botox treatment within 3 months of enrollment, (b) Fixed contracture of the affected limb, (c) Complete and total flaccid paralysis of all shoulder and elbow motor performance, (d) Prior injury to the vagus nerve, (e) Severe dysphagia, (f) Introduction of any new rehabilitation interventions during study, (g) Scar tissue/broken skin at stimulation site, or irremovable metal piercings that may interfere with the stimulation or the stimulation device, (h) Highly conductive metal in any part of the body, (i) Pregnant or plan on becoming pregnant or breastfeeding during the study period, (j) Significant arrhythmias, including but not limited to, atrial fibrillation, atrial flutter, sick sinus syndrome, and A-V blocks, (k) Presence of an electrically, magnetically or mechanically activated implant, an intracerebral vascular clip, or any other electrically sensitive support system.

A total of 144 subjects were screened for the study, and 102 subjects were excluded for the following reasons: multifocal or brainstem infarcts (42 subjects), significant cardiac arrhythmias (19 subjects), unrelated diagnosis (17 subjects), did not meet UE-FM motor inclusion criteria (8 subjects), transit issues (8 subjects), severe dysphagia (2 subjects), declined to participate (6 subjects). Thirty-six patients ultimately enrolled (**Table 1**). One patient dropped out for an unrelated health issue prior to completion of the intervention. One patient paused robotic intervention for greater than 3 weeks due to a family emergency, and thus this patient's data was excluded from the analysis. Five patients were lost to follow-up (1 was unable to be reached, 2 had unrelated health issues, 2 refused to return for FU amidst COVID-19 pandemic). Thirty-four patients completed the trial through discharge and were thus included in the analysis of the immediate effects of taVNS intervention (sham taVNS = 17 subjects; active taVNS = 17 subjects). Twenty-nine patients also completed the 3-month follow-up evaluation, and were included in the measures of treatment robustness over time (sham taVNS = 15 subjects; active taVNS = 14 subjects). One patient was missing Modified Tardieu Scale (MTS) measures and one patient had corrupted follow-up EMG measurements, and thus analyses of these measures included 33 and 34 patients at discharge, respectively, and 28 patients at follow-up across both measures. All subjects were naïve to taVNS.

Experimental Protocols

This was a double-blind, sham-controlled, repeated measures study evaluating whether 9 sessions of shoulder/elbow robotic therapy (3x/week for 3 weeks) paired with active taVNS or sham taVNS delivered during the onset of a visual cue for extension movements would significantly change objective EMG activation patterns and significantly improve clinical measures of upper extremity motor function. Patients underwent three clinical and instrumental EMG evaluations prior to intervention to verify the stability of their baseline scores. Clinical and instrumental EMG assessments were repeated immediately following 3 weeks of shoulder/elbow robotic training at discharge, and 3 months after study completion at follow-up. Upon admission, participants were classified according to baseline UE-FM with either severe (14–22 points) or moderate (23–48 points) motor impairment, and were randomized to receive active or sham taVNS, stratified by impairment level. The patient, treating clinician, and clinical evaluator were all blind to condition.

taVNS or sham stimulation was delivered to the left cymba conchae *via* a pair of conductive silicone electrodes affixed to an ear clip, which patients wore for the duration of each active-assist robotic intervention. During each 1-h long therapy session, the patient was seated comfortably with the affected upper limb strapped into a supportive trough, and was prompted by visual cue on a computer monitor to perform a total of 1,024 center-out flexion, extension, and rotational movements of the elbow and shoulder joints (**Figure 1**). Robotic therapy was active-assist, such that if the patient could not move, the robot would move the patient's arm after a 2 s delay. taVNS was delivered in single 500 ms bursts with a frequency of 30 Hz and a pulse width of 0.3 ms during the onset of the blinking visual cue for extension movements of the trained limb (right = 9 o'clock, 10 o'clock, 12 o'clock, 2 o'clock; left = 10 o'clock, 12 o'clock, 2 o'clock, 3 o'clock). A total of 256 stimulations were delivered per session. Current intensity was individually adjusted to a level just below the patient's reported pain threshold, with amplitudes ranging from 0.1 to 5.0 mA in steps of 100 μA. A device tolerance screening questionnaire was given to all participants before and after each session. For sham stimulation, current intensity threshold was evaluated at the beginning of each session and stimulation was then ramped to zero for the duration of robot therapy. This protocol allowed sham taVNS patients to experience the sensation of treatment without delivering adequate stimulation for a therapeutic effect (Gandiga et al., 2006; Brunoni et al., 2014).

Electrode and Stimulator Design

The transcutaneous auricular branch vagus nerve simulator device is a wireless all-in-one taVNS stimulator co-designed by engineers at the Feinstein Institutes for Medical Research and

FIGURE 1 | Subject receiving taVNS (arrow marks the placement of the stimulator; single 500 ms bursts, 30 Hz, pulse width = 0.3 ms, intensity just below pain threshold between 0.1 and 5.0 mA) during the blinking visual cue for the onset of extension movements on the InMotion ARM® robot. During each 1 h session patients performed 1,024 active-assist center-out clock movements of the shoulder and elbow, and received stimulation during a total of 256 extensions movements (right = 9 o'clock, 10 o'clock, 12 o'clock, 2 o'clock; left = 10 o'clock, 12 o'clock, 2 o'clock, 3 o'clock).

the MIDI Product Development Corporation (Smithtown, NY, United States) and fabricated by MIDI (**Figure 1**, arrow). It is designed to deliver low levels of current to the cymba conchae region of the ear using a pair of conductive silicone electrodes. The electrodes are affixed to a spring-load clip that is designed to fit over the left ear and are adjustable in both rotation and location, relative to the rest of the housing to accommodate subjects with different ear sizes. The device is controlled using a wireless Bluetooth link *via* an application run on a tablet that allows control over the amplitude of stimulation, onset, and timing parameters, including pulse width, frequency, burst patterns, and duration.

Photodiodes (BPW46, Vishay Intertechnology, Inc.) placed on a second robotic therapy monitor were used to detect the blinking signal for each new motor target generated by the active assist robot. Upon detection of the visual cue, a microcontroller (Arduino Leonardo; Arduino, Inc.) was used to trigger the auricular stimulation burst.

Robotic Intervention

Robotic training was delivered with the InMotion ARM® robot by Bionik Inc. The robot's design is based on the MIT-MANUS (the planar robot), developed in the Newman Laboratory of Biomechanics and Human Rehabilitation at the Massachusetts Institute of Technology, and provides customized, goal-directed, robot assisted shoulder/elbow therapy. The hallmark of this system is an impedance controller that modulates the way the robot reacts to mechanical perturbation from a patient, and allows for a dynamic interaction, in which the patient attempts to move independently, and after a 2 s delay, the robot provides adaptive, assistance-as-needed to complete the movement (Hogan, 1985, 1988; Colgate, 1988; Volpe et al., 2009). During planar robot therapy, the patient was seated comfortably facing a computer screen with the affected hand grasping the robotic handle and the forearm gently strapped in a rigid support affixed to the robotic arm. A blinking visual cue directed the patient to reach toward points in space that corresponded to the positions of the targets on a screen, moving through over a thousand intensive, active-assist flexions, extensions, and rotational movements of the elbow and shoulder joints per session, as described in past work (Lo et al., 2010). The safety and efficacy of robotic intervention is well established and has been recognized by the American Heart Association as an effective tool for upper limb motor rehabilitation (Winstein et al., 2016).

Instrumental Surface Electromyography Assessment

Setup

Electrical activity of the muscle was differentially recorded using surface EMG electrodes (Biometrics Ltd., United Kingdom) at three distinct time points: Baseline, Discharge, and Follow-up. The two electrodes were placed over the muscle belly of the biceps and triceps brachii. To ensure reproducibility of electrode placement for each patient, the length of the arm from the acromion process to the lateral epicondyle was recorded, and muscle belly of biceps and triceps brachii were palpated, with their circumferential coordinates recorded along that length. Patients were then instructed to perform maximum volitional contractions of the biceps and triceps to confirm electrode placement.

At each time point, patients performed 10 extension movements and 10 flexion movements on the robot. The first five of each acted as a warm-up; the analysis was performed on the final five movements. These movements were identical to the flexion (center-in to 6 o'clock) and extension (center-out to 12 o'clock) movements performed during robotic therapy, except that they were unassisted by the robot. For each movement, the robot would hold the patient at center in a relaxed position. The patient was then instructed to perform the extension or flexion movement without robot assistance and sEMG was recorded for each attempt. Ideally, the sEMG parameters extracted from all five movements were averaged together, however, for a small subset of noisy and inconsistent measures, fewer than five movements were accepted (this occurred in $< 5\%$ of the measures for either group).

Time Domain Analysis

The root mean square (RMS) was calculated and used to determine the peak amplitude of the RMS during a 2 s interval from the onset of muscle activation. To calculate muscle activation onset, a threshold was computed between 2 and 5 standard deviations, varying with each patient and muscle, from the baseline muscle activity. Among the methods used to normalize EMG recordings, we chose the isokinetic maximal voluntary contraction (MVC) which takes the peak amplitude during a dynamic movement as the reference to normalize the data (Chalard et al., 2020). The baseline activity was defined as the average of the first 500 ms of the recording while the patient was at rest, before the start of the flexion or extension task. The threshold for each individual patient was determined through visual inspection. After onset was determined, the integrated EMG (iEMG), the area under the RMS, was calculated for the 2 s time interval individually for the biceps and triceps during both flexion and extension movements. The peak amplitude of the RMS was used to derive the isokinetic maximum voluntary contraction (MVC) to normalize the data at each of the three measured timepoints (Fernández-Peña et al., 2009). The highest peak amplitude of all five flexion movements was used as the reference value for the isokinetic MVC of the biceps and, similarly, the highest peak amplitude of all five extension movements was used as the reference value for the isokinetic MVC of the triceps. Peak amplitude and iEMG are expressed as a percentage of the MVC (% MVC).

Frequency Domain Analysis

The EMG data was sampled at 1,000 Hz. First the data was detrended; the mean of the initial 500 ms of each frame was subtracted from the overall signal to remove any offset. To analyze the data in the frequency domain, a bandpass filter with cutoffs at 10 and 400 Hz was applied. A notch filter at 60 Hz was then applied to remove any electrical interference. The power spectral density was calculated using Welch's method (segment length = 0.3 s and 50% overlap). The mean and median frequency were calculated from the resulting power spectral density individually for the biceps and triceps during each flexion/extension movement and are expressed in Hertz (Hz).

Clinical Assessments

Upper Extremity Fugl Meyer Scale

The UE-FM is a valid and reliable assessment of performance-based impairment after stroke, measured on 0–3 ordinal scale (0 = cannot perform; 3 = performs faultlessly) with a maximum possible score of 66 points (Gladstone et al., 2002; Hsieh et al., 2009; Kim et al., 2012). The MDC (Minimum Detectable Change) is 1.56 points and the MCID (Minimal Clinically Important Difference) is 4.25 points (Page et al., 2012; Toluee Achacheluee et al., 2016).

Medical Research Council Motor Power Scale (MRC)

The MRC is a valid and reliable score that measures strength in isolated muscle groups of the shoulder, elbow, and wrist. It is measured on a 0–5 ordinal scale (0 = no contraction; 5 = normal strength) out of a possible 100 points (Paternostro-Sluga et al., 2008).

Wolf Motor Function Test

The Wolf Motor Function Test (WMFT) is a valid and reliable measure of upper limb function comprised of 15 motor-based tasks and two strength-based tasks (Wolf et al., 2001; Hsieh et al., 2009; Hodics et al., 2012). It is scored on both a Functional Ability Scale (WOLF-FAS) to measure the quality of the movement (0–5 ordinal scale out of 75 possible points) and a time test (WMFT time) to measure the speed of the movement (up to 120 s per task out of a maximum 1,800 s). Improvement is reflected by an increase in WOLF-FAS score and a decreased in WMFT time.

Modified Tardieu Scale

The MTS is a valid and reliable measure of spasticity to passive movement at slow (V1) and fast (V2) speeds (Paulis et al., 2011; Singh et al., 2011). Each joint is measured on a 0–5 ordinal scale (0 = no resistance, 5 = joint immobile), with higher scores indicating increased spasticity. Given that robotic intervention targeted more than one joint of the upper limb, the MTS was evaluated both as a summed score across 11 joints of the upper extremity, MTS total, and at the individual joint complexes for the shoulder, MTS shoulder (summed across 3 joints: horizontal adductors, vertical adductors, internal rotators), the elbow, MTS elbow (summed across 4 joints: elbow flexors, elbow extensors,

pronators, supinators), and the wrist, MTS wrist (summed across 4 joints: wrist flexors, wrist extensors, fingers, palmer interrossei/flexor digitorum superficilias). For studies involving whole-limb intervention, summed scores are advantageous as they may more sensitively detect changes across trained muscles groups (Pundik et al., 2014; Paget-Blanc et al., 2019). We selected to use the MTS instead of the Modified Ashworth Scale (MAS) as the MTS has been shown to be more sensitive to changes in spasticity (Mehrholz et al., 2005; Haugh et al., 2006). For MAS, the minimal clinically important difference (MCID) for a single joint is defined as a 1-point reduction (Brashear et al., 2002). As no MCID is established for summed measurements on either the MAS or MTS, we defined a response to treatment as at least a 2-point reduction for any single joint complex (shoulder/elbow/wrist), and at least a 3-point reduction the MTS total score summed across the whole upper limb.

Statistical Analysis

Statistical analysis was performed using Sigmaplot version 14.5. Acute effects of the intervention at discharge (N = 34) and robustness of the treatment effect at follow-up (N = 28) were analyzed separately. For normally distributed data, a Welch's t-test was used to compare between-groups (active vs. sham) changes from baseline to discharge (D-A) and baseline to follow-up (F-A), respectively, and One-way RM-ANOVA was used to analyze within-group changes over time (admission, discharge, follow-up). For data that violated the assumptions of parametric statistics, non-parametric comparisons were made using Mann-Whitney U Tests to examine between-groups changes from baseline at discharge (D-A) and follow-up (F-A), and Friedman RM-ANOVA was used to analyze within-group changes over time (admission, discharge, follow-up). *Post hoc* Tukey tests for multiple comparisons were applied as warranted. Results are presented as mean ± standard error of the mean (SEM), unless otherwise specified.

RESULTS

Clinical Outcomes for Motor Function

There were significant motor improvements after robotic training for both sham and active taVNS groups, and these improvements were robust at follow-up. Specifically, UE-FM scores improved for each group (Friedman RM-ANOVA, sham $P < 0.001$, Chi-square = 20.920; active $P < 0.001$, Chi-square = 16.453). *Post hoc* pairwise comparisons were significant from admission to discharge and admission to follow-up for both sham and active groups (Tukey test, sham and active: adm-dc $P < 0.001$, adm-fu $P < 0.01$; **Figure 2A**). Average improvement on the UE-FM was approximately 3 points for the active and sham groups at discharge (sham = 2.86 ± 0.50; active = 3.10 ± 0.57) and follow-up (sham = 3.22 ± 1.0; active = 2.79 ± 0.84), which is a reliable improvement above the minimum detectable change (MDC) of 1.56 points, but less that the minimal clinically important difference (MCID) of 4.25 points.

Similarly, MRC motor power scores improved for both groups (Friedman RM-ANOVA, sham $P < 0.01$, Chi-square = 13.0; active $P < 0.001$, Chi-square = 15.434). *Post hoc* pairwise comparisons showed significant improvements from admission to discharge and admission to follow-up for both the sham and active groups (Tukey test, active and sham: adm-dc $P \leq 0.01$; sham: adm-fu $P < 0.05$, active adm-fu $P < 0.001$; **Figure 2B**). Average improvement on the MRC was 4 points at discharge (sham = 4.00 ± 0.87, active = 4.07 ± 0.63) and approximately 4–5 points at follow-up, (sham = 3.69 ± 1.33, active = 4.56 ± 1.15).

Finally, Wolf FAS score improved within each group (Friedman RM-ANOVA, sham: $P < 0.05$, Chi-Square = 8.821; active: $P < 0.01$, Chi-square = 9.542), and *post hoc* pairwise comparisons revealed this change only occurred from admission to follow-up (Tukey test, sham and active: adm-fu, $P < 0.05$; sham = 2.53 ± 0.9, active = 3.00 ± 1.04). Wolf time score decreased within each group, as patients improved and performed functional tasks faster (Friedman RM-ANOVA, sham: $P < 0.05$, Chi-square = 6.933; active: $P < 0.01$, Chi-square = 11.236). *Post hoc* pairwise comparisons were significant for the active group only from admission to discharge, and the sham and active groups from admission to follow-up (Tukey test, active: adm-dc $P < 0.05$; sham: adm-fu $P < 0.05$, active: adm-fu $P < 0.01$).

Change scores were not different between active and sham taVNS groups at discharge or follow-up for the UE-FM Scale, MRC, Wolf FAS score, or Wolf time score (Mann-Whitney U-test, $P \geq 0.230$ across groups).

Tolerance of the stimulation was assessed in questionnaires and the stimulation was well tolerated with no differences reported across the sham and treated groups. The current was set to be less than the pain threshold, and this maneuver rendered the stimulation non-toxic, well-tolerated and not overtly distracting.

Clinical Spasticity Outcomes: Modified Tardieu Scale

Patients receiving active taVNS during shoulder/elbow robot training had a significant decrease in the MTS wrist score at discharge compared to patients receiving the sham treatment ($P < 0.05$, Mann-Whitney U-test, $U = 77.0$; sham = + 0.17 ± 0.26, active = –0.79 ± 0.31; **Figure 3A**). This difference was not apparent at follow-up (P = 0.207). On closer analysis, the Modified Tardieu measure of spasticity for the wrist demonstrated a significant difference for the active taVNS treated group (Friedman RM-ANOVA sham: $P = 0.420$; active: $P < 0.05$, Chi-square = 6.588). MTS total score and MTS shoulder score also decreased in the active compared to the sham condition, and these changes approached significance at discharge (MTS total: $P = 0.0616$, $t = 1.945$, Welch's t-test, sham = –0.49 ± 0.38, active = –1.60 ± 0.50; MTS shoulder: $P = 0.051$, U = 88.5, Mann-Whitney U-test, sham = –0.25 ± 0.24, active = –0.72 ± 0.25; **Figure 3B**). MTS scores measured at the elbow showed no significant change. Using a 2-point reduction in the MTS for a single joint complex as significant, responder rates at discharge for the wrist were 5.9% (1/17) for sham and 37.5% (6/16) for active taVNS, and for the shoulder were 11.8% (2/17) for sham and 18.8% (3/16) for active taVNS. Using a 3-point reduction in

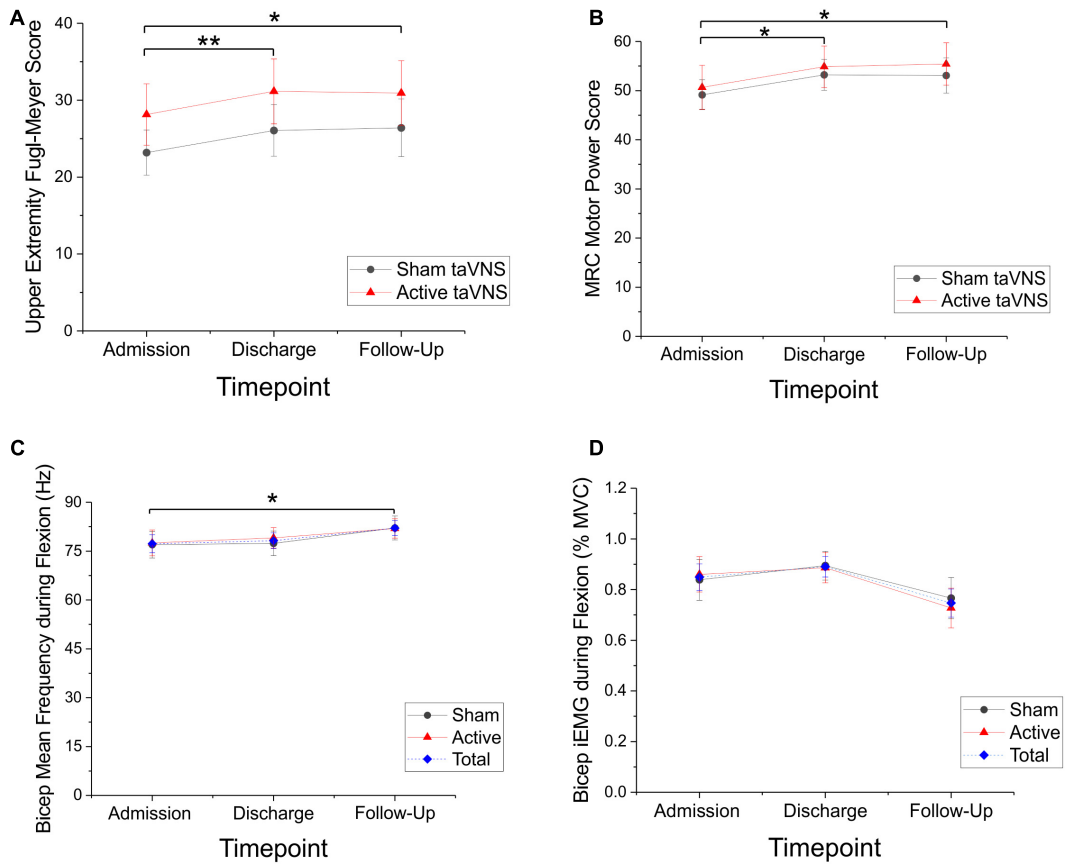

FIGURE 2 | Significant motor improvements and significant changes in sEMG measures of bicep mean frequency were seen in both the active and sham taVNS groups, suggestive of a training benefit from the robot. **(A)** Upper Extremity Fugl Meyer scores (mean ± SEM) improved for both the sham (N = 15) and active (N = 14) taVNS groups, with a mean improvement of 3 points (Friedman RM-ANOVA, sham $P < 0.001$, Chi-square = 20.920; active $P < 0.001$, Chi-square = 16.453). Improvements were significant at discharge and robust through follow-up (Tukey test, sham and active: adm-dc $^{**}P < 0.001$, adm-fu $^{*}P < 0.01$). **(B)** MRC motor power scores (mean ± SEM) were also improved for both the sham (N = 15) and active (N = 14) groups (Friedman RM-ANOVA, sham $P < 0.01$, Chi-square = 13.0; active $P < 0.001$, Chi-square = 15.434). These improvements were significant at discharge and robust through follow-up (Tukey test, active and sham: adm-dc $^{*}P \leq 0.01$; sham: adm-fu $^{*}P < 0.05$, active adm-fu $^{*}P < 0.001$). **(C)** Directional trends for sEMG are significant for both summed sham and active groups (mean ± SEM; N = 28; blue dashed line). Mean frequency of the biceps during flexion significantly increased across the combined group (One-way RM-ANOVA, $P < 0.05$, F = 3.274), between admission and follow-up (Tukey test, $^{*}P < 0.05$). **(D)** Biceps iEMG (area under the RMS curve) during flexion approached a significant reduction (in% mean voluntary contraction) across the combined group (One-way RM-ANOVA, P = 0.050).

the MTS total as significant, responder rates as discharge were 11.8% (2/17) for sham and 33.3% (5/16) for active taVNS.

Objective Surface Electromyography Outcomes: Muscle Activation

Bicep Surface Electromyography Changes During Extension Movements

Although the change in bicep peak RMS sEMG amplitude during extension movements was not significantly different between active and sham groups at discharge or follow-up (Mann-Whitney U-test, discharge: P = 0.796, U = 137.0; follow-up: P = 0.183, U = 69.0), *post hoc* comparisons of change score variance revealed significant between-group differences in the dispersion of the data at discharge (Siegel-Tukey test, $P < 0.01$; mean absolute change admission to discharge, sham = 10.63 ± 3.10, active = 26.29 ± 3.89; **Figure 4**). Within groups, Friedman RM-ANOVA revealed that bicep peak RMS amplitude during extension was significantly reduced for the active condition only (Friedman RM-ANOVA, sham: P = 0.931; active: $P < 0.05$, Chi-square = 7.0; **Figure 5**). *Post hoc* pairwise comparisons showed a significant reduction in bicep peak RMS amplitude between discharge and follow-up for the active taVNS group (Tukey test, $P < 0.05$).

There were no significant changes in the biceps iEMG or mean/median frequency during extension. There were also no significant changes across all measures for the triceps or the ratio of the biceps to triceps during extension.

Bicep Surface Electromyography Changes During Flexion Movements

When all patients (sham and active taVNS) were combined into a single group and the effect of time was assessed, there was a significant increase in bicep mean frequency during flexion

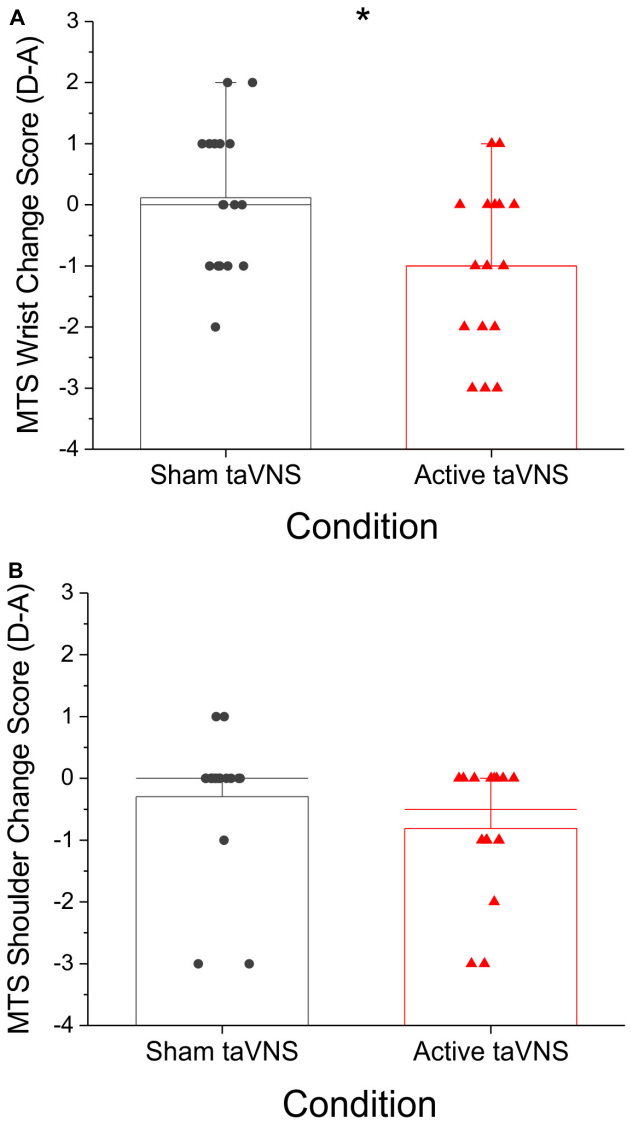

FIGURE 3 | taVNS delivered during robotic therapy extension movements significantly reduced spasticity for the active taVNS group (N = 16) compared to sham (N = 17) at discharge (mean raw change score ± SEM), but not follow-up (data not shown). Lower (more negative) scores indicate a greater reduction in spasticity. **(A)** MTS Wrist/Hand change score at discharge was significantly reduced for the active group (*P < 0.05, Mann-Whitney U-test, U = 77.0; sham = + 0.17 ± 0.26, active = –0.79 ± 0.31). **(B)** MTS shoulder change score at discharge approached a significant difference for the active taVNS group (P = 0.051, U = 88.50, Mann-Whitney U-test, sham = –0.25 ± 0.24, active = –0.72 ± 0.25).

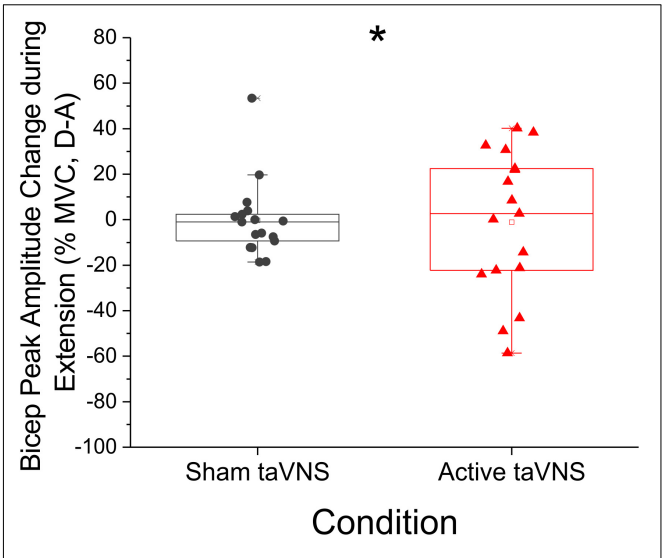

FIGURE 4 | Peak amplitude change score in antagonist (biceps) muscles during extension was notably more dispersed in active taVNS group (N = 17) compared to the sham group (N = 17) at discharge (% mean voluntary contraction change score ± SEM). The change in bicep peak RMS amplitude during extension movements was not significantly different between active and sham groups at discharge or follow-up (Mann-Whitney U-test, discharge: P = 0.796, U = 137.0; follow-up: P = 0.183, U = 69.0), but change score variance was significantly different between groups at discharge (Siegel-Tukey test, *P < 0.01).

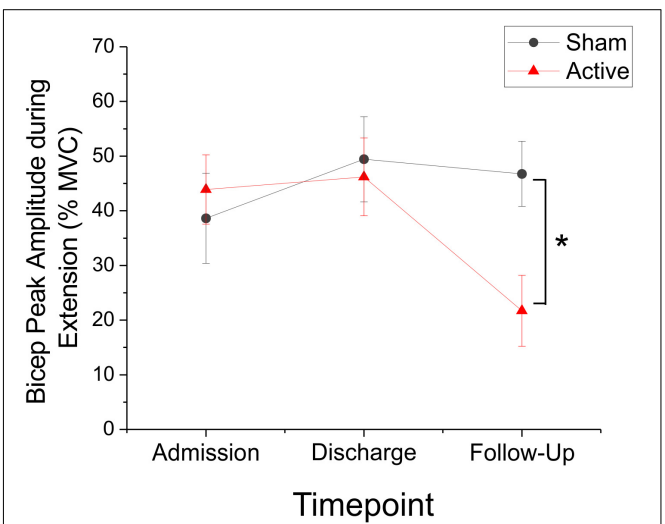

FIGURE 5 | Bicep Peak amplitude (as represented by% mean voluntary contraction ± SEM) decreased significantly during extension movements in the active taVNS group (N = 14), but not the sham group (N = 14) at follow-up (Friedman RM-ANOVA, sham: P = 0.931; active: P < 0.05, Chi-square = 7.0). *Post hoc* pairwise comparisons showed a significant reduction in bicep peak RMS amplitude between discharge and follow-up for the active taVNS group (Tukey test, *P < 0.05). There were no significant between-groups differences.

