TRANSLATIONAL STUDIES IN THE DEVELOPMENT OF NEUROSTIMULATION BASED INTERVENTIONS FOR REHABILITATION OF DYSPHAGIA AFTER STROKE

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy (PhD) in the Faculty of Medical and Human Sciences, School of Medicine

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DR DIPESH HARSHVADAN VASANT

Centre for Gastrointestinal Sciences
Institute of Inflammation and Repair

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FINAL WORD COUNT 44,784 WORDS

ABSTRACT

Neural control of swallowing is hierarchical, involving the cerebral cortex and interactions with several other brain regions including the cerebellum. Cortical control of swallowing exhibits functional asymmetry, whereby brain lesions disrupting the stronger ('dominant') hemisphere are implicated in post-stroke dysphagia. A major breakthrough has been the consistent observation that compensatory changes (neuroplasticity) in the undamaged (contralesional) hemisphere are responsible for swallowing recovery. Whilst existing therapies lack evidence-base, neurostimulation interventions capable of facilitating this natural recovery process have the potential to revolutionise swallowing rehabilitation. Whilst data using several neurostimulation modalities have been promising, translating them into much needed clinical therapies has been hampered by clinical study designs lacking homogeneity.

In a series of studies, using three different modalities I describe a step-wise approach for developing neurostimulation interventions from bench-to-bedside. Firstly, in a proof of concept experiment, targeted cerebellar repetitive Transcranial Magnetic stimulation (rTMS) was assessed in healthy subjects (n=17), confirming frequency and duration specific (250-pulses of 10-Hz) induction of long-lasting changes in pharyngeal cortical plasticity, effects which were explored with therapeutic potential in a dysphagic patient. Secondly, in a pre-clinical model of post-stroke dysphagia, optimal parameters of cortical transcranial Direct Current Stimulation (tDCS) were tested, confirming reversal of transient neurophysiological and behavioural swallowing deficits induced by a 'virtual-lesion' (10 minutes, 1-Hz rTMS to the 'dominant' hemisphere) in 15 healthy subjects. Finally, in a randomised trial, optimal parameters and dosage (5-Hz, 10 minutes daily for 3-days) of Pharyngeal Electrical Stimulation (PES) were studied in acutely dysphagic stroke patients (n=36) which despite lower than desired recruitment, trended towards reduced dysphagia severity at 2-weeks, earlier hospital discharge and nasogastric tube removal were observed.

These studies have shown for the first time that the cerebellum is a viable target for non-invasive brain stimulation swallowing studies and that cortical tDCS can reverse experimental brain lesions, with both techniques having therapeutic potential for post-stroke dysphagia. These clinical trial data add to the increasing evidence base for PES, the modality with the most evidence to date, with longer-term follow-up. The difficulties encountered in the post-stroke clinical trial in both recruitment and outcome measures highlight the importance of mechanistic studies which have often been lacking, in optimising stimulation specific factors; site, duration, intensity, dosing and controls, prior to clinical trials. An independent, larger, multi-centre, international trial of PES, with greater resources is now required to definitively determine its clinical efficacy. In summary, there may be a role for several different neurostimulation modalities in different patient sub-groups and my preliminary observations lead me to hypothesise that future translation of these therapies will depend on targeting population tailored to specific interventions.

DECLARATION

Genetic data (sampled from 16 dysphagic stroke patients) discussed in Chapter 4 has been submitted in support of a MRes degree of Manchester University by Hani Essa an undergraduate medical student who did not have a role in data acquisition during the clinical trial but was provided with genotype and dysphagia severity data limited to these 16 subjects after the trial closure for analysis as part of his research training. The data analysis and interpretation presented here have however been undertaken independently.

No other portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

COPYRIGHT STATEMENT

THE AUTHOR

I was awarded MB ChB from the University of Manchester in 2006 after completing clinical training at the University Hospital of North Staffordshire where I achieved a distinction in a gastrointestinal physiology research project on dietary factors in gastro-oesophageal reflux disease under the supervision of Mr Mark Deakin and Dr Fiona Leslie. This project stimulated my interest in research and Gastroenterology. In 2009/10, I was awarded an NIHR academic clinical fellowship in Gastroenterology and achieved membership of the Royal College of Physicians whilst completing core medical training. During my early research training as an academic clinical fellow under the supervision of Professor Shaheen Hamdy, I furthered my interest in upper gastrointestinal physiology and motility, learning techniques including intraluminal electrical stimulation and pharyngeal/oesophageal electromyography whilst investigating the association of a common genetic polymorphism and oesophageal sensitivity. I presented this work nationally and internationally, with the highlight being awarded *best oral paper presentation* at United European Gastroenterology Week (UEGW 2011, Stockholm, Sweden). This paper has since been published in the journal *Neurogastroenterology and Motility (1)*. These positive early experiences encouraged me to apply for a Wellcome Trust funded clinical research fellowship to which I was appointed in January 2011.

ACKNOWLEDGEMENT

I would like to express deep thanks to my family, in particular my wife Mili, my daughters Anjali and Nisha and my parents for their constant support and encouragement and the countless sacrifices they have made along this journey. My mother Ushma deserves a lot of credit for my academic development during my formative schooling years where she invested a lot of her own time and sacrificed career aspirations to ensure I was focussed and on the right track. Without her considerable input I know I wouldn't have been in a position to write this thesis.

I would also like to thank my colleagues and friends at the gastrointestinal sciences department, in particular Drs. Satish Mistry and Emilia Michou and my supervisor Professor Shaheen Hamdy for whom I have a great deal of respect and admiration and have learnt so much. His expert guidance has always given me a sense of direction and inspiration whenever it was required. I would also like to place on record my gratitude to Professors John McLaughlin and David Thompson whose mentorship during the early phases of my integrated academic clinical training programme proved invaluable and to my advisor Professor Pippa Tyrrell.

ACKNOWLEDGEMENT OF CONTRIBUTION OTHER AUTHORS

In the work included in this thesis I confirm that I have had the major role in all aspects of production of the papers including: data acquisition, analysis and manuscript writing. My colleagues Dr Satish Mistry and Dr Emilia Michou have made contributions including advice on experimental design and assistance with delivery of randomised interventions ensuring adequate blinding. Dr Satish Mistry initiated the data collection process in the early subjects included in the 'virtual-lesion' transcranial direct current stimulation (tDCS) study and is therefore listed as joint first author in the published paper. Dr Emilia Michou, a trained speech and language therapist, performed blinded off-line videofluoroscopic analyses in the stroke patient clinical studies. Prof. Shaheen Hamdy and Prof. John Rothwell assisted with conceptualisation and advised on study

designs. University of Manchester statisticians Mr Andy Vail and Dr Neil O'Leary were the appointed trial statisticians that independently performed statistical analyses on the randomised control trial of pharyngeal electrical stimulation (PES) data (Chapter 4). Dr Tony Payton and colleagues at the Centre for Integrated Genomic Medical Research at the University of Manchester genotyped the salivary DNA samples which were obtained from a sub-group of patients in the randomised controlled trial of PES.

PRESENTATIONS AND PUBLICATIONS

Oral presentations:

1. “The novel brain stimulation intervention of Transcranial Direct Current Stimulation restores brain and swallowing function after ‘virtual-lesion’ to human pharyngeal motor cortex” Digestive Disease Week (DDW), San Diego, USA, 20 May 2012
2. “Contralesional application of the novel brain stimulation intervention transcranial direct current stimulation (TDCS) restores brain and swallowing function in the human pharyngeal motor cortex” British Society of Gastroenterology (BSG) / Digestive Disorders Federation 2012, Liverpool, 19 June 2012
3. "Neuronavigated repetitive cerebellar stimulation produces long-lasting activation of human cortical swallowing projections", BSG 2013, Glasgow. 25 June 2013
4. *Prize awarded for Best Basic Science Research Oral Paper Presentation*: "High-frequency neuronavigated cerebellar stimulation modulates human cortical swallowing projections", North West Gastroenterology 2013, Lythm St Annes, 5 July 2013.
5. "Long-term improvement in Dysphagia Severity following Pharyngeal Electrical Stimulation (PES) after acute stroke: A Phase II Double-blinded Randomised Controlled Trial, European Society for Swallowing Disorders 2013, Malmo, Sweden, 13 September 2013
6. "Frequency and duration specific modulation of human swallowing motor pathways induced by MRI-guided cerebellar repetitive Transcranial Magnetic Stimulation (rTMS)", DDW 2014, Chicago, USA, May 2014

7. "Pharyngeal Electrical Stimulation (PES) expedites swallowing recovery in dysphagia post acute stroke : A Phase II Double-blinded Randomised Controlled Trial", DDW 2014, Chicago, USA, May 2014
8. "Pharyngeal Electrical Stimulation (PES) in dysphagia post-acute stroke: A Double-blind, Randomised Trial", BSG 2014, Manchester, UK, June 2014

Poster Presentations:

1. “Pharyngeal Electrical Stimulation: an ongoing phase II randomised control trial for dysphagia after acute stroke” World Stroke Day Event, North West Stroke Research Network, 28 October 2011
2. “Neuronavigated repetitive magnetic cerebellar stimulation induces long-lasting changes in human swallowing motor pathways” Neurogastroenterology and Motility, Bologna, Italy, 7 September 2012
3. *Poster/ Mini-oral Presentation (best posters section):*“Sustained frequency dependent changes in human pharyngeal motor cortex excitability can be induced by neuronavigated cerebellar repetitive transcranial magnetic stimulation (rTMS)”, ESSD 2012 Barcelona, Spain, 27 October 2012
4. "High-frequency neuronavigated cerebellar repetitive Transcranial Magnetic stimulation (rTMS) increases human pharyngeal motor cortex excitability", International Conference on Non-invasive Brain Stimulation 2013, Leipzig, Germany, 20 March 2013
5. *Poster of Distinction:* "Activation of human cortical swallowing projections by high-frequency neuronavigated cerebellar stimulation", DDW 2013, Florida, USA, 20 May 2013
6. *Poster of Distinction:* "The excitatory effects of repetitive cerebellar brain stimulation on human swallowing motor pathways are critically dependent on stimulus duration", BSG 2014, Manchester, June 2014

7. *Best Poster Prize award:* "The excitatory effects of repetitive cerebellar brain stimulation on human swallowing motor pathways are critically dependent on stimulus duration", University of Manchester Postgraduate Research Showcase, September 2014

Journal Papers:

1. Contralateral transcranial Direct Current Stimulation (tDCS) reverses the neurophysiological and behavioural swallowing effects after focal cortical inhibition of the human pharyngeal motor cortex Vasant DH, Mistry S, Michou E, Jefferson S, Rothwell JC, Hamdy S. The Journal of Physiology 2014 592(4): 695–709, 2014
2. High-frequency focal repetitive cerebellar stimulation induces prolonged increases in human pharyngeal motor cortex excitability Vasant DH, Michou E, Mistry s, Rothwell JC, Hamdy S. Prepared for submission to The Journal of Physiology

Book Chapters:

1. “Direct and indirect therapy: Neurostimulation for the treatment of Dysphagia after Stroke” Medical Radiology, Mistry. S, Michou, E, Vasant DH, Hamdy. S, 2012, 519-538.

2. “Central Control of Swallowing”, Vasant DH and Hamdy S, Principles of Deglutition: A Multidisciplinary Text for Swallowing and its Disorders, Springer Science 2012, 55-65.

LIST OF ABBREVIATIONS

APB	Abductor Pollicis Brevis
BDNF	Brain Derived Neurotrophic Factor,
CPG	Central Pattern Generator
DSR	Dysphagia severity rating scale,
EMG	Electromyography
FEES	Fibreoptic endoscopic evaluation of swallowing
FMRI	Functional Magnetic Resonance Imaging
ISI	Interstimulus Interval
LTD	Long-term Depression
LTP	Long-term Potentiation
MEG	Magnetoencephelography
(P)MEPs	(Pharyngeal) motor evoked potentials
(T)MEPs	(Thenar) motor evoked potentials
MRS	Modified Rankin scale
NIHSS	National Institutes of Health stroke scale
NGT	Nasogastric feeding tube
PAs	Penetration-aspiration scale
PEG	Percutaneous Endoscopic Gastrostomy
PES	Pharyngeal Electrical Stimulation
rMT	Resting Motor Threshold
rTMS	Repetitive Transcranial Magnetic Stimulation
SALTs	Speech and language therapists
SEM	Standard error of the mean
SRT	Swallowing Reaction Time
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
TOR-BSST	Torronto bedside swallowing test
UOS	Upper oesophageal sphincter
VFS	Videofluoroscopy

:,

CHAPTER 1

INTRODUCTION

Through advances in neuroscientific research there is now increased understanding of the pathophysiology of dysphagia after unilateral stroke and the natural recovery process of swallowing function. There is however an unmet clinical need for targeted therapies (such as neurostimulation interventions) capable of facilitating the natural swallowing recovery process. Experience from our department over the past decade in a series of studies using a peripheral modality of neurostimulation; pharyngeal electrical stimulation (PES) (2-6), suggests a step-wise translational approach from the research laboratory towards the bedside is required in developing a potential neurostimulation-based therapy. Figure 1.1 summarises the translational step-wise model which has been followed in developing PES and this figure will be cross-references throughout the text that follows. To varying extents, a similar approach has been applied to characterise different modalities of cerebral cortical stimulation (7-11) and a combination of peripheral and cortical stimulation (intermittent Paired Associative Stimulation (iPAS) (12-14)). The efficacy of all the neurostimulation techniques trialled have been found to be critically dependent on factors such as stimulation parameters (frequency, number of stimuli, duration and intensity), stimulation site used (electrode placement, hemisphere selected (cortical methods)) and dose (number of stimulation sessions). Unfortunately, despite this dictum, several small clinical studies using cortical stimulation have progressed to clinical trials without using evidence-based parameters and varying stimulation sites (15-22) which have unsurprisingly proved inconclusive and confusing and have inevitably delayed adoption of these techniques. Given that stroke patients often have significant morbidity, it is important that evidence-based parameters and efficacy are confirmed in healthy subjects prior to progression to studying patient populations. Disrupting swallowing neurophysiology and behaviour in healthy subjects using focal inhibitory brain stimulation (‘virtual-lesion’) has been shown to be a useful model for trialling optimised parameters of neurostimulation in a controlled environment. Swallowing neurophysiology and behaviour can be studied in health and disease using a variety of non-invasive techniques that will be introduced in this chapter.

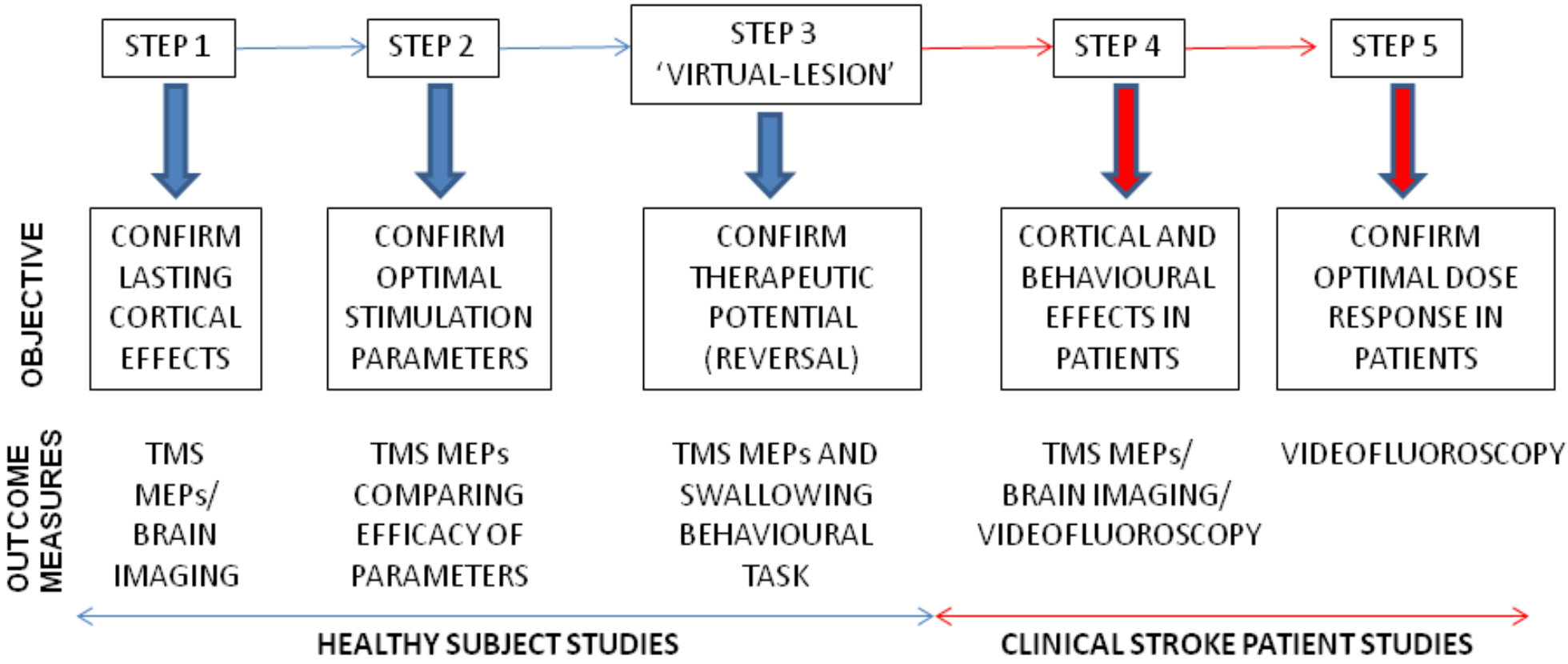


Figure 1.1 A translational model for developing neurostimulation interventions for post-stroke dysphagia. A step-wise approach prior to randomised clinical trials (based on experience from Pharyngeal Electrical Stimulation studies).

The aims of this thesis are to develop three different promising neurostimulation techniques at three different translational stages of development. Cerebellar stimulation is a completely novel approach in the swallowing literature which has not previously been characterised, therefore I completed steps 1 and 2 of the algorithm (Figure 1.1) before attempting a proof-of-concept case-study (step 4) in a stroke patient. Transcranial Direct Current stimulations (tDCS), is a promising brain stimulation modality (steps 1 and 2 completed). I therefore applied evidence-based parameters after a 'virtual-lesion' (step 3) in healthy subjects. Finally, pharyngeal electrical stimulation has been extensively studied (steps 1-5 completed) but there are currently no long-term (beyond 2-weeks) follow-up data in randomised controlled trials in acute stroke patients.

Before considering the pathophysiology and rehabilitation of oropharyngeal dysphagia after stroke, it is important to understand normal deglutative anatomy, physiology and neural control; which are discussed at the beginning of the chapter.

1.1 NORMAL SWALLOWING

Swallowing, otherwise known as deglutition, is an essential neuromuscular process involving co-ordination of 26 pairs of muscles, four cranial nerve motor nuclei and peripheral afferent inputs. This co-ordination in healthy individuals is vital for ensuring safe transport of ingested material from the mouth to the stomach for digestion without compromising the airway. Swallowing is a complex process which divided into four phases for descriptive purposes; preparatory, oral, pharyngeal and oesophageal phases (23).

