SUPPLEMENTAL MATERIAL

Safety, Feasibility and Efficacy of Vagus Nerve Stimulation Paired with Upper Limb Rehabilitation Following Ischemic Stroke

Supplementary Methods

Complete List of Inclusion Criteria:

1. History of unilateral supratentorial ischemic stroke that occurred at least 6 months prior
2. Age >18 years and <80 years
3. Right or left sided weakness of upper extremity
4. ARAT score of 15 to 50 (inclusive of 15 and 50)
5. At least 5 degrees of active wrist extension
6. Modified Rankin Score of 2, 3, or 4

Note patients above the age of 80 years were excluded from this first study for safety reasons and as we recognise patients above the age of 80 years have more variable outcome and may respond differently to stroke treatments.

Complete List of Exclusion Criteria:

1. Hemorrhagic stroke
2. Any deficits in language or attention that interferes with reasonable study participation
3. Presence of significant apraxia
4. Profound sensory loss
5. Active major neurological or psychiatric diagnosis that would likely interfere with study protocol including alcohol or drug abuse
6. Patient receiving any therapy (medication or otherwise) at study entry that would interfere with VNS (e.g. drugs that interfere with neurotransmitter mechanisms – such

as anticholinergics, adrenergic blockers, etc.). Additionally, no psychoactive medications – including nicotine – may be used during the acute study. *

7. Prior injury to vagus nerve (e.g., injury during carotid endarterectomy)

8. Severe depression (Beck Depression Scale > =29)

9. Not considered candidate for a device implant surgery (history of adverse reactions to anaesthetics, poor surgical candidate in surgeon's opinion, etc.)

10. Any other implanted device such as a pacemaker or other neurostimulator; any other investigational device or drug

11. Medical or mental instability (diagnosis of personality disorder, psychosis, or substance abuse)

12. Pregnant or plan on becoming pregnant or breastfeeding during the study period

13. Currently require, or likely to require, diathermy during the study duration

14. Any health problem requiring surveillance with MRI imaging

15. Active rehabilitation within 4-weeks prior to therapy

16. Botox injections or any other non-study active rehabilitation of the upper extremity 4-weeks prior to and during therapy

17. Severe spasticity (Modified Ashworth ≥3)

*Excluded drug classes included muscarinic antagonists, norepinephrine re-uptake inhibitors, centrally acting beta-blockers, benzodiazepines, selective serotonin re-uptake inhibitors, nicotinic antagonists, centrally active alpha-blockers, tricyclic anti-depressants, anti-psychotic drugs, anti-epileptic drugs.

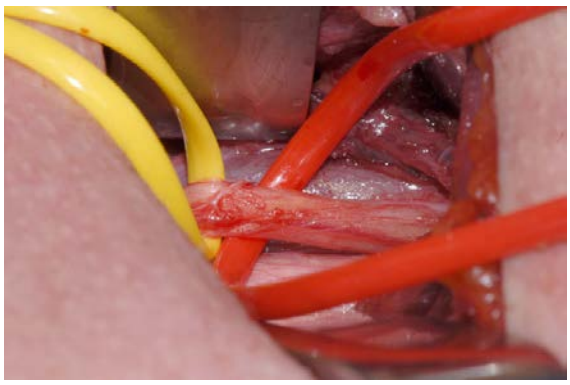
MRI Imaging and Data Analysis

MRI data was acquired on a Siemens Verio 3T MRI scanner (Erlangen, Germany) in Glasgow and on a Philips Intera Achieva 3T MRI scanner (Eindhoven, Netherlands) in Newcastle. The MRI protocol included 3D T1-weighted, T2-weighted fluid suppressed and susceptibility weighted imaging sequences. Diffusion Tensor Imaging (DTI) data was also acquired with a b-value of 1000s.m-2 and 21 diffusion directions (Glasgow) and a b-value of 800s.m-2 and 16 diffusion directions (Newcastle). Infarct volume was measured and corticospinal tract (CST) integrity was assessed using the fluid suppressed T2-weighted imaging data and the DTI respectively. We defined injury to the corticospinal tract by the CST fractional anisotropy (FA) ratio and mean diffusivity (MD) ratio between the stroke and non-stroke side, the mean diffusivity ratio and the volume of the stroke lesion within the CST.