(One-way RM-ANOVA, P < 0.05, F = 3.274; **Figure 2C**) and a reduction in bicep iEMG during flexion, which approached significance (One-way RM-ANOVA, P = 0.050; **Figure 2D**). *Post hoc* pairwise comparisons revealed a significant increase in bicep mean frequency between admission and follow-up for the combined (active and sham) group (Tukey test, P < 0.05). Both sham and active taVNS groups trended in the same directions for these measures, but neither reached significance independently.

There were no significant changes in the biceps median frequency, the ratio of biceps to triceps, or in the triceps across all sEMG measures during flexion.

Stimulation Safety and Device Tolerance

Stimulation was well-tolerated and there were no serious adverse events. The average tolerated intensity of taVNS current was 4.5 mA ± 0.06 (mean ± SEM).

DISCUSSION

The present study demonstrates that taVNS delivered prior to extension movements in a shoulder/elbow robotic training task significantly reduced spasticity in the affected arm, and significantly changed bicep peak sEMG amplitudes during extension. Motor improvements, on all clinical scales, were significant for both the active and sham taVNS groups and robust through follow-up and are indicative of a benefit from robot training. Using an MCID of a 2-point or greater reduction in spasticity, improvements in the wrist and hand were clinically significant for more than a third (6/16 = 37.5%) of the active taVNS group compared with 5.9% (1/17) of the sham group after the training period, though this improvement, unlike the motor improvements, was not maintained in follow-up. The decreased spasticity measure at discharge was an unexpected result, given that robot training was focused on the shoulder and elbow. It may be that the requirement for robotic training that places the hand around a joystick to perform shoulder/elbow movements is similar to a splinted stretch treatment and may have contributed to a relaxing of muscles in the distal forearm. Nonetheless, spasticity improvements were significant only for the active group, suggestive that taVNS targeted to extensor movements augments reduction of spasticity.

Objective sEMG measures of bicep peak amplitude during extension were significantly different for the active treatment group, however, neither this peak amplitude nor the increased variance of the peak amplitude at discharge and its resolution at follow-up, led to differential motor improvement. sEMG measures of the triceps and any of the reciprocal relationships to biceps sEMG were also unrevealing. Others have reported abnormal and unpredictable antagonist-agonist relationships in patients recovering from stroke (Burke, 1988) that, at times, correlated with stroke severity (Levin et al., 2018). Nevertheless, the EMG findings in the antagonist biceps of the treated group, begs the question of whether a longer treatment stimulation period or a higher or more frequent dose of stimulation would have led to a separation of motor performance between the groups.

Motor improvements on the UE-FM, the MRC motor power scale, and the Wolf Motor Function Test were significant for both the sham and active taVNS groups and robust through follow-up. The average UE-FM improvement was 3 points across both groups at discharge and follow-up, which is above the MDC, indicating a reliably measured improvement, but is below the MCID threshold of 4.25 points that have been taken to indicate a functionally significant change. Similarly for the objective EMG measures, when the active and sham taVNS groups were combined, there was a significant increase in the mean frequency of the biceps during flexion and a reduction of the iEMG that approached significance. Consequently, these results present a potential dichotomy for future taVNS studies between sEMG measures of general motor improvements in comparison to early biomarkers of distinct motor change attributed to taVNS.

Unlike the results in our study, other trials of VNS-paired motor training following stroke have reported significant improvements in UE-FM measures for those treated with VNS (Capone et al., 2017; Dawson et al., 2021). The difference appears to depend on increased cumulative dose of stimulation and training, and the implanted stimulator may have additional advantages (Dawson et al., 2021). Specifically, compared to 9 training sessions and ∼250 stimulated movements per session in the present study, Dawson et al. report that patients received 18 sessions (3x/wk for 6 weeks) of in-clinic therapy paired with > 300 stimulated movements (0.8 mA, 30 Hz) per session. Additionally, that trial continued with a 30-min/day home exercise program coupled with continuous VNS delivered every 10 s for 30 min until the 90-day follow-up. In another study that employed taVNS also using higher doses of stimulation, Capone et al. (2017) report that patients received 10 consecutive daily taVNS sessions in a single block that delivered pulse trains lasting 30 s (0.8 mA, 30 Hz), every 5 min for 1 h, prior to wrist or shoulder/elbow training. Thus, the combined results suggest that patients in our study were undertreated.

The novelty of the present study was the selectivity of current delivery in a closed-loop during visual cues for active-assist extension movements. Although the higher doses of stimulation and the extended treatment in other studies trumped treatment timing, it remains remarkable, given the low dose of stimulation and short duration of intervention in our study, that patients in the active taVNS group showed distinct improvements in upper limb spasticity of the wrist and hand at discharge and greater changes in bicep peak sEMG amplitude for trained extension movements. This suggests that selection of impairment-focused motor targets (e.g., extension movements) with taVNS may improve efficiency of training. Future studies of taVNS targeted to impairment-focused training should be longer duration, with a higher dose of stimulation to determine if changes in antagonist control may induce functional improvements.

CONCLUSION

Our results showed that 3 weeks of upper limb robotic training combined with taVNS delivered selectively during extension movements demonstrated significant reductions in spasticity at the wrist and hand and significant changes in bicep sEMG peak amplitude during extension movements. Similar improvements in clinical scales were seen in both active and sham groups. Changes in bicep peak sEMG amplitude may be a sensitive early biomarker of taVNS-induced improvements.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board at Northwell Health. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JC, MSa, AP-B, AC, and BV designed the study. MSt, JW, and TD-C contributed to the design of the taVNS stimulation and current delivery software. JC, MSa, AP-B, and AC performed research. JC, AC, and BV analyzed, interpreted data, and wrote the article. MSa, AP, MSt, JW, TD-C, and SZ provided additional comments and contributed to finalizing the article. All authors read and approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Effect of vagus nerve stimulation paired with rehabilitation for upper limb function improvement after stroke: a systematic review and meta-analysis of randomized controlled trials

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Vagus nerve stimulation (VNS) could potentially facilitate arm function recovery after stroke. The aim of this review was to evaluate the effect of VNS paired with rehabilitation on upper limb function recovery after stroke. We considered randomized controlled trials (RCTs) that used VNS paired with rehabilitation for the improvement of upper limb function after stroke and were published in English. Eligible RCTs were identified by searching electronic databases, including MEDLINE, Web of Science, Embase, CENTRAL and PEDro, from their inception until June 2021. Quality of included studies was assessed using PEDro score and Cochrane's risk of bias assessment. A meta-analysis was performed on the collected data. Five studies with a total of 178 participants met the inclusion criteria. Overall, the present meta-analysis revealed a significant effect of VNS on Fugl–Meyer Assessment for Upper Extremity (FMA-UE, MD = 3.59; 95% CI, 2.55–4.63; $P < 0.01$) when compared with the control group. However, no significant difference was observed in adverse events associated with device implantation between the invasive VNS and control groups (RR = 1.10; 95% CI, 0.92–1.32; $P = 0.29$). No adverse events associated with device use were reported in invasive VNS, and one was reported in transcutaneous VNS. This study revealed that VNS paired with rehabilitation can facilitate the recovery of upper limb function in patients with stroke on the basis of FMA-UE scores, but the long-term effects remain to be demonstrated. *International Journal of Rehabilitation Research* 45: 99–108 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Stroke is the leading cause of death and years lived with disability globally [1]. Upper limb impairment is one of the most prevalent dysfunctions after stroke, which results in poor health-related quality of life [2,3]. Approximately, 80–85% of patients with acute stroke present with upper limb motor impairment, and 60% of the stroke survivors still experience persistent impaired upper limb function 6 months after stroke [4,5]. Improving upper limb function is a priority for both stroke survivors and caregivers [6]. However, recent studies have revealed that the effects of current interventions for improving upper limb impairment are not satisfactory [3,7,8]. Therefore, novel and more effective methods are required to maximize upper limb recovery and ensure a high quality of life among stroke survivors [9].

Vagus nerve stimulation (VNS), which has been used for the treatment of epilepsy, headache and depression [10–12], can potentially enhance and facilitate the reorganization potential of cortical networks [13–15]. Several studies have investigated the efficacy of VNS paired with rehabilitation for upper limb function improvement in adults with stroke, but the results were conflicting and controversial. A meta-analysis of VNS and stroke published previously reported the potential effect of VNS on stroke [16]. The authors stated that additional high-quality studies, with large sample sizes, were required to validate their findings. Furthermore, the authors did not distinguish between invasive VNS and noninvasive VNS (transcutaneous VNS, tVNS), and the adverse events associated with device implantation and stimulation [16]. A previous study with a large sample size investigated

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the role of VNS in adults with stroke and was published in the Lancet recently [17]. Consequently, the present systematic review and meta-analysis of randomized controlled trials (RCTs) aimed to integrate new evidence presented in recent years to evaluate the efficacy and safety of VNS paired with rehabilitation for upper limb function improvement and to compare its effect with that of rehabilitation only or with sham VNS in adults with stroke.

Methods

The present systematic review and meta-analysis were performed and reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2020 statement (PRISMA 2020), and *Cochrane Handbook for Systematic Reviews of Interventions* [18,19]. In addition, the present systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO): CRD42021268269.

Data sources and search strategy

We systematically searched for relevant articles available in English in electronic databases, including MEDLINE (via PubMed), Web of Science, Embase (via Ovid), CENTRAL (Cochrane library) and Physiotherapy Evidence Database (PEDro) from their inception until June 2021. Search terms included keywords associated with stroke, VNS and the upper limb. The specific search strategy of MEDLINE used is presented in Table 1 (see Supplementary Table, Supplemental digital content 1, *http://links.lww.com/IJRR/A21* which presents the search strategies of the other four databases). Furthermore, manual screening of reference lists of the articles was performed to identify additional relevant studies. No ethical approval or patient consent was required because all analyses were on the basis of previously published studies.

Table 1 Search strategy of MEDLINE

MEDLINE (via PubMed)
1. Stroke [mh] or Cerebrovascular disorders [mh] or Basal ganglia cerebrovascular disease [mh] or Brain ischemia [mh] or Carotid artery diseases [mh] or Cerebral small vessel diseases [mh] or Intracranial arterial diseases [mh] or Intracranial embolism and thrombosis [mh] or Intracranial hemorrhages [mh] or Brain infarction [mh] or Stroke, lacunar [mh] or Vasospasm, intracranial [mh] or Vertebral artery dissection [mh] or Hemiplegia [mh] or Paresis [mh] or Brain injuries [mh] or Brain injury, chronic [mh]
2. Stroke* [tiab] or Poststroke [tiab] or Post-stroke [tiab] or Cerebrovasc* [tiab]
3. 1 or 2
4. Vagus nerve [mh]
5. Vagus nerve [tiab] or Vagal nerve [tiab] or Vagus nerve stimul* [tiab] or Vagal nerve stimul* [tiab]
6. 4 or 5
7. Upper extremity [mh]
8. Upper limb* [tiab] or upper extremit* [tiab] or arm* [tiab] or shoulder* [tiab] or hand* [tiab] or elbow* [tiab] or forearm* [tiab] or wrist* [tiab] or finger* [tiab] or axilla* [tiab]
9. 7 or 8
10. 3 and 6 and 9

Study selection

Endnote software was used to check for duplicated studies. Two investigators reviewed the studies independently and selected studies on the basis of the predetermined criteria. All potentially relevant articles were retrieved from the databases for the assessment of their full text on the basis of titles and abstracts. Studies that did not meet the inclusion criteria were excluded. Discrepancies between two reviewers were resolved through discussions with a third reviewer and a consensus was reached. The included studies were required to meet the following criteria: (1) studies were RCTs published in English, (2) patients were diagnosed with ischemic or hemorrhagic stroke by computerized tomography or MRI, (3) intervention treatments were VNS (transcutaneous VNS or invasive VNS) paired with rehabilitation versus rehabilitation only and (4) with regard to outcome measures, at least one outcome associated with function of the upper limb was measured.

Data extraction and quality assessment

Two reviewers independently extracted relevant data onto a predeveloped data extraction sheet, and disagreements were adjudicated by a third reviewer. The data extracted from selected studies included basic information (first author, year of publication), study design, demographic characteristics of patients (sample size, age, sex, time from stroke), details of interventions applied to the experimental and control groups, relevant outcome measures and time of evaluation.

Eligible articles were scrutinized for methodological quality by two independent reviewers using PEDro scale. The PEDro scale comprises 11 items with a total score ranging from 0 to 10 (except for item 1). The methodological quality of studies scoring 9–10 was considered to be of 'excellent' quality, studies scoring 6–8 were considered to be of 'good' quality, studies scoring 4–5 were considered to be of 'fair' quality, and studies scoring below 4 were considered to be of 'poor' quality [20]. Discrepancies between two reviewers were resolved through discussions with a third reviewer. Additionally, risk of bias assessments was performed using the criteria described in the *Cochrane Handbook for Systematic Reviews of Interventions* [19]. The evaluation entries included the following aspects: random sequence generation, allocation concealment, masking, incomplete outcome data and selective outcome reporting among others. The included articles were evaluated as 'low risk', 'high risk' or 'unclear risk'. Quality assessment was not used as a selection or exclusion criterion.

Data synthesis and analysis

The results of all included studies were pooled using standard meta-analytic methods to estimate the effect of VNS paired with rehabilitation versus rehabilitation only for upper limb function improvement after stroke. On the basis of the nature of extracted data, we assessed the mean differences (MDs) and 95% confidence intervals

(CIs) for continuous outcomes, and risk ratios (RRs) at 95% CIs for adverse events. A P value <0.05 (two-sided) was considered statistically significant in the estimation of effects. Statistical heterogeneity was evaluated using chi-square test and I^2 statistic. P value <0.05 or I^2 value >50% was considered high heterogeneity. A fixed-effects model was used when P value was >0.05; otherwise, a random-effects model was used. Sensitivity analyses were performed by excluding each study from the analysis when heterogeneity was detected, and the subgroup analyses were performed on the basis of the different methods of VNS (tVNS or invasive VNS). Publication bias was not assessed due to the limited number of included studies (fewer than ten). All statistical analyses were performed using RevMan software (Version 5.3; Cochrane Collaboration, Copenhagen, Denmark).

Results

Search results

The initial electronic search resulted in a total of 278 studies, of which 175 unique articles were retrieved after duplicates were removed. After screening the titles, abstracts and full text of the articles on the basis of the inclusion and exclusion criteria, five studies [17,21–24] with a total of 178 participants were identified as eligible for the systematic review. The five studies were also used for the meta-analysis. A detailed flowchart of the search process for the studies is included in the systematic review and meta-analysis Fig. 1.

Description of studies

The studies included in the analysis were published between 2016 and 2021. The sample size ranged from 12 to 108 participants. The primary characteristics of the selected studies, including study design, baseline characteristics of enrolled participants, details of interventions and outcomes are summarized in Table 2.

The studies included in the current systematic review and meta-analysis satisfied specific inclusion and exclusion criteria. All participants in the selected studies were diagnosed with different stages of stroke [25]. One study reported subacute or chronic phase of stroke [23], one study reported acute or subacute phase of stroke [24] and three studies reported the chronic phase of stroke [17,21,22].

All experimental groups received VNS paired with rehabilitation. Two studies used tVNS [22,24] and three studies used surgically implanted VNS [17,21,23]. The intervention period ranged from 10 days to 6 weeks. One study compared VNS paired with rehabilitation to rehabilitation only [21], one study compared tVNS combined with robotic-assisted therapy to sham tVNS combined with robotic-assisted therapy [22], two studies compared VNS paired with rehabilitation to sham VNS combined with rehabilitation [17,23] and one study compared tVNS paired with rehabilitation to sham tVNS combined with rehabilitation [24].

Outcomes were measured at baseline and at the end of the intervention. The Fugl-Meyer Assessment for Upper Extremity (FMA-UE) Score was the main outcome in the evaluation of the effect of intervention and it was measured in five studies [17,21–24]. Additionally, three trials employed the Wolf Motor Function test (WMFT) [17,23,24] and Stroke Impact Scale (SIS) [17,21,23]; two trials used the Box and Block test and Nine-Hole Peg test [21,23], and five trials reported adverse events [17,21–24].

Quality

PEDro scores of the included studies ranged from 6 to 10, with a mean score of 8. The methodological quality of two studies was considered to be of 'excellent' quality [17, 23], while that of three studies was considered to be of 'good' quality [21,22,24]. A detailed evaluation of the PEDro scores is presented in Table 3. All included studies reported adequately with regard to their methods of blinding outcome assessors and random sequence generation, except for one study [22]. Only two studies satisfied the concealed allocation criterion. Subject blinding was satisfied in three of the selected studies [17,22,23]. Risk of bias assessment of the studies included in the present systematic review and meta-analysis is illustrated in Figs. 2, 3.

Effect of intervention

Fugl–Meyer assessment for upper extremity scores

A fixed-effects model was used for the analysis of FMA-UE scores. The variations in FMA-UE scores before and after intervention in five studies [17,21–24] indicated that FMA-UE scores increased significantly as a result of VNS paired with rehabilitation when compared to rehabilitation with or without sham VNS (MD = 3.59; 95% CI, 2.55–4.63; $P < 0.01$). On the basis of subgroup analyses, three studies [17,21,23] reported that the variations in FMA-UE scores between invasive VNS paired with rehabilitation and the control groups were significantly different (MD = 3.62; 95% CI, 1.75to–5.48; $P < 0.01$). Furthermore, two studies [22, 24] revealed that the variations in FMA-UE scores between tVNS paired with rehabilitation and control groups were significantly different (MD = 3.58; 95% CI, 2.33–4.82; $P < 0.01$). No heterogeneity was detected among the studies ($I^2 = 0\%$; $P = 0.78$; Fig. 4).

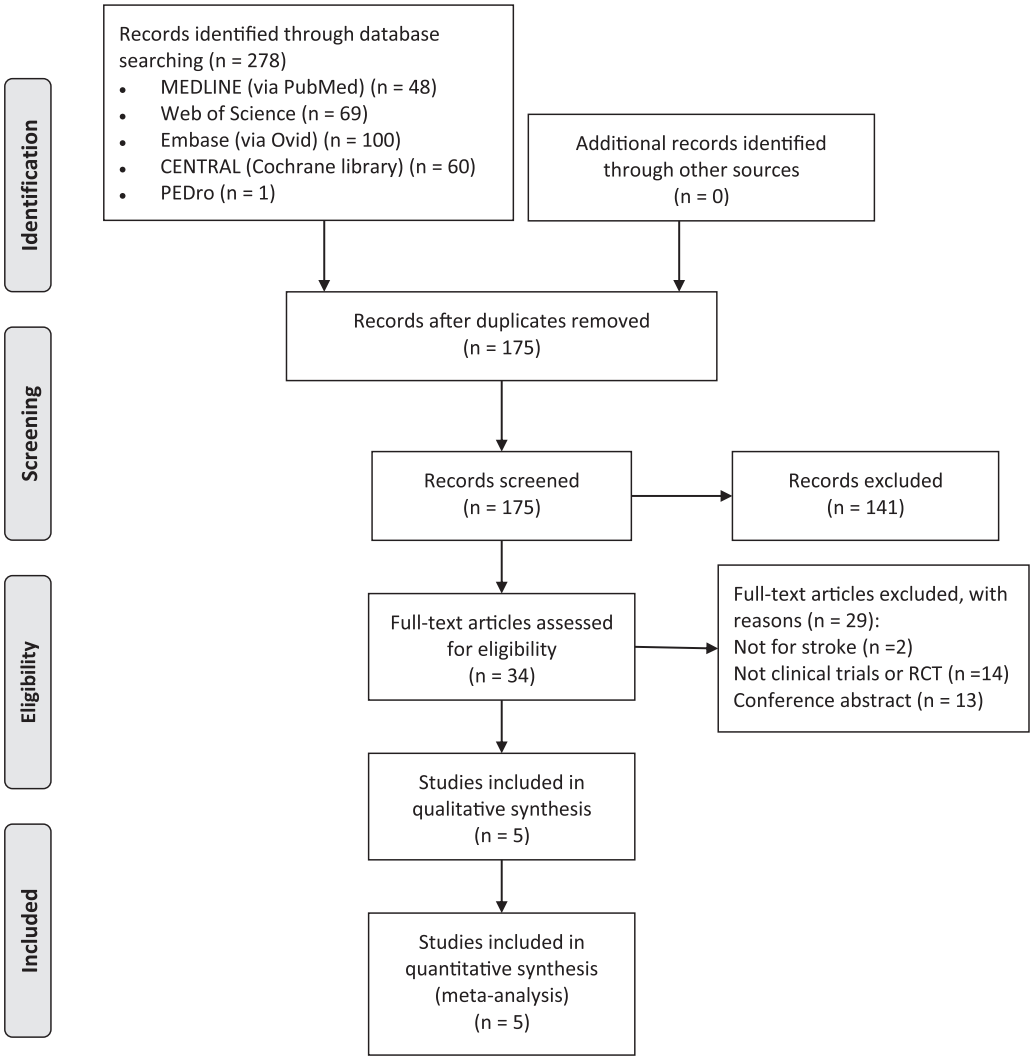
Wolf motor function test scores

A fixed-effects model was used to analyze WMFT scores. Two studies [17,23] revealed a significant difference in the variations of WMFT scores between invasive VNS and control groups (MD = 0.30; 95% CI, 0.18–0.43; $P < 0.01$), and no heterogeneity was observed among the studies ($I^2 = 0\%$; $P = 0.88$; Fig. 5). One study [24] revealed a significant difference in the variations of WMFT scores between the tVNS and control groups (MD = 3.59; 95% CI, 1.97–5.21; $P < 0.01$; Fig. 5).

Stroke impact scale (hand function)

Pooling data from two studies in the fixed-effects model [17,23] revealed no significant difference in SIS (Hand

Fig. 1



PRISMA flow diagram.

function) scores between the invasive VNS and control groups (MD = 1.07; 95% CI, −6.06 to 8.20; P = 0.77). Pooled studies were homogenous (I^2 = 0%; P = 0.83; Fig. 6).

Box and block test

No significant difference was observed between invasive VNS and control groups on the basis of Box and Block test scores (MD = −0.31; 95% CI, −3.48 to 2.87; P = 0.85) in the fixed-effects model when data from two studies were pooled [21,23]. Pooled studies were homogenous (I^2 = 0%; P = 0.94; Fig. 7).

Nine-hole peg test

No significant difference was observed in the Nine-Hole Peg test scores between invasive VNS and control groups (MD = 2.77; 95% CI, −31.40 to 36.95; P = 0.87) in the fixed-effects model when data from two studies were pooled [21,23]. Pooled studies were homogenous (I^2 = 38%; P = 0.20; Fig. 8).

Adverse events

The invasive VNS and control groups did not differ significantly in terms of adverse events associated with device implantation (RR = 1.10; 95% CI, 0.92–1.32; P = 0.29) in the fixed-effects model when data from three studies were pooled [17,21,23]. Pooled studies were homogenous (I^2 = 0%; P = 0.43; Fig. 9). Moreover, no adverse events associated with device use were reported in three studies with regard to invasive VNS [17,21,23]. One study [22] regarding tVNS did not report adverse events, while one study [24] reported that one patient in the tVNS group developed skin redness at the point of contact of the auricular skin with electrodes.

Table 2 Characteristics of included studies

Study	Design	Participants	Interventions	Outcomes
Dawson [21], 2016	RCT	*N* = 20 EG (*n* = 9) Age: 57.9 ± 17.2 years Onset: 1.8 ± 1.0 years CG (*n* = 11) Age: 60.7 ± 10.7 years Onset: 1.7 ± 1.3 years	EG: VNS paired with rehabilitation. (VNS: 0.8 mA, 100 μs, 30 Hz, lasting 0.5 s) CG: Rehabilitation alone (the rehabilitation-only group did not have a device implanted). Both groups: All participants received a 6-week course of 2-h therapy sessions 3× per week.	FMA-UE ARAT Grip and pinch strength SIS Box and Block test Nine-hole peg test At pre-, and post-Tx (6 weeks)
Capone [22], 2017	RCT	*N* = 12 EG (*n* = 7) Age: 53.71 ± 5.88 years Onset: 93.91 ± 38.81 months CG (*n* = 5) Age: 55.60 ± 7.12 years Onset: 46.00 ± 21.85 months	EG: tVNS and robotic-assisted therapy. Electric stimulator was placed in the left external acoustic meatus at the inner side of the tragus. tVNS was delivered as trains lasting 30 s and composed by 600 pulses (pulse frequency = 20 Hz; pulse duration = 0.3 ms) repeated every 5 min for 60 min. CG: Sham tVNS and robotic-assisted therapy. Both groups: Robotic treatment was delivered daily for 10 consecutive working days, immediately after the end of real or sham tVNS.	FMA-UE At pre-, and post-Tx (10 days)
Kimberley [23], 2018	RCT	*N* = 17 EG (*n* = 8) Age: 59.5 ± 7.4 years Onset: 18 (11-43) months CG (*n* = 9) Age: 60.0 ± 13.5 years Onset: 18 (6.3–53) months	EG: VNS paired with rehabilitation. VNS (0.8 mA). CG: Sham VNS paired with rehabilitation. VNS (0 mA) Both groups: Both groups were surgically implanted with the VNS device. All participants received 6-week in-clinic rehabilitation (≈3×a week for 6 weeks) followed by a home exercise program.	FMA-UE WMFT Box and Block test Nine-hole peg test SIS Motor Activity Log At pre-, and days 1, 7, 30, and 90 days after in-clinical therapy
Wu [24], 2020	RCT	*N* = 21 EG (*n* = 10) Age: 64.50 ± 9.97 years Onset: 36.30 ± 9.23 days CG (*n* = 11) Age: 61.82 ± 10.63 years Onset: 35.55 ± 6.47 days	EG: tVNS paired with rehabilitation. Parameters: 600 pulses (pulse frequency = 20 Hz; pulse duration = 0:3 ms), lasting 30 s each time, stimulating once every 5 min. CG: Sham tVNS paired with rehabilitation. Both groups: Rehabilitation training, lasting approximately 30 min, was performed immediately after the end of real or sham tVNS per day for 15 days.	FMA-UE WMFT FIM BS At pre-, and post-Tx.
Dawson [17], 2021	RCT	*N* = 108 EG (*n* = 53) Age: 59.1 ± 10.2 years Onset: 3.1 ± 2.3 years CG (*n* = 55) Age: 61.1 ± 9.2 years Onset: 3.3 ± 2.6 years	EG: VNS paired with rehabilitation (VNS: 0.8 mA, 100μs, 30 Hz stimulation pulses, lasting 0.5 s). CG: Sham VNS paired with rehabilitation. Both groups: Both groups were surgically implanted with the VNS device. Participants received 6 weeks of in-clinic therapy (three times per week; total of 18 sessions) followed by a home exercise program.	FMA-UE WMFT SIS

ARAT, arm research arm test; SIS, Stroke Impact Scale; BS, Brunnstrom stage; CG, control group; EG, experimental group; FIM, functional independence measurement; FMA-UE, Fugl-Meyer Assessment for Upper Extremity scale; Tx, treatment; VNS, vagus nerve stimulation; WMFT, Wolf motor function test.

Table 3 PEDro assessment quality results of included studies

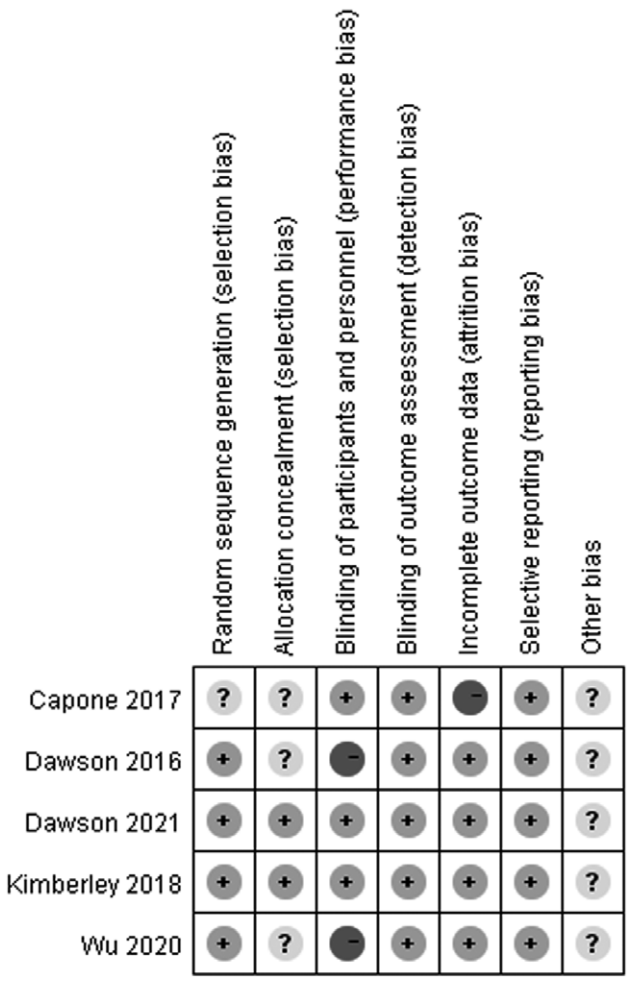
Study	Eligibility*	Random allocation	Concealed allocation	Baseline comparability	Blind subjects	Blind therapists	Blind assessors	Adequate follow-up	Intention-to-treat analysis	Between-group comparisons	Point estimates and variability	Total score	Quality
Dawson [21], 2016	YES	1	0	1	0	0	1	1	1	1	1	7	GOOD
Capone[22], 2017	YES	1	0	1	0	1	1	0	0	1	1	6	GOOD
Kimberley [23], 2018	YES	1	1	1	1	1	1	1	1	1	1	10	Excellent
Wu [24], 2020	YES	1	0	1	0	0	1	1	1	1	1	7	GOOD
Dawson [17], 2021	YES	1	1	1	1	1	1	1	1	1	1	10	Excellent

*Eligibility criteria is not included in the scoring of PEDro scale.