1.1.1 Anatomical Structures

Important anatomical regions involved in swallowing include the upper gastrointestinal tract (oral cavity, pharynx and oesophagus) and upper respiratory tract (larynx). Figure 1.2 shows the anatomical landmarks of the mouth, larynx and pharynx which are involved in swallowing and the pharyngeal musculature.

The oral cavity, the site of mastication, begins at the lips and contains 32 teeth and is separated from the pharynx by the faucial pillars. The key anatomical structure for swallowing in the oral cavity is the anterior two thirds of the tongue, which is under voluntary control via the cerebral cortex. The posterior third of the tongue is within the pharynx and is mostly under the influence of the brain stem. The tongue has intrinsic muscles which enable changes in the shape of the tongue and movements such as retrusion, protrusion or lateral displacement. Extrinsic muscles enable tongue movement in relation to the mandible, hyoid bone and petrous aspect of the cranium (24).

The functional pathway of the ingested bolus as it progresses on its journey from the mouth to through to the oesophageal opening is often known as the 'foodway'. The bolus passes in sequence from the mouth posteriorly to the oral cavity towards the oropharynx before passing inferiorly into the hyopharynx (25). The pharyngeal palate, pharyngeal portion of the tongue, the oropharynx and the hypopharynx are the mobile pharyngeal structures which are actively involved in swallowing and intersect the food passage and airway (24).

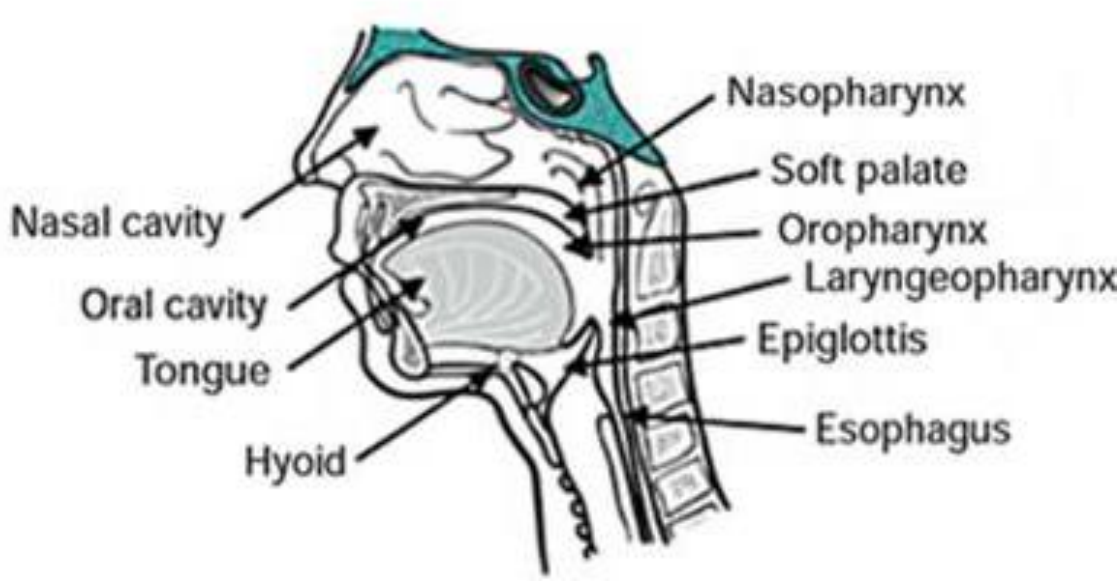


Figure 1.2 Anatomical landmarks and musculature of swallowing (*GI Motility online* | doi:10.1038/gimo5)

The superior, middle and inferior constrictor muscles of the pharynx are circular muscles, which serve to propel the bolus into the oesophagus. These constrictor muscles are attached to the cranium and hyoid bone and thyroid cartilage anteriorly, and insert on a posterior median raphe (26). The inferior constrictor muscle (cricopharyngeus) is attached to the sides of the cricoid cartilage anteriorly and closes the upper Oesophageal sphincter (UOS) by compressing it against the back of the cricoid cartilage (26).

1.1.2 Physiology of swallowing

Understanding normal swallowing physiology is vital to accurate diagnosis of swallowing disorders (27). Initiation of the oral phase of swallowing is under voluntary control. During the oral preparatory phase, the process of mastication sends sensory afferent information from the dorsum of the tongue and periodontal region that are important in regulating bolus consistency as well as lingual propulsive forces to aid transport to the pharynx (28). By the end of the preparatory phase a cohesive bolus is formed and mixed with saliva. (Figure 1.3a) The oral stage only lasts between 1-1.5 seconds and the oral portion of the tongue rolls posteriorly, generating peristaltic forces by making contact with the hard and soft palate, propelling the bolus into the pharynx (Figure 1.3b) (23, 27). The obicularis oris anteriorly and the elevation of the posterior tongue provide a seal to guard against leakage of ingested material during the oral phase.

The pharyngeal phase typically occurs within 1 second and has two purposes, firstly transport of the bolus through the pharynx and UOS and secondly to protect the airway during passage of food material (26). Arrival of the bolus in pharynx triggers a sequence of co-ordinated muscular events involving stabilisation of the closed mandible, hyoid bone movement and posterior tongue movements. After the pharyngeal wall meets the tongue, the bolus is then propelled by waves of contraction. Laryngeal and hyoid movements serve to protect the airway and laryngeal movement facilitates opening of the UOS (Figure 1.3c) (29).

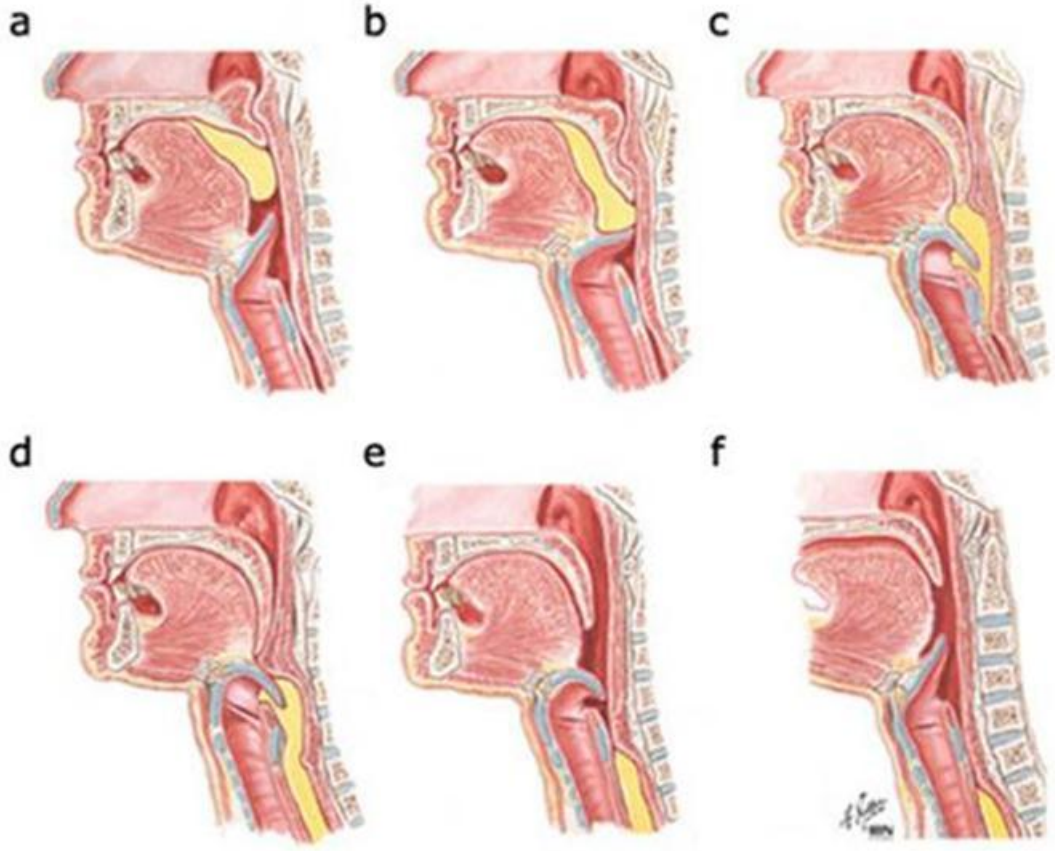


Figure 1.3 Schematic Diagram showing bolus and palatal movements during (a)- oral phase of swallowing. (b-c)- pharyngeal phase (d-f) Oesophageal phase

GI Motility online | doi:10.1038/gimo2

The oesophageal phase of deglutition is also reflexive and is triggered by bolus stimulation at the proximal oesophagus. Sensory receptors here are thought to transmit this information via the Superior Laryngeal Nerve (SLN) and Recurrent Laryngeal Nerve (RLN) (30). Once the cricopharyngeal muscles relax the bolus passes into the oesophagus and primary peristaltic waves then transmit the bolus to the stomach (Figure 1.3 d-f). The Brain Stem Central Pattern Generator (CPG) has a crucial role in co-ordinating the time interval between the pharyngeal and oesophageal phases of swallowing, to ensure efficient bolus transfer. This is achieved by separate neurally mediated excitatory and inhibitory mechanisms (30). The inhibitory mechanism affects both the longitudinal and circular muscle layers of the oesophageal wall (31). This inhibition is important particularly after rapid sequential swallowing where it prevents the potentially obstructive situation of two unsynchronised oesophageal peristaltic waves occurring at the same time (30).

1.1.3 Cerebral cortical control of swallowing

Historically, it was believed that the central neural control of swallowing was almost entirely dependent on brain stem reflexive mechanisms (29). We now understand that deglutition is a dynamic process which can be initiated volitionally via the cerebral cortex and controlled by the central nervous system in a "multi-dimensional fashion" (29, 32-33) with both volitional and reflexive components as illustrated by Figure 1.4. In particular there is emphasis on descending cortical inputs to the brain stem, in association with sensory feedback which can influence cortical activity and hence motor output to swallowing musculature via the CPG.

Early evidence implicating regions of the cerebral cortex and the brain stem in the neural control of swallowing were based on neurophysiological observations in animals such as the seminal studies by Miller and Sherrington (34-35). Thereafter, Penfield et al. using direct electrical brain stimulation in anaesthetised humans during neurosurgery demonstrated that stimulation to certain parts of the cerebral cortex can induce swallowing (36). In more recent times, studies using non-invasive cortical stimulation techniques have mapped areas of the cortex representing human swallowing musculature (37-38). Other evidence indicating cortical involvement came from reports of dysphagic stroke patients without brainstem disease with only unilateral cortical involvement (39-41). In the past decade, significant advances in neuroscientific research using functional brain imaging techniques have taken this further and improved our understanding of swallowing neurophysiology and the functional neuroanatomy of the brain structures involved in swallowing. These important techniques will be discussed in detail later in this chapter.

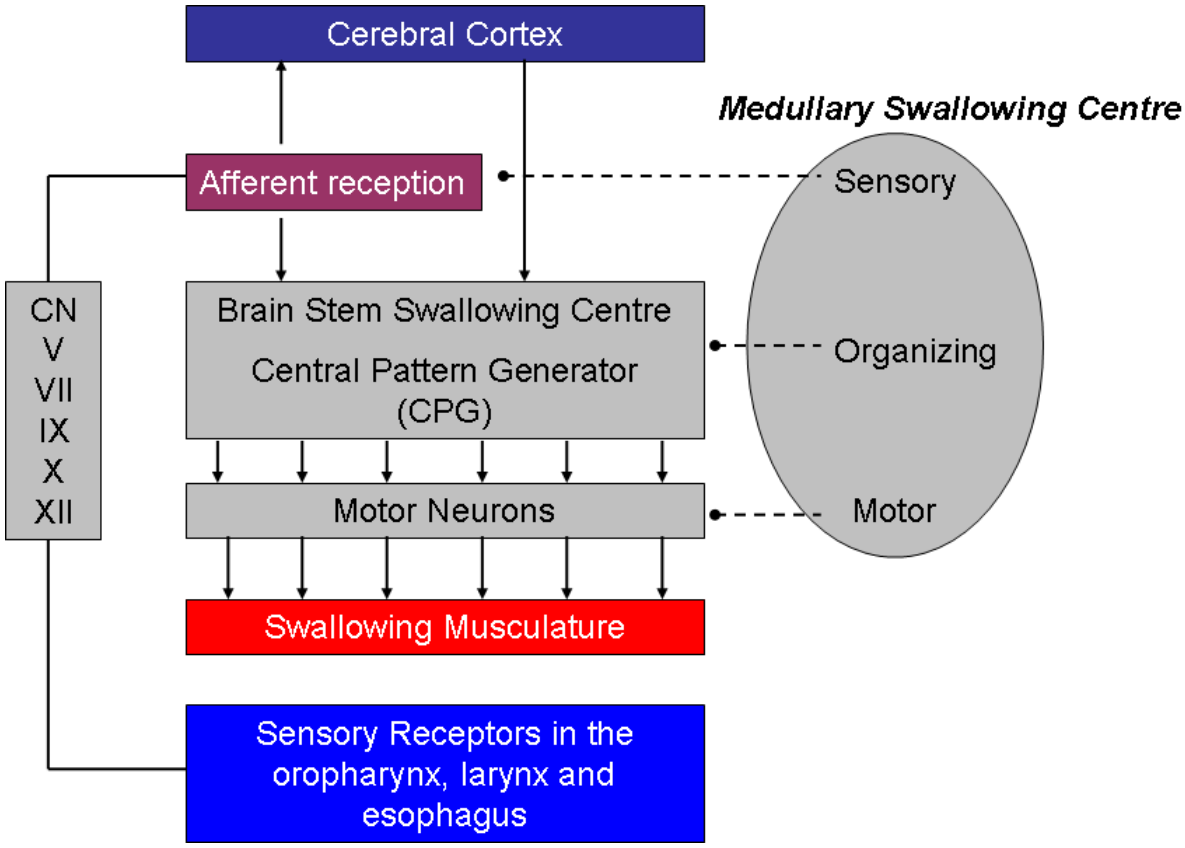


Figure 1.4 The multidimensional model of central neural control in human swallowing (modified with permission from Diamant N et al) (42)

1.1.4 The role of the cerebellum in swallowing

Until recently our understanding of the role of the cerebellum in the control of swallowing has been limited, with no previous neurophysiological studies published.

Previous links in the animal literature which suggested the cerebellum may be implicated in swallowing, included observations that throat contractions and overt swallowing occurred following cerebellar stimulation in cats (Mussen 1927; Mussen 1930). Cerebellar stimulation using neurosurgically implanted cerebellar electrodes in cats has been shown to facilitate chewing, swallowing and predatory attack of prey at high intensities (43). A more recent study by Zhu et al. involving cerebellar stimulation in rats reported altered feeding regulation. The authors have

suggested that interactions between the cerebellum and gut hormones may modulate feeding behaviour via projections to the hypothalamus (44).

Bilateral cerebellar activation has been consistently identified during swallowing in several functional brain imaging studies (Table 1.2) (45-49). Meanwhile there is some evidence that cerebellar activation during oral tasks such as throat clearing and tongue tapping is minimal, suggesting cerebellar involvement may be more in the co-ordination of sensory input with motor output during the pharyngeal phase of swallowing and modulating the pharyngolaryngeal muscles (49). Observations from instrumental swallowing studies in dysphagia post acute stroke support this hypothesis, where 5 cerebellar stroke patients were found to have no delay of the pharyngeal swallow but abnormal pharyngeal control (50).

Other pathological correlations come from the association of neurodegenerative diseases affecting the cerebellum such as spinocerebellar ataxia with dysphagia and increased probability of transient post operative dysphagia in children after removal of posterior fossa tumours (51-52).

The largely circumstantial evidence presented above does suggest a role for the cerebellum, particularly in the pharyngeal phase of deglutition but highlights a gap in the literature. Important to the context of the work presented in this thesis, recent experiments exploring the effects of non-invasive cerebellar stimulation on swallowing motor pathways have recently provided further evidence for a role of the cerebellum in swallowing neurophysiology (53) and these findings will be discussed in detail in the next section of this chapter.

1.2 BRAIN IMAGING AND SWALLOWING NEUROPHYSIOLOGY

1.2.1 Pharyngeal Motor Evoked Potentials (PMEPs) to cortical and cerebellar Transcranial Magnetic Stimulation (TMS)

In 1831, Michael Faraday discovered electromagnetic induction, when he observed that electrical voltage could be induced by a circuit in a changing magnetic field (54). One hundred and fifty years later, Barker et al. applied these principles when they introduced TMS (55), discovering that discharging a large pulse of current through copper coils placed tangentially on the scalp altered the magnetic field, inducing electrical current in the brain. This has proved to be a major advance compared to electrical stimulation and direct brain stimulation techniques, allowing painless stimulation of the human brain and deep peripheral nerves. Typically, TMS stimulator units consist of an energy storage capacitor with a low series resistance that is discharged into a stimulation coil by a solid-state switch (56).

Cortically applied Transcranial Magnetic Stimulation

TMS is a non-invasive tool, which can be used to investigate human motor corticospinal tracts and determine cortical excitability and brain re-organisation after injury (56). When applied to regions of the primary motor cortex which produce electromyographic (EMG) responses, motor evoked potentials (MEPs) can be recorded from the target muscle. There is a delay from the discharge of the stimulus until the onset of the response (response latency). The peak-to-peak amplitude is recorded as a measure of excitability. Figure 1.5 illustrates the use of cortically applied TMS to record pharyngeal MEPs via an intraluminal catheter with implanted ring electrodes and shows an example of a pharyngeal MEP (PMEP) response.