Device implantation

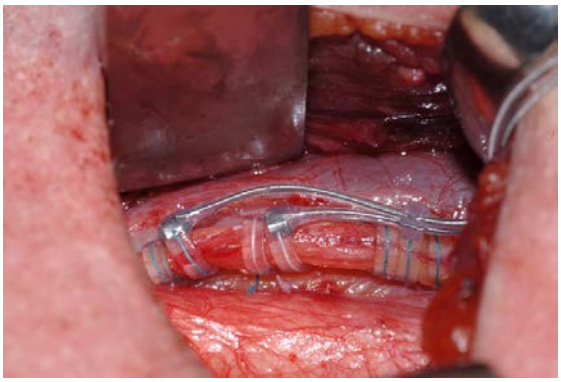
In this study, a single Otolaryngologist, Head and Neck Surgeon with an interest in vagus nerve stimulation performed device implantation at the Glasgow site and a single Neurosurgeon performed implantation at the Newcastle site. Detailed operative procedures from the Glasgow site are included for reference. Normal vocal cord movement was confirmed by flexible laryngoscopy prior to procedure. The procedure was performed under general anaesthesia. The subject was positioned on the operating table in the supine position with their neck extended through use of a shoulder roll, with the head supported on a head ring. The skin was prepared using surgical antiseptic solution and the operative area was square draped. A horizontal neck crease incision was created left of the midline over the anterior edge of the sternocleido-mastoid (SCM) muscle at level of the cricoid cartilage. The left vagus nerve was chosen as it has fewer cardiac efferent fibres going to the sino-atrial node,

thus minimising risk of arrhythmias. The level of cricoid was chosen for incision to allow dissection of an adequate length of nerve for placement of the probes without encountering significant branches. The platysma muscle was divided and subplatysmal flaps are raised. The anterior border of the SCM was identified and lateralised. Omohyoid was divided and the carotid sheath was dissected to expose the vagus nerve. The vagus nerve was identified and confirmation of an intact functional nerve was confirmed with a monitoring probe. A section of the vagus nerve was cleared of fascia. (Supplementary figure I)



Supplementary figure I: Identification of Vagus Nerve

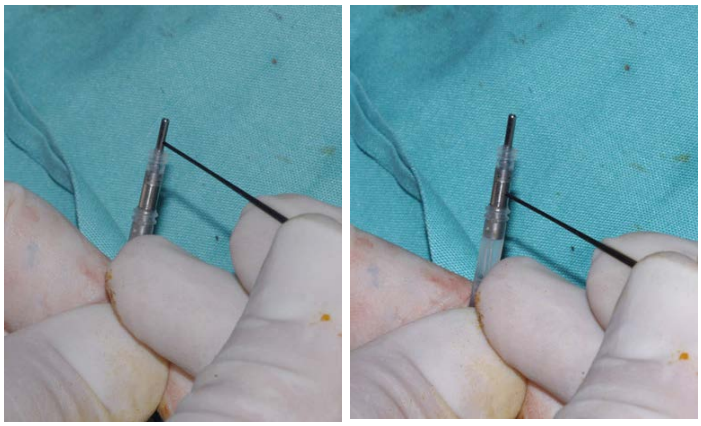
The helical nerve stimulation leads (Model 304, Cyberonics Inc., Houston, TX) was then coiled around the nerve. (Supplementary figure II)



Supplementary figure II: Placement of Stimulation probes on Vagus nerve

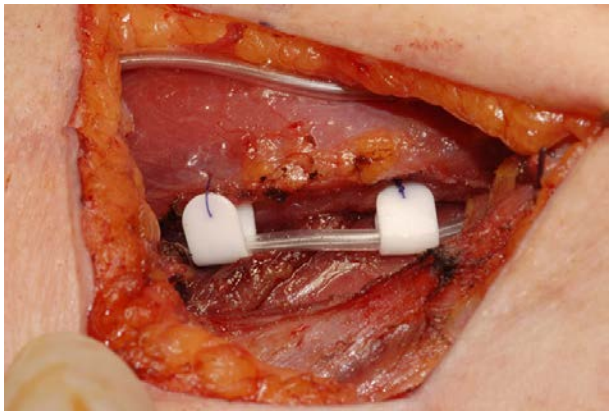
Adequate contact of both leads with the nerve was confirmed by application of the Neural Integrity Monitor (Medtronic) probe to the end of the stimulator wire at two

positions. Each position correlated with a single vagus nerve stimulator lead. (Supplementary figure III)



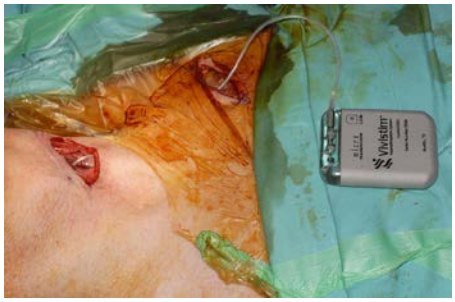
Supplementary figure III: Confirmation of lead contact with NIM probe to wire

Teflon cuffs were placed around the wire and sutured to soft tissue within the neck thereby securing it in place with a release loop to allow neck movement without tension being applied to the nerve which could cause lead displacement. (Supplementary figure IV)



Supplementary figure IV: Teflon cuffs sutured in place

A subcutaneous pocket was then created in the left pectoral region and the wire was tunnelled to allow the wire to connect to the pulse generator (Model 1000 Vivistim System, MicroTransponder Inc., Austin, TX). Contact of the leads was re-checked with the NIM probe to prior to securing the wire to the pulse generator. The pulse generator was placed within the subcutaneous pocket and sutured in place. (Supplementary figure V)



Supplementary figure V: Wire tunnelled and connected to pulse

Activity of the pulse generator was calibrated by a wireless programming device connected to computer running proprietary software (MicroTransponder) and impedance was measured across the leads. Integrity of the circuit was confirmed by observing corresponding nerve activity on NIM monitor when a stimulation pulse is triggered by the wireless device. (Supplementary figure VI).