Discussion

The present systematic review and meta-analysis reviewed the findings of previous studies to evaluate the safety and determine the effect of VNS paired with rehabilitation on upper limb function recovery in patients with stroke. The outcome measures were evaluated on the basis of the difference in performance between the baseline and immediately after the intervention. The results of the present meta-analysis revealed that the increases in FMA-UE and WMFT scores of patients in the VNS group were significantly greater than those in the control group. However, the increases in SIS (hand function), Box and Block test and Nine-Hole Peg test scores were similar in both groups. The results are consistent with the findings of a previous review [16]. Our findings have presented moderate statistical evidence

Fig. 2



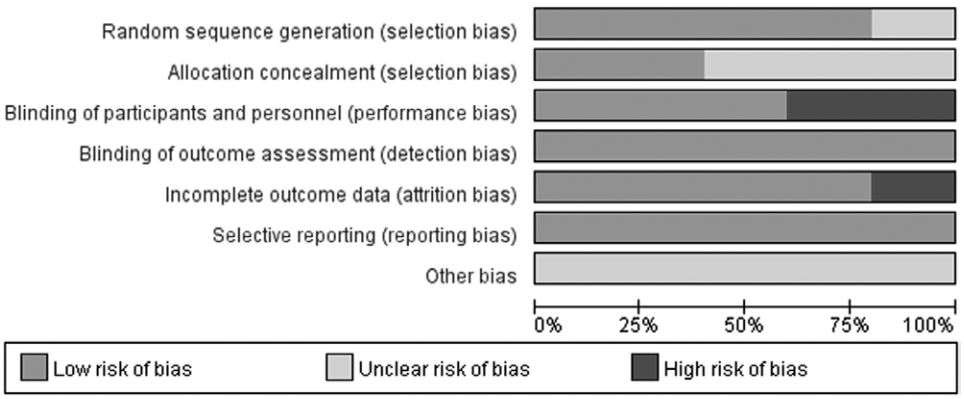
Risk of bias summary according to the Cochrane risk of bias tool: '–', '+' and '?' indicate high, low and unclear risk of bias, respectively.

for improved efficacy of VNS paired with rehabilitation when compared to the efficacy of convenient rehabilitation on the basis of FMA-UE and WMFT scores.

With regard to the FMA-UE, an increase of 3.59 was recorded across all included trials on average. One study revealed that the clinically important difference (CID) for FMA-UE in individuals with minimal to moderate impairment due to chronic stroke ranged from 4.25 to 7.25 points [26]. However, the variations in scores observed in the present systematic review were lower than the CID threshold, which suggest that there was no clinical significance. One study that investigated invasive VNS defined a clinically meaningful response as a 6-point or greater improvement in FMA-UE score and reported that more participants in the VNS group reached a threshold of clinically meaningful response when compared with the control group (23 [47%] of 53 vs. 13 [24%] of 55, $P = 0.0098$) [17]. Similarly, an increase of 0.3 was observed in invasive VNS on average on the basis of WMFT scores and an increase of 3.59 was observed in tVNS on average. Lin *et al.* reported that the CID of WMFT in patients with stroke varied from 0.2 to 0.4 points [27]. Both variations reached the CID threshold, which indicated a clinical significance.

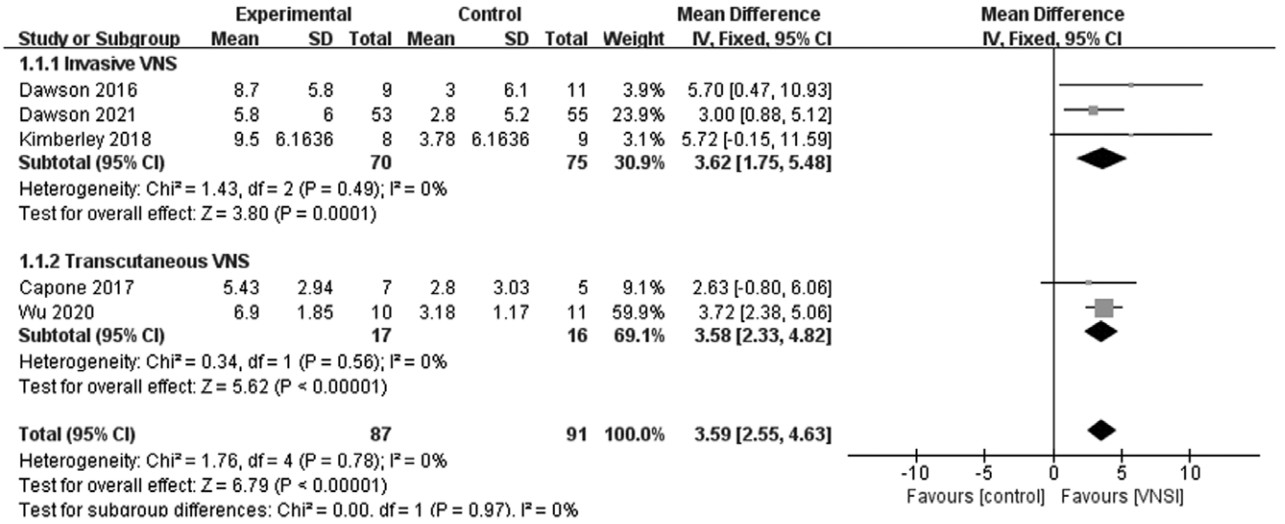
The primary safety outcome measure was the number of adverse events associated with device implantation or stimulation. The results of the present meta-analysis revealed no significant difference in adverse events associated with device implantation between the invasive VNS and control groups. Only one study reported that one patient in the tVNS group developed skin redness at the point of stimulation [24]. In addition, no adverse events associated with therapy were reported. tVNS is a relatively safe intervention as a result of surgical-related complications caused by invasive VNS, such as left vocal cord palsy and dysphagia; however, no study has compared the effect

Fig. 3



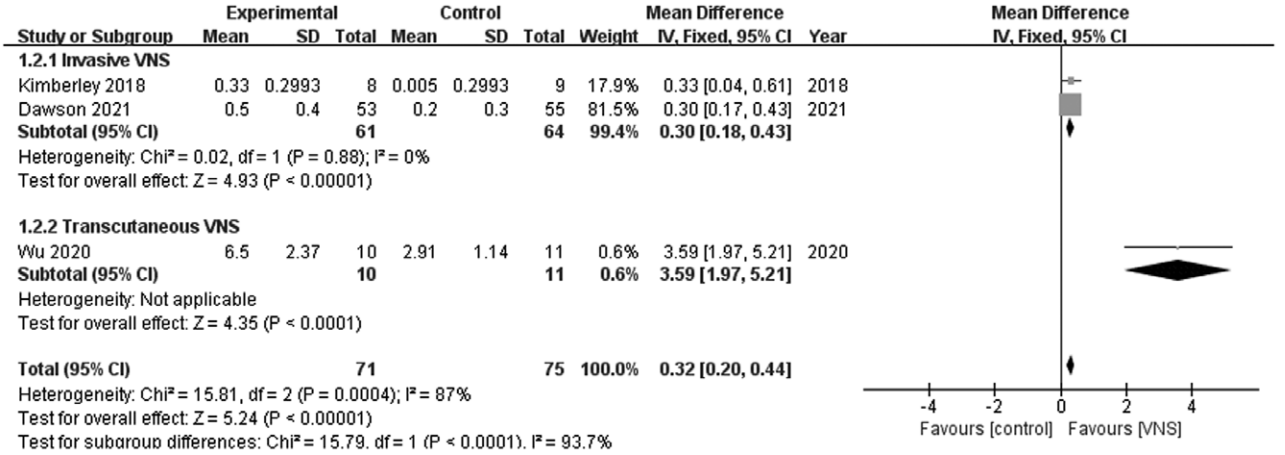
Risk of bias graph according to the Cochrane risk of bias tool.

Fig. 4



Fugl–Meyer assessment for upper extremity scores.

Fig. 5



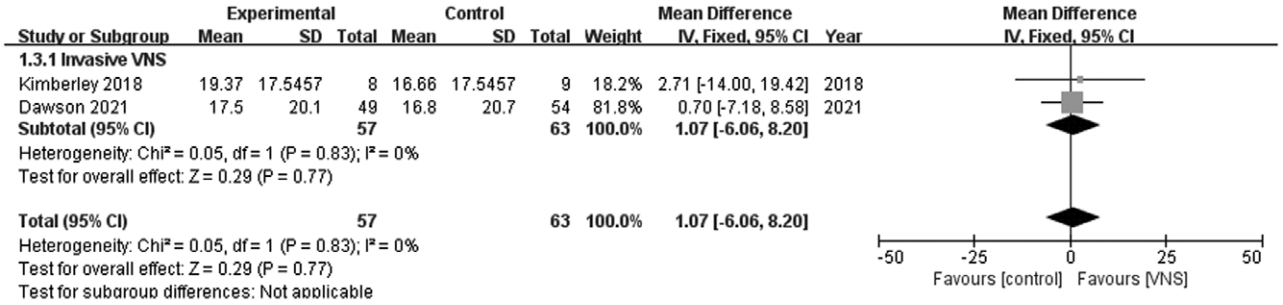
Wolf motor function test scores.

of invasive VNS to that of tVNS. Although there is increasing interest in tVNS, concerns regarding the degree of activation of vagal fibers, optimal stimulation site and stimulation parameters, and potential effects of stimulation on other nerves in the region have been raised.

The patients in the selected studies were diagnosed with stroke in the subacute or chronic phase, which suggests that the mechanism of VNS improvement occurs through the upregulation of neuroplasticity. Furthermore, VNS could have a potential benefit in improving acute stroke performance due to its participation in pathophysiological processes associated with anti-glutamate effects, anti-inflammatory activity, attenuating spreading depolarizations and decreasing intracranial pressure [28]. Further studies are required to elucidate the mechanisms and therapeutic effects of VNS.

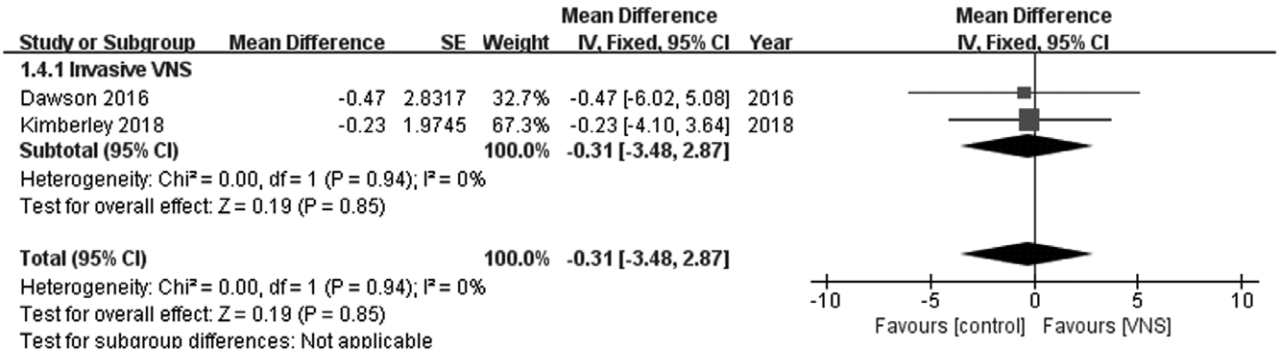
The stimulation parameters of invasive VNS for three studies that were included in the present systematic review and meta-analysis were the same; that is, burst of 500 ms with a constant current of 0.8 mA, pulse duration of 100 μs, and frequency of 30 Hz, which were derived from hypothesis-driven research in human and animal models [14,15,29,30]. The stimulations of invasive VNS

Fig. 6



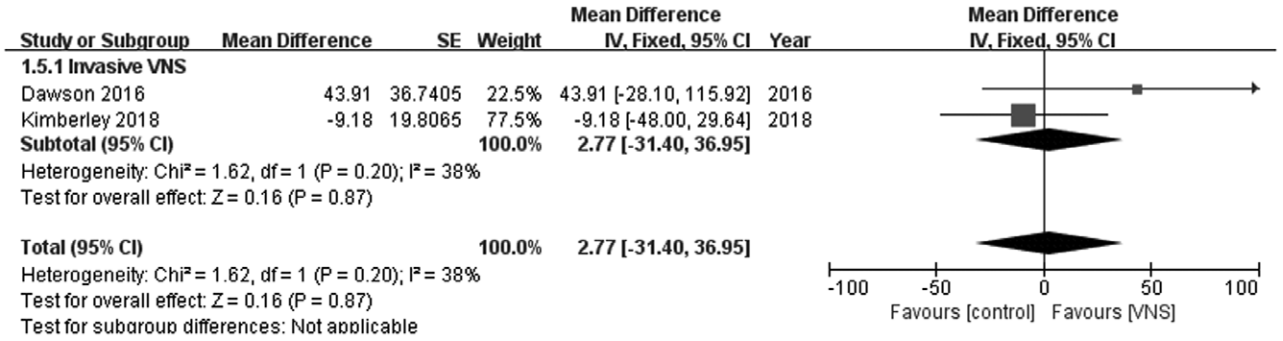
Stroke Impact Scale (hand function).

Fig. 7



Box and Block test.

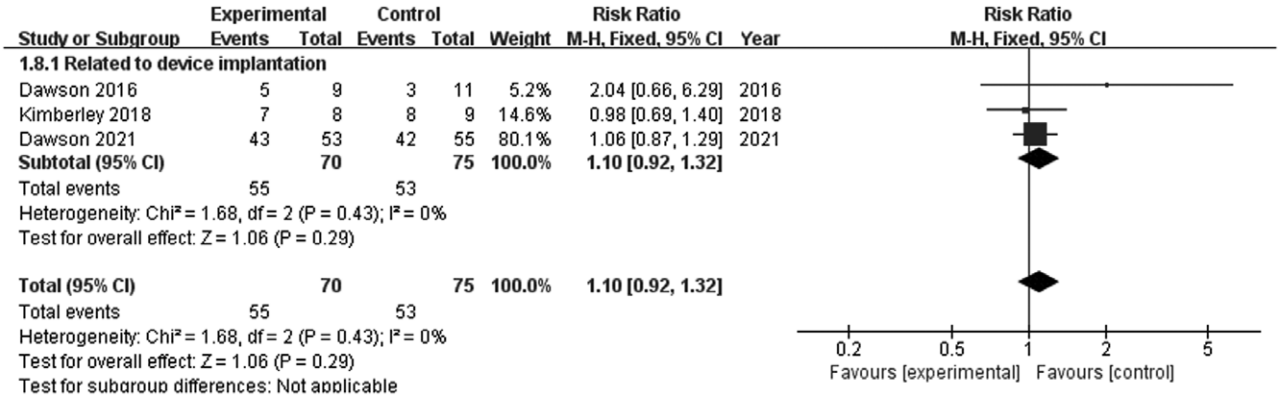
Fig. 8



Nine-Hole Peg test.

were delivered to the left vagus nerve to avoid activation of the sinoatrial node. The stimulation site for tVNS was the left external acoustic meatus on the inner side of the tragus, and the stimulation intensities for two studies were adjusted independently (above the detection threshold and below the pain threshold) to a pulse duration of 0.3 ms and frequency of 20 Hz repeated every 5 min for 60 min. However, there was no relevant basis for the stimulation parameters of tVNS, and the specific range of parameters that influence cortical plasticity remain unknown. Therefore, further studies regarding tVNS should be conducted.

Fig. 9



Adverse events.

Consequently, on the basis of the evidence provided by the current systematic review and meta-analysis, invasive VNS and tVNS paired with rehabilitation are effective in improving upper limb performance in patients with stroke. VNS could be used as adjuvant therapy for patients with subacute or chronic stroke in clinics. However, further research regarding the adverse events associated with device implantation in invasive VNS should be conducted.

Study limitations

The limitations of the current systematic review and meta-analysis were as follows. First, studies published in languages other than English were excluded. Second, quality assessment was not used as a selection or exclusion criterion. Third, the lack of concealed allocation and blinding in a few of the studies selected could have influenced the results. Fourth, outcomes of selected studies were measured immediately after treatment without any long-term follow-up. Finally, the number of included studies and patients were relatively small and may not provide sufficient statistical power to support the results.

Conclusion

VNS paired with rehabilitation is a promising strategy to promote upper limb function recovery for patients with stroke. The results of this systematic review and meta-analysis indicate that VNS paired with rehabilitation could improve upper limb function in patients with stroke on the basis of FMA-UE and WMFT scores. More studies with a focus on the long-term effect are needed.

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K.Z., J.Y. and Y.Q. helped with conceptualization. K.Z. and J.Y. helped with the design search. K.Z. helped with writing. Z.Z. and J.H. helped with data extraction/quality assessment. K.Z. helped with data analysis. K.Z., J.Y. and Y.Q. helped with the consultation and project management.

Conflicts of interest

There are no conflicts of interest.

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Systematic Review and Meta-Analysis

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Vagus nerve stimulation for upper limb motor impairment after ischemic stroke

A meta-analysis

Yu-lei Xie, MD, Shan Wang, MD*, Qing Wu, MD, Xin Chen, MD

Abstract

Background: Upper limb motor impairment is a common complication following stroke. Although few treatments are used to enhance motor function, still approximately 60% of survivors are left with upper limb motor impairment. Several studies have investigated vagus nerve stimulation (VNS) as a potential technique for upper limb function. However, the efficacy and safety of VNS on upper limb motor function after ischemic stroke have not been systematically evaluated. Therefore, a meta-analysis based on randomized controlled trial will be conducted to determine the efficacy and safety of VNS on upper limb motor function after ischemic stroke.

Method: We searched PUBMED, MEDLINE, EMBASE, Cochrane Library, Web of Science, China National Knowledge Infrastructure Library (CNKI), and Wan Fang Database until April 1, 2021.

Results: Six studies consisting of 234 patients were included in the analysis. Compared with control group, VNS improved upper limb function via Fugl-Meyer Assessment-Upper Extremity (mean difference = 3.26, 95% confidence interval [CI] [2.79, 3.74], $P < .00001$) and Functional Independence Measurement (mean difference = 6.59, 95%CI [5.77, 7.41], $P < .00001$), but showed no significant change on Wolf motor function test (standardized mean difference = 0.31, 95%CI [–0.15, 0.77], $P = .19$). The number of adverse events were not significantly different between the studied groups (risk ratio = 1.05, 95%CI [0.85, 1.31], $P = .64$).

Conclusion: VNS resulted in improvement of motor function in patients after ischemic stroke, especially in the sub-chronic stage. Moreover, compared with implanted VNS, transcutaneous VNS exhibited greater efficacy in poststroke patients. Based on this meta-analysis, VNS could be a feasible and safe therapy for upper limb motor impairment.

Abbreviations: CI = confidence interval, FIM = Functional Independence Measurement, FMA-UE = Fugl-Meyer Assessment-Upper Extremity, MD = mean difference, RCT = randomized controlled trial, SMD = standardized mean difference, VNS = vagus nerve stimulation, WMFT = Wolf motor function test.

Keywords: ischemic stroke, meta-analysis, randomized controlled trial, upper limb motor impairment, vagus nerve stimulation

1. Introduction

Stroke is a primary cause of mortality and associated morbidity worldwide.[1] Approximately 60% of survivors after stroke suffer from upper limb motor impairment, which consecutively lead to loss of independence with poor quality of life.[2,3] Therefore, it is essential to identify novel treatments for stroke survivors. Vagus nerve stimulation (VNS) either implanted or transcutaneous, is a neuromodulation therapy, which sends impulses into the neural center to generate corresponding nervous activity by stimulating the cervical vagus nerve.[4,5] VNS has been widely applied to the clinical treatment of many diseases such as epilepsy, drug-refractory depression, pain, chronic tinnitus, and so on.[6–10] Furthermore, VNS gradually shows a positive effect for the treatment of motor impairment after the stroke.[11–13]

Although the specific mechanism of VNS is not fully understood, studies have shown that VNS may activate the nucleus basalis neuron and locus coeruleus neuron, resulting in the widespread release of acetylcholine and norepinephrine in the cerebral cortex, respectively. The release of neurotransmitters eventually enhances the synaptic plasticity and the reorganization of cortical networks which ultimately improves motor function.[14,15] Several randomized controlled trials (RCTs) both on animals and human have shown that VNS paired with rehabilitation training can be a potential option in terms of efficacy and safety on upper limb motor impairment after ischemic stroke.[16–19] However, Dawson et al[20] reported no significant change in motor function after VNS in the intention to treat analysis. Besides, a meta-analysis[21] investigated the efficacy of VNS as the rehabilitation following stroke, which revealed a significant effect of VNS on Fugl-Meyer Assessment-Upper Extremity (FMA-UE). However, the conclusion was based

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The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are publicly available.

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on 3 RCTs with a small sample size with mixed models of ischemic and hemorrhagic stroke. Recently, some new researches evaluating the effect and safety of VNS on the motor function of ischemic stroke has emerged.

This meta-analysis aims to evaluate the efficacy and tolerability of VNS for upper limb motor impairment after ischemic stroke based on RCTs and attempted to provide clinical evidence for the VNS in the treatment of upper limb motor impairment after ischemic stroke.

2. Methods

This systematic review protocol was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis Protocol (PRISMA-P). This is a literature based study, so ethical approval is not necessary.

2.1. Study search strategy

The methodology of this meta-analysis was done as recommended by the Cochrane Collaboration.[22] The databases such as PUBMED, MEDLINE, EMBASE, Cochrane Library, Web of Science, China National Knowledge Infrastructure Library (CNKI), and Wan Fang Database were searched from inception until April 1, 2021, with the following keywords: vagus nerve stimulation and stroke. There were no restrictions on the language, region, race, or publication types.

2.2. Selection criteria

Patients diagnosed with ischemic stroke; Only RCTs comparing VNS paired with rehabilitation training and with only rehabilitation training; Studies having available completed valid data.

2.3. Data extraction and outcome measures

All of data were extracted independently by the 2 examiners, any disputes were settled by the consensus. In case of incomplete data, authors were contacted for details. For crossover trials, we only took the data for the first period (before crossover) into consideration.

The primary outcome included FMA-UE and the adverse events related to the therapy or devices, evaluating the efficacy and safety of VNS for upper limb impairment, respectively. The secondary outcomes included the Wolf motor function test (WMFT) and Functional Independence Measurement (FIM).

2.4. Quantitative and statistical analysis

All statistical analysis was performed by Review Manager 5.3 (The Cochrane Collaboration, Copenhagen, Denmark). Two independent examiners evaluated the quality of each RCT to estimate the risk of bias with the Cochrane risk of bias tool including sequence generation, allocation concealment, masking, incomplete outcome data, selective reporting, and other issues.[23] We also utilized risk ratio to assess dichotomous outcomes and calculated 95% confidence intervals (CIs). Besides, mean difference (MD) and standardized mean difference (SMD) with 95%CI were assessed for continuous variables.

Heterogeneity in data of the selected study was assessed using the χ^2 test and the I^2 statistics. When I^2 was less than 50% with a P value more than .1, there was no heterogeneity and therefore a fixed-effect model was used. On the contrary, if there was heterogeneity, we used a random-effect model to test the robustness of the results for the possible explanations. Furthermore, sensitivity and subgroup analysis was performed to find out the source of heterogeneity. However, due to the small number of included studies (n = 6), the publication biases could not be assessed.

3. Results

3.1. Study inclusion

Figure 1 shows the flow chart of PRISMA. For the total of 502 studies identified by the predefined search strategy, 216 studies were selected after excluding the 286 duplications. Failing to meet the inclusion criteria, 193 studies were excluded through screening the abstracts and titles. Of the remaining 23 studies, 10 were sorted out after reading through the full text. One RCT was excluded for participants with both ischemic and hemorrhagic stroke,[19] eventually, 6 studies were included in the analysis.[17,20,24–27]

3.2. Study characteristics

The characteristics of included studies are described in Table 1. A total of 234 patients were included in this meta-analysis. The sample size in the included studies, varied from 17 to 108. In each study, patients were randomly assigned to 2 groups: VNS paired with upper limb rehabilitation and upper limb rehabilitation alone. For 3 studies of implanted VNS, only 1 did not perform VNS device implantation in rehabilitation-only participants.[20] For 3 studies of transcutaneous VNS, electrodes were fitted to the cymba conchae of the left ear, the sham group without electrical stimulation. While the stroke durations ranged from 1 month to years, the intervention lasted from 15 days to 6 weeks. Although more males than females were enrolled in the included studies, groups seemed balanced from sex. There was a 5-point significant difference (VNS group 40.10 ± 9.70 versus control group 45.30 ± 8.40) in the baseline of FMA-UE in the study of Dawson et al.[20] Three studies[25–27] employed transcutaneous VNS whereas 3 studies[17,20,24] adopted implanted VNS as intervention. The stimulation parameters of VNS were different each study, such as stimulation intensity (mA), frequency (Hz), pulse width (μs), and duration (ms). Three studies[17,20,24] employed the same stimulation settings of 0.8 mA, 30 Hz frequency, 100 μs pulse width with pulse train of 0.5 seconds. The measurements of effect mainly included FMA-UE, with other parameters such as WMFT, FIM, Brunnstrom stage, Ashworth, Box and Block Test, Nine-Hole Peg Test, and so on. The number of adverse events related to devices or therapy was chosen to evaluate safety of the employed VNS.

3.3. Study quality

All included studies were RCTs. All of the included studies described the sequence generation method. Three studies illustrated the allocation concealment covering via email, phone call and/or an interactive voice response system. One study[27] did not report the completeness of outcome data. The study of Wei[26] did not describe the blinding and also had a high risk of bias on allocation concealment. Figure 2 describes the risk of bias in detail.

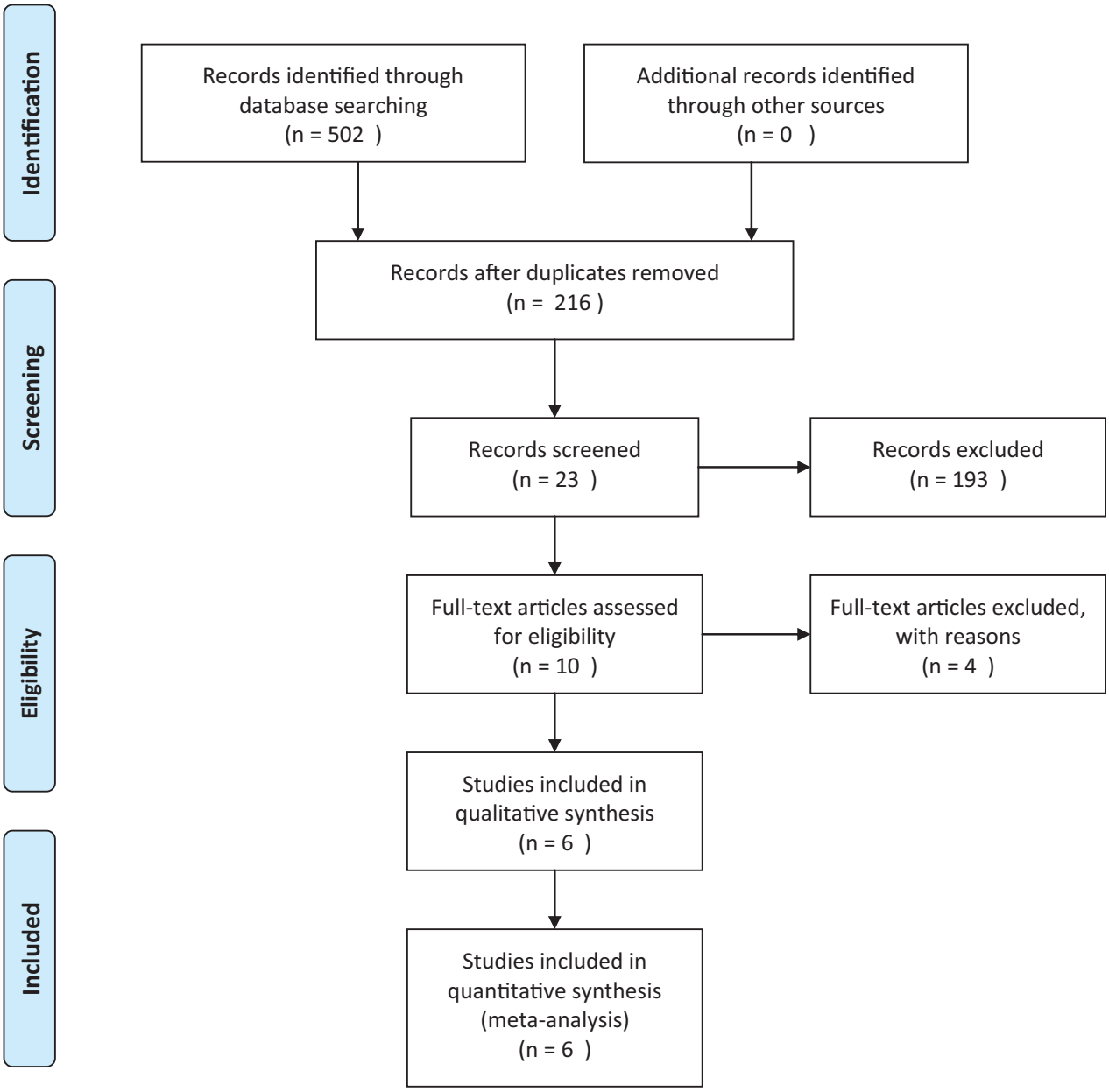

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Figure 1. The PRISMA flow chart of the study selection process.

Table 1

Characteristics of included studies in the meta-analysis.

Study	Design	Patients		Duration of stroke		Gender (m/f)		Age(yr)		FMA-UE at baseline		Methods		Device parameters	Outcome measures		Adverse events
		Real	Sham	Real	Sham	Real	Sham	Real	Sham	Real	Sham	Real	Sham		Effect	Safety	
Wu, 2020	A single-blinded, RCT	10	11	36.30±9.23 (d)	35.55±6.47 (d)	5/5	8/3	64.50±9.97	61.82±10.63	17.50±4.91	16.82±3.89	Transcutaneous VNS plus rehabilitation training for 15 days	Sham VNS plus rehabilitation training for 15 days	Optimum intensity, 20 Hz, 300 μs, lasting 30 s per time of every 5 minutes, total of 1600 pulses	FMA-U,WMFT,FIM, Brunnstrom stage	HR; BP	Skin redness
Dawson, 2016	A blinded, open, RCT	9	11	1.8±1.0 (y)	1.7±1.3 (y)	7/2	9/2	57.9±17.2	60.7±10.7	40.1±9.7	45.3±8.4	Left VNS plus rehabilitation training for 6 weeks (18 times)	Only rehabilitation training for 6 weeks (18 times)	0.8 mA, 30 Hz, 100 μs, duration of 0.5 s	FMA-UE, ARA T, grip, and pinch strength	The number of serious adverse events related to therapy	Left vocal cord palsy and dysphagia; nausea; taste disturbance; hoarseness; neck tingling
Kimberley, 2018	A fully blinded, RCT	8	9	18±0.5 (m)	18±11.68 (m)	4/4	5/4	59.5±7.4	60±13.5	29.5±6.4	36.4±9.4	VNS plus rehabilitation training for 6 weeks (18 times)	Sham VNS plus rehabilitation training for 6 weeks (18 times)	0.8 mA, 30 Hz, 100 μs, duration of 0.5 s	FMA-UE, WMFT, Box and Block Test, Nine-Hole Peg Test, Stroke Impact Scale, and Motor Activity Log	The number of serious adverse events related to the device or therapy	Implantation wound infection; shortness of breath and dysphagia; hoarseness
Dawson, 2021	Triple-blinded, RCT	53	55	3.1±2.3 (y)	3.3±2.6 (y)	34/19	36/19	59.1±10.2	61.1±9.2	34.4±8.2	35.7±7.8	VNS plus rehabilitation training for 6 weeks (18 times)	Sham VNS plus rehabilitation training for 6 weeks (18 times)	0.8 mA, 30 Hz, 100 μs, duration of 0.5 s	FMA-UE, WMFT, MAL, SIS, SS-QOL, EQ-5D, BDI	NA	Vocal cord palsy
Wei, 2020	RCT	13	13	48.77±24.74 (d)	50.38±22.07 (d)	4/9	3/10	61.31±11.54	57.23±10.17	32.85±12.13	28.31±13.55	Transcutaneous left auricular VNS plus rehabilitation training for 4 weeks	Sham VNS plus rehabilitation training for 4 weeks	Optimum intensity, 25 Hz, 100 μs, lasting 30 s per time of every 30s	FMA-UE, Brunnstrom stage, MFAS, Ashworth	Electrocardiogram	Mild nausea and vomiting; mild pain in the left ear
Zhang, 2020	A triple-blinded, RCT	21	21	38±1 5(d)	36.86±2(d)	11/10	8/13	66.1±1.49	64.1±1.03	18.76±0.94	17.9±0.76	Transcutaneous left auricular VNS plus rehabilitation training for 3 weeks	Sham VNS plus rehabilitation training for 3 weeks	0.5 mA, 20 Hz, lasting 30s per time of every 2 mintues, total of 30 mintues for 3 weeks	FMA-UE, WMFT, FIM	The number of serious adverse events related to therapy; BP; HR	No adverse events

ARAT = action research arm test, BDI = the Beck depression inventory, BP = blood pressure, FIM = Functional Independence Measurement, FMA-UE = Fugl-Meyer Assessment-Upper Extremity, HR = heart rate, MAL = motor activity log, MFAS = motor function assessment scale, RCT = randomized control trail, SIS = Stroke Impact Scale, SS-QOL = stroke specific quality of life, VNS = vagus nerve stimulation, WMFT = Wolf motor function test.