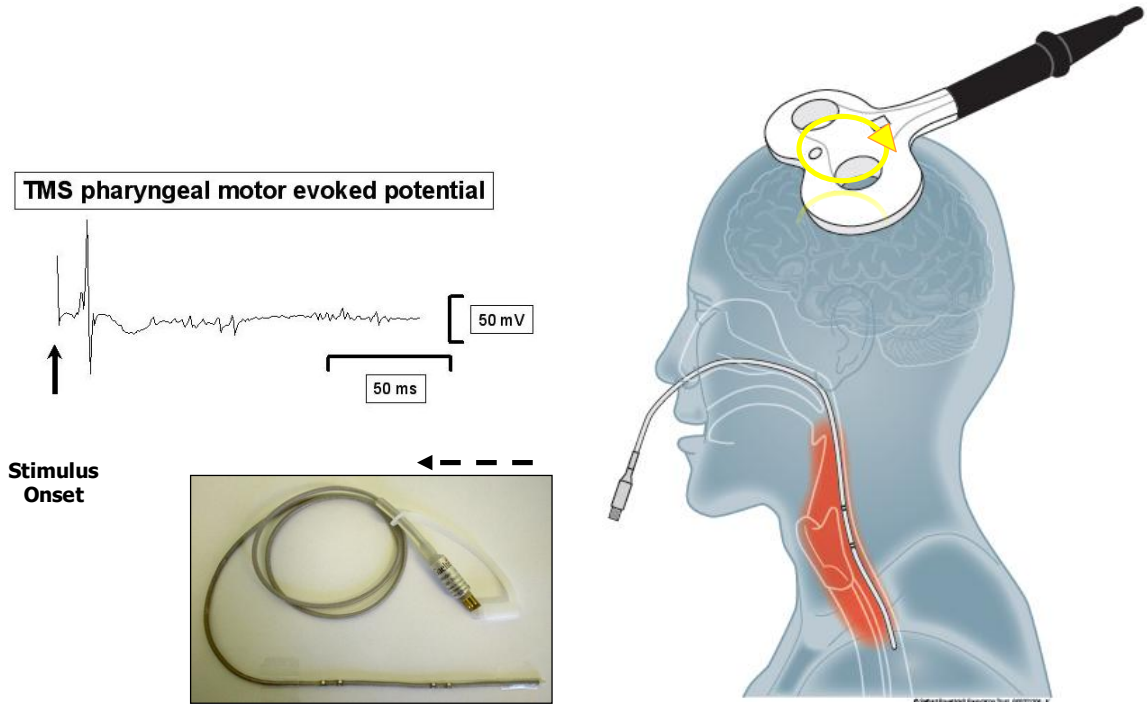


Figure 1.5 Schematic illustration of a cortically evoked pharyngeal motor evoked potential (Reproduced with permission from University of Manchester GI sciences department archive. Image created by Medical illustrations department Salford Royal Foundation Trust)

Studies probing cortical projections to the pharynx have shown that muscle groups involved in swallowing are represented bilaterally, but asymmetrically in the human motor and premotor cortex areas in somatotopic fashion with the mylohyoid lateral and the pharynx more medial (38, 57). Figure 1.6 illustrates this pharyngeal functional asymmetry in an individual with a stronger right swallowing hemisphere (38). This clinically relevant finding of asymmetric bilaterality, independent of handedness, suggests that humans have a 'dominant' swallowing hemisphere.

These TMS studies for the first time provided a clear description of cortical maps of cerebral areas involved in the corticobulbar pathway; demonstrating that multiple regions of the cerebral cortex could be stimulated to induce motor responses in swallowing musculature. Subsequent studies have confirmed that TMS cortical mapping of pharyngeal musculature is a highly reproducible technique(58). TMS has frequently been used to study cortico-pharyngeal representation and excitability in health and diseased states including post stroke dysphagia(59). Given that changes

in cortical excitability as measured by changes in the amplitude of MEPs have been demonstrated to correlate closely with changes in cortical activation on functional brain imaging scans (4-5, 11, 13, 60) and swallowing function (4, 6, 12, 17, 61-63), TMS has proven to be an extremely important tool for evaluating the efficacy of interventions for post-stroke dysphagia.

Cerebellar Transcranial Magnetic stimulation

Non-invasive Cerebellar stimulation, had been previously used safely to elicit upper limb responses, where initial studies using electrical stimulation by Ugawa et al. showed that cerebellar stimulation in the appropriate position inhibited hand motor responses to cortical stimulation (64). The same group and others replicated these findings using a paired-pulse paradigm of Cerebellar Transcranial Magnetic Stimulation (TMS) followed by Cortical TMS to assess evoked motor potentials (65-66). These studies have shown an inhibitory effect of cerebellar stimulation on motor cortex excitability. Using a similar paired-pulse experimental design, Jayasekeran et al. recently assessed the effects on PMEPs and discovered that not only could they evoke distinctive pharyngeal responses by directly stimulating the cerebellum (Figure 1.7 a and b), with cerebellar-cortical TMS pulses at interstimulus intervals of 50-200ms, they were able to increase excitability in the pharyngeal motor cortex (53). In summary, this novel study has shown that cerebellar stimulation produces excitation of the pharyngeal motor cortex and suggested that the neural network from the cerebellum to the pharyngeal muscles is different to those that supply the hand, given that inhibitory changes have been seen in the hand following cerebellar stimulation, triggering our hypothesis that cerebellar neurostimulation could be applied to facilitate the recovery of dysphagia after stroke. This will be one of the main focuses of this thesis and will be addressed in the next chapter.

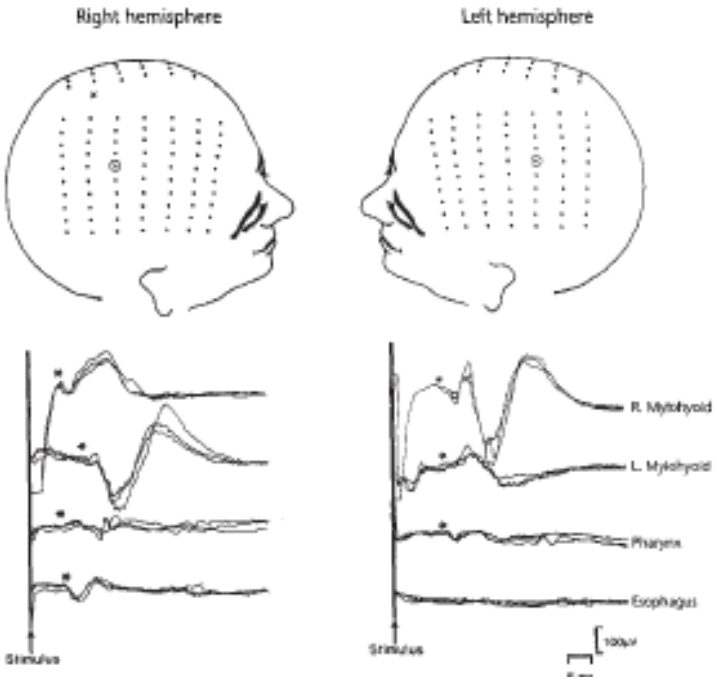


Figure 1.6 Functional asymmetry in the pharyngeal motor cortex. Reproduced with permission (38). Pharyngeal Electromyography (EMG) responses after the TMS stimulus are larger on the right hemisphere, indicating that this individual has right pharyngeal hemispheric dominance. (Cross on scalp indicates vertex position and dot indicates the site of stimulation where a TMS pulse was delivered)

A)

B)

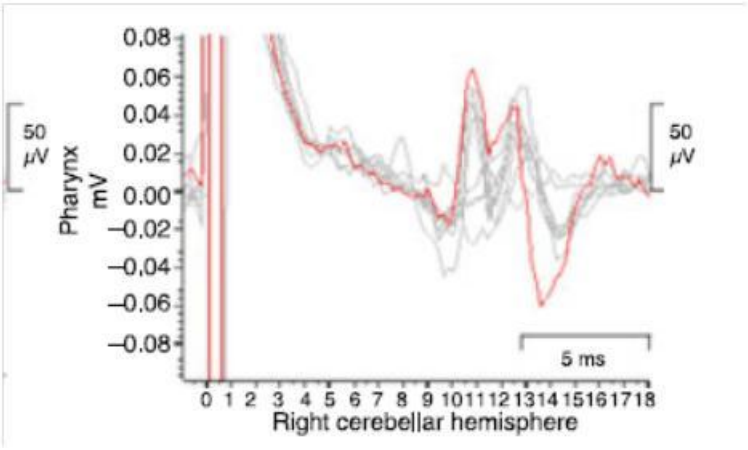


Figure 1.7 – Cerebellar evoked PMEPs; A) photograph showing coil targeted over the cerebellar midline B) Typical biphasic morphology of a cerebellar evoked pharyngeal motor evoked potential (PMEP) reproduced with permission (53).

Navigated TMS (neuronavigation) using frameless stereotaxy

MEPs from swallowing musculature and TMS mapping of cortical swallowing representation have previously been shown to be a highly reproducible and reliable technique(58, 67). One of the main perceived challenges in TMS studies in general has been accurate positioning of the magnetic coil above the relevant brain region and controlling for individual variance in neuroanatomy (68). Since the initial cortico-pharyngeal topographic mapping work by Hamdy et al., where optimal coil positions for PMEPs ('hot-spots') were reported at a mean coil position of 5cm anteriorly and 7cm laterally from the cranial vertex (38), consistent with the anatomical variation argument, a number of subsequent pharyngeal cortical TMS mapped studies have generally reported a more posterio-medial mean 'hot-spot', with lateral distances often closer to 4cm and anterior distances ranging from 2-5cm from the cranial vertex (9, 12-14, 69), whereas a study from a different group reported a more lateral pharyngeal representation (58). Additionally, Jayasekeran et al. found that the mean cerebellar 'hot-spots' for evoking PMEPs were ~4cm lateral to the inion for either cerebellar hemisphere. One way of ensuring that anatomical variance is controlled for is to perform cortical TMS mapping in every subject to determine individual 'hot-spots' for the subject instead of using mean 'hot-spot' data from previous studies.

Modern neuronavigation systems based on devices used by neurosurgeons have now been developed to target TMS coil placement to within millimetres of an intended cortical target. These devices utilise frameless stereotaxy to monitor head position, relative to the coil position and the stimulated brain region visualised over a 3 dimensional reconstruction of the subjects' brain scan and head surface on the computer screen. Briefly, this is achieved via an optical tracking system, with three LEDs fixed to both the head (via a head band) and the TMS coil (Figure 1.8). The camera then calculates the spatial position of the LEDs from both head and coil relative to the MRI scan and submits these co-ordinates to the common reference system. A referencing procedure is performed to co-register various anatomical landmarks with the 3D reconstruction of the head (68). Whilst a neuronavigated approach is expensive, studies have shown that in 'standard TMS' mapping is anatomically imprecise (70), with a minority of subjects (10%) can erroneously be stimulated in adjacent functionally distinct cortical regions, generally within a 2cm of the target (71). A few subsequent comparative studies have shown superior neuronavigated stability of EMG traces, MEPs amplitudes and superior efficacy of targeted cortical interventions (72-74). A neuronavigated TMS approach is particularly useful when targeting specific brain regions such as the cerebellum. Until now, there have been no previous studies adopting neuronavigated TMS in the swallowing literature. In Chapter 2 we attempted to characterise cerebellar neurostimulation using a neuronavigated approach for the first time.

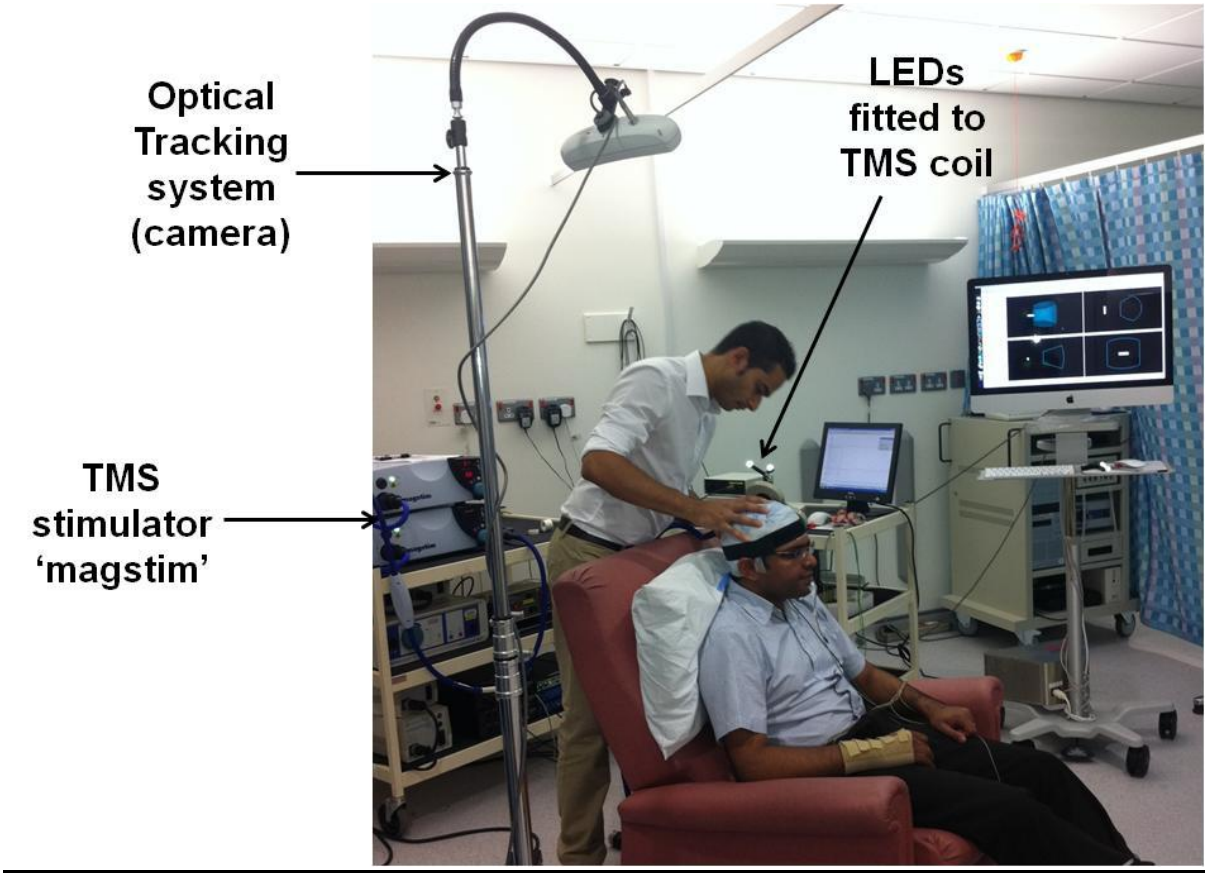


Figure 1.8 Laboratory set-up for neuronavigated-TMS mapping studies

1.2.2 Functional Brain imaging techniques

One of the main limitations of TMS is that it cannot determine the functional relevance of these corticofugal projections to swallowing function. Table 1.1 lists the different brain imaging techniques available to study swallowing neurophysiology, briefly outlining how each method works.

FMRI is now widely available to researchers and has been extensively used to assess cortical regions involved in swallowing (Figure 1.9) (45, 47, 75-79). Techniques have now improved to reduce motion artefact. Diffusion-weighted fMRI is a highly sensitive, relatively new modality, with the added advantage of being more resistant to motion artefact (80) and is being increasingly utilised in stroke medicine and brain connectivity studies. The principles of this technique primarily involve detection of signals generated by water motion in voxels of tissue (81). Early data from the first study of its kind in the swallowing literature using this technique has mapped in detail the

neural fibre tracts activated during tongue movements, volitional water swallowing and saliva swallows. Particular areas of activation included Primary motor cortices (Brodmann area (BA) 4) during water swallowing. Whereas tongue elevation and saliva swallows BA 6 (pre and supplementary motor cortices) and BA 20, 22 and 44. This work has provided further evidence for cerebral asymmetry with larger tract volumes seen in the dominant swallowing hemisphere (82).

Both fMRI and Positron Emission Tomography (PET, Figure 1.10) studies also demonstrate changes in cortical function by way of altered regional cerebral blood flow but fMRI is often preferred given the absence of exposure to ionising radiation and it has excellent spatial resolution. The main limitation of fMRI is that due to poor temporal resolution it is not possible to accurately follow the sequence of activations during execution of a sequential task such as swallowing. This is where Magnetic Encephalography (MEG) has proved useful.

Numerous studies using the aforementioned functional brain imaging techniques have consistently reported functional asymmetry in the swallowing cortex, with the left hemisphere being most frequently cited (45, 47, 75-79).

Table 1.1 compares the advantages and limitations for each of these brain imaging techniques in investigating human swallowing pathways. Table 1.2 shows the main cortical and subcortical regions that have been identified as active during swallowing by each of these scanning modalities.

A meta-analysis including 10 studies and a total of 98 subjects studied during volitional swallowing using functional brain imaging techniques and interpretation of voxel activity recently reported on local activity likelihood (ALE) in cortical regions (83). It was determined that during volitional water swallows 12 areas had significant ALE (Figure 1.11). The left and right sensorimotor cortex, right inferior parietal lobe and right insula were found to have the highest ALE. A systematic review including 14 studies using fMRI in healthy subjects during swallowing showed similar data (84).

The primary motor cortex was again found to be the most prevalent region of activation, followed by the primary sensory cortex (S1, Brodmann's area (BA) 3, 2, 1). The insula and anterior cingulate cortex (BA 32, 33) were also commonly activated during swallowing. Other cerebral sites are activated during swallowing but not consistently with some variability in studies and in individuals (85).

Table 1.1 Advantages and disadvantages of the different modalities used to assess swallowing neurophysiology in health and disease (86)

Imaging Modality	Mode of detecting cortical activity during swallow	Advantages	Limitations
Transcranial Magnetic Stimulation (TMS)	• Electromagnetic fields used to induce activity in neural tissue below stimulator site • Pharyngeal response measured using Electromyography (EMG)	• Non-invasive • Can be performed at bedside • Easier in dysphagic patients (no swallow required)	• Unable to assess functional neuroanatomy • Unable to study cortical activity during a swallowing task
Functional Magnetic Resonance Imaging (fMRI)	• Alterations in cortical blood flow reflect changes in cortical activity • Blood-Oxygen-Level-Dependent (BOLD)	• Detailed neuroanatomy (Spatial resolution 2 mm) • Single-event related approach gives specific cortical activity during a task and reduced motion related artefact. • No exposure to ionising radiation	• Limited temporal resolution • Swallowing during scan can be difficult for dysphagic subjects
Positron Emission Tomography (PET)	• Alterations in cortical blood flow reflect changes in cortical activity • H2 15O injection to estimate blood flow	• Better spatial resolution in subcortical areas than FMRI	• Unable to use single event related approach • temporal resolution inferior to fMRI • Ionising radiation exposure
Magnetoencephalography (MEG)	• Cortical neuronal activity shown by detection of postsynaptic magnetic fields	• Similar spatial resolution to fMRI and PET • Superior temporal resolution (milliseconds) • Can be used during motor task. • No exposure to ionising radiation	• Availability

Table 1.2 Summary of the main cortical and sub-cortical activations associated with swallowing, as identified by functional brain imaging studies.