Supplementary figure VI: Testing integrity of circuit with wireless device and NIM monitor

The omohyoid was repaired and the neck and chest incisions closed.

Participants were either discharged later that day or on the following day.

Details of Stimulation Parameters and SAPS Software

The Vivistim® system is an active implantable device consisting of an IPG, an implantable lead, external controller wand (for communication) and custom programming software. A netbook computer is loaded with the Stroke Application Programming Software (SAPS) and is used to set the stimulation parameters and load them into the patient's IPG via the wand. SAPS is a custom software used to program the stimulation parameters and imitate VNS via a pushbutton.

During therapy the IPG was programmed to output a 0.5 second burst of stimulation. The stimulation consisted of a 30 Hz signal of 100 μsecond constant current pulses with an amplitude of 0.8 mA.

Supplementary figure VII: The Vivistim IPG.

Therapy Tasks

The therapy tasks consisted of the following:

1. Reach and grasp objects

The task involves the ability to reach, grasp, manipulate and release a variety of objects along with a variety of strength and range of motion requirements and some degree of endurance (i.e., being able to stand during task performance).

2. Turning doorknobs and turning keys

The patient reaches and manipulates a variety of styles of doorknobs and latches. The patient also must insert and turn a key in a variety of locks.

3. Gross Movement Task

The patient performs a series of movements to reach overhead and behind their back.

4. Flip objects

This tasks involves pronation and supination to flip objects.

5. Eating

The patient performs common eating motions such as cutting, securing, and scooping while simulating eating.

6. Insert objects into wells

This tasks requires the patient to pick up small objects and place them in jars using the thumb and at least one additional digit.

7. Open and close a bottle or jar

The patient opens and closes various jars and bottles using finger dexterity and/or wrist motions.

In addition participants performed additional (at least one) tasks. One example was fastening and unfastening a belt buckle.

Long-Term Follow-Up and Post Implantation Care in VNS Group

Participants were able to choose to have the device removed at any point during the study or to enter a long-term phase and receive further treatment at 6 monthly intervals. In the event of removal, both the generator and the portion of the lead up to the electrode would be removed. The electrode would likely also be removed, unless in the opinion of the surgeon, after viewing the electrode and nerve, removal of the electrode would convey a risk of nerve damage.

Supplementary Results

Supplementary Table I

MRI Variable	VNS Group (n=9)	Rehabilitation Only Group (n=10)
Infarct volume, mm^3	85706.8 (76191.27)	55614.87 (86028.24)
Lesion to CST overlap volume, mm^3	8708.89 (5522.34)	5735.07 (5929.89)
CST FA ratio	0.72 (0.09)	0.81 (0.12)
MD ratio	1.45 (0.29)	1.28 (0.28)

Baseline MRI data by study group. MRI = magnetic resonance imaging. All values are mean (SD). CST = corticospinal tract. FA = fractional anisotropy. MD = mean diffusivity.

Supplementary Table II

	VNS Group (n=9)	Rehabilitation Only Group (n=10)
Infarct volume, mm^3	r = 0.22, p=0.567	r = -0.16, p=0.663
Lesion to CST overlap volume, mm^3	r = -0.03, p=0.936	r = -0.39, p=0.264
CST FA ratio	r = 0.19, p=0.631	r = 0.38, p=0.274
MD ratio	r = -0.02, p=0.967	r = -0.33, p=0.357

Correlation between change in FMA-UE score and baseline MRI variables in each study groups. Values shown are the Pearson correlation co-efficient and p value. CST = corticospinal tract. FA = fractional anisotropy. MD = mean diffusivity.

Supplementary Table III

	VNS Responder (n=6)	Rehabilitation Only Responder (n=4)	P Value
Infarct Volume, mm3	109666 (79767)	3779 (3391)	0.023
Lesion to CST Overlap Volume, mm3	1534 (1159)	1128 (1223)	0.036
FA ratio	0.79 (0.06)	0.85 (0.11)	0.014
MD ratio	1.39 (0.05)	1.17 (0.09)	0.034

Baseline MRI data in VNS treated responders compared to rehabilitation only responders. Response is defined as a > 6 point improvement in the FMA-UE score. Values shown are mean (SD). P values are for a 2-sample t test.