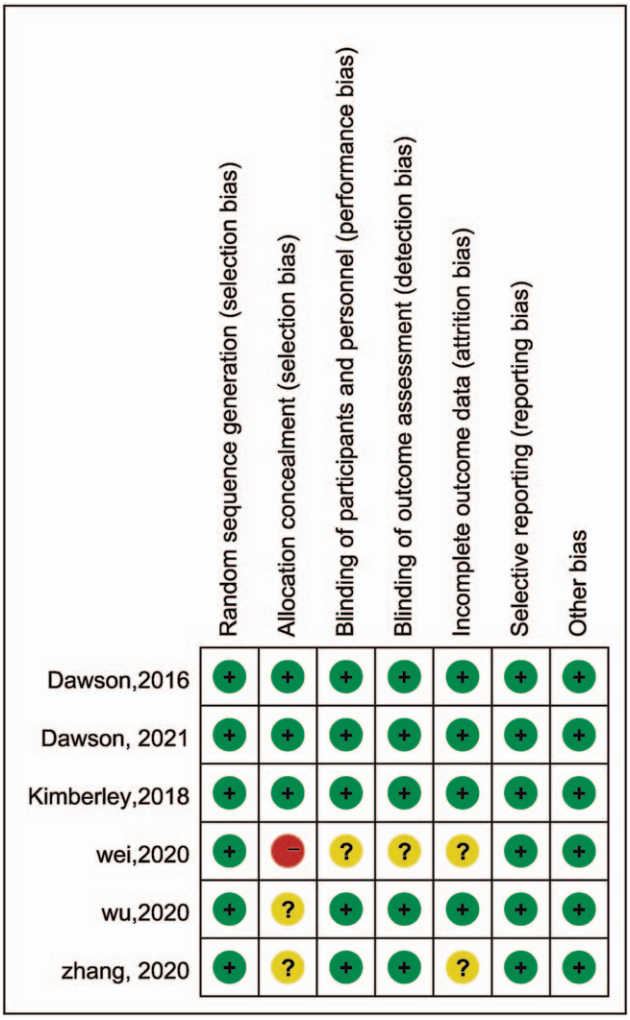

Figure 2. Risk of bias summary of included studies in this meta-analysis.

3.4. The primary outcomes

3.4.1. Fugl-Meyer Assessment-Upper Extremity. FMA-UE primarily reflects the change of upper limb function. FMA-UE scores at the endpoint were available for all the selected studies. The simulated results were comparable with the control group, where VNS group has shown the higher change on FMA-UE scores (MD = 3.26, 95%CI [2.79, 3.74], $P < .00001$) with acceptable heterogeneity ($\chi^2 = 7.97$, $P = .16$, $I^2 = 37\%$) (Fig. 3A).

3.4.2. The adverse events related to therapy or devices. The adverse events associated with the therapy were reported in 5 studies[17,20,24–26] as shown in Table 1. The simulated result revealed that the VNS was feasible and safe (risk ratio = 1.05, 95%CI [0.85, 1.31], $P = .64$) with no obvious heterogeneity in the obtained data ($\chi^2 = 2.9$, $P = .57$, $I^2 = 0\%$) (Fig. 4).

3.5. The secondary outcomes

Four studies[17,24,25,27] reported WMFT, including 188 patients, however, significant heterogeneity was detected among the studies ($\chi^2 = 48.10$, $P < .00001$, $I^2 = 94\%$). Heterogeneity remained even after transferring the data into the random-effect model (Fig. 5) ($\chi^2 = 48.10$, $P < .00001$, $I^2 = 94\%$). Moreover, the simulated result revealed significant heterogeneity with no statistical difference among the groups (SMD = 1.32, 95%CI [–0.27, 2.91], $P = .10$). Each study was excluded orderly following the sensitivity analysis. After removing the study of Zhang et al, although the heterogeneity changed but no significant difference in simulated result ($\chi^2 = 2.76$, $P = .25$, $I^2 = 28\%$) (SMD = 0.31, 95%CI [–0.15, 0.77], $P = .19$) (Fig. 6).

Two studies[25,27] including 63 patients reported FIM. The simulated results were comparable with control group, however VNS significantly improved limb motor function via FIM with no obvious heterogeneity (MD 6.59, 95%CI [5.77, 7.41], $P < .00001$) ($\chi^2 = 0.01$, $P = .92$, $I^2 = 0\%$) (Fig. 7).

3.6. Subgroup analysis

Subsequently, subgroup analysis was performed based on the intervention and duration of stroke to identify possible factors that might affect the efficacy of VNS on ischemic stroke.

In the subgroup of intervention, the group of transcutaneous VNS included 89 patients whereas the group of implanted VNS included 145 patients. It was observed that transcutaneous VNS (MD = 4.14, 95%CI [1.51, 6.77], $P = .002$) showed greater effect on patients after ischemic stroke than the implanted VNS (MD = 0.55, 95%CI [–2.59, 3.69], $P = .73$) (Fig. 8).

The stroke durations of all the included patients were longer than 2 weeks. Hence, the value of 6 months was taken as the cutoff point, while dividing the durations into recovery and sequelae stages. The recovery stage group included 89 patients and the sequelae stage group included a total of 145 patients. The subgroup analysis of stroke duration indicated that the patients within recovery stage (MD = 4.14, 95%CI [1.51, 6.77], $P = .002$) demonstrated better enhancement in motor function in comparison with the sequelae stage (MD = 0.55, 95%CI [–2.59, 3.69], $P = .73$) (Fig. 9).

4. Discussion

Following the stroke, the recovery of upper limb impairment is relatively slower than that of the lower limb. Although a series of therapies have been applied to the clinical treatment, there is still a large number of patients suffering from upper limb impairment.[28–30] Several RCTs are reporting VNS, as a promising tool for a feasible and effective gain of motor function after stroke, although there are only a few meta-analysis that have been done on this subject. There is a growing need for the simulated analysis underlying RCTs to ascertain the effect of VNS on poststroke motor impairment.

In the current meta-analysis, 6 studies including 234 patients were analyzed. We used FMA-UE, WMFT, FIM, and the number of adverse events to evaluate our simulated results. There was only a significant difference in the FMA-UE score between the groups, which further validates the use of VNS. Based on the pooled results, subgroup analysis on the intervention and duration of stroke were performed. The efficacies of both implanted and transcutaneous VNS on ischemic stroke have been proven in the pre-clinical and clinical trials, with emphasis on the importance on pairing VNS with rehabilitative exercises.[2,18,31,32] It is speculated that transcutaneous VNS shares a similar pathway or mechanism with that of implanted VNS. The VNS causes stimulation mediated activation of brainstem

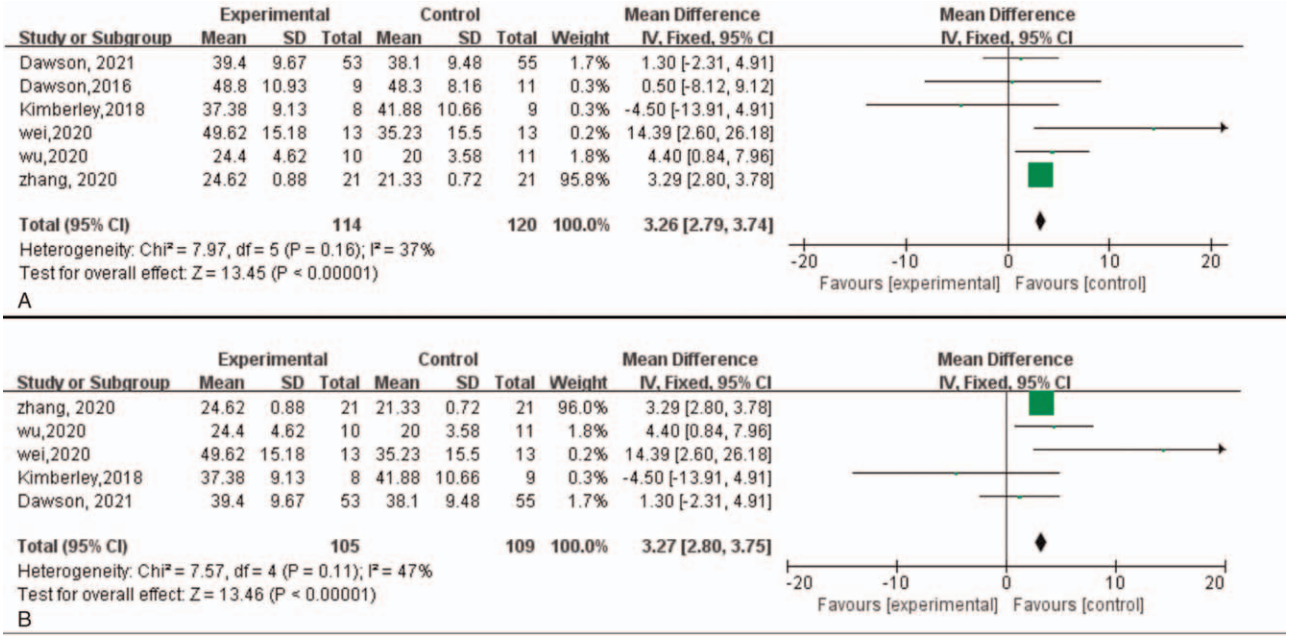

Figure 3. Forest plot of efficacy of VNS on motor function with FMA-UE. FMA-UE = Fugl-Meyer Assessment-Upper Extremity, VNS = vagus nerve stimulation.

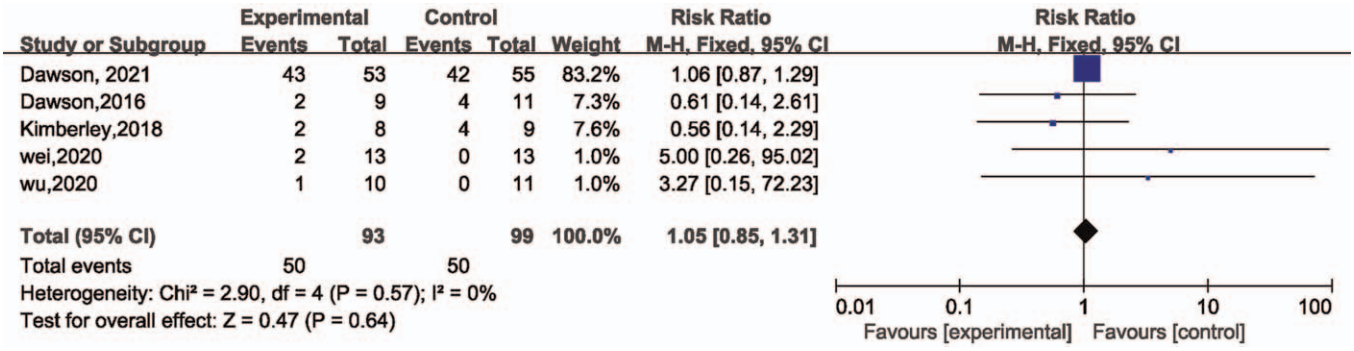

Figure 4. Forest plot for meta-analysis of safety of VNS on motor function. VNS = vagus nerve stimulation.

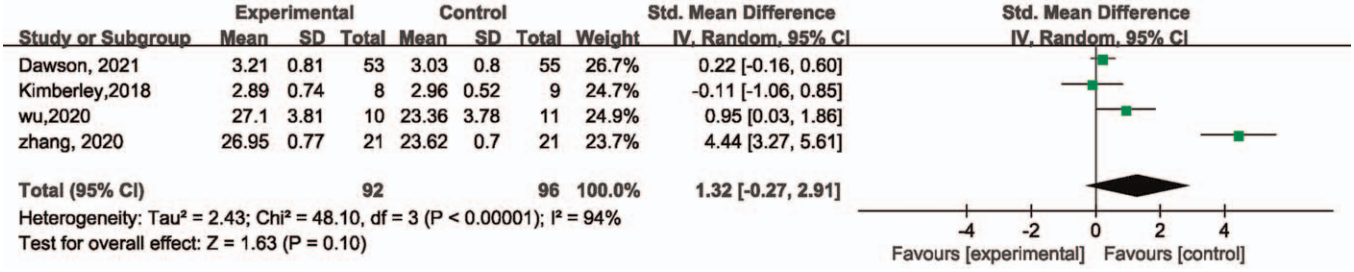

Figure 5. Forest plot for meta-analysis of efficacy of VNS on motor function with WMFT. VNS = vagus nerve stimulation, WMFT = Wolf motor function test.

vagi nuclei via afferent fibers of the vagus nerve, though there was no evidence to show whether the intensity of activated vagus nerve was maintained consistently between both the VNS.[25,33] However, there is scarcity of studies which compares the efficacy of both VNS. The result of subgroup analysis revealed that the implanted VNS did not affect the motor function after ischemic stroke. Notably, the FMA-UE scores of reports by Dawson et al and Kimberley et al at baseline had a 5-point and 6-point difference between the studied groups, respectively. Therefore, this meta-analysis indicated that the transcutaneous VNS has

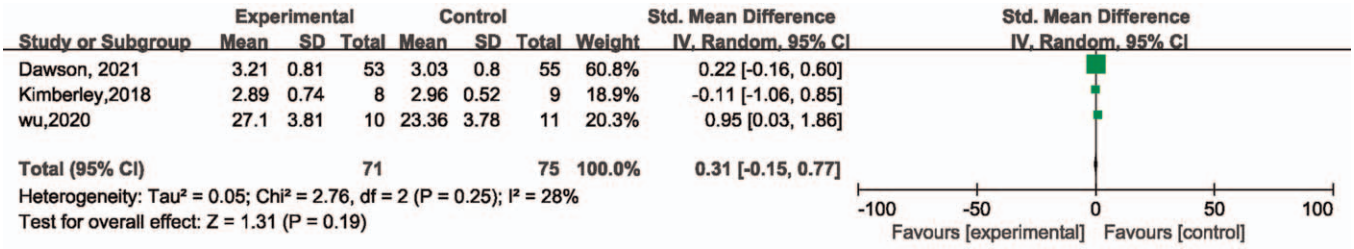

Figure 6. Forest plot for sensitivity analysis of efficacy of VNS on motor function with WMFT. VNS = vagus nerve stimulation, WMFT = Wolf motor function test.

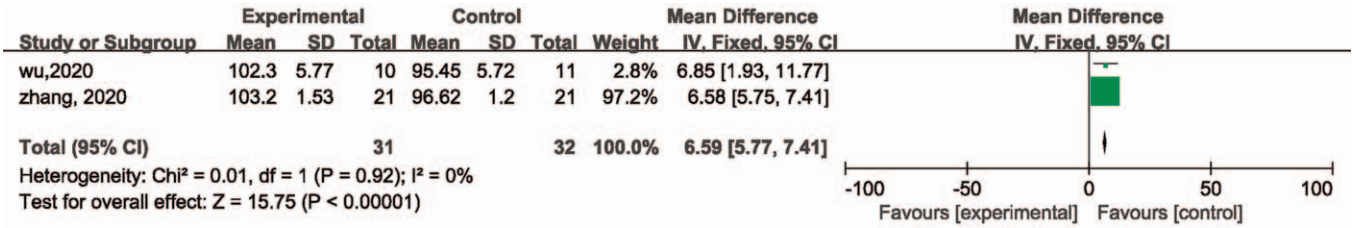

Figure 7. Forest plot for meta-analysis of efficacy of VNS on motor function with FIM. FIM = Functional Independence Measurement, VNS = vagus nerve stimulation.

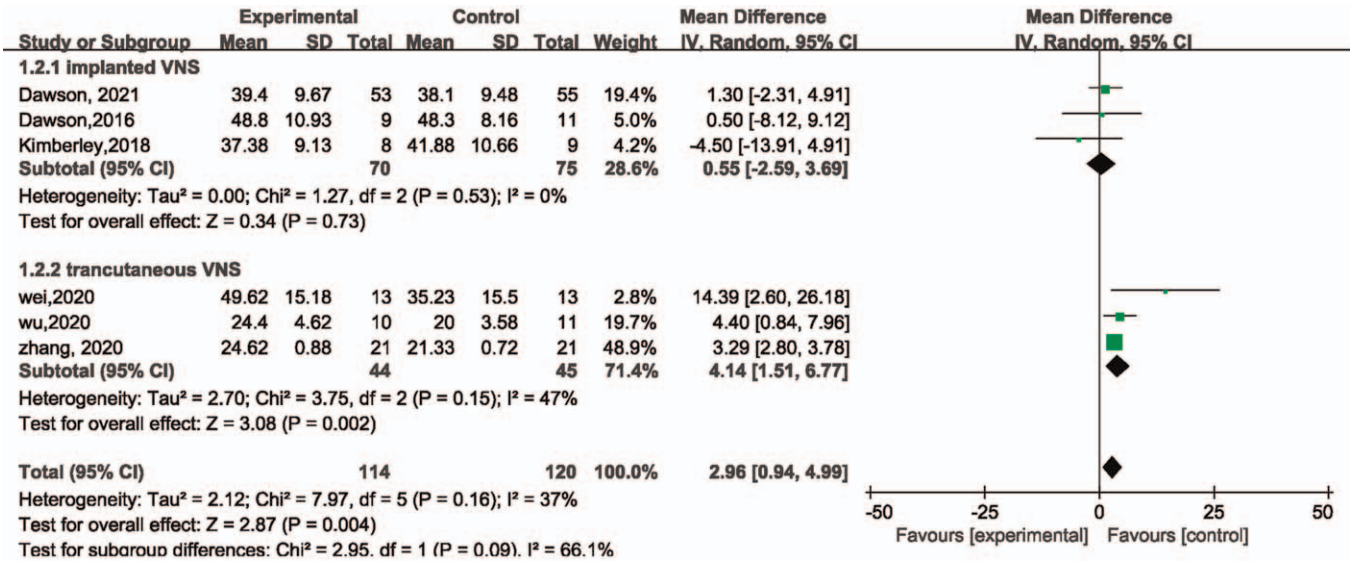

Figure 8. Forest plot for within intervention subgroup analysis of efficacy of VNS on motor function. VNS = vagus nerve stimulation.

superior benefits in improving the motor function in patients after ischemic stroke, whereas the implanted VNS might also be effective.

For implanted VNS groups, 1 study did not implant the device related VNS as the control group.[20] Although the population weight of this one in included studies was small, to eliminate the effect of placebo, the other 5 studies were analyzed. The simulated result still showed the significant change on FMA-UE scores (MD = 3.27, 95%CI [2.80, 3.75], $P < .00001$) with acceptable heterogeneity ($\chi^2 = 7.57$, $P = .11$, $I^2 = 47\%$) (Fig. 3B) among groups, which seemed to identify the effectiveness of VNS.

Within-subgroup analysis of stroke duration suggested that compared with patients in the sequelae stage, those in the recovery stage had a significant change in motor function. A series of trials have identified that enhancement of neuroplasticity mediated by VNS paired with rehabilitation training, for the basis of motor function recovery poststroke.[15,17,20,31] Interestingly, in comparison with chronic stroke, patients with sub-chronic stroke often demonstrate greater improvement in motor function.[34]

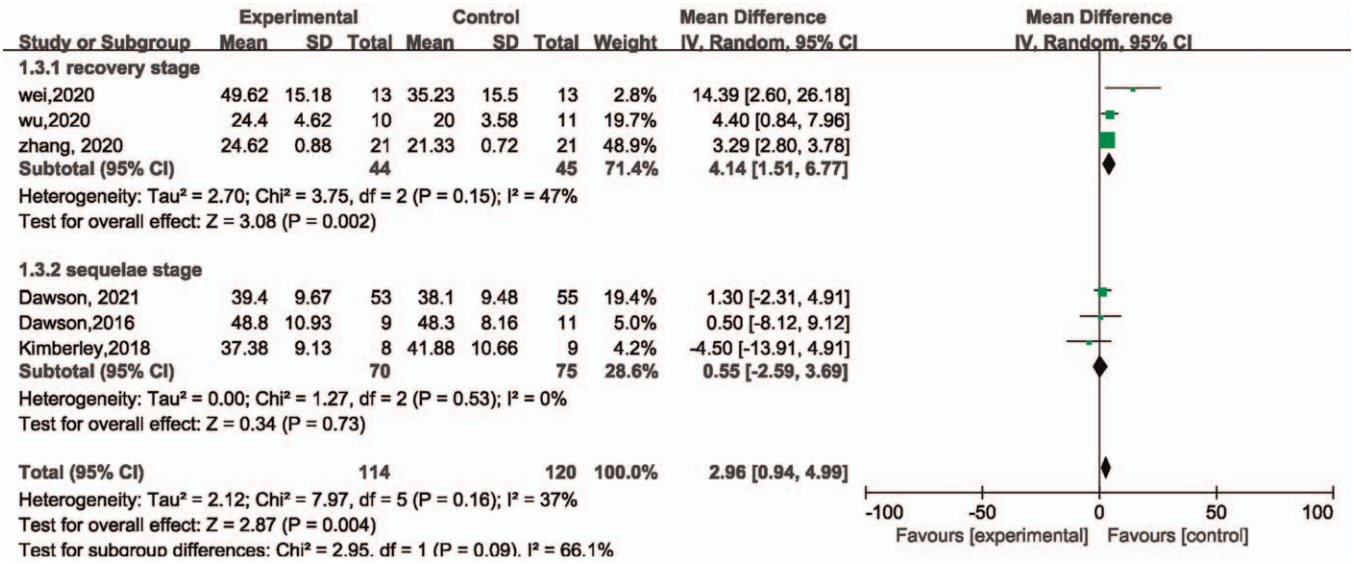

Figure 9. Forest plot for within stroke duration subgroup analysis of efficacy of VNS on motor function. VNS = vagus nerve stimulation.

Similarly, taking the difference of FMA-UE at baseline in the study of Dawson et al and Kimberley et al into consideration, VNS can improve motor function in patients with sub-chronic stroke, and might also be effective for those with chronic stroke.

Based on subgroup analysis, the Chinese cohorts were given transcutaneous VNS during the recovery stage while the White cohorts treated by implanted VNS during the sequelae stage. In view of different religious beliefs, economics, sociology, and cultures, the acceptance of VNS varied among each race. Previous study showed the racial disparities in access to VNS devices.[35] Therefore, ethnicity might be an influence factor for these outcome measures.

VNS also showed positive effects on WMFT and FIM. Based on the sensitivity analysis of WMFT, the study of Zhang et al was considered as the source of heterogeneity, due to the different stimulation parameters, unclear allocation concealment, and sample size.

There was no significant difference in safety between the studied groups. According to the data from 6 studies, only a few patients reported the occasional slight discomfort, whereas none of the severe events were reported associated with the device. Hence, VNS was deemed significantly safe for upper limb impairment after ischemic stroke.

In the current analysis, there was a great difference in the proportion of male and female although no significant differences among groups. Failing to obtain valid data, the different effects of VNS on sexuality could not be analyzed. Fortunately, there were studies reporting the sex differences in hemodynamic and autonomic regulation of cardiovascular systems both on animal and human trials.[36,37] In terms of adverse effects of VNS, female subjects were more likely to express side effects than that of males, and this difference may originate from discrepancy in the sensitivity of certain nuclei following the cardiac branch pathway in female and male subjects.[38,39] On the difference of the curative effects of VNS, female subjects also performed less effectiveness.[40] These findings might be the basic evidence for future researches exploring the response of sexuality to VNS.

While the mechanisms of VNS are still unclear, it is speculated that it may be associated with the neuroprotection within the acute stage[41–44] and enhancement of neuroplasticity during poststroke.[14,45] The neuroprotection included: induction of neoangiogenesis to reduce infarct volume, alleviate neuron damage and enhance neurofunction.[46] Suppression of inflammation via activating the cholinergic anti-inflammatory pathway.[47] Adjustment in the level of malondialdehyde, glutathione, and superoxide dismutase in brain regions for suppressing the cellular responses to oxidative stress.[48] As discussed earlier, VNS could also enhance synaptic plasticity via release of neurotransmitters.[15] Furthermore, studies have reported that the VNS promoted the level of brain-derived neurotrophic factor, which in turn triggered nerve regeneration and enhances synaptic plasticity.[49]

The optimal parameters of VNS are explored to increase the degree of VNS-dependent neuroplasticity. Pruitt et al[50] reported an inverted-U relationship between stimulation intensity with the motor function recovery, therefore suggesting the moderate-intensity VNS (0.8 mA) paired with rehabilitation for a significant yield of greater functional recovery than lower (0.4 mA) and higher stimulation intensity (1.6 mA), although the mechanism underlying this relationship was not defined. The same relationship was detected for the stimulation frequency, where the moderate-frequency (30 Hz) enhanced the cortical plasticity than the slower (7.5 Hz) and faster (120 Hz) pulse rate.[51] Overall, the above studies elucidated the influence of different stimulation parameters on motor function recovery. Although, more studies are required to explore and validate the most optimal program.

There were some limitations in this meta-analysis. First, considering the number of included studies, the sample size of each study, the quality of studies, and simulated synthesis, the conclusions from simulated results must be interpreted with caution. Second, the dose parameters were varying for the included studies such as stimulation intensity, frequency, and training duration of VNS. At present, there is no standard recommendation for the parameters for using VNS,[50] therefore,

the efficacy of VNS may vary with the change in parameters. Lastly, it is worth noting that the patients enrolled in the included studies might not be the true representation of patients with upper limb impairment after ischemic stroke worldwide.

5. Conclusion

Overall, this meta-analysis demonstrated that the VNS is feasible and safe for patients with upper limb impairment after ischemic stroke. Poststroke, use of VNS showed an improvement in motor function in patients, and especially for those in the sub-chronic stage. Moreover, compared with the implanted VNS, transcutaneous VNS was more effective for patients after ischemic stroke. However, due to the above-mentioned limitations, future multicentric studies with larger sample RCTs are required to optimize the stimulation parameters and to identify the efficacy of VNS on motor function after stroke.

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Vagus Nerve Stimulation Paired with Rehabilitation for Upper Limb Motor Function After Ischaemic Stroke (VNS-REHAB): A Randomised, Blinded, Pivotal, Device Trial

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JD, NE, TJK, DP, BT, SC designed the study protocol. The first draft of the manuscript was written by the Publication Committee, which included JD, TJK, CL and NE. The publication committee have had access to all study data and outputs from statistical analysis. The publication committee took the primary responsibility for crafting the manuscript text and provided the overall principal leadership for the study. Figures were created by NE. Statistical analysis was performed independently by David Ng, Ph.D. from WuXi Clinical. All authors provided critical revisions to the manuscript text.

Data Sharing Statement

Data collected for the study, including deidentified individual participant data, data dictionary defining each field in the set, study protocol, and statistical analysis plan will be available after the completion of the post market study requirements of regulatory approval. Data will only be shared upon the approval of the proposal with the principal investigators, the sponsor of the study, and requires a signed data access agreement with specific funding to access the database without any support from investigators. Requests should be sent to VNSdatarequest@gmail.com.

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Abstract

Background: Long-term loss of arm function after ischaemic stroke is common and may be improved by Vagus Nerve Stimulation (VNS) paired with rehabilitation.

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Methods: In this pivotal, randomised, triple-blind, sham-controlled trial, we assigned participants with moderate to severe arm weakness, at least nine months after ischaemic stroke, to receive rehabilitation paired with active VNS or rehabilitation paired with sham stimulation (Control). All participants were implanted with a VNS device and received six weeks of in-clinic therapy followed by a home exercise program. The primary outcome was the change in impairment measured by the Fugl-Meyer Assessment Upper Extremity (FMA-UE) score on the first day after completion of in-clinic therapy. All analyses were by intention to treat. The trial was registered on ClinicalTrials.gov (NCT03131960).

Findings: We randomised 108 participants between Oct 2, 2017 and Sept 12, 2019 (53 to VNS and 55 to Control). A total of 106 completed the study. On the first day after completion of in-clinic therapy, the mean (±SD) FMA-UE score increased by 5.0 points (SD 4.4) in the VNS group and by 2.4 points (SD 3.8) in the Control group (p=0.001, between group difference 2.6, 95% CI 1.03 to 4.2). Ninety days later, a clinically meaningful response on the FMA-UE score was achieved in 47% with VNS versus 24% in controls (p=0.01; between group difference 24%, 95% CI 6 to 41%). The Wolf Motor Function Test (WMFT) functional score increased by 0.46 (±0.40) points in the VNS group compared to 0.16 (±0.30) points in the Control group (p<0.0001, between group difference 0.30, 95% CI 0.16 to 0.43). The FMA-UE score increased by 5.8 points (±6.0) from baseline with VNS and by 2.8 points (±5.2) in controls (p=0.008, between group difference 2.96, 95% CI 0.83 to 5.08). There was one serious adverse event related to surgery (vocal cord paresis).

Interpretation: Participants with moderate to severe arm impairment after ischaemic stroke showed clinically meaningful improvements in motor impairment and function with paired VNS compared to rehabilitation with sham VNS.

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Keywords

vagus nerve; stroke; rehabilitation; neuromodulation; physical therapy; occupational therapy; plasticity; upper extremity

Introduction

Approximately 80% of people with acute stroke have upper limb motor impairment and as many as 50%−60% of these survivors still have persistent problems six months later.[1,2] Persistent arm impairment is linked with poorer quality of life and reduced well-being.[3] Identifying new treatments to improve upper limb function after stroke is a research priority for both stroke survivors and caregivers.[4]

There are few effective treatments to enhance upper limb recovery after stroke. Trials of increased therapy dose and of adjuvant drug or brain stimulation therapies have not been effective[5–8]. Constraint induced movement therapy has been shown to improve measures of upper limb impairment and function in selected people with stroke, possibly through helping them re-learn how to use intact motor pathways[9].