Table Reproduced from *GI Motility Online* (2006) with permission

Brain region	PET	fMRI	MEG
Sensorimotor cortex	✓	✓	✓
Insula	✓	✓	
Anterior cingulated	✓	✓	✓
Posterior cingulated		✓	✓
Supplementary motor cortex	✓	✓	✓
Basal ganglia	✓	✓	
Cuneus	✓	✓	
Precuneus	✓	✓	✓
Temporal pole	✓	✓	
Orbitofrontal cortex	✓	✓	
Cerebellum	✓	✓	
Brainstem	✓	✓	

PET, positron emission tomography;

fMRI, functional magnetic resonance imaging;

MEG, magnetoencephalography

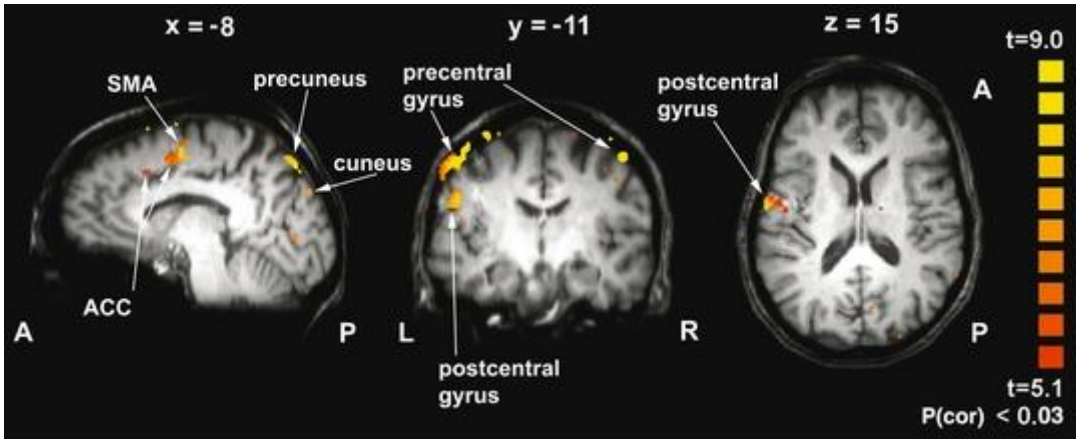


Figure 1.9 Functional MRI during volitional swallowing. Reprinted with permission from *Martin RE et al (76).* This image shows brain activation associated with voluntary water bolus swallows using fMRI.

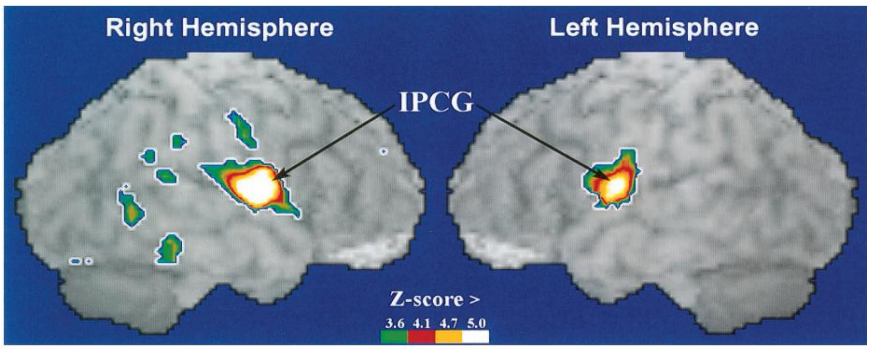


Figure 1.10 Positron Emission Tomography (PET) Image. reproduced with permission from *Zald D et al (87).* Cortical activation seen at the precentral gyrus and other lateral cortical regions. The strongest activations localize to the inferior precentral gyrus (IPCG). In the right hemisphere, this focus extends into the adjacent inferior postcentral gyrus. Additional foci are seen in the right hemisphere within the inferior, superior and middle temporal gyri.

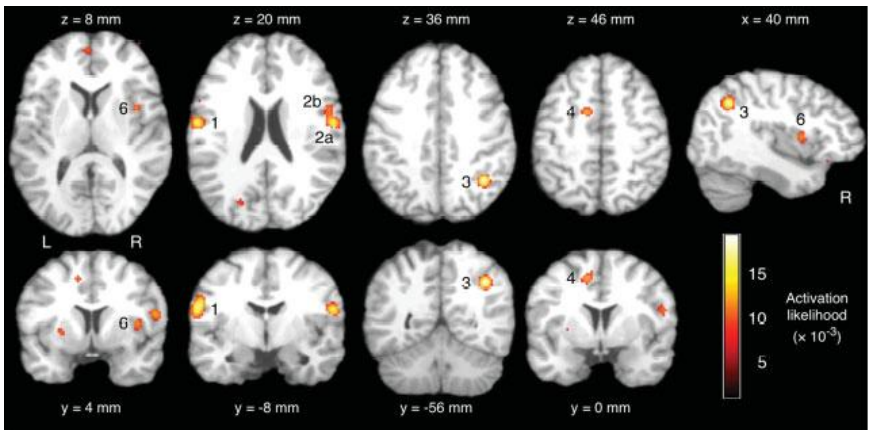


Figure 1.11 Brain activity associated with water swallowing. Significant activation clusters included the left precentral gyrus (1), right postcentral (2a) and inferior frontal gyrus (2b), right inferior parietal lobule (3), left cingulate gyrus (4), and right insula (6). Reprinted with permission from; *Soros. P et al (83).*

1.3 DYSPHAGIA

The term dysphagia comes from two greek words (‘dys’ and ‘phage’) which translate to “difficulty eating”. Dysphagia can be classified anatomically depending on the anatomical phase affected (i.e. oropharyngeal, pharyngeal or oesophageal).

1.3.1 Signs and symptoms of dysphagia

Dysphagic patients present with a variety of symptoms. Accurate history taking may help the astute clinician differentiate between neurological and structural causes of dysphagia by establishing the progressiveness and order of difficulty swallowing for liquids, solids or both. Common presenting symptoms include regurgitation, choking, coughing, difficulty chewing, food sticking in the throat, difficulty initiating swallow and weight loss. Signs of dysphagia which indicate in increased aspiration risk include change in the character of the voice (wet voice), indication of laryngeal dysfunction and weak voluntary cough. Currently a bedside water swallow screening test is recommended for all stroke patients (88). Cervical auscultation of swallowing sounds is a controversial technique which has been shown to have variable reproducibility rates dependant on the observer and is not currently in the national clinical guidelines (89).

1.3.2 Instrumental tests for dysphagia diagnosis

Stroke patients with suspected dysphagia after bedside screening tests all receive more detailed bedside clinical evaluation by trained speech and language therapists (SALTs) . Bedside examinations are not highly specific or highly sensitive (90). Instrumental examinations are therefore an important diagnostic tool for the deglutologist, allowing a more detailed assessment with direct visualisation of oropharyngeal structures and the swallow mechanism allowing detection of 'silent' aspiration events which may be difficult to confidently diagnose at bedside examination.

Videofluoroscopy (VFS) is a highly specific instrumental test allowing detailed imaging of the upper aerodigestive tract capturing the dynamics of oropharyngeal physiology during the swallow and is often considered the gold-standard diagnostic test in oropharyngeal dysphagia (91). Fibreoptic endoscopic evaluation of swallowing (FEES) is an alternative instrumental examination, which can be performed more easily at the bedside and does not require ionising radiation exposure. This technique involves nasoendoscopy, with the endoscope positioned in the hypopharynx allowing

direct visualisation of the vocal cords before, during and after swallowing. This method has a very similar sensitivity for detecting aspiration and laryngeal penetration to VFS (91). FEES does not however allow a detailed examination of swallowing structures and physiology and requires endoscopic expertise and specialised equipment.

1.3.3 Causes of dysphagia

Given the intricate neurological control of swallowing described earlier, it is of little surprise that deficits from neurological disorders (including Parkinson's disease, multiple sclerosis, motor neurone disease, myaesthenia gravis and stroke) are the major causes of dysphagia. Other main causes include structural pathologies suggested by clinical history often detected endoscopically. Rarer causes include connective tissue disorders, iatrogenic and psychogenic dysphagia.

1.3.4 Stroke and oropharyngeal dysphagia

Stroke is recognised is a leading cause of death and disability worldwide and is associated with multiple medical complications leading to prolonged hospital admissions and health care costs (92). Oropharyngeal dysphagia is a very common complication with an incidence of between 37% - 78% and can often be detected in the early phase of acute stroke (93). Many stroke patients do recover their swallowing spontaneously, however in between 11-50% of cases problems can persist up to 6 months (94-95). Studies have shown that presence of dysphagia after stroke at three months is an independent predictor of poor outcome and institutionalisation (96).

1.3.5 Complications of dysphagia after stroke

Dysphagia, leading to aspiration of ingested foods, liquids or oral secretions, is thought to be the primary risk factor for pneumonia after stroke (97). Dysphagic stroke patients are three times more likely to suffer with pneumonia, whilst those with confirmed aspiration are eleven times more likely to develop pneumonia (93). A retrospective study aimed to quantify the cost of pneumonia and associated mortality in a stroke patients. The estimated cost of a single patient developing

pneumonia after stroke was $21,338. In terms of mortality, the authors reported a high relative risk of in hospital death in stroke patients with pneumonia of 5.7 (95% CI, 5.4-6.0) (97). One of the main focuses of clinical care and research should therefore be early detection of dysphagia after stroke, management strategies to prevent pneumonia, malnutrition and restorative swallowing rehabilitation techniques.

1.3.6 Current management of post-stroke oropharyngeal dysphagia

Current clinical practice involves mainly compensatory strategies to try and prevent complications, with very little evidence base for currently available therapies (98-100). As a result patients frequently become increasingly dependent during lengthy hospital stays, whilst the natural recovery process takes place.

Enteral feeding either via nasogastric tube or percutaneous endoscopic gastrotomy (PEG) has been shown to have no benefit in reducing the risk of pneumonia or aspiration or in improving patient outcomes(99). There is a role for enteral feeding in patients with restricted oral intake in order to maintain nutritional and hydration status, particularly if oral intake is restricted.

A variety of behavioural interventions involving head and neck exercises (chin tuck, head turn or Mendelsohn manoeuvre) have been trialled by speech and language therapists in dysphagia rehabilitation. These measures can be used clinically but there is a lack of evidence to support their efficacy (98, 100). One exercise described by Shaker et al. which promotes opening of the upper oesophageal sphincter by reinforcing the actions of the suprahyoid muscles has been shown to reduce pharyngeal residue after swallowing (101).

1.4 RECOVERY OF SWALLOWING FUNCTION AFTER STROKE

1.4.1 Brain Plasticity

Brain plasticity is an experience driven process which leads to long-term morphological or functional changes in the central nervous system and can result in behavioural changes (102). Environmental changes, conditioning stimuli and brain lesions can evoke such plastic changes. Brain injury, such as hemispheric stroke affecting the pharyngeal motor cortex is an example of this, where as discussed above, plastic changes in the unlesioned hemisphere occur during recovery of swallowing function (103).

Traditionally, it has been believed that plastic changes occur at a synaptic level, when neurons fire together with co-existing activation of pre and post synaptic membranes leading to strengthening of the synapse. In contrast synapses weaken when this close correlation is absent (104). This is the “Hebbian” theory (105). Plastic changes may occur by activity dependent alterations in the efficacy of existing synapses or by morphological changes such as dendritic branching, formation of new synaptic contacts and collaterals (106).

Long term potentiation (LTP) and Long term Depression (LTD) are important “intermediate” processes that cause pre and post synaptic changes at excitatory Glutamatergic synapses. These changes can occur whilst new synapses are still growing. The terms refer to the fact that short duration patterned activation of pre or post synaptic membranes can producer longer term changes in the transmission performance across the synapse (107).

Long term potentiation (LTP) is a process which results in an increase in synaptic strength, lasting greater than one hour after short duration of high frequency stimulation (108). LTP only occurs when concurrent depolarisation of sufficient magnitude of both pre-synaptic and post-synaptic terminals resulting in post synaptic depolarisation. In addition to structural and metabolic changes, Glutamate via its NMDAR (N-methyl D-amphetamine Receptors) located on post synaptic dendritic processes plays an important role. NMDAR receptor depolarisation ultimately permits

the flux of ions (including Ca2+), facilitating LTP (109-110). By contrast, long term depression (LTD) is a long-lasting decrease in synaptic strength and occurs after low frequency stimulation. The key mediator in this process is the inhibitory neurotransmitter gamma aminobutyric acid (111).

Neuroplasticity can be influenced by external interventions which provide sensory experience, motor skill acquisition and electrical or magnetic stimulation (102). If the subject receives such an intervention passively then this can be thought of as a “Non-Behavioural” intervention. Figure 1.12 shows a summary of three different types of non-behavioural interventions (peripheral electrical stimulation, peripheral sensory stimulation and Transcranial magnetic stimulation (TMS)), which have all been previously been used in the context of dysphagia after stroke with a view to manipulating neuroplasticity (102). “Behavioural” interventions involve a motor task as part of the intervention protocol. Repetitive electrical stimulation of neural pathways can artificially induce LTP and LTD, with several hundred low frequency stimuli reducing synaptic strength and high frequency stimulation enhancing synaptic strength (107).

Inter-individual variation in induced cortical plasticity following such interventions is becoming increasingly recognised and there is some emerging evidence that genetic variation may play a role. Brain Derived Neurotrophic Factor (BDNF), a type of neurotrophin that contributes to LTP, LTD, short-term synaptic plasticity and neuronal excitability (112) has a genetic polymorphism at codon 66 which produces the substitution of valine to methionine (val66met) that is present in approximately 33% of the Caucasian population (113). Functional brain imaging and studies of TMS MEPs in the limb literature have shown altered cortical excitability responses post-training (114-115) and after non-invasive brain stimulation modalities such as repetitive Transcranial Magnetic Stimulation (rTMS) and transcranial Direct Current Stimulation (tDCS) (116) in subjects carrying the met66 allele compared to those without the polymorphism. A subsequent TMS study in the healthy human pharyngeal motor cortex found that induction of plastic responses in met66

carriers significantly varied with different neurostimulation interventions using parameters known to be effective in the swallowing motor system (117). Compared to val66val counterparts, Met66 carriers had significantly inferior PMEPs responses to peripheral (pharyngeal) electrical stimulation but responded more favourably to cortical stimulation (repetitive TMS (rTMS)) (117). These data suggest that BDNF val66met polymorphisms may be clinically useful in predicting responses to different neurostimulation interventions in swallowing, however there is currently no published data on this in the dysphagic stroke literature.

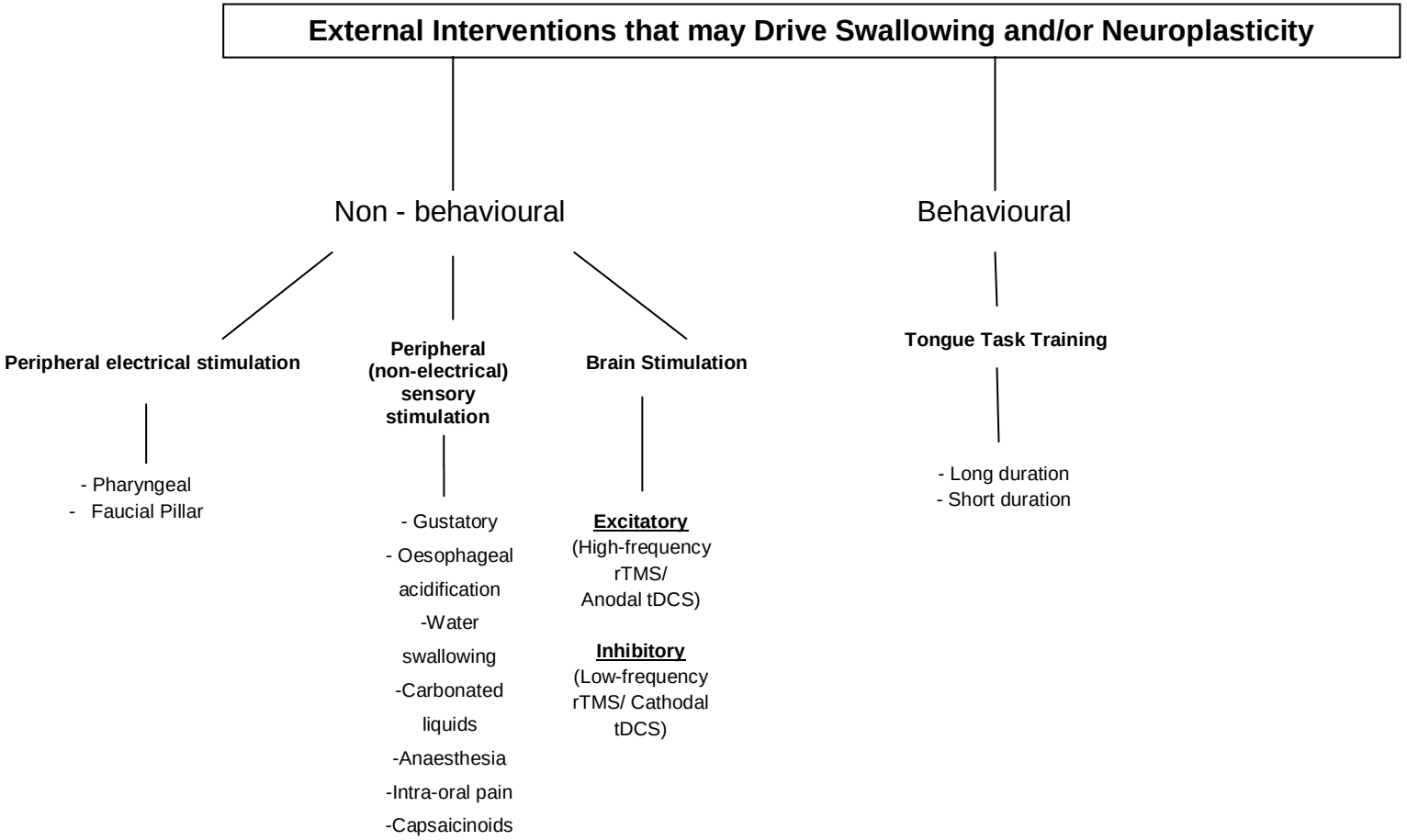


Figure 1.12 Experiences that may drive neuroplasticity in the pharyngeal motor cortex (Modified and adapted from (102))

1.4.2 The role of neuroplastic changes in swallowing recovery

As described above, original TMS studies by Hamdy et al. showed that pharyngeal musculature is represented bilaterally, but very asymmetrically in the cerebral cortex and that using this technique these areas can be mapped in healthy volunteers (38). These findings led the authors to hypothesise that a stroke lesion affecting the 'dominant' swallowing hemisphere may be responsible for dysphagia following unilateral hemispheric stroke. Subsequent TMS studies on 20 hemispheric stroke patients, 8 of which had dysphagia, highlighted that dysphagic patients had smaller pharyngeal responses from the unaffected hemisphere compared to non-dysphagic patients. These findings suggested that in dysphagic patients, the 'non-dominant' (unaffected hemisphere) which has a smaller pharyngeal representation may not be able to maintain swallowing after stroke (118).

In an attempt to understand the mechanism for recovery of swallowing after stroke, Hamdy et al. followed up 28 hemispheric stroke patients and studied their swallowing at baseline (71% were dysphagic), at 1 month (46% dysphagic) and at 3 months (41% dysphagic). All subjects were studied with TMS to examine the pharyngeal cortical representation at each time point and with videofluoroscopy (VFS). Subjects who were non-dysphagic at baseline after hemispheric stroke had greater pharyngeal cortical representation on the contralesional hemisphere compared to dysphagic subjects. TMS follow up data at 1 month and 3 months indicated that subjects that recovered swallowing function had significantly greater pharyngeal representation in the unaffected hemisphere compared to the baseline when dysphagic. These findings suggest that re-organisation in the contralesional hemisphere is key in swallowing recovery (Figure 1.13) (61). An fMRI study comparing cortical activations during swallowing tasks between dysphagic hemispheric stroke patients and healthy subjects has confirmed compensatory recruitment and activation of regions of the cerebral cortex in the intact hemisphere, supporting the theory that plastic changes in the unlesioned hemisphere are crucial in the recovery of swallowing after stroke (119). Similarly,

an MEG study by Teismann et al. imaged swallowing activations in subacute stroke patients with and without dysphagia and compared findings with a group of healthy controls (120). The authors reported increased pharyngeal motor representation in the contralesional hemisphere in hemispheric stroke patients without dysphagia, consistent with findings by Hamdy et al. In contrast, MEG studies in the acute phase dysphagic stroke patients revealed almost abololished cortical activation in the contralesional hemisphere during swallowing (120).

A trial of Electrical Stimulation to the neck musculature in a diagnostically undefined group of 8 dysphagic patients by Oh et al. have also shown expansion of the cortical pharyngeal representation on TMS mapping in the 3 stroke subjects who recovered their swallowing function (63). These findings are consistent with the aforementioned studies, however the influence of the neck muscle stimulation intervention in driving this reorganisation versus spontaneous recovery are unclear as the study is severely limited by a lack of any control intervention, a small sample size and TMS responses not being obtained in 3 of the 8 patients meant that the data set was incomplete. Further evidence comes from a recently published sham controlled trial of a single dose of neurostimulation interventions in chronic post-stroke dysphagia, where videofluoroscopic improvements in swallowing behaviour were correlated with increased cortical excitability in the undamaged hemisphere(6).

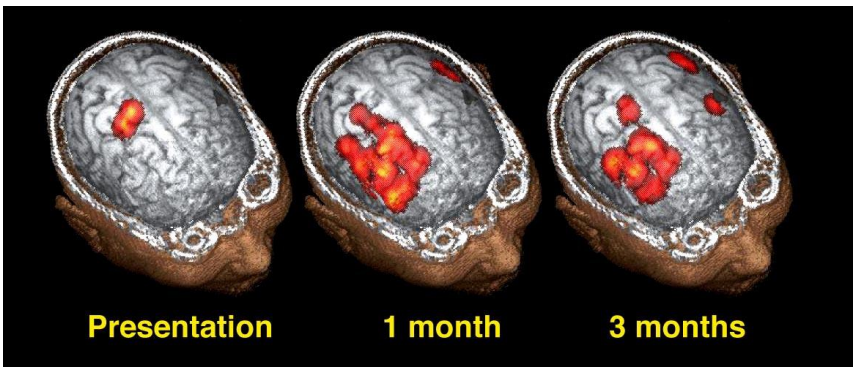


Figure 1.13 Expansion of pharyngeal motor cortex on unlesioned hemisphere during swallowing recovery after stroke. Reproduced with permission (61)

1.5 NEUROSTIMULATION AND THE TREATMENT OF DYSPHAGIA AFTER STROKE

As described in section 1.1.3, we know that swallowing musculature is represented bilaterally within the human motor cortex. The excitability and topography of these representations can be altered by practice, skill acquisition and injury. These plastic changes are likely to be the basis of learning and recovery after brain injury such as stroke (106). As we now understand more about the mechanisms of plasticity there is understandably a considerable amount of interest in strategies to manipulate this phenomenon and attempt to improve patient outcomes. Figure 1.12 gives examples of device based and non-device based neurostimulation interventions which may have neuromodulatory effects on the pharyngeal motor system. This thesis will however focus on developing device based neurostimulation techniques to modulate plasticity in the pharyngeal motor cortex. Several neurostimulation based modalities have been studied in stroke patients with promising data, however clinical trials have proven challenging, with sample sizes being small and as a result none of these modalities are currently recommended for clinical use (121). The types of patients that would benefit most from these interventions (i.e. those with persistent post-stroke dysphagia) have been shown to be patients with higher stroke severity scores (NIHSS ≥ 12), greatest lesion volume and those with bilateral infarctions (122-123), equating to significant disabilities and intercurrent illnesses which make this group a difficult group to consent and study with medical device based interventions. Before use in dysphagic patients, there are several important concepts that should be addressed by healthy volunteer studies (Figure 1.1). Several studies characterising the application of the various neurostimulation based therapies that have been piloted thus far have shown that the effects on cortical excitability are dependent on timing, frequency, duration and number of pulses of the intervention delivered (3-4, 7-9, 124). This background work in establishing the optimal parameters in healthy volunteers is essential before trialling interventions in patients. This next section aims to review current evidence by modality for neurostimulation techniques that have been studied in human swallowing in health and disease.

Non-invasive Brain Stimulation (Cortical and Cerebellar approaches)

As described in detail earlier in this chapter, there is clear evidence to suggest that neuroplastic compensatory changes in the undamaged hemisphere are important in recovery of function in the bilaterally innervated swallowing motor system. This contrasts the situation in the unilaterally innervated hand motor system, where the lesioned hemisphere appears to be most important during recovery (125) and where maladaptive cortical inhibition in the unlesioned hemisphere appears to inhibit the ipsilesional hemisphere transcallosally (126). These differences have important clinical implications for cortical stimulation studies. In the hand system for example researchers have successfully targeted the unlesioned hemisphere with inhibitory brain stimulation (127), or targeted the injured hemisphere with excitatory brain stimulation, to obliterate the competition between the hemispheres (128). However, in the swallowing system, where there is no transcallosal inhibition (129), the most plausible target is the unlesioned hemisphere with excitatory paradigms.

1.5.1 Repetitive Transcranial Magnetic Stimulation (rTMS)

Based on the same principles as single-pulse TMS (1.2.1), repetitive trains of transcranial magnetic stimulation (rTMS) can evoke lasting changes in cortical excitability and neuroplasticity in cortical motor pathways (130-134). Cortical rTMS has also been extensively studied to condition the pharyngeal motor system in health and disease.

Figure 1.14 shows the experimental set up for rTMS swallowing studies using this form of stimulation cortically. Here, the subject has a pharyngeal catheter secured in place and this is connected to the computer, conveying EMG information from the pharyngeal electrode pair. After undergoing single-pulse TMS this subject's pharyngeal motor cortex has been mapped and marked on the surface of the surgical cap. The figure of 8 coil, which is seen on the photograph secured in place over the pharyngeal motor cortex and is connected to the magnetic stimulator

(seen behind the subject). Using computer software the frequency of stimulation can be controlled and delivered.

Figure 1.14 rTMS being administered to the Pharyngeal Motor Cortex

Translational studies in the development of high-frequency rTMS interventions for post-stroke dysphagia

Step 1: confirmation of lasting cortical effects

The initial rTMS swallowing studies by Gow et al. showed that specifically only 5-Hz stimulation increased cortical excitability, with bilateral effects, at 120% of resting motor threshold (7) and no effects after 10-Hz intervention.

Step 2: confirming the optimal parameters

Jefferson et al. have since determined that a 250-pulses 5-Hz rTMS regime is superior to 100-pulses, concluding that at this duration there was no added benefit of a higher intensity (120% rMT) over a lower intensity intervention (90% rMT) (8).

Step 3: Reversal of a 'virtual-lesion'

These optimal parameters, applied contralaterally, have been shown to reverse the neurphysiological and behavioural effects of a 'virtual-lesion' in healthy subjects (next section)(8).

Step 4: Cortical and behavioural effects in dysphagic stroke patients

Interestingly, a small trial using these parameters in chronic hemispheric post-stroke dysphagia (n=6) after a single application of 5-Hz rTMS over the unaffected hemisphere, produced non-significant increases in cortical excitability (p=0.08) and did not improve swallowing at videofluoroscopy (VFS) measured for 30 minutes post-intervention compared to sham(6).

Step 5: A randomised dose-response study in dysphagic stroke patients

Thus far the optimal number and dosing regimen of this intervention has not been investigated.

Step 6: Randomised controlled trials using optimal parameters

However, despite the lack of dose-response data, a published randomised control trial in dysphagic stroke patients (n=18) with purely behavioural outcome measures, showed that only active contralesional 5-Hz rTMS improved VFS PAs compared to baseline, with effects at 2 and 4-weeks post-intervention(21). This data suggests that this intervention may require more than one session to produce therapeutic effects in a patient population. Unfortunately, the authors selected some untested parameters for this study, using a 500-pulse regime and arbitrarily assigned patients to 10 treatment sessions (over 2 weeks)(21). Interestingly, a recently published case report in a single patient with chronic dysphagia post lateral medullary infarction reported swallowing improvement compared to pre-treatment status, following bilateral 5-Hz rTMS (135) at parameters that were identical to those used by Park et al. (21) apart from using a higher intensity intervention (120% of rMT). Whilst these findings are of interest, natural spontaneous recovery could not be excluded in the case reported and the additive effects of bilateral cortical stimulation

need to be studied using the model outlined in Figure 1.1 and comparing effects to unilateral stimulation and sham before further evaluation in patients.

Inhibitory rTMS and the 'virtual-lesion' model

Focal application of low frequency stimulation has been shown to have inhibitory effects with ability to induce a 'virtual-lesion' with temporary and reversible changes in behaviour (136-139). Mistry et al. have shown that with 1-Hz rTMS for 10 minutes to the pharyngeal motor cortex of the 'dominant' swallowing hemisphere (at 120% of pharyngeal resting motor threshold (rMT)) cortical excitability could be suppressed for up to 45 minutes with altered swallowing behavioural consequences as determined by increased error rates in a swallowing reaction time task (69). A videofluroscopic study in healthy subjects has confirmed that whilst there are measureable subtle changes in swallowing behaviour after this intervention, the 'virtual-lesion' does not induce aspiration or penetration in the healthy subject (140). Therefore this experimental model safely mimics the situation in stroke, where lesions affecting the 'dominant' hemisphere for swallowing result in dysfunction and has proven to be a useful laboratory tool for assessing the ability of potential neurostimulation modalities to reverse cortical inhibition and restore normal swallowing behaviour(3, 8, 12).

Other therapeutic trials of rTMS in dysphagic stroke patients using non-evidence based approaches

A number of rTMS studies have progressed directly to clinical trials without completing any of steps 1-5 in Figure 1.1. These studies have varied in terms of hemisphere stimulated, frequency and duration of stimulation, number of interventions, outcome measures with some studies being open-label and uncontrolled. Interpretation of the data is very difficult due to the heterogeneity of the study designs as the data are not directly comparable.

a) Non-evidence based Inhibitory rTMS clinical data in post-stroke dysphagia

In one such example of an uncontrolled study of 7 chronically dysphagic stroke patients, Verin et al. used the hand stroke restorative model, applying 1-Hz rTMS (Inhibitory) to the unaffected hemisphere for 20 mins at 100% resting motor threshold for 5 days and performed videofluoroscopy and functional swallowing assessments at baseline and 3 weeks. The authors reported that these patients only had “mild” dysphagia at baseline as defined by dysphagia handicap index (DHI) and that total DHI score “significantly” improved 3 weeks after rTMS. However, the DHI takes into account quality of life factors and when swallowing impairment values are analysed in isolation this improvement was not statistically significant. On VFS analysis, only swallowing reaction time and aspiration/residue scores for paste and liquids improved, with none of the other measurements (oral transit time, pharyngeal transit time and Laryngeal Closure duration) showing any change (16). In this design of study, it is difficult to interpret any effects of the intervention as there was no control used and therefore the modest improvements seen could be due to the natural recovery process. Similar to the study by Verin et al., two inhibitory rTMS studies using identical parameters (1-Hz 1200 pulses, 100% rMT) in acute dysphagic stroke patients over the unlesioned hemisphere, showed improved swallowing at VFS compared to baseline (15, 22). In the study by Kim et al. given that there was no reported comparison with sham with the 1-Hz intervention, natural swallowing recovery cannot be ruled out in this study (15).

b) Non-evidence based excitatory cortical rTMS clinical data in post-stroke dysphagia

In two controversial studies, Khedr et al. studied hemispheric (17) and brain stem dysphagic stroke patients (141) using previously untested excitatory rTMS parameters (missing out steps 1-5 in our suggested model Figure 1.1) with 300 pulses of 3-Hz rTMS over the lesioned hemisphere, targeting oesophageal instead of pharyngeal motor cortex at 120% (17) and 130% (141) resting motor threshold, for 10 minutes assigned for 5 consecutive days arbitrarily. The primary outcomes of these studies were the dysphagia outcome and severity scale (DOSS) rating. In both studies

there was improvement in dysphagia score in the active group compared with sham (17, 141). However, the rationale for measuring oesophageal MEPs and stimulating oesophageal motor cortex in oropharyngeal dysphagia patients is unclear and the outcome measures lacked standardisation of the behavioural aspects of swallowing assessment and the absence of a control site of cortical stimulation (142). Given that the study by Jefferson et al. has shown that excitatory paradigms of rTMS provoke bilateral cortical excitability possibly via transcallosal mechanism(8); it is possible that the swallowing recovery seen in the studies by Khedr et al, reflect the changes in cortical excitability of the unlesioned hemisphere that they demonstrated in a subset of their patients via single-pulse TMS(17).

Similar to the model used by Khedr et al. in acute hemispheric stroke dysphagia, Kim et al. studied 10 patients applying 10 sessions of 5-Hz rTMS (1000 pulses at 100% rMT) over the lesioned hemisphere, an intervention which did not improve swallowing at the end of treatment compared to baseline within the same group(15). This data contrasts the effects of 5-Hz stimulation over the unlesioned hemisphere(21).

A recent uncontrolled case series in chronic stroke patients (n=4) applied 3-Hz rTMS bilaterally (300-pulses per hemisphere) twice daily for 6 days at 130% hand rMT with some suggestion of modest improvement from baseline swallowing status in each subject but given the sample size and lack of controls it is impossible to distinguish any changes from natural swallowing recovery and no statistics were presented(143).

In summary, rTMS has been shown to increase pharyngeal cortical excitability bilaterally, the optimal number of pulses, frequency and intensity have been established for unilateral stimulation and these parameters are able to reverse 'virtual-lesions' applied to the contralateral hemisphere in healthy subjects. There is some debate amongst research groups which hemisphere should be selected (lesioned or unlesioned) and which side is more important in recovery.

The next step in the development of unilateral cortical rTMS would be a dose-response study (Figure 1.1 - step 5) using the optimal parameters, before a further, more definitive, randomised trial in patients. Cerebellar targeted rTMS is a completely novel approach and has not been investigated before and Chapter 2 will focus on determining if this approach produces lasting cortical effects and determining the optimal parameters in healthy subjects (Steps 1 and 2).

1.5.2 Intermittent and continuous Theta Burst Stimulation (iTBS and cTBS)

Theta burst stimulation uses rapid (50-Hz frequency), short duration (2 second) protocols of rTMS (600 pulses in total), which have been shown to facilitate when delivered intermittently (repeated every 10 seconds) and inhibit cortical excitability when delivered continuously (139, 144).

Mistry et al. performed the first studies investigating both inhibitory (cTBS) (69) and excitatory protocols (iTBS) (10) applied to pharyngeal motor cortex (Figure 1.1 step 1). Whilst cTBS could not suppress pharyngeal motor cortex excitability(69), iTBS only excited the contralateral hemisphere, with these effects taking over an hour post-intervention to build-up (10). This technique has not been developed further given the modest effects observed and safety guidelines with such a high-intensity protocol (145-146) which limit the investigator to using stimulator outputs well below the resting motor threshold of the pharynx (80% rMT hand).

1.5.3 Transcranial Direct Current Stimulation (tDCS)

Transcranial Direct Current Stimulation (tDCS) is a relatively new, non-invasive brain stimulation modality, which produces shifts in neuronal excitability induced by delivery of weak direct current (147-148). Recent data from the stroke literature suggests that tDCS may have a role in expediting recovery of motor function (149-150). This modality has advantages making it an attractive option as a neurostimulation modality in that it is safe and very easily portable compared to rTMS. Over the past two years there have been several studies (Table 1.3) examining the effects of tDCS on the pharyngeal motor cortex and swallowing and some evidence for effects of cortical tDCS on oesophageal motility (151). Figure 1.15 shows a tDCS study in progress. This

subject is having anodal tDCS. The anode is the pink electrode pad and is sitting over the right pharyngeal motor cortex in this subject. The blue pad (cathode) is seen at a reference point on the contralateral supraorbital region. These pads are secured and water is continually infused to maintain low impedence levels. The pads are linked to the DC stimulator box which is controlled by a personal computer which controls the intensity output. The direction of current flow between the electrodes determines the excitability changes seen. Cathodal tDCS is inhibitory and anodal excitatory (148).

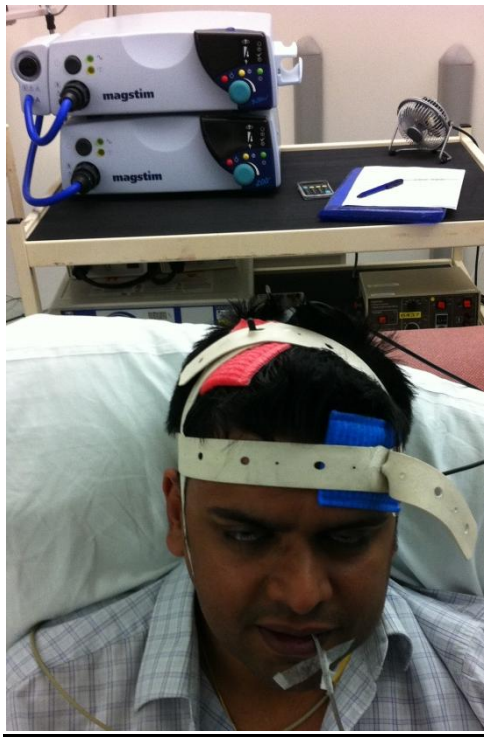


Figure 1.15 Typical experimental set-up and electrode montage for Anodal tDCS applied to pharyngeal motor cortex

Translational studies in the development of tDCS for post-stroke dysphagia

Steps 1 and 2 - Confirmation of lasting cortical effects and determining the optimal parameters

Jefferson et al. assessed TMS PMEPs before and at four time points after either anodal low intensity tDCS (1m A for 20 mins), anodal high intensity tDCS (1.5 mA for 10 mins), cathodal low intensity tDCS (20 mins 1 mA) or high intensity cathodal tDCS (10 mins 1.5mA) or sham(9). The results showed that in order to excite or inhibit the pharyngeal motor cortex longer durations or higher intensities were required compared to in the limb literature. The optimal cortical excitability

responses were seen with 20 mins of 1 mA or 10 minutes of 1.5 mA Anodal tDCS (9). Interestingly, there were no changes in the cortical excitability of the contralateral hemisphere (9). Cortical effects of tDCS over the swallowing motor system have since been confirmed on a functional brain imaging study using MEG (11). Anodal tDCS at these optimal parameters, also appears to enhance swallowing related behaviours in healthy subjects (11, 152).