One potential method to enhance the reorganisation potential of the brain following stroke is via cholinergic and monoaminergic modulation of motor cortex neurons[10,11]. This may be achieved by Vagus Nerve Stimulation (VNS). VNS paired with sensory input or motor training has been shown to result in input-specific reorganization of rat cortical neurons[12,13]. In rodent models of ischemic stroke, VNS combined with movement training significantly improved forelimb motor recovery and tripled the synaptic connectivity of motor cortex neurons compared to movement training alone[14]. Two pilot studies of VNS paired with intensive upper limb rehabilitation have been conducted in people with long-term moderate to severe arm weakness after stroke. VNS-treated participants had greater improvement in Fugl-Meyer Assessment-Upper Extremity (FMA-UE) score compared to participants that received intense rehabilitation alone[15,16].

We performed a pivotal, randomised, blinded, controlled trial comparing active VNS paired with rehabilitation versus sham stimulation paired with rehabilitation in people with moderate to severe arm impairment after ischaemic stroke. The purpose of this trial was to determine whether VNS paired with rehabilitation is a safe and effective treatment for improving arm function after stroke.

Methods

Further details regarding the design of the trial have been published previously.[17] The study was approved by the review boards at each institution and subject to appropriate regulatory approvals (FDA Investigational Device Exemption (IDE, #G170031) and UK MHRA No #CI/2015/0011). The study was registered on clinicaltrials.gov (NCT03131960). Written informed consent was obtained from all participants. The study was conducted according to the Declaration of Helsinki and was undertaken in 19 sites in the UK and USA.

Participants

Study participants were male and female adults aged ≥ 22 years and ≤ 80 years old with a history of unilateral supratentorial ischaemic stroke that occurred between nine months to ten years prior to enrollment. People with moderate to severe arm impairment defined as a FMA-UE score between 20–50 were eligible for inclusion. Full inclusion and exclusion criteria are provided in the supplement.

Randomisation and Masking

We randomised participants at the time of VNS implant surgery to either rehabilitation paired with active VNS (VNS group) or rehabilitation paired with sham stimulation (Control group) on a 1:1 basis. Randomisation was done by ResearchPoint Global (USA) using SAS PROC PLAN, with stratification by region (US/UK), age (≤30, >30), and baseline FMA-UE score (20–35, 36–50). The randomisation allocation was sent via email to an unblinded clinical engineer at each site who tested and programmed the device with the appropriate stimulation settings for group assignment during implantation. Participants, outcomes assessors, and treating therapists were blinded to group assignment. In an effort to maximize masking of treatment allocation, all participants were implanted with the VNS device. In addition, both treatment groups participants received 5 stimulations in reducing

strengths (0.8 mA and then lower) at the beginning of each therapy session followed by stimulation according to randomised allocation. This was designed to minimize risk of participants being able to guess treatment allocation by exposing all participants to the same stimulation parameters at the start of each session. After the primary endpoint assessment, participants were asked to rate their certainty regarding group allocation by picking one of five options; knew they received VNS; thought they received VNS; knew they were in the sham stimulation group; thought they were in the sham stimulation group; or had 'no idea.'

Study Procedures

A pre-surgery assessment was performed. Device implantation was done under general anaesthesia. A horizontal neck crease incision was created left of the midline at the level of the cricoid cartilage. After the vagus nerve was identified, the stimulation lead was wrapped around the vagus nerve. The lead was then tunnelled subcutaneously to the pulse generator device which was contained in a subcutaneous pocket in the pectoral region.[18]

Baseline assessments were performed one week after device implantation. Stimulation was tested in increments of 0.1 mA to assess if participants felt and tolerated stimulation. If stimulation at 0.8 mA was uncomfortable, stimulation settings were lowered to a comfortable level, and this level was used in the study. This process was performed in both groups regardless of treatment allocation. In two participants, stimulation settings were lowered to 0.7 mA and 0.6 mA.

In-clinic rehabilitation therapy began the next day and was provided three times per week for six weeks (total of 18 sessions). Details about the upper limb rehabilitation delivered in the trial have been reported previously.[16] Briefly, in-clinic rehabilitation consisted of high repetition, task-based, functional, individualised, and progressive upper limb exercises. All participants received the same goal-oriented and intense upper limb rehabilitation following specific guidelines[16]. Therapy tasks were divided into six categories: reach and grasp, gross movement, object flipping, simulated eating tasks, inserting objects, and opening/closing containers. For a given task, the object, movement direction and/or environment factors were adjusted to maintain difficulty level and subject motivation. Since participants had varying degrees of impairment and functional deficit, the exact number of repetitions and tasks per session varied. However, it was expected that six tasks would be performed in the same order at each session and that approximately 30–50 repetitions would be performed on each task giving >300 repetitions per session. The therapist timed the VNS pulse with each repetition of movement (Appendix Figure S1). The VNS group received 0.8 mA (or 0.7 and 0.6 mA in two participants as described above), 100 μs, 30 Hz stimulation pulses, lasting 0.5 seconds, during each movement repetition. The Control group received 0 mA pulses.

Following the six weeks of in-clinic therapy, all participants began daily, therapist-prescribed home exercises. The home therapy session lasted 30 minutes and included tasks following the same principles as the in-clinic therapy. During home exercises, participants activated the VNS device via a single magnet swipe over the device and 30 minutes of either active or sham VNS was then delivered according to their randomised allocation. The stimulation output current was kept the same as during in-clinic therapy. Bi-monthly

phone calls between the therapist and participant were conducted to ensure compliance and adequate exercise intensity.

Study Outcome Measures

Outcome assessments were performed on days one and 90 after the completion of the six weeks of in-clinic therapy. These included the FMA-UE, Wolf-Motor Function Test (WMFT function and time score), Motor Activity Log (MAL), Stroke Impact Scale (SIS) score, Stroke Specific Quality of Life (SS-QOL), EuroQol-5D (EQ-5D), and the Beck Depression Inventory (BDI). The WMFT and FMA-UE were also assessed at day 30 following completion of in-clinic therapy. A description of each of the measures is provided in the supplement. Assessments were performed by the same assessor at baseline and at follow-up.

The primary outcome was change in FMA-UE score from baseline to the first day following completion of in-clinic therapy[19,20]. The secondary outcomes measures were 1) clinically meaningful response on FMA-UE score at day 90, 2) change in day 90 WMFT-Functional score, and 3) change in day 90 FMA-UE score. We defined a clinically meaningful response as a six 6 point or greater improvement in FMA-UE score based on previous research demonstrating that a 5.25-point change was associated with an excellent improvement (greater than 50% improvement) in arm function[21].

Tertiary outcome measures were the MAL, SIS score, SS-QOL score, EQ-5D score and the BDI score. We added WMFT response rate as a post-hoc outcome measure to assess response on a functional outcome measure. A clinically meaningful response was defined as a ≥ 0.4-point change in WMFT-Functional score at day 90.[22]

Safety reporting

Data on all adverse events and serious adverse events were recorded prospectively. Events were coded with the use of the *Medical Dictionary for Regulatory Activities* (MedDRA, version 22). Severity and causality/relationship to study treatment (rehabilitation and VNS) or implant surgery was assigned by the site Principal Investigator.

Sample Size

The a priori sample size calculation was based on data from our pilot studies.[15,16] A sample size of 100 participants (50 per group) was determined to provide 80% power (alpha = 0.05) to detect a FMA-UE difference of 2.3 (SD 4) points between the two treatment groups. We enrolled 108 participants to allow for drop-outs.

Statistical Analysis

Statistical analyses were independently performed by ResearchPoint Global using SAS Version 9.4 or higher.

A pre-defined futility analysis was performed by the Data Safety Monitoring Board (DSMB) based on data from the first 40 participants. The criteria for futility were not met and DSMB determined that the trial could continue.

All efficacy and safety summaries were performed on the intent-to-treat (ITT) population, defined as all participants who have any surgical portion of the implant procedure attempted, regardless of the treatment to which they are assigned, and regardless of the amount of intervention completed. A Per Protocol (PP) population was a priori defined to include participants who completed at least 12 sessions without major protocol violations that could impact and/or compromise the safety or efficacy of the treatment.

For the primary outcome measure, an analysis of covariance (ANCOVA) model was used, with the change from baseline to day one following completion of in-clinic therapy as the dependent variable, and treatment arm, region (UK or USA), treatment by region interaction as factors, and with age and baseline FMA-UE score as covariates. A significance level of 0.05 was used. The region by treatment interaction was to be removed from the final model if it was not significant ($p>0.1$). For the responder analysis at day 90 post completion of therapy, we used a logistic regression model with treatment arm, region, age and baseline FMA-UE score as factors. An ANCOVA model, with the change from baseline as the dependent variable, and treatment / randomisation strata as factors was used for the analysis of the WMFT-functional change and the FMA-UE change at day 90 following completion of in-clinic therapy. The three secondary outcomes measures were tested for significance in a hierarchical manner in the order listed. Significance was declared for the first secondary outcome at 0.05, and each subsequent outcome only if all higher ranked endpoints were significant at 0.05. For the responder analyses, a number needed to treat to achieve an additional clinically meaningful response was calculated. For the post-hoc outcome measure of WMFT response rate at day 90 we used a Fisher Exact test to assess the between-group difference. Summary statistics for tertiary measures were tabulated but formal statistical analysis was not performed. In additional post hoc analyses we compared response rates on the FMA-UE score at 3 additional levels (≥4 points, ≥5 points and ≥7 points). We also compared the proportion who guessed they received VNS and who correctly guessed their treatment allocation.

A ‘last observation carried forward’ approach was used if an assessment was missing after baseline. We assessed the effect of missing data by first performing a Mixed Model Repeated Measures test (SAS PROC MIXED) on the full data set. We then performed multiple imputation with missing at random assumptions (SAS PROC MI).

Trial management and role of the funding source

An independent data safety monitoring board (DSMB) reviewed adverse events, safety information and the planned futility analysis. The funder, MicroTransponder Inc, supported the writing committee in the writing of the manuscript. MicroTransponder played no role in data collection, data interpretation or the decision to submit the manuscript. The decision to submit the manuscript was the responsibility of JD, TJK, and CL. The corresponding author had full access to all the data in the study.

Results

108 participants were randomised between Oct 2, 2017 and Sept 12, 2019. A total of 195 participants consented and were screened for eligibility. 140 people met eligibility criteria

and 32 withdrew prior to device implantation and randomisation. Of the 108 randomised participants, 53 were assigned to the active VNS group and 55 to the Control sham stimulation group. A total of 107 completed the study intervention and were included in the per-protocol population, and 106 attended for primary endpoint assessment (see trial profile, Figure 1). There were no significant protocol deviations that affected the rights, safety, or well-being of participants or the scientific integrity of the study (Appendix text and Appendix Table S1). Baseline demographics are shown in Table 1. Groups were well matched at baseline. Enrollment by site is shown in the supplement (Appendix Table S2).

Participants in the VNS and Control groups received a similar number of stimulations per therapy session (VNS: 422 (SD 99) stimulations, Control: 419 (SD 86) sham stimulations). The mean duration of each in-clinic rehabilitation session was 90 (SD 16) minutes.

103 participants (49 VNS and 54 Control) rated their certainty regarding treatment allocation (Appendix Table S3). Nine VNS (18%) and 9 Control participants (18%) in each group believed they received VNS (p>0.999). Nine VNS (18%) and 13 (24%) Controls participants guessed their treatment allocation correctly (p=0.631).

The primary outcome (change in FMA-UE score from baseline to the first day after in-clinic therapy) was significantly higher in the VNS group than the Control group (VNS: 5.0, SD 4.4, Control: 2.4, SD 3.8; p=0.001; between group difference 2.60, 95% CI 1.03 to 4.2) (Figure 2, Appendix Table S4). There was no significant interaction between treatment allocation and geographic region (p>0.1).

A clinically meaningful response on the FMA-UE score occurred in more participants in the VNS group compared to the control group at day 90 following completion of in-clinic therapy (47% versus 24%, p=0.01; between group difference 24%, 95% CI 6 to 41%), resulting in a number needed to treat of 4.3 for VNS. Response rates defined as a ≥4 point, ≥5 point and ≥7 point increase on the FMA-UE score were consistent higher with VNS and are shown in Appendix table S5.

The WMFT-functional score was significantly increased in the VNS group compared to the Control group at 90 days after the end of in-clinic therapy (VNS: 0.46, SD 0.40, Control: 0.16, SD 0.30; p<0.0001; between group difference 0.30, 95% CI 0.16 to 0.43). The FMA-UE score was also significantly increased in the VNS group compared to the Control group at 90 days (VNS: 5.8, SD 6.0, Control: 2.8, SD 5.2; p=0.008; between group difference 2.96, 95% CI 0.83 to 5.08). A clinically meaningful response on the WMFT-Functional test occurred in significantly more participants in the VNS group than the Control group (57% vs 22%, p=0.01), resulting in a number needed to treat of 2.8 with VNS.

A total of 334 adverse events (163 VNS, 171 Control) were reported in 85 (78%) participants. The majority of these (n=242) were mild. A total of 21 (40%) participants in the active VNS group and 24 (55%) controls reported an adverse event rated as either possibly, probably, or definitely related to device implantation. These were mostly due to post-operative pain. A total of 13 participants in the active VNS group and 9 controls reported an adverse event rated as either possibly, probably or definitely related to device use. The number of events, the number of participants reporting at least one event, and the

number of severe events were similar in both groups (Appendix Table S5 and Table S6). There were no unexpected adverse events or serious adverse device events reported. There was one case of vocal cord palsy in a control participant, which resolved after five weeks.

For tertiary outcomes, there was a numerically greater difference between baseline and follow-up in the VNS group than in the Control group for the MAL, SIS, SS-QoL, EQ-5D and BDI scores (Appendix Table S7).

Results for all outcomes were similar on the per-protocol analysis and sensitivity analyses revealed no significant effect of missing data (Appendix Table S8).

Discussion

In our trial involving participants with moderate to moderately-severe arm impairment after chronic ischaemic stroke, participants who were assigned to VNS paired with rehabilitation demonstrated clinically meaningful improvements in motor impairment and function compared to participants assigned to rehabilitation and sham stimulation. The number of participants achieving a clinically meaningful improvement in upper limb impairment in the active VNS group was approximately double that of the Control group, with nearly half of the participants in the active VNS group achieving a clinically meaningful response. Notably, the responder rate was also significantly higher in the VNS group for the WMFT, a measure of arm function and was consistent across different FMA-UE score thresholds. The greater improvement in the VNS group was consistent across the primary outcome measure and all secondary outcome measures.

All participants were at least nine months post stroke, with a mean time from stroke of over three years. Treatment options for people with arm impairment at this stage typically focus on treatment of complications, rather than concerted efforts to improve function. Our data show it is possible to achieve meaningful improvements many years after stroke. Any improvements are unlikely to be attributable to spontaneous or expected recovery; indeed, many stroke survivors suffer functional decline at this time point.[23] Many recent large clinical trials have not found additional clinically important improvements in arm impairment or function with intensive rehabilitation treatment, despite the use of rehabilitation devices, when compared to usual care.[5,24] We saw a small improvement in the Control group, consistent with other trials. However, the amount of improvement was 2–3 times higher across multiple measures of arm function in participants who received active VNS paired with therapy. These findings are consistent with improvements seen in numerous experimental studies of motor recovery after stroke and in our clinical pilot studies (Appendix Figure S2).[10,15,16,25]

Nearly half of participants receiving VNS had a clinically meaningful improvement assessed by the FMA-UE score.[26] We found a similar rate of clinically meaningful response rate for the WMFT.[22] In addition, tertiary outcome measures, including the MAL,[27], SIS-ADL,[28] and SS-QoL,[29] suggested greater improvement in the VNS group. The consistency of findings across WHO outcome dimensions provides further evidence that the VNS-related

improvements demonstrated are important to stroke survivors. Further, responses were maintained at 90 days after completion of in-clinic therapy.

In preclinical models of ischaemic and hemorrhagic stroke, VNS paired with task-specific rehabilitation significantly enhanced post-stroke recovery compared to rehabilitation alone.[10] When VNS was dissociated from rehabilitation or when rehabilitation was delivered alone, rats showed relatively less motor improvement, suggesting that task-specific rehabilitation paired with VNS is key to driving plastic changes in the motor cortex.[30] Pairing VNS with rehabilitation has been shown to triple the synaptic connectivity in the corticospinal tract networks controlling the impaired forelimb compared to rehabilitation alone.[14] This task-specific neuroplasticity is believed to result from molecular and neuronal mechanisms induced by VNS that include activation of noradrenergic, cholinergic and serotonergic systems.[31] It is possible that VNS-mediated heterosynaptic neuromodulation facilitates long-term synaptic changes in motor neurons during a temporal learning window for spike-timing dependent plasticity.[32,33] This pre-clinical evidence would suggest that VNS as used in this clinical human trial may exploit similar neuroplastic mechanisms[34], although this remains to be verified.

This intervention requires surgical device implantation. VNS devices are used for the treatment of epilepsy and depression, and over 100,000 devices have been implanted worldwide for such clinical indications. The risk of implantation and side effects of stimulation have been well described.[35,36] We found a similar low rate of vocal cord palsy, as has previously been documented, suggesting that the risk of vocal cord palsy is not substantially increased in well-selected people with a history of chronic ischaemic stroke. We saw no serious adverse device events. The stimulation parameters of 0.8 mA, 100 μs, 30 Hz and 0.5 second duration were used in all our preclinical stroke studies and in our two pilot studies of VNS for post-stroke rehabilitation[10]. These settings have been shown to cause desynchronization of the rat cortical EEG[12] suggesting activation of cholinergic and noradrenergic neurons[37,38] and to be associated with cortical plasticity and motor recovery[39,40]. Non-invasive methods of stimulating the vagus nerve are now available[41]. However, it is unclear whether non-invasive VNS activates the nerve to the same degree as with cervical implantable VNS[42]. The optimum site to deliver non-invasive VNS and which, if any, stimulation parameters cause task specific plasticity is unclear.

In this trial, the risk of bias was low and groups were well matched at enrolment. All participants were implanted with a VNS device; and blinding of therapists, participants, and outcome assessors was achieved. There was no evidence of expectation bias or unmasking of participants. The majority of participants were uncertain or incorrect regarding their treatment allocation and there was no difference between groups in the number who guessed they received VNS or who guessed correctly. This suggests that the study was well-blinded. Randomisation was performed by an independent service with allocation concealment. The outcome measures used here are common in stroke rehabilitation trials and are valid, reliable, and sensitive to change. There were low levels of missing data and all but two participants completed the study to day 90. While the long-term data from this study are not yet available, our earlier pilot study suggests that benefits of paired VNS therapy are maintained over time[43].

Our study has some limitations. We cannot generalise our findings to people who do not meet trial eligibility criteria or to people with other types of stroke or other neurological disorders. In particular it is unclear whether VNS paired with rehabilitation improves motor outcomes in people with a more severely affected upper limb, spasticity and severe sensory loss. Although improvements were maintained for at least 90 days, we cannot be certain that the benefits of VNS paired with rehabilitation will be maintained in the longer-term and this should be investigated in future research. The sample size of our study limits our ability to assess the effect of VNS treatment in different sub-groups and two-thirds of participants in our study were male.

Participants with arm impairment, an average of three years after ischaemic stroke, who received rehabilitation showed clinically meaningful improvements in impairment and function that were 2–3 times greater with VNS compared to sham VNS. Improvements with paired VNS therapy were also reflected in quality-of-life measures. VNS combined with rehabilitation is a novel strategy to help people achieve improvement in arm and hand function after stroke.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

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Research in Context Panel

Evidence before this study

Intense task-specific rehabilitation has a limited effect on upper limb impairment in people with long-term problems after ischaemic stroke. Vagus nerve stimulation paired with rehabilitation has been shown to improve forelimb function after experimental stroke and showed promise in two clinical pilot studies. However, no large adequately powered clinical study has been performed.

Added value of this study

VNS-REHAB is the first multicenter trial with adequate statistical power to compare rehabilitation plus active VNS paired with rehabilitation and sham stimulation. Participants treated with VNS had clinically meaningful improvements in measures of upper limb function and impairment on the first day after completion of in-clinic therapy and similar improvements 90-days later after a period of home exercise. The clinical response rate with active VNS was double that of sham stimulation on both the FMA-UE and WMFT, and almost 50% of active VNS treated participants achieved a clinical response. Improvements were also reflected in quality of life measures. The rate of surgical complications due to VNS implantation was similar to that seen with use of VNS in epilepsy.

Implications of the available evidence

The results of this trial support the use of VNS paired with rehabilitation for the treatment of selected people with upper limb impairment at least 9 months after ischaemic stroke. Further research should explore how to implement this approach in clinical practice and whether VNS can be used to improve other impairments after stroke, including more severe degrees of arm impairment.

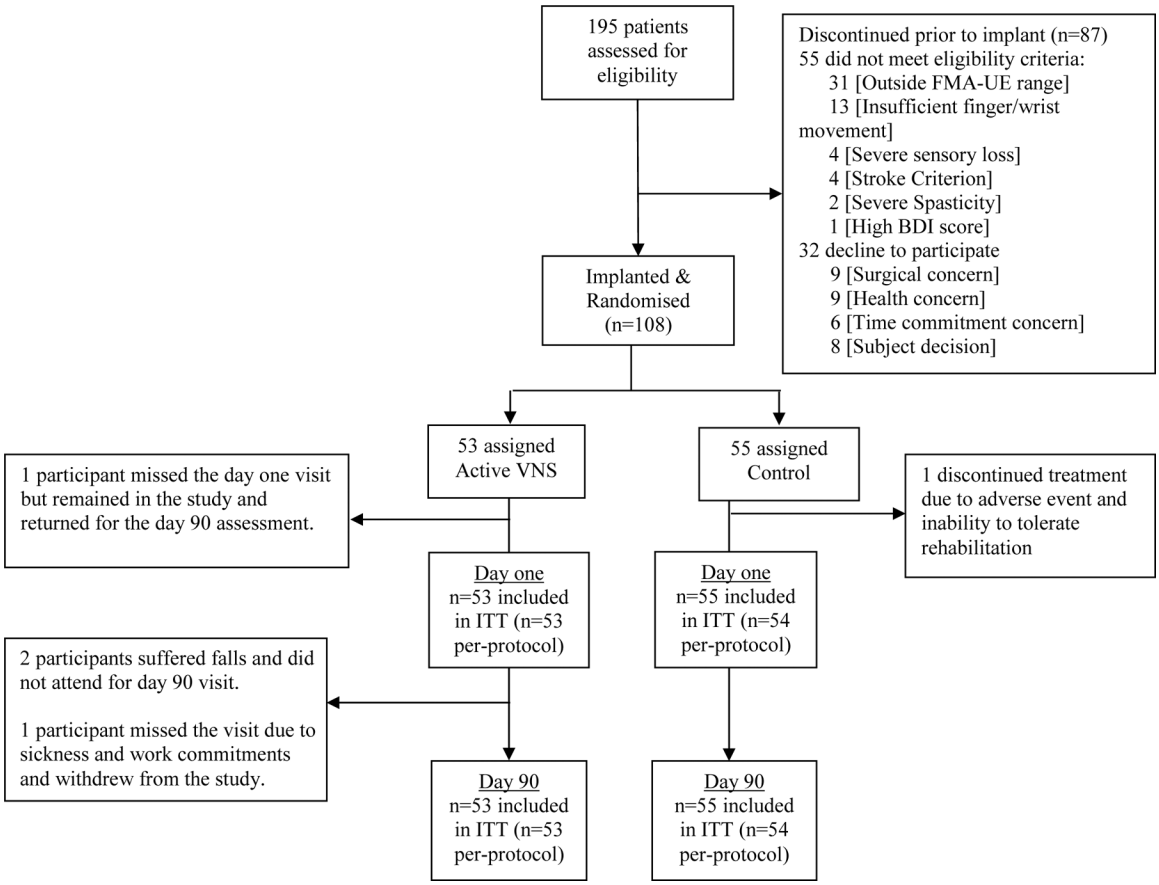

Figure 1:
Trial profile

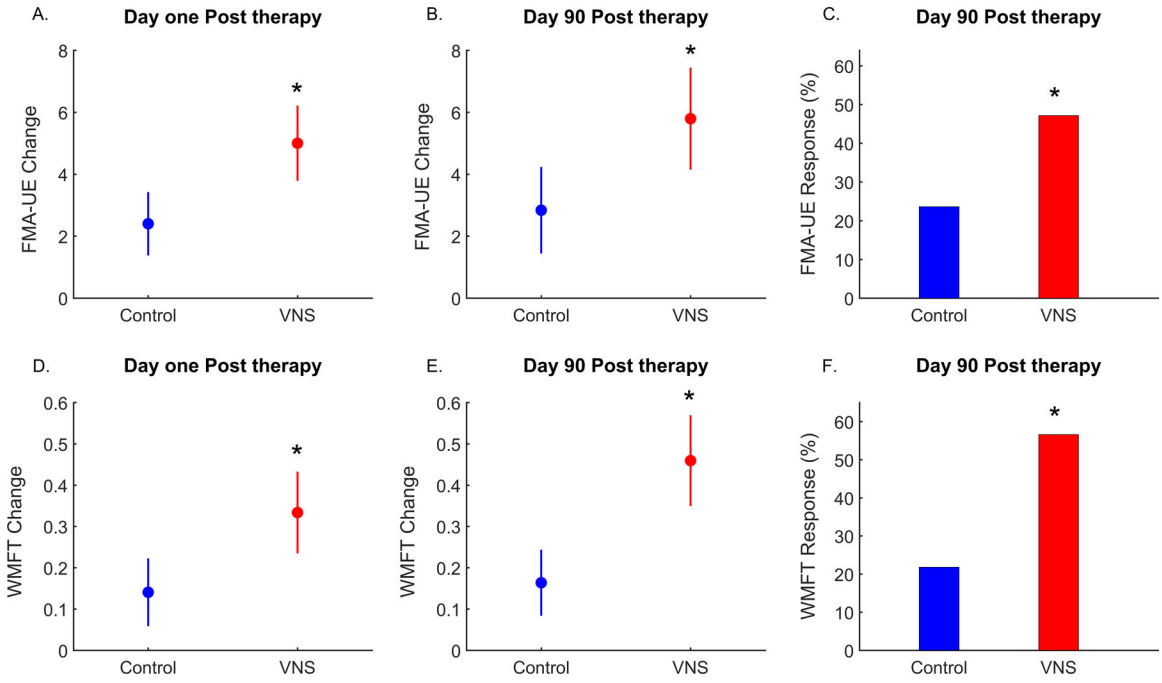

Figure 2. Change in Primary and Secondary Outcome Measures.

A. Change in Fugl-Meyer Assessment-Upper Extremity (FMA-UE) score between baseline and day one post completion of in-clinic therapy. (Primary End-point). B. Change in FMA-UE score between baseline and day 90 post completion of in-clinic therapy. C. FMA-UE response rate (≥6 point change from baseline) at day 90 post completion of in-clinic therapy. D. Change in Wolf Motor Function Test-Functional (WMFT) score between baseline and day one post completion of in-clinic therapy. E. Change in WMFT score between baseline and day 90 post completion of in-clinic therapy. F. WMFT response rate (≥ 0.4 point change from baseline) at day 90 post completion of in-clinic therapy. The circle is the mean group value and the vertical lines denote 95% confidence intervals. * denotes $p<0.05$ for the between group difference. Red: VNS group; Blue: Control group.

Table 1.

Baseline Characteristics

	VNS (n=53)	Control (n=55)
Gender (N, %)		
Male	34 (64%)	36 (65.5%)
Female	19 (37%)	19 (35%)
Ethnicity (N, %)		
Caucasian	42 (79%)	43 (78%)
African-American	9 (17%)	9 (16%)
Asian, Indian, Other	1 (2%)	4 (7%)
Not Reported	1 (2%)	1 (2%)
Age (years, Mean ± SD)	59.1 ± 10.2	61.1 ± 9.2
Time since stroke (years.)	3.1 ± 2.3	3.3 ± 2.6
Handedness (Right/Left/Ambidextrous)	48 (91%) / 4 (8%) / 1 (2%)	50 (91%) / 5 (9) / 0
Side of Paresis (Right/Left)	25 (47%) / 28 (53%)	26 (47%) / 29 (53%)
FMA-UE Baseline Score (Mean ± SD)	34.4 ± 8.2	35.7 ± 7.8
WMFT Functional Score	2.71 ± 0.70	2.83 ± 0.65

Baseline demographic and clinical characteristics by randomisation group in the intention to treat population. FMA-UE is Fugl-Meyer Assessment Upper Extremity. WMFT is Wolf Motor Function Test. Participants could select more than one option for ethnicity.

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SYSTEMATIC REVIEW
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Efficacy and Safety of Vagus Nerve Stimulation on Upper Limb Motor Recovery After Stroke. A Systematic Review and Meta-Analysis

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Background: Upper limb motor impairment is one of the main complications of stroke, affecting quality of life both for the patient and their family. The aim of this systematic review was to summarize the scientific evidence on the safety and efficacy of Vagus Nerve Stimulation (VNS) on upper limb motor recovery after stroke.

Methods: A systematic review and meta-analysis of studies that have evaluated the efficacy or safety of VNS in stroke patients was performed. The primary outcome was upper limb motor recovery. A search of articles published on MEDLINE, CENTRAL, EBSCO and LILACS up to December 2021 was performed, and a meta-analysis was developed to calculate the overall effects.

Results: Eight studies evaluating VNS effects on motor function in stroke patients were included, of which 4 used implanted and 4 transcutaneous VNS. It was demonstrated that VNS, together with physical rehabilitation, increased upper limb motor function on average 7.06 points (95%CI 4.96; 9.16) as assessed by the Fugl-Meyer scale. Likewise, this improvement was significantly greater when compared to a control intervention (mean difference 2.48, 95%CI 0.98; 3.98). No deaths or serious adverse events related to the intervention were reported. The most frequent adverse events were dysphonia, dysphagia, nausea, skin redness, dysgeusia and pain related to device implantation.

Conclusion: VNS, together with physical rehabilitation, improves upper limb motor function in stroke patients. Additionally, VNS is a safe intervention.

Keywords: vagus nerve, vagus nerve stimulation, transcutaneous vagus nerve stimulation, stroke, rehabilitation

INTRODUCTION

Stroke is a neurological condition caused by vascular problems such as cerebral infarction and/or intracerebral or subarachnoid hemorrhage (1). In 2019, more than 12 million strokes occurred worldwide, making it one of the leading causes of morbidity. Stroke is considered the second leading cause of mortality overall and one of the leading causes of disability worldwide, ranking

first in people over 50 years of age (2). In the United States, stroke occurs in more than 7 million people, with a prevalence of 2.5 % (3).