In summary, tDCS is a very promising intervention which has the added advantage of being easy to provide sham stimulation, is easily portable and non-invasive. It seems to only excite the cortical hemisphere which is stimulated and therefore transcallosal involvement is unlikely. Further mechanistic work in the form of a 'virtual-lesion' study (Chapter 3) is next step in the development of this technique followed by a dose-response study in patients. Kumar et al., in a small pilot study with limited follow-up lacking neurophysiological or behavioural outcome measures in dysphagic stroke patients, used different parameters (2mA Anodal tDCS for 30 minutes) contralesionally and showed some improvement in functional dysphagia scores (19). Two further studies used evidence-based parameters but stimulated the lesioned instead of unlesioned hemisphere and only reported modest effects of intervention (18, 20). These inconclusive studies arbitrarily assigned subjects to five (19) or ten (18, 20) sessions without any evidence base. Interestingly, Kumar et al. have recently published a protocol where the authors have declared an intent to study 99 hemispheric dysphagic stroke patients, yet again using different parameters (2mA for 20 minutes of anodal tDCS) and have included an arm comparing high-dose (10 sessions) versus low-dose (5 sessions) tDCS and control (153). The results of this work and other similar studies using evidence based parameters will be of importance to future applications of this technique.

Table 1.3 Summarises the translational studies in the literature which have used rTMS, tDCS and other forms of cortical neurostimulation to modulate the pharyngeal motor cortex.

Stimulation modality	**Healthy subject cortical excitability and parameter defining studies (Steps 1 & 2)**	**Brain Imaging studies (Step 2)**	**Reversal of a 1-Hz rTMS 'virtual lesion' (Step 3)**	**Pilot trials in dysphagic patients (Step 4)**	**Dose Response Data (patients) (Step 5)**	**Randomised trials dysphagic stroke (Step 6)**
Repetitive Transcranial Magnetic Stimulation (rTMS)	*Gow et al. 2004 (7)* *Jefferson et al. (8)*		*Jefferson et al. 2009 (8)*	*Michou et al. 2014(6)* *Verin et al. 2009 (16)* *Khedr et al. 2009 (17)* *Khedr et al. 2010 (141)* *Kim et al. (15)* *Rhee et al 2013* *Momosaki et al 2014 (135, 143)* *Lim et al. 2014(22)*		*Park et al. 2013* *NB - did not define dose-response (step 5) and used different duration (500-pulses) (21)*
Transcranial Direct Current Stimulation (tDCS)	*Jefferson et al. 2009 (9)*	*Suntrup et al. 2013 (11)*	***Vasant et al. (Chapter 3*) (154)***	*Kumar et al. 2011 (19)* *Yang et al. 2012 (18)* *Shigematsu et al 2013 (20)*		
Theta Burst Stimulation (TBS)	*Mistry et al. 2007 (69)* *Mistry et al. 2012 (10)*					

1.5.4 Pharyngeal Electrical Stimulation (PES)

Pharyngeal Electrical Stimulation (PES) is a form of peripheral neurostimulation that has been worked up fairly extensively over the past decade. This stimulation technique involves placement of a thin (3.2mm diameter) intraluminal catheter with bipolar pairs of electrodes in the pharynx with connections to a trigger and electrical stimulating device.

Translational studies in the development of PES for post-stroke dysphagia

Step1: Confirming cortical effects

The original healthy volunteer studies by Hamdy et al. used 10 minutes of 10 Hz Pharyngeal stimulation and assessed pharyngeal and oesophageal motor evoked potentials (MEPs) to single pulse TMS, pre-stimulation, immediately post, post 30 minutes and post 60 minutes (2). The results showed significantly increased pharyngeal MEP responses to single-pulse TMS for 30 mins post-intervention, with these excitatory effects lasting for an hour before returning to pre-

stimulation levels. In contrast oesophageal MEP responses decreased over the same time period (2). Using single-pulse TMS in the same study, the authors also compared the size of pharyngeal and oesophageal cortical representation pre and post stimulation in 5 volunteers. This interesting TMS mapping data demonstrated that the after PES the pharyngeal motor cortex expanded in association with reduced oesophageal motor representation (2).

Step 2: Confirming the optimal parameters

Fraser et al. have since shown that 5-Hz Pharyngeal Stimulation, with an intensity of 75% maximum tolerated are the optimal parameters for inducing cortical excitability after PES. In the same study, the authors studied 8 healthy subjects before and 1 hour after PES using fMRI. These imaging results showed the PES compared to sham was associated with bilateral increase in activity in primary sensory and primary motor cortices (4).

Step 3: Reversal of a 'virtual-lesion'

Building on from this work Jayasekeran et al. tested PES in healthy subjects, using the 'virtual-lesion' model as described above, confirming that PES could reverse the cortical inhibition and altered swallowing behaviour (3).

Step 4: Confirmation of cortical and behavioural effects in dysphagic stroke

Cortical and behavioural effects of PES have been proven in dysphagic stroke patients, with increased cortical excitability of predominantly the unlesioned hemisphere being observed in both acute (4) and chronic (6) stroke being strongly associated with improved swallowing behaviour at VFS.

Step 5: Optimal dose-response in dysphagic patients

A dose-response trial using PES in a group of 22 acutely dysphagic stroke patients compared the efficacy of several regimens of PES; once daily (3 and 5 days), three times a day (3 and 5 days) and sham (3). Greatest reduction in PAs was seen with a three day regimen once daily (3).

Step 6: Randomised trial in dysphagic patients

Using the optimal parameters and dosing regimen, 28 dysphagic stroke patients (16 randomised to active and 12 sham PES) were followed up at 2-weeks post-interventions. Active PES significantly reduced the number of aspirative swallows at VFS and improved feeding status (based on reduced dysphagia severity rating scale scores (DSR)) compared with sham (3). Whilst the interventional groups had similar functional status (Barthel Index) the active group had a shorter length of hospital stay.

In summary, PES has been shown to increase pharyngeal motor cortex excitability bilaterally, increasing cortical activity as seen on fMRI and can reverse 'virtual-lesions' applied to the swallowing motor cortex. The optimal dosing regimen has been identified and a small randomised control trial has shown that this treatment is safe and compared to placebo can improve feeding status, reduce aspiration scores and reduce hospital inpatient stays. The next translational step would be to reproduce this evidence in a larger, (Phase IIc) multi-centre Randomised Control trial with longer-term follow-up (Chapter 4).

1.5.5 Oral Electrical Stimulation

Steps 1 and 2: Confirming cortical effects and defining optimal parameters

In a TMS cortical excitability study combined with swallowing behavioural measurements at VFS in healthy subjects, Power et al. confirmed that lasting increases in cortical excitability could be induced with frequency specific electrical faucial pillar stimulation (0.2-Hz for 10 minutes), without altering swallowing behaviour(124).

Step 4: Examining effects in dysphagic stroke patients

In the pre 'virtual-lesion' era, these optimal parameters were evaluated in a randomised study in hemispheric stroke patients (n=16), where it was found to be ineffective in terms of improving swallowing behaviour(155).

1.5.6 Transcutaneous Neuromuscular electrical stimulation (NMES)

NMES involves passing a small electrical current via electrodes at supramotor thresholds to stimulate the neuromuscular junction and create a muscle contraction. In the swallowing system this has been delivered via two bipolar pairs of electrodes placed over the submental and laryngeal regions of the neck. The example of NMES illustrates the importance of the evidence based translational model we are proposing in this thesis for developing such interventions in post-stroke dysphagia.

Since the technique was originally introduced in 2001 it proceeded directly to non-placebo controlled trials in dysphagic stroke (156) and it has been available commercially for clinical use in a wide range of dysphagic aetiologies despite a lack of standardisation of parameters, no understanding of mechanism, difficulties with conflicting results in small clinical studies and as a result the lack of evidence base has resulted in professional and ethical debates for speech and language therapists (157). Speech and language therapy training courses incorporating NMES currently recommend parameters of 80-Hz stimulation for an hour consisting of 59 seconds of stimulation per minute, where clinicians are trained to ask patients to swallow forcibly for an hour during intervention (158). It is unclear whether the forcible swallowing or the NMES itself may be responsible for any beneficial effects. To reiterate the problems with the approach to developing NMES, almost a decade after its launch, the first studies assessing the effects of this intervention on cortical excitability in healthy subjects and dysphagic stroke patients (i.e. steps 1 and 4 in our proposed model, Figure 1.1) confirmed that NMES does not increase corticobulbar excitability in dysphagic stroke patients (159) and in healthy subjects effects on cortical excitability could not be separated from effects forcible swallowing (160). Therefore effects on cortical plasticity from this intervention are doubtful. Whilst clinical trials using this technique have largely been uncontrolled, using small heterogenous patient groups (158, 161), it is clear that the most plausible mechanism for this technique involve biomechanical changes causing lowering of the hyo-laryngeal complex (162-164), forcing a stronger swallow from the patient to overcome the resisted laryngeal

elevation. Given that some stroke patients may not be able to overcome this there are concerns this could increase the risk of aspiration in a proportion of patients (162). Results from small randomised studies to date have been mixed with some studies indicating no significant benefit over standard therapy (165-167) and some showing some benefit on swallowing behaviour when combined with traditional swallowing therapies(22, 168-169). Further work is required to determine the physiological and neurological effects of this intervention in stroke patients.

Table 1.4 Summarises the translational development of peripheral electrical neurostimulation techniques in health and disease

Stimulation modality	**Healthy subject cortical excitability and parameter defining studies** **(Steps 1 & 2)**	**Brain Imaging studies** **(Step 2)**	**Reversal of a 1-Hz rTMS 'virtual lesion'** **(Step 3)**	**Pilot trials in dysphagic patients** **(Step 4)**	**Dose Response Data (patients)** **(Step 5)**	**Randomised trials dysphagic stroke** **(Step 6)**
Pharyngeal Electrical Stimulation (PES)	*Hamdy et al. 1998 (2)* *Fraser et al. 2002(4)* *Fraser et al. 2003 (170)*	*Fraser et al. 2002 (4)* *Suntrup et al. 2015(5)*	*Jayasekeran et al. 2010 (3)*	*Fraser et al. 2002 (4)* *Michou et al. 2014 (6)*	*Jayasekeran et al. 2010 (3)*	*Jayasekeran et al. 2010 (3)* ***Vasant et al. 2014 (Chapter 4*)***
Oral Stimulation	*Power et. al 2004 (124)*			*Power et al. 2006 (155)* *Park et al. 1997 (171)*		
Neuromuscular Electrical Stimulatiion (NMES)	*Doeltgen et al 2010. (160)*			*Gallas et al 2010 (159)* *Freed et al. 2001 (156)* *Leelamanit et al. 2002(172)* *Kiger et al. 2006 (173)* *Ludlow et al. 2007 (162)* *Carnaby-Mann et al. 2008 (174)*		*Bulow et al. 2008 (165)* *Lim et al. 2009(166)* *Ryu et al. 2009 (175)* *Permsirivanic et al. 2009 (167)* *Lim et al. 2014 (22)* *Huang et al. 2014 (169)* *Lee at al. 2014 (168)*

Combined Cortical and Peripheral neurostimulation

1.5.7 Paired Associative Stimulation (PAS)

Paired associative stimulation (PAS) is a technique whereby the target motor cortex is stimulated by two stimuli in synchrony. Both cortical stimuli and peripheral stimuli can independently excite the pharyngeal motor cortex as discussed above and by stimulating the pre-synaptic and post synaptic membrane in synchrony in theory this should facilitate the plastic mechanism according to Hebbian theory.

Step 1: confirmation of lasting cortical effects

Singh et al. combined pharyngeal electrical stimulation with rTMS and tested paired pulses at four different inter stimulus intervals (ISI) between 50-125ms. They delivered a total of 90 pulses of PAS over 30 minutes (13). The optimal ISI *was* 100ms, producing the maximum increase in cortical excitability. The increased cortical excitability compared with baseline was sustained for 2 hours. Both the cortically stimulated and unstimulated pharyngeal motor cortex were excited after PAS, with long lasting effects. Singh et al. also studied 7 subjects before and after real or sham PAS with magnetic resonance spectroscopy and found that in the stimulated pharyngeal motor cortex, active PAS caused a focal decrease in glutamate. This provides further evidence that glutamate is involved in modulating plastic changes seen after PAS (13).

Step 2: Defining the optimal parameters

Michou et al. confirmed that 10 minutes duration PAS intervention produced the optimal effects on cortical excitability when compared to a longer intervention (30 minutes) and sham (12). In a separate study the authors found additive effects of a second dose of stimulation in subjects who did not 'respond' to a single-session(14).

Step 3: Reversal of a 'virtual-lesion'

Interestingly, optimal parameters of PAS only reversed the neurophysiological and behavioural effects of the 'virtual-lesion' when it was applied contralesionally, contrasting the effects of ipsilesional PAS after 'virtual-lesion' (12).

Step 4: Confirmation of cortical and behavioural effects in dysphagic patients

In patients with dysphagia post chronic stroke effects of a single dose of PAS have been confirmed to excite the pharyngeal motor cortex bilaterally and significantly improve swallowing behaviour at VFS post-intervention (6, 12).

In summary, PAS is a very promising neurostimulation technique, which producing lasting increases in cortical plasticity, reverses effects of a 'virtual-lesion' and cortical and swallowing behavioural improvements have been confirmed in stroke patients. Whilst this technique is not used in this thesis the next translational step would be a dose-response study in post-stroke dysphagia before proceeding to a randomised trial.

Table 1.5 Summary of published translational work in developing combined peripheral and cortical neurostimulation for post-stroke dysphagia

Stimulation Modality	**Healthy subject cortical excitability and parameter defining studies** **(Steps 1 & 2)**	**Brain Imaging studies** **(Step 2)**	**Reversal of a 1-Hz rTMS 'virtual lesion'** **(Step 3)**	**Pilot trials in dysphagic patients** **(Step 4)**	**Dose Response Data (patients)** **(Step 5)**	**Randomised trials dysphagic stroke** **(Step 6)**
Intermittent Paired Associative Stimulation (iPAS)	*Singh et al. 2009(13)* *Michou et al. 2012, 2013 (12, 14)*	*Singh et al. 2009 (13)*	*Michou et al 2012 (12)*	*Michou et al. 2012 (12)* *Michou et al. 2014 (6)*		

In this Chapter I have summarised current understanding of the neural control of swallowing, the pathophysiology of dysphagia post hemispheric stroke and the limitations of current therapies available for this condition. I have also introduced various techniques including TMS, neuronavigation and VFS which will be used in this thesis to study swallowing neurophysiology and behaviour in health and disease. In the latter part of the chapter evidence for brain plasticity in swallowing recovery is discussed together with the potential of neurostimulation techniques to revolutionise post-stroke dysphagia therapy by augmenting this natural recovery process. Based on the swallowing literature, current evidence for each modality is summarised and the rationale for a step-wise translational approach required to develop these neurostimulation techniques is described.

In the next three chapters, translational studies covering the entire spectrum of the outlined developmental model will be presented using three different approaches. In Chapter 2, I evaluate the effects of non-invasive cerebellar stimulation using rTMS to determine if lasting cortico-pharyngeal effects can be induced and if so to determine the optimal parameters of this intervention. In Chapter 3, I tested optimal parameters of tDCS in a 'virtual-lesion' study to confirm its therapeutic potential in healthy subjects. Finally in Chapter 4, a randomised controlled trial of PES in acutely dysphagic post stroke patients was performed with 3-months follow-up.

CHAPTER 2

CEREBELLAR STIMULATION DRIVES HUMAN CORTICO-PHARYNGEAL PLASTICITY WITH THERAPEUTIC POTENTIAL IN POST-STROKE DYSPHAGIA

2.1 ABSTRACT

Background & Aims: Brain neurostimulation can modulate cortical swallowing neurophysiology with therapeutic promise in post-stroke dysphagia. Furthermore, cerebellar neurostimulation is a novel, unexplored approach to modulation of swallowing pathways as a prelude to therapy for dysphagia.

Methods: Healthy subjects (n=17) underwent MRI-guided single-pulse Transcranial Magnetic Stimulation (TMS) to co-localise pharyngeal and thenar representation in the cortex and cerebellum (midline and hemispheric). Following acquisition of baseline motor evoked potentials (MEPs) recordings from each site, subjects were randomised to receive one of five cerebellar repetitive TMS (rTMS) interventions (Sham, 1-Hz, 5-Hz, 10-Hz and 20-Hz) on separate visits to the cerebellar site with strongest pharyngeal activity. Additionally, a subset of subjects randomly received each of three different durations (50, 250, 500-pulses) of optimal frequency versus sham cerebellar rTMS on separate visits. Post-intervention MEPs were recorded for an hour and compared to sham. To demonstrate therapeutic plausibility of these effects in stroke, we randomized to separate days the optimal cerebellar intervention versus sham stimulation to a chronically dysphagic cerebellar stroke patient, and evaluated the short-term effects on post-interventional MEPs and videofluoroscopic cumulative Penetration-Aspiration scores (cPAs).

Results: Only 10-Hz cerebellar rTMS increased cortico-pharyngeal MEPs amplitudes (mean bilateral increase 52%, P=0.007) with effects lasting 30 minutes post-intervention with an optimal train-length of 250-pulses (P=0.019). These optimal parameters also increased cortico-pharyngeal MEPs amplitudes (maximum 55%) in the dysphagic stroke patient, improving swallowing safety (cPAs: Active -15%, Sham +42%) on post-intervention videofluoroscopy.

Conclusions: Optimised parameters of cerebellar rTMS produce sustained increases in pharyngeal cortical excitability which may have therapeutic benefit in post-stroke dysphagia.

2.2 INTRODUCTION

The cerebellum is important in planning and executing complex motor tasks. Evidence from animal studies (43-44, 176-179) and human functional brain-imaging literature (45-49, 87, 180-181) infers cerebellar involvement in the neurophysiologic control of swallowing. Furthermore, additional evidence on the role of the cerebellum in swallowing comes from pathological associations between oropharyngeal dysphagia and cerebellar stroke (50, 182-184), degenerative cerebellar diseases,(185-186) partial cerebellectomy (187) and pre- and post-operative dysphagia in posterior fossa tumours.(52, 183, 188) Despite apparent neurophysiological importance, the physiologic relevance of the cerebellum in swallowing remains relatively unexplored. Recently, Jayasekeran et al.(53) systematically probed this relationship using single-pulse Transcranial Magnetic Stimulation (TMS) and discovered that distinctive cerebellar-evoked pharyngeal Motor Evoked Potentials (cb-PMEPs) with similar response latencies to cortically-evoked pharyngeal Motor Evoked Potentials (cortical-PMEPs) could be evoked from cerebellar sites (both the cerebellar midline and hemispheres).(53) Interestingly, when paired-pulses of cerebellar-cortical conditioning were delivered at short inter-stimulus intervals (ISI) (50, 100 and 200ms); this strongly excited pharyngeal corticobulbar projections.(53) These findings led us to hypothesize that longer-trains of high-frequency (5, 10 and 20-Hz) cerebellar repetitive TMS (rTMS) would produce long-lasting excitatory corticobulbar effects. Such changes could have therapeutic potential given that this type of brain plasticity has been shown to improve swallowing in post-stroke dysphagia.(3, 12, 17, 19, 21). Moreover, the effects of both high (8) and low-frequency cortical rTMS (7, 69) applied to pharyngeal motor cortex are critically dependent on the train-length of interventions. Given the evidence for frequency specific modulation of the swallowing neural network, we further hypothesized that repetitive cerebellar stimulation would show evidence for frequency and durational dependency on its effects on swallowing corticobulbar projections. Finally, we tested our optimal parameters of cerebellar rTMS in a chronic post-stroke gastrostomy tube fed

dysphagic patient, to examine if functional swallowing improvements could be induced by cerebellar stimulation.