Motor impairment occurs in 85% of patients with stroke, and it is considered one of the main problems resulting from this condition (4). Motor affectation in these subjects is characterized by a decreased capacity and strength of muscles, mainly of the upper extremities, diminishing the quality of life of both the patients and their families (5). Recovery of motor function occurs spontaneously during the 1st months after stroke (6) as a result of brain plasticity processes in the sensory and motor systems (7), however, 50 to 75% of these patients persist with significant motor sequelae limiting daily activities (8).

Efforts have been made to develop therapies that can improve motor impairment in stroke patients (9). Among these therapies are: constraint-induced movement (10), mirror therapy (11), and resistance training (12), however, these interventions have a low level of adherence (13) and the evidence supporting their effects is still weak (10–12). Recently, Vagus Nerve Stimulation (VNS) has been proposed as an intervention that could have beneficial effects in the recovery of motor function in these patients, since it contributes to the generation of adaptive neuroplasticity and the activation of neuromodulators that reduce brain inflammation (14, 15).

VNS consists in the activation of the vagus nerve using electrical current, either through the use of implants or extracorporeal electrodes. As the vagus nerve is composed mainly of afferent fibers, it allows the modulation of different brain structures receiving vagal afferent information, such as the nucleus of the solitary tract, locus coeruleus, raphe nuclei and the hypothalamus (16). In experimental animal stroke models, VNS has been shown to reduce infarct volume and improve neurological outcomes (17, 18). It has been proposed that one the mechanisms mediating these neuroprotective effects of VNS in acute cerebral ischemia is the modulation of the cholinergic anti-inflammatory pathway, and more specifically the α7 nicotinic acetylcholine receptor (α7nAChR) (19), a neurotransmitter gated ion channel expressed widely in the brain and on immune cells (20, 21). Activation of these receptors by the vagus nerve leads to a reduction in the release of pro-inflammatory cytokines (21), with beneficial effects on the reduction of infarct size and cerebral edema on experimental models of stroke (19).

In addition, it has been shown that VNS paired with motor training of the extremities, may upregulate cortical plasticity mechanisms that result in motor function recovery after a stroke (22). Following brain injuries affecting the motor or sensory cortices, nearby cortical regions partially regenerate to provide some of the lost functionality (23, 24). The size of the regenerated motor or sensory representation in surrounding cortical areas correlates with functional recovery, however the result gain in functionality is only a fraction of the observed pre-injury levels (24, 25). Previous studies have demonstrated that repeatedly pairing VNS with specific movements results in increased representation of these movements in the primary motor cortex (22). Further animal experiments have provided evidence that the administration of VNS paired with repeated movements of affected limbs after motor cortex damage is associated with a significant recovery of forelimb function that is superior to that observed with physical training alone (26, 27). These potentiating effects of VNS on cortical reorganization mechanisms may be related with the activation of nuclei such as locus coeruleus, raphe nuclei and nucleus basalis. These nuclei generate an increase in neuromodulators important in neuroplasticity, such as noradrenaline, serotonin, brain-derived neurotrophic factor and acetylcholine (28, 29). When these neurotransmitters are simultaneously released during neural activity related with motor rehabilitation, synaptic plasticity is promoted in motor-specific circuits (30). Thus, VNS paired with motor rehabilitation can cause an increased specific reorganization of the motor cortex, resulting in an enhanced motor recovery after cerebral ischemia (27).

VNS has mainly been administered by using implanted electrodes, but more recently, a non-invasive technique, known as transcutaneous VNS (cervical or auricular) has been proposed (31). VNS has traditionally required the implantation of an electrical pulse generator at the left subclavicular level, which is connected to electrodes in the left cervical branch of the vagus nerve (32). Its insertion is performed by a surgical procedure, which presents a higher risk of adverse events (33), the most frequent being dysphonia during stimulation, due to its proximity to the laryngeal nerve (34). On the other hand, transcutaneous VNS works through the placement of non-invasive electrodes on the neck or auricle for stimulation of the cervical or auricular branch of the vagus nerve, respectively (32). Transcutaneous VNS has a lower risk of adverse events, is reversible and easy to implement (32). In addition, experimental evidence suggests that the effects of transcutaneous VNS on brain function are comparable to those obtained with VNS (33). Diverse studies using electrical stimulation of the auricular branch of the vagus nerve in experimental models have shown a significant effect of this technique on the reduction of brain infarct volume (35–37). The magnitude of reduction in infarct size has been similar to the one reported for implanted VNS (18). In addition to these effects, transcutaneous VNS has shown to regulate other mechanisms that can promote recovery of neurological function after ischemic stroke (38, 39). These include upregulation of angiogenesis, which can improve perfusion of the tissue surrounding the injury promoting recovery (40), regulation of blood brain barrier permeability, which could improve cerebral edema after stroke (41), and inhibition of neuroinflammation resulting in neuroprotective effects against ischemic cerebral injuries (37). No animal studies have evaluated the effects of transcutaneous VNS paired with rehabilitation on the recovery of motor function after brain ischemic injury. However, multiple studies have shown beneficial effects of this technique on upregulation of mechanisms involved in neuroplasticity, such as upregulation of brain-derived neurotrophic factor (42). Transcutaneous VNS has also shown to improve axon regeneration and re-organization in experimental models of cerebral ischemia (37), suggesting that this technique may have similar effects to VNS on the mechanisms underlying its beneficial effects on motor recovery after a stroke.

There have been multiple clinical studies that have evaluated the safety and efficacy of implanted and transcutaneous VNS

in the recovery of motor function after stroke (34, 43–49), and recently, meta-analyses have suggested that VNS has a positive effect on upper limb function in stroke patients (50–52), however these reviews did not evaluate the effect vagus nerve stimulation according to time since stroke. The aim of this systematic review is to summarize and analyze the scientific evidence of the safety and efficacy of both implanted and transcutaneous VNS for the management of upper limb motor impairment after stroke.

METHODS

Search Strategy

The search was performed using the following databases: MEDLINE, CENTRAL, EBSCO and LILACS, without date restriction and was focused on studies conducted in humans. A combination of MeSH terms was used for the search, which were: ((Vagus Nerve Stimulation) OR (Vagus Nerve)) AND (Stroke). The search was conducted in December 2021 and was restricted to articles published in English or Spanish.

Selection Criteria

Studies of patients with acute or chronic stage stroke, where VNS was the intervention, compared to usual care or placebo stimulation, were included. The main outcome was the efficacy of VNS on upper limb motor recovery. Information on mild, moderate and severe adverse events of VNS was also collected to assess safety aspects. Clinical trials were included. Editorials, protocols, letters to the editor, commentaries, and case reports were excluded. Studies that only evaluated neuroplasticity mechanisms, neuromodulator production, cytokine inhibition, or brain infarct volume were also excluded. In order to include all relevant research, we reviewed the references of the included studies and also published abstracts from scientific conferences. In addition, we searched the clinicaltrial.gov website to identify clinical trials of VNS in stroke patients. The first author (JAR) independently reviewed the titles and abstracts for an initial assessment of eligibility criteria. Once the titles and abstracts were reviewed, JAR and DL reviewed the full-text articles to evaluate the inclusion of studies in the analysis. Discrepancies and doubts on the inclusion of articles were resolved by a third investigator (RG).

Data Extraction

Information from the articles was extracted by two reviewers (JAR, DL), using an established format containing the following variables: lead author, year of publication, outcome assessed, type of study, population, intervention assessed, comparison group,

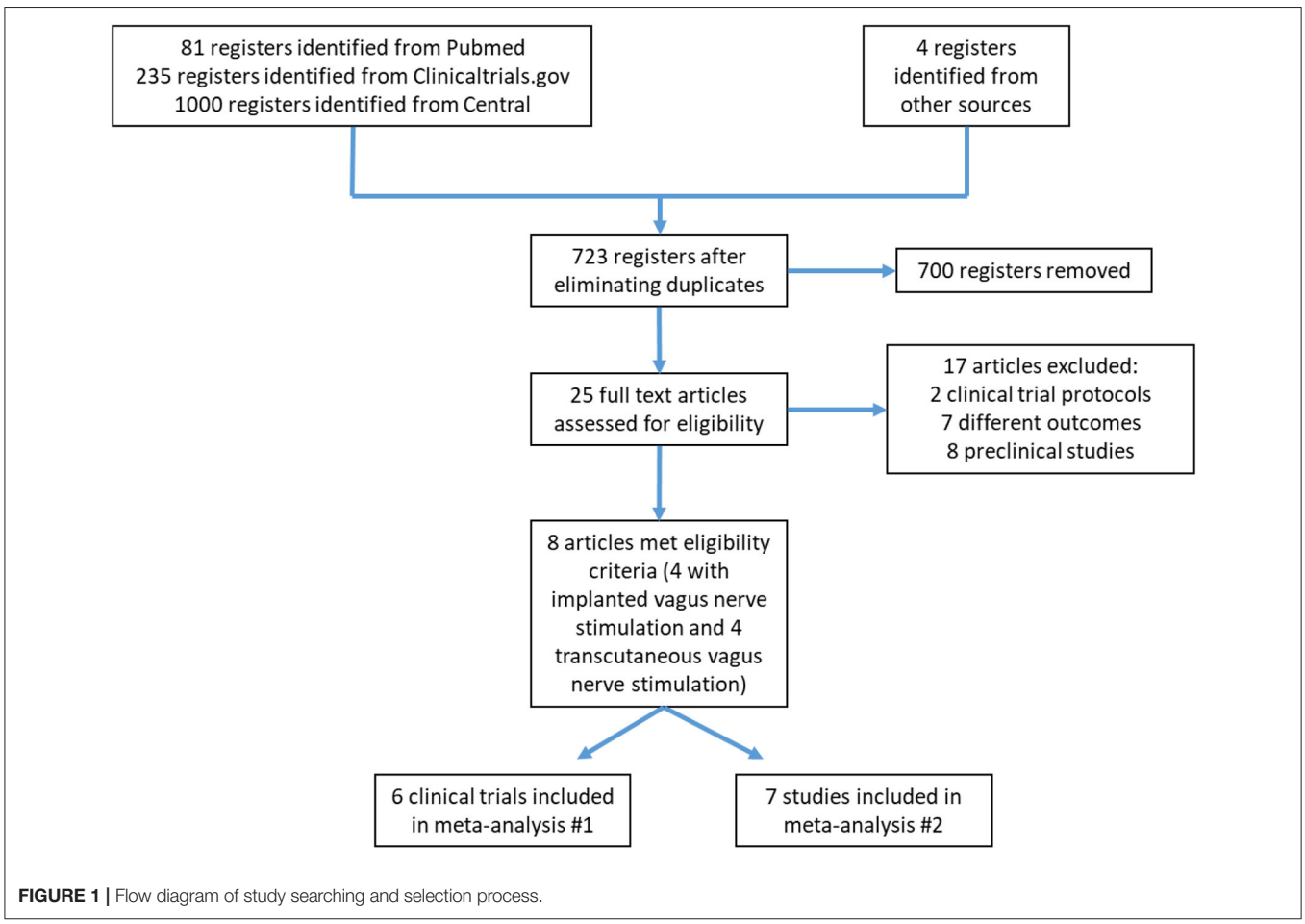

FIGURE 1 | Flow diagram of study searching and selection process.

TABLE 1 | Characteristics of the studies included in the systematic review.

Author (year)	Outcome	Population	Intervention group	Control group	Results
Dawson et al. (47) (NCT01669161)	Upper limb motor function	Twenty patients with a history of unilateral supratentorial ischemic stroke that occurred at least 6 months before inclusion.	Nine patients with implanted VNS on the left vagus nerve (0.5 s of charged balanced pulses with 0.8 mA amplitude, 100 μs pulse width, 30-Hz frequency, delivered during each movement repetition) + rehabilitation therapy (6-week course of 2-h therapy sessions, 3x week, and at least 300 to 400 movements per session).	Eleven patients with rehabilitation therapy only (6-week course of 2-h therapy sessions, 3x week, at least 300 to 400 movements per session). This group did not have an implanted device.	The mean change in the Fugl-Meyer Assessment-Upper Extremity (FMA-UE) score in the VNS group was 8.7 (SD 5.8) vs. 3.0 (SD 6.1) in the control group (between group difference = 5.7, 95% CI−0.4; 11.8, $p = 0.064$)
Kimberley et al. (49) (NCT02243020)	Upper limb motor function	Seventeen patients with a history of unilateral supratentorial ischemic stroke that occurred between 4 months to 5 years before randomization	Eight patients with implanted VNS on the left vagus nerve (0.5 s of charged balanced pulses with 0.8 mA amplitude, 100 μs pulse width, 30-Hz frequency, delivered during each movement repetition) + rehabilitation therapy (6-week course of 2-h therapy sessions, 3x week, and 300 to 500 movement repetitions per session). After 6 weeks of in-clinic therapy, participants began daily therapist-prescribed home exercises. For the first 30 days of at-home therapy, participants received 0 maVNS and active VNS thereafter.	Nine patients with sham stimulation (0 mA) + rehabilitation therapy (6-week course of 2-h therapy sessions, 3x week, and 300 to 500 movements per session). After 6 weeks of in-clinic therapy, participants began daily therapist-prescribed home exercises.	**Day 1 after therapy:** The mean change in FMA-UE score in the VNS group was 7.6 vs. 5.3 in the sham group (between group difference = 2.3, 95% CI−1.8; 6.4, $p = 0.20$). **Day 90 after therapy:** The mean change in FMA-UE score in the VNS group was 9.5 vs 3.8 in the sham group (between group difference = 5.7, 95% CI−1.4; 11.5, $p = 0.055$). The FMA-UE response rate at day 90 (≥6-point change from baseline) in the VNS group was significantly higher (88.0%) compared with the control group (33.0%) ($p = 0.03$)
Dawson et al. (48) (NCT02243020)	Upper limb motor function	Seventeen patients with a history of unilateral supratentorial ischemic stroke that occurred between 4 months to 5 years before randomization	Eight patients with implanted VNS initially underwent 6 weeks of in clinic rehabilitation therapy + active VNS followed by home exercises paired with self-administered active VNS.	Nine patients with implanted VNS initially underwent 6 weeks of in clinic rehabilitation therapy + sham VNS followed by home exercises with control VNS through day 90. Subjects in this group then crossed over and received 6-weeks of in-clinic rehabilitation paired with active VNS and continue a home exercise program paired with self-administered active VNS	**1-year follow-up of VNS paired with rehabilitation for all participants:** The FMA-UE score increased by 9.2 points (95% CI = 4.7; 13.7; $P = 0.001$). 73% demonstrated a clinically meaningful improvement (≥6 points) in FMA-UE
Dawson (2021) (34) (NCT03131960)	Upper limb motor function	Hundred and eight patients with history of unilateral supratentorial ischemic stroke that occurred between 9 months and 10 years before enrolment.	Fifty-three with implanted VNS on the left vagus nerve (0.5 s of charged balanced pulses with 0.8 mA amplitude, 100 μs pulse width, 30-Hz frequency, delivered during each movement repetition) + rehabilitation therapy (6-week course of 2-h therapy sessions, 3x week, and > 300 movement repetitions per session). After 6 weeks of in-clinic therapy, participants began daily therapist-prescribed home exercises. For the first 30 days of at-home therapy, participants received 0 maVNS and active VNS thereafter.	Fifty-five patients with sham stimulation (0 mA) + rehabilitation therapy (6-week course of 2-h therapy sessions, 3x week, and >300 movement repetitions per session). After 6 weeks of in-clinic therapy, participants began daily therapist-prescribed home exercises.	**Day 1 after therapy:** The FMA-UE score was significantly increased in the VNS group compared with the control group (5.0 [SD 4.4] vs. 2.4 [SD 3.8]); between group difference = 2.6, 95%CI 1.0; 4.2, ($p = 0.0014$). **Day 90 after therapy:** The FMA-UE score was significantly increased in the VNS group compared with the control group (5.8 [SD 6.0] vs. 2.8 [SD 5.2]); between group difference = 3.0, 95%CI 0.8; 5.1, ($p = 0.0077$). The FMA-UE response rate (≥6-point change from baseline) in the VNS group was significantly higher (47.0%) compared with the control group (24.0%) (between group difference 24.0%, 95%CI 6; 41, $p = 0.0098$).

(Continued)

TABLE 1 | Continued

Author (year)	Outcome	Population	Intervention group	Control group	Results
Capone et al. (46)	Upper limb motor function	Fourteen patients with either ischemic or hemorrhagic stroke that occurred at least 1 year before inclusion.	Seven patients with transcutaneous auricular VNS (location = left external acoustic meatus, frequency = 20 Hz, pulse width = 0.3 ms, duration = 20 s, intensity = level between the detection and pain thresholds) repeated every 5 min for 60 min + robot-assisted therapy (three sessions of 320 assisted movements per day) Immediately after the stimulation. The intervention was delivered daily for 10 consecutive working days	Seven patients with sham stimulation (location = left ear lobe, frequency = 20 Hz, pulse duration = 0.3 ms, duration = 20 s, intensity = level between the detection and pain thresholds) repeated every 5 min for 60 min + robot-assisted therapy (three sessions of 320 assisted movements per day) Immediately after the stimulation. The intervention was delivered daily for 10 consecutive working days.	The FMA-UE score was significantly increased in the VNS group compared with the control group (5.4 vs 2.8; Mann–Whitney U = 5 00, $p = 0.048$)
Redgrave et al. (45) (NCT03170791)	Upper limb motor function	13 patients with an anterior circulation ischemic stroke at least 3 months before enrolment	13 patients with transcutaneous auricular VNS (location = left cymba concha, frequency = 25 Hz, pulse width = 0.1 ms, intensity = maximum tolerable level) delivered during each movement repetition + rehabilitation therapy (6-week course of 1-h therapy sessions, 3x week consisting of upper limb repetitive task practice: 30–50 repetitions of 7–10 arm movements)	No control group	The mean (SD) improvement in FMA-UE was 17.1 (SD 7.8). Ten patients (83%) achieved a clinically relevant increase of >10 points with an overall effect size of 0.68
Wu (57) (registration no. ChiCTR1800019635)	Upper limb motor function	Twenty two patients with a history of ischemic stroke that occurred between 0.5 and 3 months before enrollment	Ten patients with transcutaneous auricular VNS (location = left cymba concha, frequency = 20 Hz, pulse width = 0.3 ms, intensity = maximum tolerable level, lasting 30 seconds each time, stimulating once every 5 min) performed for 30 min + rehabilitation therapy (30 min, performed after the end the stimulation) per day for 15 consecutive days	Eleven patients with sham stimulation (electrodes were fixed to the cymba conchae of the left ear without electrical stimulation) performed for 30 min + rehabilitation therapy (30 min, performed after the end the stimulation) per day for 15 consecutive days	**Day 1 after therapy:** The FMA-UE score was significantly increased in the VNS group compared with the control group (6.9 [SD 1.85] vs 3.18 [SD 1.17]); between group difference = 3.72, 95%CI 2.32; 5.12, $p < 0.001$). **Week 4 after therapy:** The FMA-UE score was significantly increased in the VNS group compared with the control group (7.70 [SD 1.49] vs. 3.36 [SD 1.75]); between group, $p < 0.001$)
Chang et al. (44)(NCT03592745)	Upper limb motor function	Thirty-four patients with unilateral supratentorial stroke and chronic (>6 months) upper limb hemiparesis	Seventeen patients with transcutaneous auricular VNS (location = left cymba concha, frequency = 30 Hz, pulse width = 0.3 ms, intensity = maximum tolerable level) ~ 250 stimulated movements per session + shoulder/elbow robotic therapy (total of 1,024 flexion, extension, and rotational movements of the elbow and shoulder joints) 3 days per week for 3 weeks (9 sessions)	Seventeen patients with sham stimulation (location = left cymba concha, intensity = 0 ma) + shoulder/elbow robotic therapy (total of 1,024 flexion, extension, and rotational movements of the elbow and shoulder joints) 3 days per week for 3 weeks (9 sessions)	**At discharge:** The FMA-UE score was increased in the VNS group compared with the control group (3.10 [SEM 0.57] vs. 2.86 [SEM 0.50]). **Follow up (3 months after intervention):** The FMA-UE score was increased in the VNS group compared with the control group (2.79 [SEM 0.84] vs. 3.22 [SEM 1.0])

SEM, Standard error of the mean.

results in terms of primary and secondary outcomes, adverse event reporting, and stimulation parameters.

Assessment of Methodological Quality

We assessed the risk of bias of the included clinical trials using the Cochrane Collaboration's domain-based scale, which evaluates allocation concealment, randomization, blinding of participants and investigators, blinding in outcome assessment, selective outcome reporting, and incomplete outcome data.

Synthesis of Information

Qualitative analysis of each of the articles was performed, taking into account the characteristics of the studies, population, intervention, control group, outcomes and adverse event reporting.

Quantitative Analysis

An initial meta-analysis of 6 clinical trials was performed (three implanted and three transcutaneous VNS studies), where the mean difference in upper limb motor recovery between the active and control interventions was assessed. A second meta-analysis evaluated the average increase in motor recovery from baseline and included six clinical trials and one intervention study that had no comparison group, for a total of seven studies (three evaluating implanted VNS and four evaluating transcutaneous VNS). In each meta-analysis, the mean with its 95 % confidence interval was calculated. Care was taken not to duplicate data from clinical trials with more than one publication. A subgroup analysis was performed to determine the difference in the effects according to the VNS technique (implanted vs. transcutaneous) and the mean time since the stroke (more than 3 years versus <3 years). A random-effects model was used for the meta-analysis and heterogeneity was assessed using the I^2 statistic, where I^2 >60% was considered as significant heterogeneity. All analyses were performed in the RStudio program using the meta library.

RESULTS

The database search yielded 1,316 records; after eliminating duplicates, 723 were selected for title and abstract review.

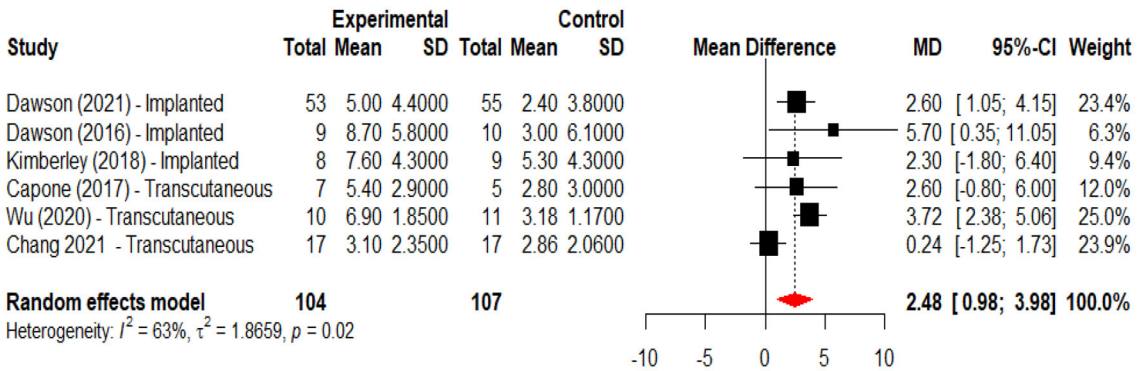

FIGURE 2 | Forest plot for the meta-analysis of vagus nerve stimulation effects on upper limb motor function (FMA-UE score increase) when compared to a control intervention. Dawson et al. (34), Dawson et al. (47), and Kimberley et al. (47) used implanted stimulation, Capone et al. (46), Wu et al. (43), and Chang et al. (44) used transcutaneous stimulation.

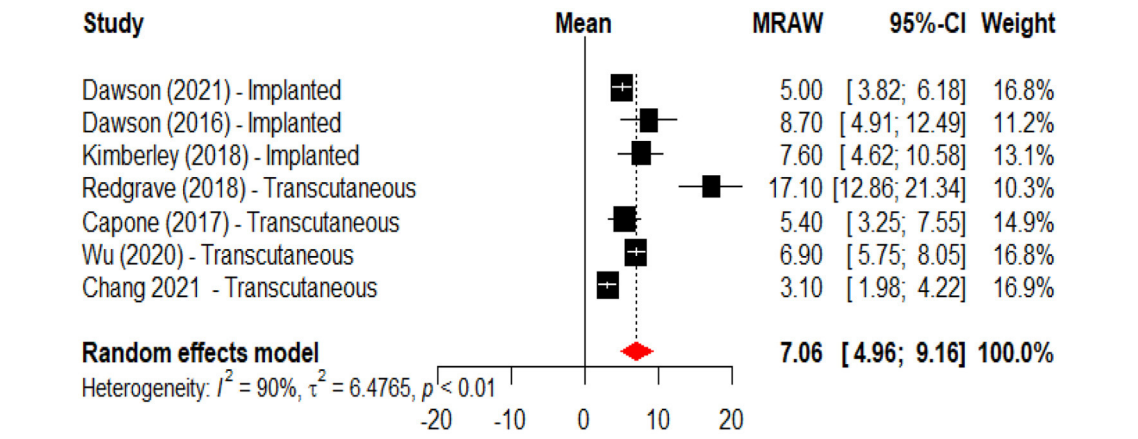

FIGURE 3 | Forest plot for the meta-analysis of vagus nerve stimulation effects on upper limb motor function (FMA-UE score increase) when compared to baseline. Dawson et al. (34), Dawson et al. (47), and Kimberley et al. (47) used implanted VNS, Capone et al. (46), Redgrave et al. (45), Wu et al. (43), and Chang et al. (44) used transcutaneous stimulation.

Of these, 700 were excluded mainly because of study design and the lack of stroke as a studied event. In total, 25 research articles were reviewed in full. From these, eight articles met the eligibility criteria for the systematic review (**Figure 1**). The main reasons for exclusion were the evaluation of outcomes other than those stated in the selection criteria of this review (e.g., evaluation of physiological mechanisms of neuroplasticity or impact on cerebral infarct size).

Study Characteristics

Eight studies evaluating VNS were included, all of them published in English between 2016 and 2021, of which four used implanted and four transcutaneous VNS [cervical (n = 0), auricular stimulation (n = 4)]. The implanted VNS studies analyzed the efficacy of stimulation plus physical rehabilitation compared with patients who received physical rehabilitation therapies plus placebo stimulation or physical therapy alone (34, 47–49). The implanted VNS protocol in all evaluated studies had a duration of 18 sessions distributed over 6 weeks, where stimulation was administered in conjunction with rehabilitation training and used the following parameters: an amplitude of 0.8 mA, a pulse duration of 0.1 ms, a frequency of 30 Hz, and a duration of 0.5 seconds; with stimuli administered during each movement repetition. In general, all patients who received the intervention had a significant improvement in motor function, as assessed by the Fugl-Meyer scale (**Table 1**). This improvement in motor function persisted significantly up to 90 days after the end of the intervention in two studies (34, 49) (**Table 1**).

Regarding transcutaneous VNS, all studies used auricular stimulation. The study by Redgrave et al. (45) included 13 patients that had an anterior circulation ischemic stroke at least 3 months previously and had residual upper limb dysfunction. These subjects underwent 18 x 1-hour sessions over 6 weeks in which they received stimulation on the cymba conchae of the left ear concurrently with upper limb repetitive task practice (30–50 repetitions of 7–10 arm movements). Subjects received transcutaneous VNS with a frequency of 25 Hz, a pulse width of 0.1 ms, at maximum tolerated intensity (median intensity = 1.4 mA) during each movement repetition. This study found that transcutaneous VNS improved mean motor mobility at visit 18 (upper limb Fugl-Meyer mean increase = 17.1, SD 7.8), and that 10 patients (83 %) achieved a clinically relevant increase of >10 points on the Fugl-Meyer scale (45).

The study by Capone et al. (46) was a controlled clinical trial with a sample of 14 patients. Patients were randomized to robot-assisted physical therapy sessions associated with active transcutaneous auricular VNS or sham stimulation during 10 consecutive working days. Stimulation consisted of pulse trains lasting 20 s, with a pulse width of 0.3 ms and a frequency of 20 Hz, repeated every 5 min for 60 min. Patients in the transcutaneous auricular VNS group received the stimulation with electrodes placed in the left external acoustic meatus at the inner side of the tragus, whereas for those in the control group, electrodes were attached to the left ear lobe. The intensity of the stimulation was adjusted to a level between the detection and pain thresholds. Robotic-assisted therapy was delivered immediately after the end of real or sham transcutaneous VNS. In this study, the active intervention was found to improve upper extremity motor mobility (Fugl-Meyer scores) after 2 weeks of treatment as compared to the sham group (Mann-Whitney U = 5.00, p = 0.048) (34) (**Table 1**). Additionally, no adverse events were reported, and patients reported comfort and convenience during the intervention.

Wu et al. (43) evaluated the efficacy of transcutaneous auricular VNS in 10 patients with a history of ischemic stroke that occurred between 0.5 and 3 months compared with 11 patients that received sham stimulation. The active transcutaneous auricular VNS was delivered with electrodes fitted to the left

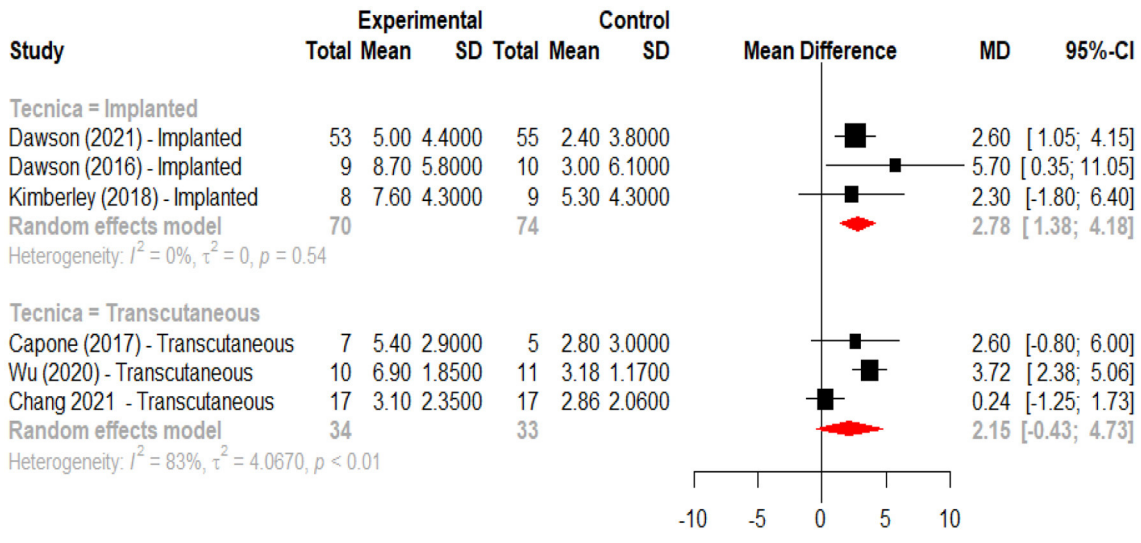

FIGURE 4 | Forest plot for the meta-analysis of vagus nerve stimulation effects on upper limb motor function (FMA-UE score increase) when compared to a control intervention according to intervention modality (implanted vs. transcutaneous).

cymba concha and consisted of pulse trains lasting 30 s, with a pulse width of 0.3 ms and a frequency of 20 Hz, repeated every 5 minutes for 30 minutes. The control group received sham stimulation (location= left cymba concha, intensity = 0 mA). Both groups received rehabilitation therapy performed after the end of the stimulation. In the study, the intervention group was found to significantly increase on FMA-UE score compared with the control group (6.9 [SD 1.85] vs. 3.18 [SD 1.17]); between group difference= 3.72, 95%CI 2.32; 5.12, $p < 0.001$) (**Table 1**).