2.3 METHODS

2.3.1 Subjects

Power calculations: Based on a 40% effect size (suggested by similarly designed neurostimulation-based studies in swallowing literature (7, 53)), we determined that at least 12 full data sets would be required to achieve power of 80% and statistical significance of 5%. This would also be a large enough sample to determine interventional differences compared to sham (154).

Healthy subjects (n=17, 11 males, 6 females, mean age 30 ± 3 years) and one dysphagic-stroke patient (female, aged 67 years, 56 days after right posterior inferior cerebellar artery territory infarction) were recruited. All subjects complied with exclusion criteria including; pregnancy, epilepsy, cardiac pacemaker, previous brain surgery, claustrophobia, previous swallowing problems, use of centrally acting medication and implanted metal. Written informed consent was obtained prior to participation. All components of the study were approved separately by two Greater Manchester Research Ethics Committees (GM East and North) and were conducted at Salford Royal NHS Foundation Trust in accordance with the World Medical Association Declaration of Helsinki.

2.3.2 Experimental Techniques

2.3.2.1 Magnetic Resonance Imaging (MRI): Each healthy subject had a whole-brain anatomical MRI scan (T1* weighted image, Phillips 3T Intera-Achiva, Netherlands). The subject's head was immobilised in a cradle, within the head coil, using foam padding to minimise movement.

2.3.2.2 Neuronavigation: All subjects' MRI whole-brain anatomical scans were uploaded onto neuronavigation software (Brainsight^2, Rogue Research, Canada) on a personal computer (iMac, Apple MacIntosh, USA). Neuronavigated-TMS has advantages when targeting specific brain regions with some evidence for improved accuracy and reliability of neurophysiological data. Given the potential advantages, we adopted neuronavigation in our healthy subject protocols. At the beginning of each session, anatomical co-registration was performed using a remote controlled pointer and an optical tracking system (Polaris Vicra, NDI, Ontario, Canada). Subsequently, following single-pulse TMS, cortical and cerebellar sites were co-registered with the subjects' own MRI brain-scan using frameless stereotaxy and a calibrated TMS coil to confirm the optimal sites. Once identified, these hot-spots were saved as targets for future mapping sessions. The coil position of each hot-spot was visualised, sampled, captured with a screen shot and saved as a target for future sessions. If any TMS hot-spots appeared anatomically sub-optimal on MRI brain-scan, the hot-spot was refined with a combination of real-time neuronavigation and single-pulse TMS.

2.3.2.3 Electromyography: *Pharyngeal:* Volunteers swallowed a 3.2mm diameter intraluminal catheter (Gaeltec Ltd, Isle of Skye, Scotland) housing a pair of bipolar platinum ring-electrodes such that the electrodes were positioned at mid-pharyngeal level (15-17cm from the nasal flare or 13-15cm ab oral depending on subject preference) enabling recording of pharyngeal motor evoked potentials (PMEPs). *Thenar:* As a secondary control, thenar motor evoked potentials (TMEPs) from the abductor pollicis brevis muscle contralateral to the hemisphere giving the largest cortical PMEPs were also recorded via surface electrodes (H69P, Tyco Healthcare, UK). The catheter, thenar and two earth electrodes were connected via a preamplifier (CED 1902; Cambridge Electronic Design, Cambridge, UK) with high and low pass filter settings of 200Hz and 2kHz, respectively. Response signals were processed through a 50/60Hz noise eliminator (‘HumBug’; Quest Scientific, North Vancouver, Canada) to remove unwanted electrical interference collected

through a laboratory interface (CED micro 1401) at a sampling rate of 5kHz and recorded using Signal software (v4.0, CED) running on a personal computer.

2.3.2.4 Cortical and cerebellar single-pulse TMS: Single-pulse TMS was applied to all sites using a figure-of-eight coil (outer diameter 7cm) with a maximum output of 2.2 Tesla (The Magstim Company, Wales). For cortical PMEPs and TMEPs, the coil handle was held in antero-posterior direction at an angle of 45º tangential to the scalp (38), whereas for cerebellar (cb)-PMEPs, the coil was positioned over the posterior fossa, tangentially to the scalp with the handle pointing superiorly (53).

2.3.2.5 Videofluoroscopy (VFS): Research VFS procedures(6) were conducted on the dysphagic stroke patient in the Radiology Department. Examinations involved 6 swallows of 5ml boluses of liquid barium (60% w/v, EZ-HD®, E-Z-EM Limited, UK) and lateral view images acquired (Siemens Fluorospot® H SIRESKOP SX Unit, Germany).

2.3.2.6 Cerebellar rTMS: Trains of stimuli were delivered through a figure-of-eight coil connected to a Magstim super-rapid stimulator (The Magstim Company) with a maximum output of 1.8 Tesla. The cerebellar site evoking the largest amplitude cb-PMEPs (strong cerebellar site) was selected as the stimulation site for all interventions. Signal software was programmed to generate the pre-determined stimulation parameters according to randomisation.

Protocols

2.3.3 Experimental protocol 1: Effects of low (1-Hz) and high-frequency (5, 10 and 20-Hz) cerebellar rTMS conditioning on pharyngeal motor cortex and cerebellar excitability

The subjects wore a tightly-fitted disposable cap upon which anatomical landmarks including the cranial-vertex and inion were identified and marked. Subjects were intubated with the pharyngeal

catheter to allow mapping of the pharyngeal cortical representation by discharging TMS at suprathreshold intensities bilaterally to identify the optimal sites for pharyngeal responses. To enhance targeting accuracy, neuronavigation was used to validate coil positioning for each hot-spot on each subject's MRI brain-scan. At these marked sites, the pharyngeal resting motor threshold (rMT) for each hemisphere was determined by the lowest intensity of single-pulse TMS required to evoke cortical PMEPs of >20 μV on 50% of occasions. Thenar cortical representation and rMT were determined on the hemisphere with stronger pharyngeal cortical representation (the side that produced the largest amplitude cortical PMEPs at the lowest intensity). In order to determine the optimal sites for evoking cb-PMEPs at the midline and both cerebellar hemispheres, previously described optimal cerebellar sites(53) were marked as reference points. Single-pulses of TMS were then sequentially discharged over the posterior fossa to confirm the optimal coil positions to evoke cb-PMEPs at all three cerebellar sites (midline and both hemispheres). The strongest cerebellar site and the cerebellar rMT (stimulator output (%) required to evoke cb-PMEPs >20μV on 50% of trials) were determined.

Baseline measurements of cortical excitability at all three sites (both pharyngeal cortices and hand (thenar) motor cortex) were obtained by delivering 10 pulses of single-pulse TMS at rMT+20% stimulator output (total 30-stimuli). Baseline measurements of cerebellar excitability at all three sites (right, midline and left cerebellum) were made by delivering 5 single-pulses of TMS at rMT+10% stimulator output (total of 15 stimuli).

Each of five different rTMS interventions were then randomly assigned (StatsDirect v2.7.8, StatsDirect Ltd, UK) and delivered over the strongest cerebellar site, on separate visits, at least one week apart. Prior to delivery of intervention, rMT was confirmed using the super-rapid stimulator. To assess the effects of stimulation frequency the following interventions were trialed:

a) Sham: delivered at 5-Hz parameters (below) but with the coil tilted to 90˚ ensuring that only the edge of one wing of the figure-of-eight coil was in contact with the head (53).
b) 1-Hz: Single continuous train of 600-stimuli delivered at an intensity of 90% cerebellar rMT.
c) 5-Hz: 5 trains of 50-stimuli (each lasting 10 seconds) with an intra-train interval of 10 seconds delivered at 90% of thenar rMT.
d) 10-Hz: 5 trains of 50-stimuli (each lasting 5 seconds) with an intra-train interval of 10 seconds delivered at 90% of thenar rMT.
e) 20-Hz: 10 trains of 25-stimuli (each lasting 1.25 seconds) with an intra-train interval of 10 seconds delivered at 90% of thenar rMT.

The different intensities and durations selected for high and low-frequency interventions in this study were based on experience using these frequencies in pharyngeal cortical rTMS studies; where 1-Hz interventions have been administered at higher intensities (based on pharyngeal rMT) (69) than high-frequency interventions (based on thenar rMT) (8). All the rTMS parameters applied were compliant with international safety guidelines on the use of TMS (145, 189). High-frequency interventions were limited to 250-pulses to equalise energy dissipation between interventions and based on the study by Jefferson et al (8).

Post-intervention, cortical and cerebellar excitability was followed-up with single-pulses of TMS as per baseline; immediately and repeated every 15 minutes for an hour.

2.3.4 Experimental protocol 2: Effects of varying duration of optimal frequency cerebellar rTMS on pharyngeal motor cortex and cerebellar excitability

A subset of subjects from protocol 1 (n=12, 7 male, 5 female, mean age 31 ± 4 years) underwent the same neuronavigated-TMS procedures to confirm hot-spots before baseline assessments of cortical and cerebellar excitability. Each of 4 different optimal frequency rTMS interventions were then randomly assigned and delivered over the strong cerebellar site on separate visits at least

one week apart, to assess the effects of stimulation duration. The following train-lengths of optimal-frequency rTMS were trialed:

a) Sham: pulses delivered at intermediate train-length (below) but with coil tilted to 90˚ (as in protocol 1a).
b) Short: 1 train of 50-stimuli at 90% thenar rMT.
c) Intermediate: 5 trains of 50-stimuli with intra-train interval of 10 seconds delivered at 90% of thenar rMT.
d) Long: 10 trains of 50-stimuli with intra-train interval of 10 seconds delivered at 90% of thenar rMT.

As with protocol 1, cortical and cerebellar excitability was measured before, immediately and every 15 minutes for 1 hour post-intervention.

2.3.5 Experimental protocol 3: Proof-of-concept study of optimal parameter cerebellar rTMS intervention in chronic post-stroke dysphagia

The recruited patient (female, 67 years, 56 days after posterior-circulation infarction, National Institutes of Health Stroke Scale=1, gastrostomy-fed with VFS confirmed dysphagia), attended the laboratory twice. On both occasions, VFS was performed initially to obtain baseline measurements. As in protocols 1 and 2, the patient was intubated with the pharyngeal catheter. Single-pulse TMS was used to identify cortical pharyngeal and thenar hotspots, whilst the mean cerebellar lateral and inferior distances from the inion in protocol 1 (Figure 2.1) were used to guide single-pulse TMS mapping of cerebellar hot-spots. Baseline cortical excitability (bilateral pharyngeal and thenar) was assessed as in protocols 1 and 2. The patient was randomised to receive sham or active cerebellar rTMS intervention (at the optimal parameters defined by the results of protocols 1 and 2) on separate visits, one week apart. Stimulation was given to the unaffected cerebellar hemisphere (i.e. contralateral to the infarction site). Excitability (TMS)

measurements were repeated immediately and 30 minutes post-intervention. A follow-up VFS was performed after the final TMS measurements on both visits.

2.4 DATA ANALYSIS

2.4.1 Electromyographic analysis: For each site, mean latency and peak-to-peak amplitudes of MEPs were determined from each group of 10 traces for pharynx and thenar, and from each group of 5 traces for cb-PMEPs. In order to minimise variability (and eliminate the effects of age and sex within our population), data were normalised to percentage change from baseline.

2.4.2 Videofluoroscopy analysis (Protocol 3 only): Frame-by-frame analysis of the VFS data took place in a blinded manner off-line.(6) The safety of all swallows was assessed and scored using the 8-point penetration-aspiration scale (PAs), describing the severity of airway compromise (190).

2.5 STATISTICAL METHODS

Cortical and cerebellar MEPs were analysed separately using a standard statistical software package (SPSS 20.0, SPSS Inc, Chicago, Illinois, USA). Initially, raw baseline MEP data were compared non-parametrically (Kruskal–Wallis) to avoid bias resulting from studies being conducted on separate days. Based on previous studies (8, 12, 154) percentage change from baseline MEP amplitudes and latencies were compared to sham using separate general linear model repeated-measures analysis of variance (rmANOVA) excluding baseline values. Significant-effects were followed-up with post-hoc analyses using Bonferroni's correction for multiple comparisons to explore the strength of the main effects. Non-sphericity was corrected using Greenhouse-Geisser where necessary. Data are displayed as mean (± standard error of the mean) unless stated otherwise.

2.6 RESULTS

2.6.1 Protocol 1: Effects of varying cerebellar rTMS frequency on PMEPs

In all 17 healthy subjects, reproducible MEPs were evoked from all cortical and cerebellar neuronavigated hot-spots (Figures 2.1 - 2.3). Cerebellar rTMS was well tolerated at all parameters without any adverse effects. 12/17 subjects had larger cb-PMEPs from left cerebellar hemisphere, whereas 5/17 had stronger right cerebellar pharyngeal representation. Therefore, no subject received interventions at the cerebellar midline. The majority of subjects (14/17) had strongest cerebellar-pharyngeal representation ipsilaterally to the strongest pharyngeal cortical representation.

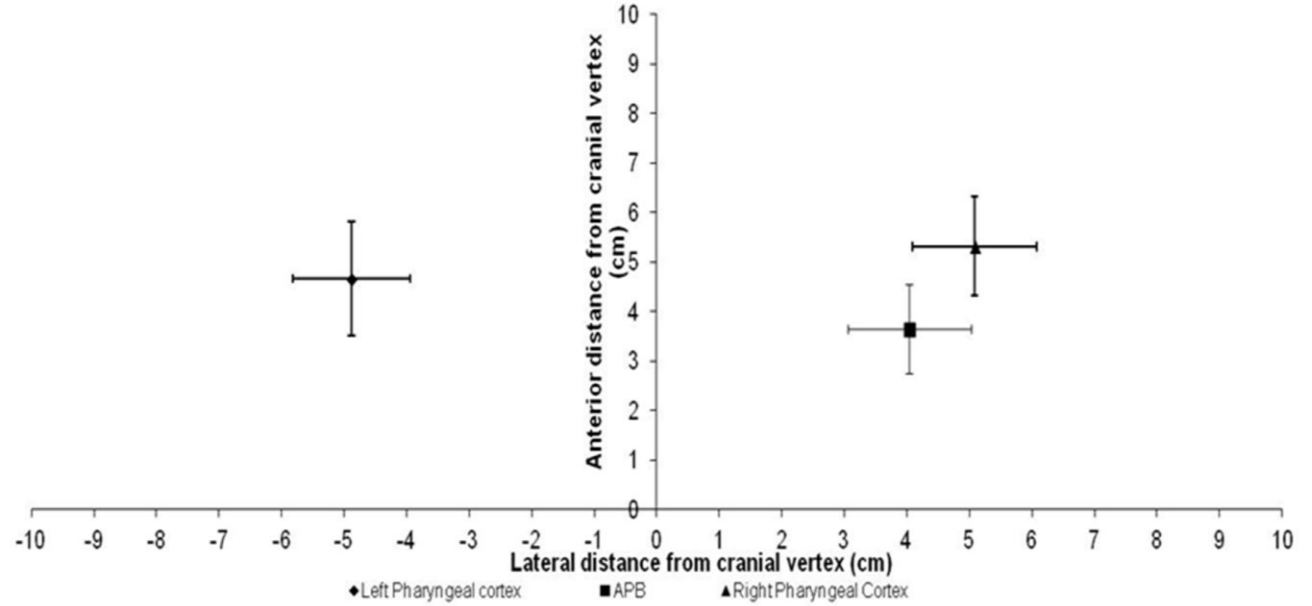
Mean cortical rMTs of ipsilateral and contralateral pharynx and thenar were 68 ± 1%, 71 ± 1% and 41 ± 1% respectively while mean cerebellar rMT was 58 ± 1% at all three cerebellar sites.

Mean baseline cortical PMEPs amplitudes were: ipsilateral 128 ± 11 μV and contralateral 113 ± 8 μV (with mean latencies: 8.4 ± 0.1 and 8.7 ± 0.3 milliseconds respectively). Mean TMEPs amplitudes were 874 ± 56 μV (latency 21.9 ± 0.1 milliseconds). Mean cb-PMEPs amplitudes were; strong hemisphere 148 ± 16 μV, midline 70 ± 6 μV and weak hemisphere 99 ± 10 μV with similar mean latencies (8.0 ± 0.1, 8.1 ± 0.1 and 8.0 ± 0.1 milliseconds respectively).

Baseline pharyngeal, thenar and cerebellar MEPs did not vary between experimental sessions (Kruskal-Wallis; ipsilateral pharynx: chi-square=0.9, df=4 *P*=0.93; contralateral pharynx: chi-square=0.2 df=4 *P*=1.0; thenar: chi-square=2.4 df=4 *P*=0.66; strong cerebellum: chi-square=4.3 df=4 *P*=0.37; mid cerebellum chi-square=3 df=4 *P*=0.57 and weak cerebellum: chi-square=0.6 df=4 *P*=0.96) and latencies (ipsilateral pharynx: chi-square=3.2, df=4 *P*=0.53; contralateral pharynx: chi-square=0.1 df=4 *P*=1.0; thenar: chi-square=0.5 df=4 *P*=.97; strong cerebellum: chi-square=0.8 df=4 *P*=0.94; mid cerebellum chi-square=0.9 df=4 *P*=0.93 and weak cerebellum: chi-square=0.3 df=4 *P*=0.99).

One-hertz cerebellar rTMS was delivered at a mean intensity of 61 ± 3% whilst high-frequency (5, 10 and 20-Hz) interventions were delivered at 53 ± 1% of stimulator output.