Chang et al. (44) is the most recent study that evaluated the efficacy of the transcutaneous auricular VNS on the upper limb motor function. In this clinical trial, the authors included 34 patients with unilateral supratentorial chronic (>6 months) stroke. The intervention consisted of transcutaneous auricular VNS (location = left cymba concha, frequency = 30 Hz, pulse

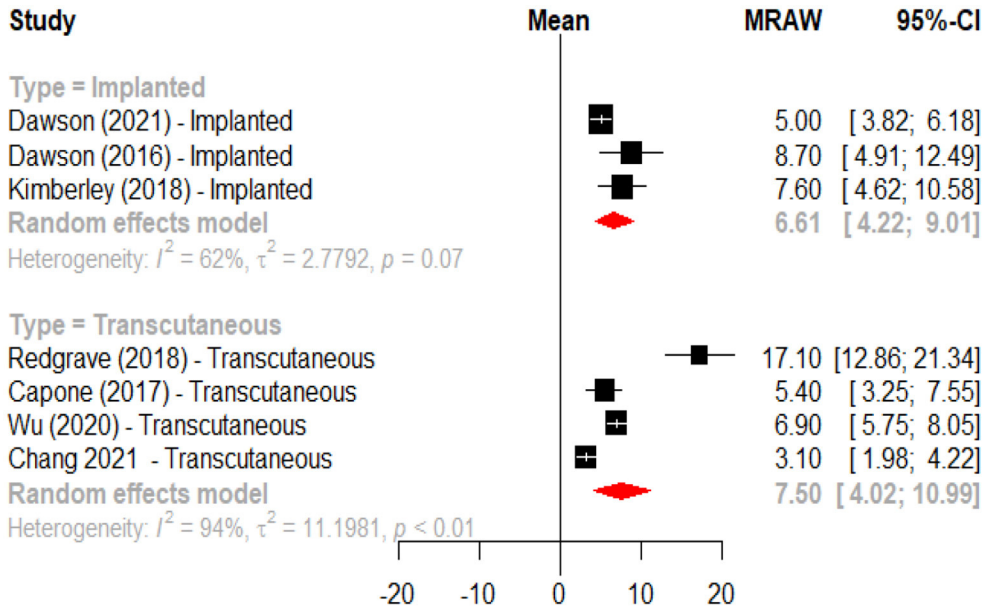

FIGURE 5 | Forest plot for the meta-analysis of vagus nerve stimulation effects on upper limb motor function (FMA-UE score increase) when compared to baseline according to intervention modality (implanted vs. transcutaneous).

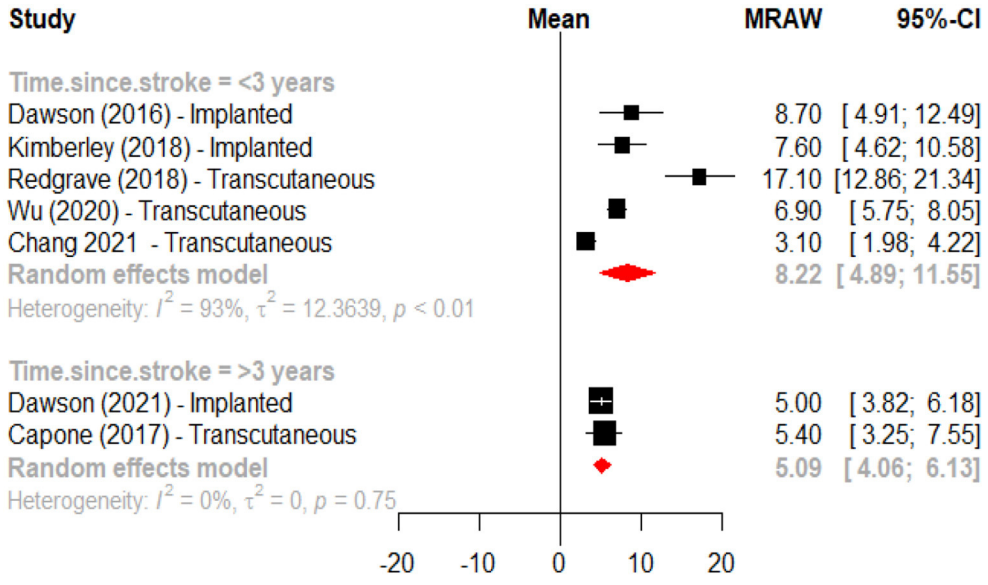

FIGURE 6 | Forest plot for the meta-analysis of vagus nerve stimulation effects on upper limb motor function (FMA-UE score increase) when compared to baseline according to time since stroke (<3 years vs. ≥3 years).

width = 0.3 ms, intensity = maximum tolerable level) with robotic therapy 3 days per week, for 3 weeks (nine sessions). The control group received sham stimulation (location = left cymba concha, intensity = 0 ma) with robotic therapy 3 days per week, for 3 weeks (nine sessions). The study found that at discharge, the FMA-UE score was increased in the intervention group compared with the control group (3.10 [SEM 0.57] vs. 2.86 [SEM 0.50]) (**Table 1**).

An initial meta-analysis was performed to evaluate the effects of active VNS vs. a control intervention on motor recovery after stroke. From the eight studies one was excluded because it did not have a control group (45), and one more (48) because reported data from clinical trials with a publication already included in the analysis. This meta-analysis revealed that motor recovery, as measured by change in the Fugl-Meyer assessment score, was significantly greater in the active group when compared to those subjects receiving the control intervention (mean difference 2.48, 95%CI 0.98; 3.98) (**Figure 2**). In a second meta-analysis, including the study that did not have a control group (45), it was observed that the intervention increased upper limb motor function (Fugl-Meyer scores) by an average of 7.06 (95%CI 4.96; 9.16) points when compared to baseline (**Figure 3**). An analysis by subgroups in meta-analysis #1 did not show clear differences (**Figure 4**). The analysis by subgroups in meta-analysis #2 showed that studies where transcutaneous VNS (**Figure 5**) was used or included participants with a lower average time since stroke (<3 years) (**Figure 6**) were associated with greater effects in motor recovery.

Safety

No intervention-related deaths or serious adverse effects (AEs) were reported. The most frequent moderate AE associated with implanted VNS was left vocal cord paralysis associated with or without dysphonia (11.11 % in Dawson et al. and 5.88 % in Kimberley et al.) (47, 49); The most frequent moderate AE associated with the device implantation procedure, present in one patient, was surgical site infection requiring intravenous antibiotic treatment (49). The most frequent mild AEs related to stimulation therapy were, in order: dysphonia, dysphagia, nausea and dysgeusia. None of the mild AEs required changes in therapy protocol and all of them self-resolved during the follow-up period (34, 47, 49). Four studies reported AEs in transcutaneous VNS, observing fatigue, dizziness, ear pain, skin redness and tiredness (43, 45, 53). In the study by Capone et al. (transcutaneous VNS) there were no adverse events and patients did not report discomfort from the procedure (46). One study that monitored heart rate and blood pressure levels showed no clinically significant change throughout the treatment sessions (43).

Risk of Bias Assessment

The risk of bias assessment was applied to the six clinical trials (Dawson et al., Dawson et al., Kimberley et al., Capone, et al., Wu et al., Chang et al.) (34, 43, 44, 46, 47, 49) upon which the initial meta-analysis for the assessment of VNS efficacy for motor rehabilitation was based. All studies had low risk of bias in the selective reporting domain. The performance bias domain had the highest risk of bias with two studies (Dawson et al., Wu et al.) (43, 47) (**Figure 7**). In the clinical trial reported by Capone et al. (46), the risk of bias in the domains of randomization, and allocation concealment was unclear, and the risk of attrition bias was high (**Figure 7**).

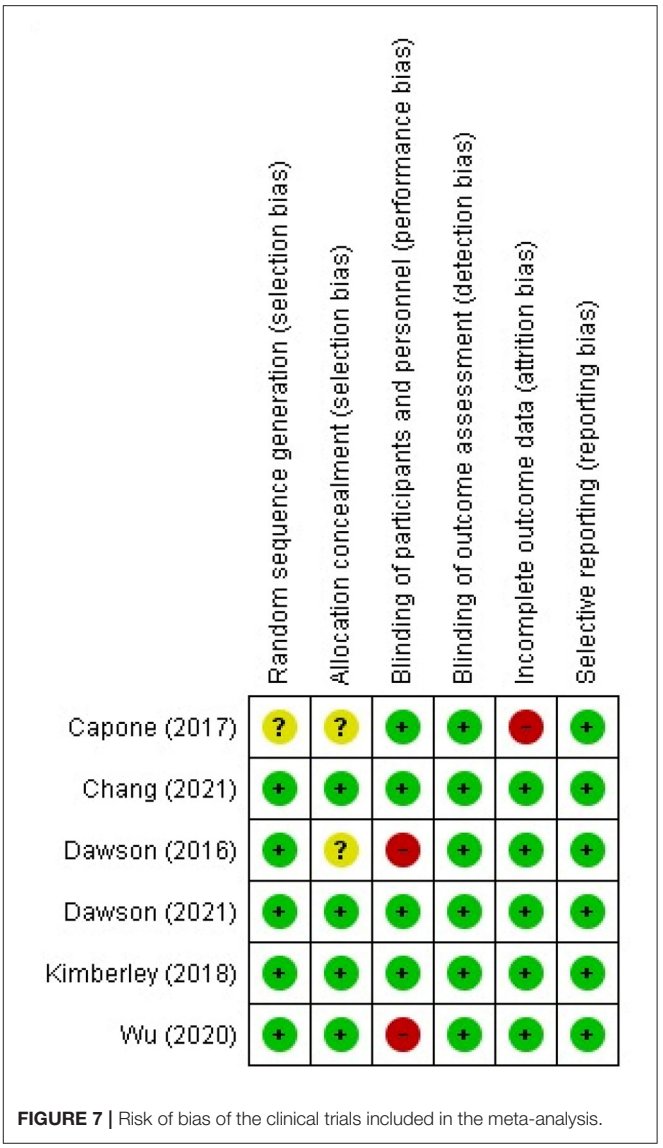

FIGURE 7 | Risk of bias of the clinical trials included in the meta-analysis.

DISCUSSION

This systematic review and meta-analysis demonstrate that VNS is an effective therapy for upper limb motor recovery in stroke patients. Factors such as the VNS technique used and the time of intervention since the event seem to have an influence on the results obtained, with greater benefits if the stimulation is performed non-invasively and prior to 3 years after the event. However, the studies performed so far with transcutaneous stimulation have included a limited number of patients, therefore more evidence is needed before a definitive conclusion can be

reached in this regard. The performed studies have shown a low rate of adverse events, so it can be concluded that VNS is a safe procedure for the management of this pathology. The incidence and severity of adverse events depend on whether the stimulation is performed with an implanted device or with a non-invasive technique, since the former has a higher risk of moderate adverse events such as vocal cord paralysis and surgical site infection associated with the implantation procedure, whereas for the transcutaneous technique the adverse events reported were all mild (e.g., fatigue, dizziness, ear pain and tiredness).

Previous clinical studies have demonstrated the efficacy of VNS for the treatment of migraine, anxiety symptoms, depression and epilepsy (54). In this systematic review, VNS together with physical rehabilitation was found to significantly improve upper limb motor function when compared with rehabilitation alone; a similar result to that reported in recently published meta-analyses (50–52). VNS together with physical therapy increases upper limb motor recovery of stroke patients by an average of 7 points in the Fugl-Meyer scale, which could be considered as a clinically significant response (55). In some of the reviewed studies with implanted VNS, a clinically significant response was found in 47 to 88% of patients up to 90 days after the end of in-clinic therapy, supporting potential sustained effects of the intervention on motor recovery after stroke (34, 49).

Results from this meta-analysis suggest that implementation of this intervention at earlier stages of the post-stroke recovery process could have a significantly greater effect in motor rehabilitation. Studies that in included participants where the intervention was, in average, initiated <3 years after the stroke had an estimated increase of eight points in the Fugl-Meyer scale after VNS and motor rehabilitation compared to an estimated increase of five points in the studies that included participants with an average of more than 3 years since the event. Only one study included patients in the sub-acute phase of stroke rehabilitation Wu et al. (43). This study found an average increase of 6.9 points in the Fugl-Meyer scale after 15 days of therapy, which increased to 7.7 points 4 weeks after therapy and was significantly greater than the change observed in the sham group. Given that most of the cortical reorganization processes are expected to occur during the sub-acute phase post stroke (56), this may be the optimal window of recovery to be modulated by the implementation of VNS in combination with physical therapy, however, future clinical studies with larger sample sizes will be necessary to confirm whether earlier administration of this intervention is associated with greater improvement in motor function.

This systematic review identifies several knowledge gaps that should be evaluated in further studies. First, although initial results from studies evaluating transcutaneous VNS are promising, more clinical trials evaluating this technique with larger sample sizes and appropriate control interventions are required to determine a more accurate effect size of this technique in motor recovery after stroke. Other variables that need to be studied include the definition of optimal stimulation parameters and treatment duration, as well as the appropriate timing for the combination of the stimuli with physical rehabilitation protocols. In addition, future studies will need to evaluate whether VNS has differential effects according to the compromised vascular region, severity of the lesion and stroke subtype (e.g., lacunar vs. non-lacunar) among other clinical characteristics that could impact the effectiveness of this intervention.

The systematic review and meta-analysis have some limitations that are important to mention. First, the number of clinical trials, was very low, and one of the included studies had no comparison group. A high statistical heterogeneity between studies was also identified. There are some sources of heterogeneity that could not be evaluated, for example, the day of primary outcome evaluation, physical rehabilitation protocol parameters, the severity of the lesion, and the vascular region affected by the stroke, among others.

We conclude that VNS together with physical rehabilitation improves upper limb motor function in stroke patients. Additionally, VNS is a safe intervention. More studies are needed to evaluate the efficacy and effectiveness of transcutaneous VNS in patients with stroke and to evaluate optimization of its effect according to the timing of the intervention and the use of more effective stimulation parameters.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JR-C independently reviewed the titles and abstracts for an initial assessment of study eligibility criteria. Once the titles and abstracts were reviewed. JR-C and DL-F reviewed the full-text articles to evaluate the inclusion of studies in the analysis. Discrepancies and doubts on the inclusion of articles were resolved by a third investigator RG. JR-C and RG wrote the initial version of the manuscript. JR-C, DL-F, SS-B, and FS-S reviewed the article and made significant contributions to the interpretation of results. All authors contributed to the article and approved the submitted version.

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Non-invasive Vagus Nerve Stimulation in Cerebral Stroke: Current Status and Future Perspectives

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Stroke poses a serious threat to human health and burdens both society and the healthcare system. Standard rehabilitative therapies may not be effective in improving functions after stroke, so alternative strategies are needed. The FDA has approved vagus nerve stimulation (VNS) for the treatment of epilepsy, migraines, and depression. Recent studies have demonstrated that VNS can facilitate the benefits of rehabilitation interventions. VNS coupled with upper limb rehabilitation enhances the recovery of upper limb function in patients with chronic stroke. However, its invasive nature limits its clinical application. Researchers have developed a non-invasive method to stimulate the vagus nerve (non-invasive vagus nerve stimulation, nVNS). It has been suggested that nVNS coupled with rehabilitation could be a promising alternative for improving muscle function in chronic stroke patients. In this article, we review the current researches in preclinical and clinical studies as well as the potential applications of nVNS in stroke. We summarize the parameters, advantages, potential mechanisms, and adverse effects of current nVNS applications, as well as the future challenges and directions for nVNS in cerebral stroke treatment. These studies indicate that nVNS has promising efficacy in reducing stroke volume and attenuating neurological deficits in ischemic stroke models. While more basic and clinical research is required to fully understand its mechanisms of efficacy, especially Phase III trials with a large number of patients, these data suggest that nVNS can be applied easily not only as a possible secondary prophylactic treatment in chronic cerebral stroke, but also as a promising adjunctive treatment in acute cerebral stroke in the near future.

Keywords: non-invasive vagus nerve stimulation, transcutaneous cervical VNS, transcutaneous auricular VNS, rehabilitation, stroke, parameters

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INTRODUCTION

It is estimated that there will be approximately 200 million stroke patients in the world by 2050 (Brainin et al., 2020). Despite extensive therapeutic advances in recent years, stroke including ischemic and hemorrhagic (roughly 87 and 13%) (Kuriakose and Xiao, 2020), is still a leading cause of disability and a significant health problem worldwide. Approximately 60% of patients who suffer

stroke only partially recover or are unable to recover within 6 months (Lee et al., 2015). Therefore, it is paramount to develop novel complementary treatment approaches that can be easily applied and do not interfere with established protocols including thrombolysis and thrombectomy.

During stroke rehabilitation, developing effective and evidence-based therapies to reduce impairment, improve functional activities, and enhance participation in activities are important goals. Neurostimulation techniques have been used increasingly in clinical and fundamental neuroscience. Vagus nerve stimulation (VNS), a Food and Drug Administration (FDA)-approved addition to medication for the treatment of partial epilepsy, depression, and primary headache disorders, is one potential therapy (Ben-Menachem, 2002; Yuan and Silberstein, 2016; Carreno and Frazer, 2017). It has also recently been recognized that VNS has the potential to enhance the recovery from neurological injuries, including stroke (Khodaparast et al., 2014, 2016; Capone et al., 2017; Dawson et al., 2021). The VNS-REHAB study, which was recently published in the Lancet, supports the use of VNS as a new therapeutic option for limb paralysis caused by an ischemic stroke (Dawson et al., 2021). In clinical practice, two methods of stimulation are used: invasive vagus nerve stimulation (iVNS) and non-invasive vagus nerve stimulation (nVNS) (Mertens et al., 2018; Wang et al., 2021c). nVNS are non-invasive devices that have been developed to stimulate the vagus nerve transcutaneously. By which, unique risks and adverse events associated with implants such as medical care, infection, peritracheal hematoma, damaged vocal cords, and dyspnea are precluded or reduced (Ben-Menachem et al., 2015; Zhao X.-P. et al., 2019; Li et al., 2020b). Furthermore, nVNS delivery systems may be more suitable for emergency patients who have suffered bursts of ischemic stroke. These systems may not require a surgical procedure, thereby improving patient safety.

As nVNS continues to rapidly grow in popularity and application in stroke, the field generally lacks a consensus on optimum initial time, stimulation cites, and stimulation parameters. The question of whether the nVNS can have the same effects in stroke recovery, as well as the underlying mechanisms and future research directions, needs to be addressed further. Therefore, this critical review aims to explore the reported studies on nVNS in stroke to present narrative accounts of its therapeutic potential and mechanisms of action that may facilitate its therapeutic effects. The abbreviations in this review are listed in **Table 1**.

TABLE 1 | Abbreviations.

Abbreviations			
Auricular branch of the vagal nerve	ABVN	Middle cerebral artery occlusion	MCAO
Autonomic nervous system	ANS	Myeloperoxidase	MPO
Blood brain barrier	BBB	Non-invasive vagus nerve stimulation	nVNS
Blood oxygen level dependent	BOLD	Non-invasive VNS	nVNS
Brain-derived neurotrophic factor	BDNF	Norepinephrine	NE
Central nervous system	CNS	Nucleus tractus solitarious	NTS
Cholinergic anti-inflammatory pathway	CAP	Percutaneous auricular VNS	paVNS
Dentate gyrus	DG	Peroxisome proliferator-activated receptor γ	PPARγ
Dynamic contrast enhanced MRI	DCE-MRI	Post-stroke insomnia	PSI
Electromyogram	EMG	Spreading depolarization	SD
Endothelial nitric oxide synthase	eNOS	Tight junction protein	TJP
Food and Drug Administration	FDA	Transcutaneous auricular vagus nerve stimulation	taVNS
Fugl-meyer assessment-upper extremity	FMA-UE	Transcutaneous cervical vagus nerve stimulation	tcVNS
Function independent measure	FIM	Transcutaneous vagus nerve stimulation	tVNS
Functional magnetic resonance imaging	fMRI	Traumatic brain injury	TBI
Growth differentiation factor 11	GDF11	Tumor necrosis factor α	TNF-α
Human high mobility group 1	HMGB1	Upper limb fugl-meyer	UFM
Hypothalamic–pituitary–adrenal axis	HPA	Vagus nerve	VN
Interleukin	IL	Vagus nerve stimulation	VNS
Invasive vagus nerve stimulation	iVNS	Vascular endothelial growth factor	VEGF
Ischemia/reperfusion	I/R	Wolf motor function test	WMFT
Matrix metalloproteinase	MMP	α7 nicotinic acetylcholine receptor	α7nAchR

VAGUS NERVE STIMULATION

History and Clinical Application of Vagus Nerve Stimulation

Vagus nerve stimulation has a history dating back to the 19th century when James Corning examined the anti-seizure effect of manual stimulation of the vagal nerve in epileptic patients (Lanska, 2002). There are two methods of stimulation in clinical practice, invasive vagus nerve stimulation (iVNS) and non-invasive vagus nerve stimulation (nVNS). According to an international consensus published recently, there are four currently accepted VNS modalities: cervically implanted VNS (iVNS), transcutaneous cervical VNS (tcVNS), transcutaneous auricular VNS (taVNS), percutaneous auricular VNS (paVNS) (Farmer et al., 2020). In iVNS, a pulse generator is implanted beneath the skin in the upper chest, along with electrodes connected to the left vagal nerve (Goodnick et al., 2001; Pruitt et al., 2016; Dawson et al., 2021). Systems for delivering nVNS utilize the distribution of vagal afferents through the skin, either at the external ear (taVNS) or in the neck (tcVNS) (Straube et al., 2015; Gaul et al., 2016; Genheimer et al., 2017; Burger et al., 2019).

Following decades of trials conducted on animals and humans. iVNS was approved by the FDA for the treatment of medically refractory partial epilepsy in 1997 (Morris et al., 2013) and severe, recurrent unipolar depression and bipolar depression in 2005 (Young et al., 2020). iVNS Therapy also received Conformite Europeenne (CE) marking in Europe for the treatment of epilepsy and treatment-resistant or

treatment-intolerant depression (DeGiorgio and Krahl, 2013; Young et al., 2020). Invasive surgeries and their unwanted side effects of iVNS have led to the development of a new, completely non-invasive stimulation way. nVNS has received special attention from basic, clinical, and translational studies due to its comparable benefits to iVNS, ease of use, higher accessibility, and fewer side effects (Ben-Menachem et al., 2015; Frangos et al., 2015; Marin et al., 2018). nVNS entered clinical treatment in 1997, its clinical effectiveness and its physiological action are similar but with greater tolerability and fewer patients reporting side effects (Redgrave J. et al., 2018). tcVNS has also been approved by the FDA to treat migraines (Martelletti et al., 2018) and cluster headaches (Gaul et al., 2016; Marin et al., 2018).

In such a long period of clinical practice, hundreds of thousands of patients have been treated for various neurological disorders, such extensive experience has provided many opportunities to explore new clinical applications for VNS in other neuropsychiatric disorders except epilepsy, migraines, depression. And Among the most intriguing potential directions of VNS is the treatment of stroke. Recent randomized controlled trials have also shown that combined with rehabilitation therapy, iVNS and nVNS may benefit upper limb recovery after stroke (Khodaparast et al., 2014; Capone et al., 2017; Dawson et al., 2021).

Anatomic Basis for Non-invasive Vagus Nerve Stimulation

The vagus nerve (VN) is a mixed cranial nerve composed of 80% sensory fibers (afferent) and 20% motor fibers (efferent). It is located on both the left and right sides of the body, acting as a two-way channel between the central nervous system and the autonomic nervous system (ANS), transmitting sensory and motor information between the systems. Its afferent fibers transmit visceral and somatic information from the body to the brainstem and thus providing a unique pathway to the brain (Groves and Brown, 2005; Kaniusas et al., 2019; Farmer et al., 2020). While its efferent fibers originate in the dorsal motor nucleus (to supply the heart, lungs, esophagus, and stomach) and in the nucleus ambiguous (to innervate the muscles in pharynx and larynx). Most of afferent fibers of VN terminate in the nucleus tractus solitarius (NTS) in the lower medulla (e.g., for visceral afferents, heart, taste, and aorta), whereas others terminate in the nucleus spinalis of the trigeminal nerve, like some laryngeal and pharyngeal afferents (Trevizol et al., 2015; Yuan and Silberstein, 2016). The right part of the vagus nerve is more closely associated with the cardiac atria and innervates the sinoatrial node that controls heart rate; whereas the left part of the vagus nerve is typically associated with the ventricles of the heart and innervates the atrioventricular node that controls contraction force (Guiraud et al., 2016). The vagus nerve is therefore essential in the maintenance of homeostasis and parasympathetic system function, regulating inflammatory, cardiovascular function, and gastric emptying efferent effects.

According to Erlanger and Gasser, the VN consists of A-, B-, and C-fibers with corresponding conduction velocities (Yuan and Silberstein, 2016). Based on anatomical research, as the VN passes caudally through their ganglia, it divides into four branches: the auricular branch, the meningeal branch, the sympathetic branch (joint with the superior cervical sympathetic ganglion), the pharyngeal branch, and the laryngeal branch (Ruffoli et al., 2011; Yuan and Silberstein, 2016; Kaniusas et al., 2019). The auricular branch of the vagus nerve (ABVN) is the only branch of vagus nerve that reaches the body surface. As the ABVN forms a cutaneous receptive field in the pinna, which is roughly located in the 1–1.5 mm gap between the skin and the auricular cartilage (Bermejo et al., 2017). ABVN can be found in both the cymba and cavum conchae, however, cymba conchae are 100% dominated by ABVN (Peuker and Filler, 2002). The ABVN afferent fiber enters the vagal trunk *via* the jugular ganglion and projects NTS, where the integration of autonomic neurons occurs. The conchae collect afferent information and activate the caudal ventrolateral medulla and dorsal motor nucleus to control central autonomic activity (Butt et al., 2020; Wang et al., 2021b). This is why the conchae have the ability to manage bodily functions. Yakunina et al. (2017) found that stimulation of the auricular canal could activate the vagus nerve pathway to the maximum extent, so this location might be the best anatomical location for transcutaneous vagus nerve stimulation.

During the first half of the twentieth century, researchers began studying the NTS of the vagus nerve, the main afferent transmission from the vagus nerve to the central nervous system, and its projections to the cortex. The areas of the brain that are activated by nVNS depending on the focus have been speculated in various studies. Empirical measures, such as fMRI, EEG, and MEG, are critical to confirm proposed hypotheses (Schulz-Stübner and Kehl, 2011; Colzato et al., 2018; Jongkees et al., 2018). Burger and Verkuil (2018) suggest that nVNS engages limbic areas, such as the hippocampal and amygdala, while Yuan and Silberstein (2016) suggest that stimulation of the vagus nerve influences the distribution of hypocretin and orexin in people with cluster headache, and Jacobs et al. (2015) suggest that nVNS enhances memory by increasing locus coeruleus activity. With fMRI. Kraus et al. (2007) demonstrated that non-invasive vagus nerve stimulation results in prominent changes in cerebral activity with marked deactivation in temporal and limbic regions. fMRI studies have shown that nVNS increases neural activity more than sham stimulation in the left prefrontal cortex, right caudate, mid-cingulate and cerebellum (Badran et al., 2018). It also decreases functional connectivity between the posterior cingulate cortex and the lingual gyrus (Zhao B. et al., 2019), and suppresses processes to generate tinnitus (Yakunina et al., 2018; Yakunina and Nam, 2021).

Stimulation of the vagus nerve may also increase synaptic plasticity in central networks after injury (Meyers et al., 2018; Collins et al., 2021). When the vagus nerve is stimulated electrically, the neuromodulatory effect is immediately triggered. A VNS pulse rapidly activates noradrenergic locus coeruleus and cholinergic nucleus basalis, two key neuromodulators in the brain (Morrison et al., 2021). When these pro-plasticity neuromodulators are simultaneously released with neural activity related to rehabilitation, synaptic plasticity in task-specific circuits is promoted.

In general, VN activity correlates with wellbeing, health, relaxation, and even emotions like empathy, while it is negatively correlated with risk factors such as morbidity, mortality, and stress (Thayer et al., 2010; Zulfiqar et al., 2010; Farmer et al., 2020). VN thus plays a critical role in brain-body interactions. These complex interactions naturally cause interest in artificial stimulation for therapeutic purposes.

NON-INVASIVE VAGUS NERVE STIMULATION IN ANIMAL MODELS OF STROKE

In the review of nine animal studies (Ay et al., 2016; Jiang et al., 2016; Ma et al., 2016; Yang et al., 2018; Zhao X.-P. et al., 2019; Li et al., 2020b,a; Lindemann et al., 2020; Zhao et al., 2022; **Table 2**), most manuscripts have settled on a frequency of 20 or 25 Hz that has been shown to be more biologically active both in implanted functional neuroimaging as well as in taVNS optimization trials (Raedt et al., 2011; Hays et al., 2014; Thompson et al., 2021). The FDA approved areas of 20 to 30 Hz stimulation frequencies because studies had shown that frequencies of 50 Hz and above can cause severe and irreversible damage to the vagus nerve during VNS (Groves and Brown, 2005). **Table 2** shows that three studies used the cervical branch of the vagus nerve and six studies used the ABVN as stimulation locations. In rodent models, although the lateral differences are not clear and may differ depending on the parameters used, most of these studies used the left vagus nerve for stimulation.

The tcVNS was initiated at variable times (30 min to 24 h) after cerebral ischemia in rats and mice. The ability of tcVNS to activate the NTS was assessed using c-Fos immunohistochemistry. tcVNS activates the vagus nerve fibers and stimulates its main afferent relay nuclei in the brainstem (NTS) (Ay et al., 2016). The main effects and mechanisms of nVNS illuminated in animal research are summarized below.

Reducing Infarct Size and Improving Neurological Outcome

Several animal studies have demonstrated that nVNS could reduce the cerebral infarction volume and improve the neurological deficit remarkably in rats with cerebral ischemia (Ay et al., 2016; Zhao X.-P. et al., 2019; Li et al., 2020b; Lindemann et al., 2020; Zhao et al., 2022). In these studies, nVNS provided approximately a 25–50% reduction in infarct size, which was similar to previously reported reductions achieved by iVNS (Ay et al., 2011; Sun et al., 2012). Ay et al. (2016) tested the effect of tcVNS at different initiated time after middle cerebral artery occlusion (MCAO) on tissue and functional outcome by changing the therapeutic window up and down by 1 h each time until a comparable effect size with 30-min stimulation was achieved. They found that the effect of tcVNS on infarct size was consistent when stimulation was initiated up to 4 h after MCAO. Furthermore, the improvement in forelimb function was so long-lasting that it continued even after the stimulation had stopped, consistent with results obtained in aged ischemic stroke rats treated with iVNS and rehabilitative training (Hays et al., 2016).

Promoting Angiogenesis

After focal cerebral ischemia, the newly formed collateral blood vessels can improve perfusion of the surrounding tissues and promote recovery of nervous system functions. Recent studies have suggested that angiogenesis, almost in parallel to neurogenesis, participates in the recovery of neurological function after ischemic stroke (Song et al., 2019; Alrafiah et al., 2021; Wang et al., 2021a). It was proposed that VNS increased hippocampal progenitor cell proliferation in the adult rat dentate gyrus, so that such progenitor cells contribute to the healing of damaged neurons from ischemic injury (Lu et al., 2017). It would appear that this plasticity is involved in VNS's efficacy as a treatment for ischemic stroke. In cerebral ischemic rats, taVNS enhanced the expression of angiogenic factors, including BDNF, eNOS, and VEGF, and increased endothelial proliferation, stimulated angiogenesis, and increased microvessel density surrounding the infarct area (Zhang et al., 2017). Another study has shown that taVNS promotes endothelial cells proliferation 7 days after cerebral ischemia, and that taVNS enhances expression of ALK5 in endothelial cells (Ma et al., 2016). The effects of taVNS on post-stroke recovery, as well as up-regulation of cerebral GDF11, and down-regulation of splenic GDF11, indicate brain-spleen communication. Following a stroke, the brain releases ischemic signals, the activated spleen released its GDF11 reserves into the blood circulation, allowing it to deposit in the damaged brain. These results indicate that taVNS may enhance the recovery after stroke by increasing GDF11 concentrations in the vasculature (Ma et al., 2016).

Regulating Blood Brain Barrier Permeability

The breakdown of the Blood Brain Barrier (BBB) and the subsequent brain edema are two of the key components of neurological dysfunction in stroke. They are associated with poor clinical outcomes during and after ischemic stroke (Cai et al., 2014). A significant association between stroke progression and BBB breakdown has been demonstrated. As early BBB permeability can be reversed with treatment, it would make sense that the VNS could be involved in regulating cerebral edema after stroke (Gaul et al., 2016).

According to a study, the use of taVNS during MCAO significantly reduced the permeability of the BBB after ischemia and reperfusion measured by DCE-MRI 24 h after stroke. taVNS treated rats with ischemic hemispheres demonstrated significantly lower levels of serum IgG leakage as detected by IHC after MRI, consistent with the findings described above (Yang et al., 2018). BBB integrity is maintained primarily by ECs sealed at tight junctions, astrocyte endfeet, pericytes, and extracellular matrix. In reperfusion injury, proteases are involved in the biphasic opening of the BBB. A number of mechanisms have been proposed to account for the degradation

TABLE 2 | Stimulation location, parameters, and therapeutic effects for all studies of nVNS in rodent models of stroke.

Authors	Rodent models	Device	Initial time	Parameters	Stimulation side and sites	Stimulation duration	Effects	Results and conclusion
Zhao et al., 2022	Rat, I/R (right ICA)	taVNS, tcvns (Hanshi Electroacupuncture Instrument, Nanjing Hanshi Co. Ltd.)	24 h post-stroke	10 Hz, 1 mA, Pulse width (not described)	Bilateral concha auricularis region or rat tragus	30 min/session, 7 days	Levels of acetylcholine, IL-1β, IL-6, and TNF-α↓; Cx43 phosphorylation↓	Improves motor function
Li et al., 2020a	Rats, MCAO/R (right)	taVNS (Grass Model S48 stimulator, Grass Technologies, Warwick, United States)	30 min post-stroke	20 Hz, 0.5 mA, 0.5 ms, square wave	Left cavum concha	60 min/session, twice daily, 14 days, 28 days	PPAR-γ↓; BDNF, VEGF, P-eNOS↑	Decreases neurological deficit scores, neuronal damage, and infarct volume. Increases microvessel density and endothelial cell proliferation.
Lindemann et al., 2020	Rats, MCAO (left)	tcVNS, iVNS (External transcutaneous stimulator, electrocore Inc.)	30 min post-stroke	iVNS: 25 Hz, 0.5 mA, 0.3 ms tcVNS:25 Hz,1 ms, 5 kHz sine waves.	Left vagus nerve (ivns), left cervical vagus nerve (tcvns)	iVNS: 60 min; tcVNS: 2 min, repeated after 15 min	Spreading depolarizations frequency↓	Improves behavioral tests. Reduces infarct volume. Both iVNS and nVNS reduce the frequency of SDs.
Li et al., 2020b	Rats, MCAO/R (right)	taVNS (Grass Model S48 stimulator, Grass Technologies, Warwick, United States)	30 min post-stroke	20 Hz, 0.5 MA, 0.5 ms, square	Left cavum concha	60 min/session, twice daily,14 days, 28 days	α7nAchR expression↓; Activation of the BDNF/cAMP/PKA/p-CREB pathway	Enhance axonal plasticity through activation of the BDNF/cAMP/PKA/p-CREB pathway
Zhao X.-P. et al., 2019	Mice, MCAO/R (right)	tcVNS (gammacore; Lectrocore, LLC, Basking Ridge, NJ, United States)	1 d before MCAO	25 Hz, 1 ms, 5 kHz sinewaves average voltage of 15 V	Right cervical vagus nerve	60 min	M2 phenotype microglia : Arg-1$^+$ cells↑; IL-17A↓; (TUNEL + NeuN+) cells↓	Reduces infarct volume. Improves neurological outcomes. Reduces neurons apoptosis. Promotes microglial M2 polarization.
Yang et al., 2018	Rats, MCAO (right)	taVNS (gammacore; Lectrocore, LLC, Basking Ridge, NJ, United States)	30 min post-stroke	25 Hz, 1 ms, 5 kHz sinewaves average voltage of 15 V	Left cervical vagus nerve	50 min	TJPs: ZO-1↑ BBB transfer rate, serum IgG leakage↓; MMP-2/9 ↓	Reduces infarct volume. Protects Blood-brain barrier.
Ma et al., 2016	Rats, MCAO/R (right)	taVNS (Grass Model S48 stimulator, Grass Technologies, Warwick, United States)	30 min post-stroke	20 Hz, 0.5 mA, 0.5 ms, square	Left cavum concha	60 min/session, twice daily,24 h, 3 days,7 days	upregulate cerebral GDF11 and downregulate splenic GDF11; increase expression of ALK5 in ECs; stimulate proliferation of ecs.	Prompts neuro behavioral recovery Stimulated proliferation of endothelial cells.
Jiang et al., 2016	Rats, MCAO/R (right)	taVNS (Grass Model S48 stimulator, Grass Technologies, Warwick, United States)	30 min post-stroke	20 Hz, 0.5 mA, 0.5 ms, square	Left cavum concha	60 min/session, 2–3 weeks	Microvessel density and endothelial cell proliferation↑; BDNF, eNOS and VEGFs↑	Prompts neuro behavioral recovery and angiogenesis. Reduces infarct volume.
Ay et al., 2016	Rats, MCAO (right)	tcVNS (gammacore; electrocore, LLC).	30 min post-stroke	25 Hz, 1 ms, 5 kHz, 12 V sine waves	Right vagus nerve in the neck	60 min	Decreaselba-1, CD68, and TNF-α positive cells and increase the number of HMGB1 positive cells.	Reduces infarct volume. Improves neurological score. Inhibits ischemia-induced immune activation.

of tight junction proteins (TJPs). Matrix metalloproteinases (MMPs) are degrading enzymes that disrupt TJPs, leading to BBB disruption during ischemic stroke. In the ischemic hemisphere, taVNS inhibited BBB breakdown, as evidenced by decreases in TJP cleavage, ZO-1, occludin, and claudin-5 in endothelial cells. Additionally, it protected tight junction

proteins in microvessels from disruption and reduced MMP-2/9 expressions in astrocytes around compromised vessels (Yang et al., 2018). In addition, taVNS improved BBB integrity after cerebral cortex microinfarcts as well as in rat models of cortical dysplasia and traumatic brain injury, indicating that it may be useful in the effects on BBB after ischemic stroke.

Inhibiting Neuroinflammation

Researchers believe that VNS can potentially modulate inflammation *via* a broader vagal neural network (Yuan and Silberstein, 2016). Recent studies suggest that VNS may act as a neuromodulator to activate certain innate, protective pathways in the central nervous system (CNS). VNS may exert its anti-inflammatory properties in a variety of diseases through its afferent fibers (activating the HPA pathway) and efferent fibers (activating the CAP pathway).

The vagus nerve system suppresses the release of proinflammatory cytokines. It was found that VNS reduced plasma levels of TNFα, IL-1β, IL-6, and MPO in colitis rats through the autonomic neural pathway (Sun et al., 2013). There have been animal and clinical studies exploring the efficacy of nVNS in the treatment of inflammation. A study found that taVNS reduced IL-6 and TNF-α release and prevented endotoxemia in mice (Hong et al., 2019). Lerman et al. (2016) found that tcVNS reduced levels of cytokines and chemokines in the blood of healthy people. Meanwhile, Clancy et al. (2014) reported that taVNS decreased sympathetic nerve activity in healthy people.

Through alpha-7 nicotinic acetylcholine receptors (α7nAChRs), central immune activation (e.g., macrophage accumulation and microglial activation) can influence acetylcholine levels and cause anti-inflammatory effects (Kalkman and Feuerbach, 2016). The α7nAChR subunit is required for the CAP to limit cytokine production, according to Wang et al. (2003). The cholinergic anti-inflammatory response is induced by the α7nAChR. Acetylcholine is released when the vagus nerve is stimulated, inhibiting the anti-inflammatory pathway *via* the α7nAChR on activated macrophages and other cytokine-producing cells. Finally, TNF and other pro-inflammatory cytokines that play a role in inflammation are suppressed (Oke and Tracey, 2009). Recent studies have also found that taVNS has anti-inflammatory effects in both the peripheral and central nervous systems, which are mediated through α7nAChRs (Zhao et al., 2012; Corsi-Zuelli et al., 2017). taVNS has also been reported to have neuroprotective effects against ischemic cerebral injuries *via* an anti-inflammatory mechanism (Li et al., 2020b).

Microglia are central nervous system resident macrophages that perform a variety of tasks such as synaptic organization, phagocytosis of apoptotic cells, and neuronal excitability regulation (Sasaki, 2017; Baig et al., 2022). Ischemia triggerS resting microglia to the M1 phenotype causing damage to functioning neural cells including neurons and astrocytes (Hu et al., 2012). Activation of microglia to the M2 phenotype, on the other hand, can stop the inflammatory process by producing anti-inflammatory cytokines like IL-4 and IL-10 (Hu et al., 2012; Liu et al., 2016; Zhao X.-P. et al., 2019). As a result, microglial M2 polarization could be a new target for fighting inflammation after cerebral I/R injury. Zhao X.-P. et al., 2019) demonstrated that tcVNS attenuated cerebral ischemic injury by promoting microglial M2 polarization. Intranasal administration of recombinant IL-17A dampened the tcVNS induced M2 polarization of microglia and its neuroprotective effects, which suggests that the effect of tcVNS might occur through IL-17A signaling inhibition. tcVNS inhibits microglia activation and normalizes altered cytokine levels after MCAO by reducing the number of Iba-1, CD68, and TNF-α positive cells and increasing HMGB1 positive cells (Ay et al., 2016). These findings underline that anti-inflammatory mechanisms play an important role in ischemic neuroprotection by nVNS.

Facilitating Post-stroke Axonal Plasticity

Axonal plasticity plays an important role in neurofunctional recovery after stroke. The neurofunctional recovery that occurs in the days to weeks following an ischemic stroke appears to be linked to axonal plasticity including axonal regeneration and reorganization (Liu et al., 2015; Bu et al., 2021). taVNS treatment enhanced α7nAchR expression in the ischemic cortex. And ischemic rats treated with taVNS demonstrated improved axonal plasticity (regeneration and reorganization of axons), in accordance with elevated levels of BDNF/cAMP/PKA/p-CREB pathway members. Thus, taVNS could effectively boost axonal plasticity in the brain after I/R injury while improving neurofunctional recovery (Li et al., 2020b).

Reducing Spreading Depolarizations

Spreading depolarizations (SDs) are sudden and sustained gray matter depolarizations that can occur in a variety of brain states, ranging from healthy brain tissue, such as the migraine aura, to different areas of an ischemic brain, such as the severely energy-depleted infarct core and its surrounding moderately ischemic tissue (Dreier and Reiffurth, 2015). SDs are caused by the failure of the sodium pump in the penumbra aftera n ischemic stroke, and they create cytotoxic edema, disrupt blood flow, and result in infarction of viable tissue, as well as affecting neuronal survival and outcome (Dreier, 2011; Rakers and Petzold, 2017; Dreier et al., 2018; Baig et al., 2022). Furthermore, they are thought to play a role in the development of ionic and vasogenic edema at later stages of ischemia (Dreier et al., 2018; Mestre et al., 2020). As a result, in experimental models and clinical cases of stroke and other acute neurological disorders, SDs are among the most important contributors to infarct generation, cell death, and injury expansion (Lauritzen et al., 2011; Dreier and Reiffurth, 2015). Lindemann et al. (2020) discovered that delivering nVNS or iVNS during permanent MCAO significantly reduced the frequency of SDs in the cortical peri-infarct area compared to sham VNS, without affecting relative blood flow changes, blood pressure, heart rate, or breathing rate. They hypothesize that either nVNS or iVNS could be a safe and effective intervention for reducing the clinical burden of SD waves in stroke.

CLINICAL TRIALS TO ASSESS SAFETY AND EFFICACY OF NON-INVASIVE VAGUS NERVE STIMULATION AFTER CHRONIC/SUBACUTE ISCHEMIC STROKE

In our review, we found four studies and one case report that investigated the influence of nVNS on upper-limb motor function, sensory function, and sleep disturbance after stroke. Among which, four studies included chronic stroke patients (Capone et al., 2017; Redgrave J.N. et al., 2018; Baig et al., 2019; Zhao B. et al., 2019) except one study included subacute ischemic stroke patients (Wu et al., 2020). Here, we summarized the mainly functional improvement, parameters, side effects and future directions of nVNS in clinical studies on stroke.

In addition, several recently completed and ongoing clinical studies are focused on the safety and effects of nVNS on stroke (Baig et al., 2022). Especially some studies are focused on acute or subacute stroke (NCT03733431; NCT04050501; NCT03292159; Clinicaltrials.gov). Instruments and procedures (MRI, CT perfusion, EMG, or force coupled to a computer monitor) that can help quantify the findings have been utilized in several studies in addition to the generally used scale for outcome evaluation. The findings should help us better understand the effectiveness, adverse effects, and ideal settings of nVNS, as well as how nVNS influences stroke.

Non-invasive Vagus Nerve Stimulation Combined With Rehabilitation Improves Upper Limb Function After Chronic Stroke

It is generally accepted that upper extremity impairment as one of the results of stroke has a deep impact on quality of life, but the clinical application of the treatment may not readily be seen until after stroke. Studies have shown that iVNS paired with rehabilitation significantly improves forelimb strength and speed in models of ischemia and hemorrhage in rats (Hiraki et al., 2012; Hays et al., 2014, 2016; Khodaparast et al., 2016). Clinical studies showed that paired rehabilitation with VNS improves motor function in patients suffering from chronic strokes. The Fugl-Meyer Assessment-Upper Extremity (FMA-UE) score of stroke patients after iVNS was clearly higher than the score of pure rehab patients who did not receive iVNS (Dawson et al., 2020). Significant improvements in the Wolf Motor Function Test (both in terms of function and timing), Box and Block Test and Nine-Hole Peg Test has also been observed (Dawson et al., 2020). Similar results have also been reported in stroke patients treated with nVNS. Redgrave J.N. et al. (2018) conducted a pilot study combining taVNS with post-stroke upper limb rehabilitation in 18 sessions (1 h), showing improvement in motor function in the pilot study. While Redgrave and Baig used therapists to conduct rehabilitation training, Capone et al. (2017) have reported that taVNS combined with robot-assisted rehabilitation may be able to promote mild improvements in arm function and promote long-term benefits for stroke recovery.

Motor Activated Auricular Vagus Nerve Stimulation (MAAVNS) was devised as a closed-loop solution to the parametric problem (Cook et al., 2020). It combines taVNS with motor activity by using pulses at 25 Hz for 500 s during a focused motor task (Cook et al., 2020). It has been shown to be an effective neurorehabilitation tool and in early studies has shown promise in helping neonates learn motor skills (Badran et al., 2018, 2020). It is being explored further to facilitate stroke rehabilitation in adults. Therefore, the continued development of nVNS may radically change the field and potentially remove the barrier of surgery for many patient populations.

Non-invasive Vagus Nerve Stimulation Improving Sensory Recovery After Chronic Stroke

Stroke survivors with sensory impairments tend to recover less functionally after their injuries. A long-term follow-up study found iVNS combined with tactile therapy improved sensory function in a man suffering from the severe sensory decline in his left hand and arm (Meyers et al., 2018). This may be related to increased neuroplasticity throughout the brain. Following the study, the authors speculated that combining VNS with sensory stimulation can be an alternative method for promoting neuroplasticity and sensory recovery for chronic stroke patients. However, this was based on only one case study. After that, Baig et al. reported the impact of taVNS paired with repetitive motor task practice on sensory recovery in a cohort of chronic stroke patients. An average of 18 sessions (1 h/session) were given over 6 weeks to twelve participants who were >3 months post-ischemic stroke and would still have residual upper limb weakness. The repetition of functional arm movements concurrently with the taVNS at the maximum level of intensity is 300 repetitions. The UFM (Upper Limb Fugl-Meyer) assessment was used to assess the light touch and proprioception of the upper limb at baseline and during post-intervention. Seven out of 11 participants (64%) who had sensory impairment at baseline regained some sensation after the intervention. Patients with the greatest improvement in motor function had the greatest increase in UFM sensation.

There is a possibility that the improvements in proprioception observed in subjects could be explained by an improvement in strength and range of motion achieved through upper limb tasks facilitated by taVNS. As a result of the increased range of joint movements, it is possible that the increased sensory feedback from the affected limb increased neuroplasticity in the cortical sensory networks. When combined with the correlation between improved motor function and sensory feedback, it is possible to hypothesize that motor and sensory recovery are positive feedback loops that mutually enhance one another.

Non-invasive Vagus Nerve Stimulation Treating Post-stroke Insomnia After Chronic Stroke

Patients with cerebrovascular disease are often affected by post-stroke insomnia (PSI). Approximately 37–59% of patients with stroke complain about insomnia (Duss et al., 2018). Studies

suggest that insomnia is also associated with an increased risk of morbidity from cardiocerebrovascular disease as well as a reduced outcome from stroke. It has been proved that taVNS is effective in treating depression with insomnia and primary insomnia (Liu et al., 2020). A case report by Zhao B. et al. (2019) examined the effectiveness and neuromechanics of taVNS in PSI patients. BOLD-fMRI was carried out before and after 4 weeks of taVNS. A 4-week taVNS intensive treatment produced significant improvement in insomnia symptoms. Falling asleep time was reduced to less than 30 min, and sleep duration was increased to 7 h. The therapeutic effect was still observed 3 months after treatment. PSQI scores dropped from 13 to 8 points.

Based on the association of the basal ganglia with the frontal lobe and thalamus, a reduced functional connectivity in the striatum and thalamus may suggest an emotional circuit disorder. Following taVNS treatment, posterior cingulate cortex and regions of basal ganglia associated with emotion showed increased functional connectivity. This case study provides evidence that taVNS therapy may provide a new, portable, self-managed, and safe technique for the treatment of PSI patients.

Clinical Trials to Assess Safety and Efficacy of Non-invasive Vagus Nerve Stimulation After Subacute Ischemic Stroke

Researchers recently published a randomized pilot study exploring the safety and effectiveness of taVNS in treating patients with subacute ischemic stroke. In this study, 21 patients with strokes in the acute or subacute phase (between 0.5 and 3 months post onset) were included (Wu et al., 2020). At the endpoint, there were significantly greater improvements in FMA-U, FIM, and WMFT scores in the taVNS group compared with the sham-taVNS group. Moreover, the taVNS group obtained a significantly higher improvement of FMA-U score as compared with the sham-taVNS group at 4and 12 weeks. Only one adverse event related to contact with the auricular skin electrodes was noted. In the present study, taVNS proved to have a beneficial effect on the rehabilitation of upper limb motor function in patients with subacute strokes. nVNS may be able to reduce ischemic brain injury as it can be easily applied within a non-hospital setting early after stroke thanks to its relatively small therapeutic window.

Side Effects of Non-invasive Vagus Nerve Stimulation

It has been shown that the nVNS was safe and well-tolerated, and those adverse events were very rare (Capone et al., 2017; Redgrave J.N. et al., 2018; Baig et al., 2019; Zhao B. et al., 2019; Wu et al., 2020). Redgrave J. et al. (2018) published a systematic review of the safety and tolerability of taVNS. Itching and redness (16.7%) around the stimulation site are common side effects, as are tingling and pain in the area (Redgrave J. et al., 2018). Some less common side effects have been noted in <1% of the study participants, including nausea and vomiting (Schulz-Stübner and Kehl, 2011; Kreuzer et al., 2014; Jacobs et al., 2015; Yuan and Silberstein, 2016), headache (Stefan et al., 2012; Kreuzer et al., 2014; Gaul et al., 2016; Yuan and Silberstein, 2016; Baig et al., 2019), facial drooping (Goadsby et al., 2014; Yuan and Silberstein, 2016), dizziness (Jacobs et al., 2015; Gaul et al., 2016; Liu et al., 2018; Baig et al., 2019), vocal hoarseness (Stefan et al., 2012; Goadsby et al., 2014).

In addition, due to the vagus nerve's influence on cardiac activity, researchers closely monitored HR and BP during nVNS sessions in to detect any potential cardiovascular harm. The HR and systolic blood pressure (SBP) do not show significant pre-post differences. All cardiovascular parameters did not change significantly throughout the treatment. Heart palpitations were reported in one research (Bauer et al., 2016). According to the systematic review by Redgrave J. et al. (2018), 7/1322 participants in total reported cardiac side effects such as palpitations, arrhythmia, bradycardia, and hypotension. Steyn et al. (2013) found that the mean heart rate in four participants with asthma decreased from 106 to 85 beats per minute following nVNS. However, all participants experienced no symptoms following the procedure. Symptomatic bradycardia was observed in a male volunteer who collapsed with bradycardia and hypotension after receiving bilateral conchal taVNS (2–100 Hz, pulse width 0.2 ms) in addition to a painful stimulus (Laqua et al., 2014). Kreuzer et al. (2012) reported two cases of cardiac arrhythmia (left bundle branch block and sinus arrhythmia), in their retrospective assessment of the cardiac safety of taVNS. No work has yet examined the relationship between stimulation parameters or dose and the rate of side effects experienced, which should be a priority of future research in the area, and clear documentation of both side effects and stimulation parameters is crucial to observe any trends.

Stimulation Parameters

For VNS, setting the optimal stimulation parameters has a huge impact on clinical efficacy. Morrison et al. (2021) found that stimulation intensity affects motor cortex plasticity. Many factors, such as the stimulation site and side, electrode and waveform configuration, continuous stimulation or pulse-synchronous stimulation, titration protocols, current amplitude and frequency, and stimulation on-and-off time can impact the clinical efficacy of VNS (De Ferrari and Schwartz, 2011). According to Helmstaedter et al. (2001), the effectors of stimulation parameters and the resulting direction of VNS's cognitive effects appear to be highly constrained by stimulation parameters. The timing and amount of VNS therapy also play a crucial role in maximizing its therapeutic benefits (Meyers et al., 2018; Nuntaphum et al., 2018).

Due to the fact that studies have been done with participants with different clinical conditions and with diverse stimulation parameters, it is hard to determine an ideal stimulation site for any specific disease (Goadsby et al., 2014; Gaul et al., 2016; Liu et al., 2018; Martelletti et al., 2018). Despite the lack of consensus on ideal parameters, nVNS researchers carried out human clinical trials using parameters similar to those administered in cervically implanted VNS analogs.

Here are the specific parameters of stimulation for nVNS in several studies (**Table 3**). Most studies used the left auricular branch of the vagus nerve as the stimulated sites, except one

TABLE 3 | Stimulation location, parameters, therapeutic effects, and side effects for all studies assessing the efficacy of nVNS in patients with stroke.

Authors	Study groups	Stimulation sites and device	Phase of stroke	Paired	Parameter settings	nVNS duration	Therapeutic effects	Side effects
Wu et al., 2020	taVNS/sham group; Randomized pilot study	taVNS; left ear concha; bhd-1a transcutaneous electrical stimulation therapy instrument (Bohua, china).	Subacute ischemic stroke	taVNS paired with conventional rehabilitation training	20 Hz; 0.3 ms; lasting 30 s each time, stimulating once every 5 min; Mean stimulation intensity 1.66 ma	15 days.	Improves upper limb motor function	Skin redness
Redgrave J.N. et al., 2018	Feasibility study with no control group.	TaVNS; left ear concha; Nemos (cerbomed)	Chronic stroke. 3 months post-stroke	taVNS paired with upper limb repetitive task-specific practice	25 Hz; 0.1 ms; Median stimulation intensity 1.4 mA	3 times a week, over 6 weeks	Improves upper limb motor function	Light-headedness in one Participant and general tiredness and fatigue in two
Baig et al., 2019	Feasibility study with no control group.	TaVNS; left ear concha; Nemos (cerbomed)	Chronic stroke. 3 months post-stroke	taVNS paired with repetitive upper limb task training	25 Hz;0.1 ms; Median stimulation intensity 1.4 mA	3 times a week, over 6 weeks	Promotes motor and sensory rehabilitation	None reported
Capone et al., 2017	Real or sham tVNS associated with Robot-assisted therapy.	taVNS; left ear concha; Twister-ebm	Chronic stroke, ischemic or hemorrhagic	taVNS paired with robot-assisted therapy	20 Hz;0.3 ms ,lasting 30 s each time, stimulating once every 5 min Mean stimulation intensity 2.0–4.5 mA	10 working days.	Improves upper limb function	None reported
Zhao B. et al., 2019	Case report	taVNS; bilateral auricular concha areas; device not mentioned	7 months post-hemorrhagic stroke	None	20 Hz; less than 1 ms; Intensity 4–6 mA	30 min, twice a day, 4 weeks	Alleviates post-stroke insomnia	None reported

case report chose bilateral auricular branches of the vagus nerve to stimulate. According to researchers, since vagal fibers to the heart are supposed to originate from the right side, only the left ear was stimulated to reduce the risk of cardiac side effects. A common frequency of 20 or 25 Hz is used in these studies. It is common for the stimulation current to be set according to a subject's sensitivity or just below their pain threshold (Frangos et al., 2015; Lerman et al., 2016; Yakunina et al., 2018; Sclocco et al., 2020; Yakunina and Nam, 2021). Studies gradually raised stimulation intensity by 0.1 mA increments until the maximum level reported by participants (Redgrave J.N. et al., 2018). The intensity of stimulation ranged between 0.5 and 6 mA. Another study adjusted stimulation intensity to levels above detection thresholds and below pain thresholds (Capone et al., 2017). The range is similar to those reported in other diseases, Stimulation amplitudes vary over a wide range [from 0.5 mA (Jongkees et al., 2018) to 12 mA (Trevizol et al., 2016)]. The amplitude or amount, of energy delivered to tissues, is also unknown despite current values for electric motors being reported, due to the significant effect of electrode and tissue impedance and the need for precise placement. In addition, the electrochemistry of the stimulation electrode has a significant impact on the maximum current tolerance of the participant, without a doubt.

CONCLUSION AND FUTURE DIRECTION

In this review, we reviewed current animal and clinical researches on non-invasive vagus nerve stimulation on cerebral stroke, emphasizing the outcomes, underlying mechanisms, stimulation parameters, sites of stimulation, and side effects.

The development of neuroscience has led to a new type of intervention, neuromodulation therapy, that targets the nervous system to achieve therapeutic results. Several studies have shown that nVNS affects the same brain regions and yields therapeutic effects similar to iVNS (Terré and Mearin, 2009; Van Leusden et al., 2015). Since nVNS is non-invasive, it has been receiving special attention in basic, clinical, and translational research for its benefits which are comparable to those of iVNS, ease of use, and reduced side effects, In addition, it is more accessible. Auricular and cervical branches of the vagus nerve are most commonly targeted by nVNS.

As nVNS continues to emerge as a promising treatment in stroke, there is still a lot to be done and a large number of literatures to be improved. Several studies have confirmed the effect of nVNS on stroke rehabilitation, however, most of the current studies focused on upper limb function, and future studies need to focus on the improvement of other functions post-stroke, such as cognition impairment, dysphagia, aphasia, and intestinal dysfunction. There is a lack of large sample RCT studies, and therefore, no strong evidence on the role of nVNS in stroke rehabilitation.

Rehabilitation effects are being demonstrated in stroke. The parameters and protocols of most of the described methods vary enormously, so there is no clear evidence on the best location to apply nVNS or the stimulation parameters that will provide the most therapeutic benefit. As nVNS research grows, we need to take a historical perspective into account and further optimize

the parameter space. In addition, study results should also be analyzed to determine the frequency of treatments, the number of doses per day, and the degree of treatment tolerance.

The precise mechanism by which nVNS exerts its therapeutic effects is still unclear. We need further studies examining the mechanical basis of nVNS to facilitate our future trials. A systematic study must be conducted to reveal the precise mechanism of action and ideal stimulation modalities of nVNS if it is to reach its full potential as a non-invasive and clinically relevant therapy. Future investigations should not be restricted by past hypotheses about the effects of nVNS on neural activation and function.

Most studies have only a small sample, some with only one participant. This makes it difficult to determine whether the findings or proposed pathways can be generalized. In order to avoid the risk of having extreme or biased results, studies with a large sample size are necessary. Further standard stimulation methods of nVNS combine electrophysiology and imaging evaluation methods are needed to reduce subjective bias during training and devise more effective rehabilitation strategies for stroke.

In addition to helping avoid costly missed opportunities for reducing ischemic brain injury, nVNS may be able to reduce ischemic brain injury as it can be easily applied within a non-hospital setting early after stroke thanks to its relatively small therapeutic window.

AUTHOR CONTRIBUTIONS

QW conceived and supervised the project. LL and DW researched literature and wrote the manuscript. HP, LH, XS, and CH contributed to the manuscript revision. All authors contributed to the article and approved the submitted version.

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