A)



B)

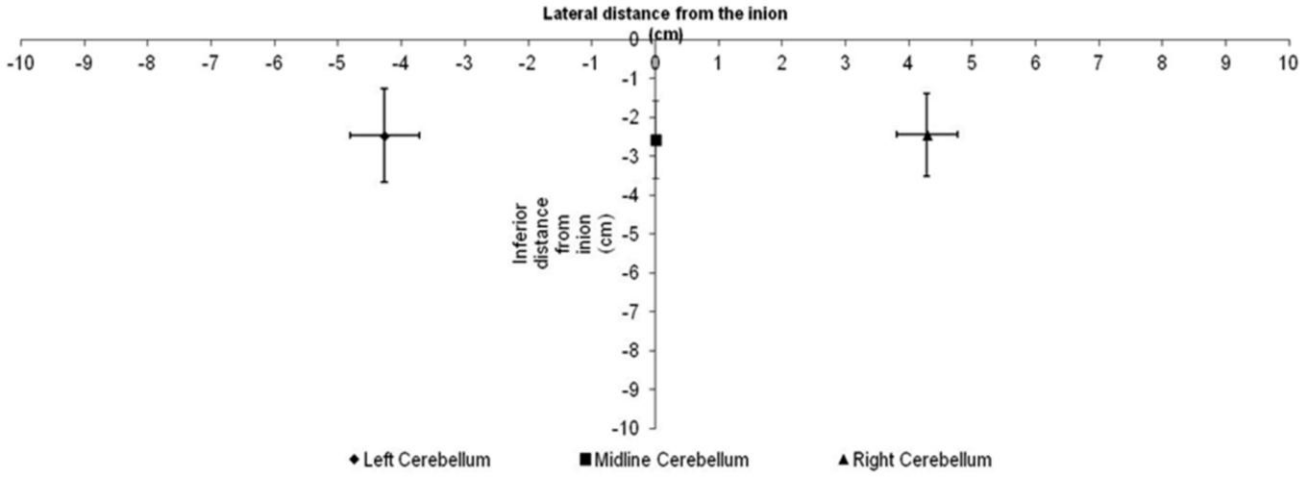


Figure 2.1: Schematic plot of motor hot-spots. *Group mean laterality and antero-posterior distances (with Standard Deviation) A) from cranial vertex to cortico-pharyngeal and cortico-thenar hot-spots B) from inion to pharyngeal motor representation in the cerebellum.*

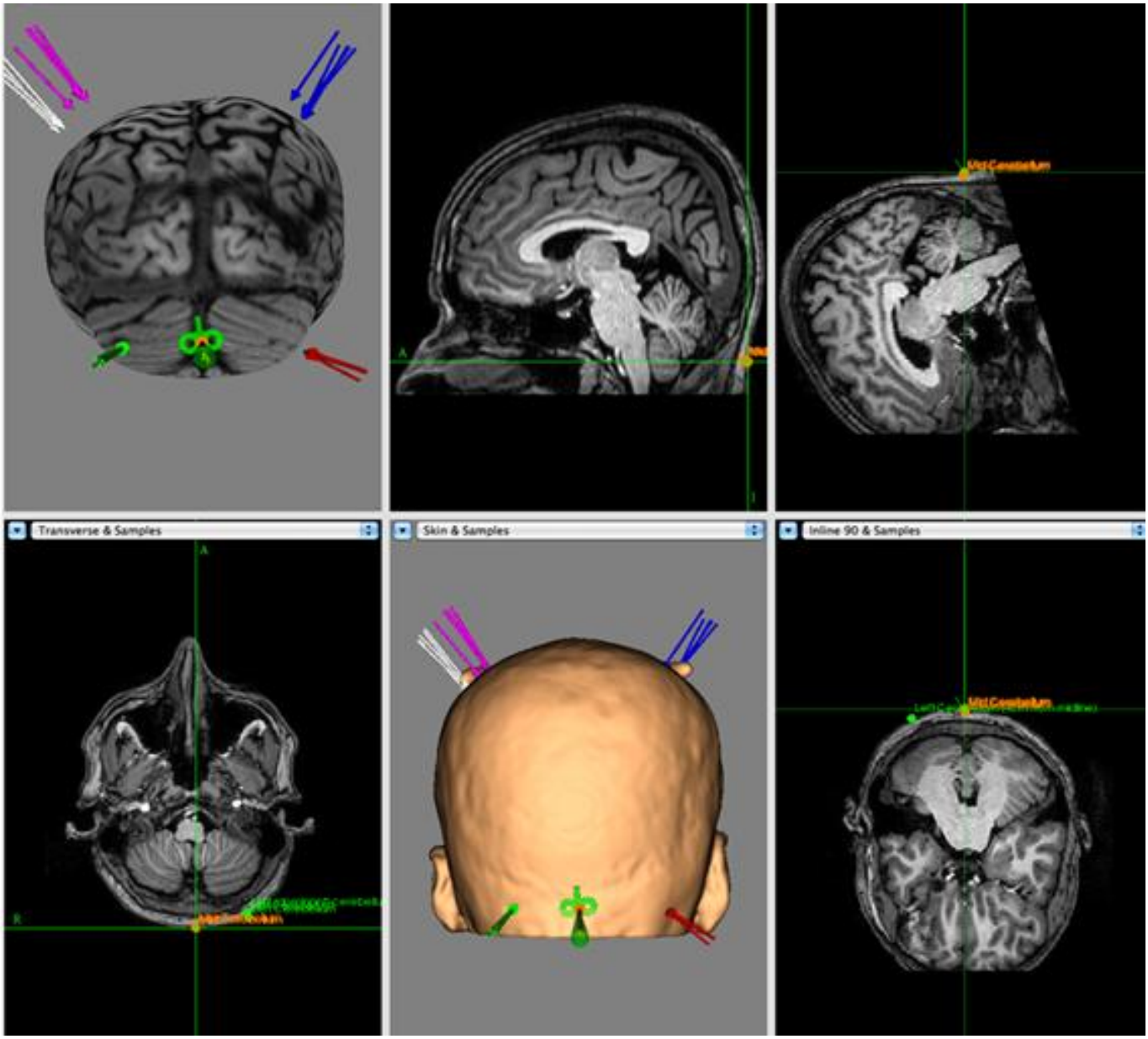
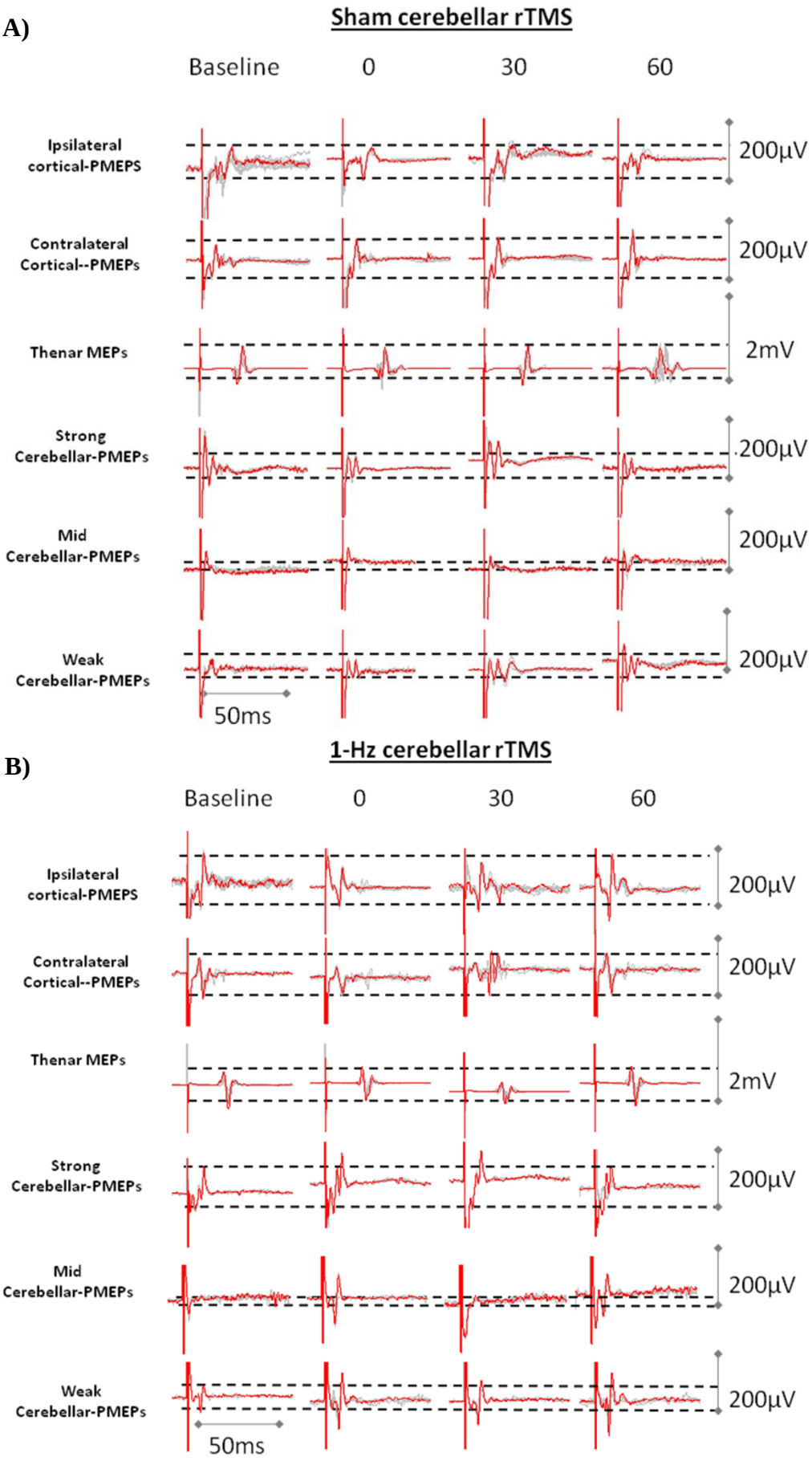
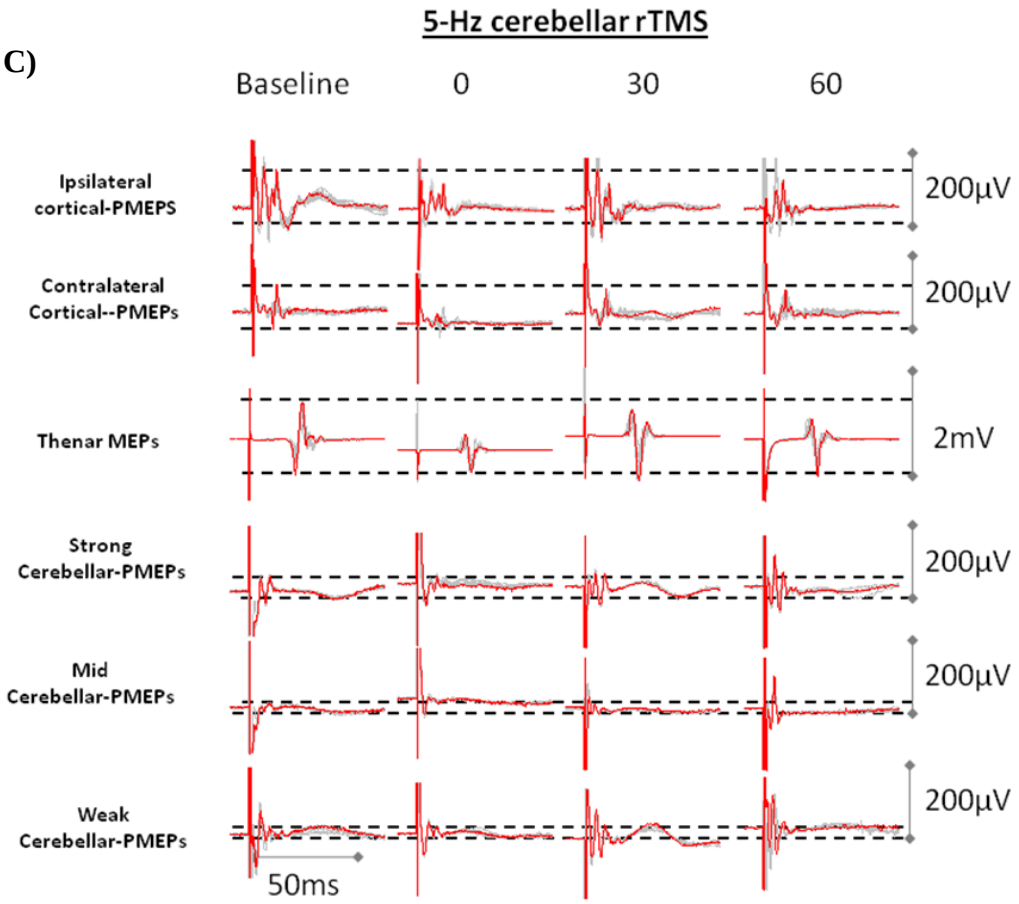


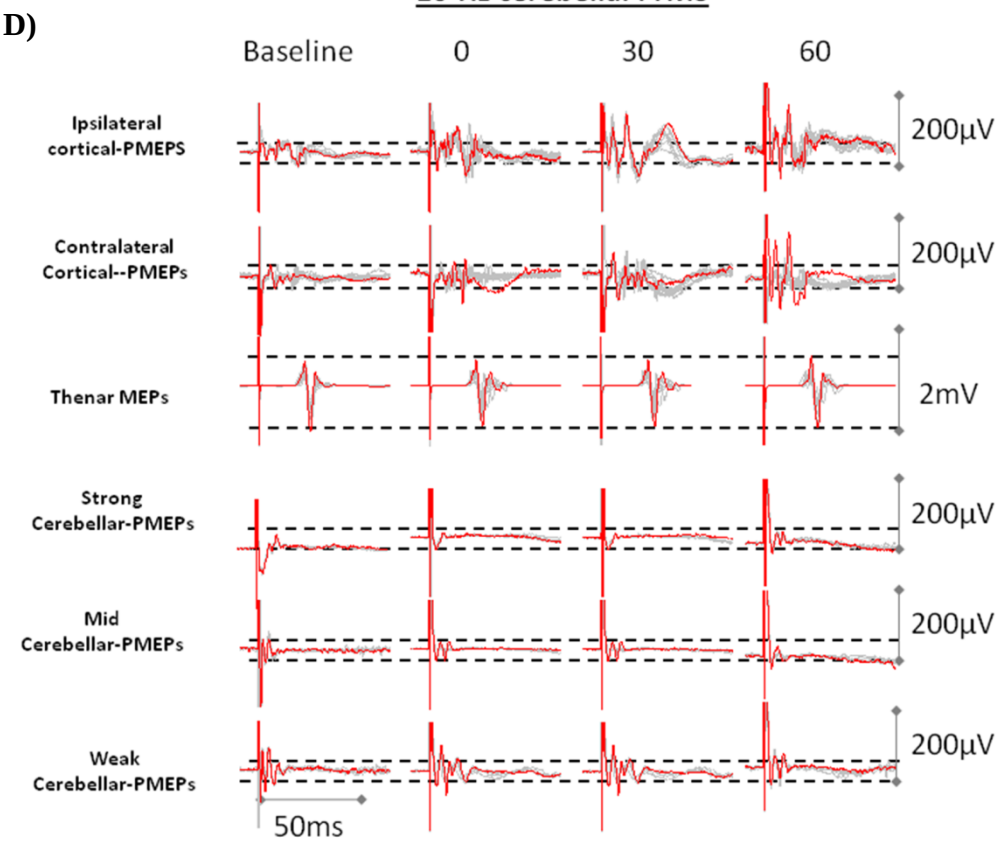
Figure 2.2: Representative neuronavigated-TMS mapping data from one subject co-registered with the subject's own MRI brain scan. *This figure shows 'hot-spot' reproducibility (each arrow at each site corresponding to one of five sessions). In this figure the coil is being targeted over the cerebellar midline.*


A)
Sham cerebellar rTMS
Baseline
0
30
60
Ipsilateral cortical-PMEPS
Contralateral Cortical--PMEPs
Thenar MEPs
Strong Cerebellar-PMEPs
Mid Cerebellar-PMEPs
Weak Cerebellar-PMEPs
200µV
200µV
2mV
200µV
200µV
200µV
50ms
B)
1-Hz cerebellar rTMS
Baseline
0
30
60
Ipsilateral cortical-PMEPS
Contralateral Cortical--PMEPs
Thenar MEPs
Strong Cerebellar-PMEPs
Mid Cerebellar-PMEPs
Weak Cerebellar-PMEPs
200µV
200µV
2mV
200µV
200µV
200µV
50ms

C)


5-Hz cerebellar rTMS
Baseline
0
30
60
Ipsilateral cortical-PMEPS
Contralateral Cortical--PMEPs
Thenar MEPs
Strong Cerebellar-PMEPs
Mid Cerebellar-PMEPs
Weak Cerebellar-PMEPs
200µV
200µV
2mV
200µV
200µV
200µV
50ms

D)


10-Hz cerebellar rTMS
Baseline
0
30
60
Ipsilateral cortical-PMEPS
Contralateral Cortical--PMEPs
Thenar MEPs
Strong Cerebellar-PMEPs
Mid Cerebellar-PMEPs
Weak Cerebellar-PMEPs
200µV
200µV
2mV
200µV
200µV
200µV
50ms

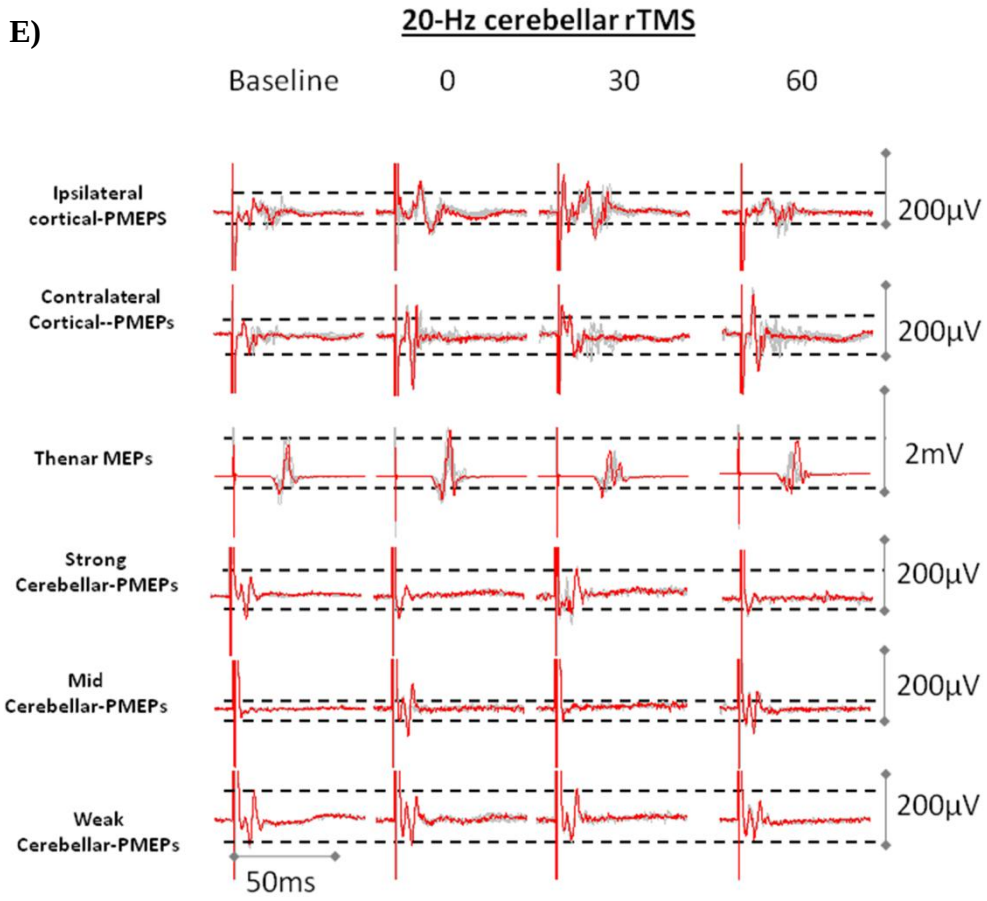
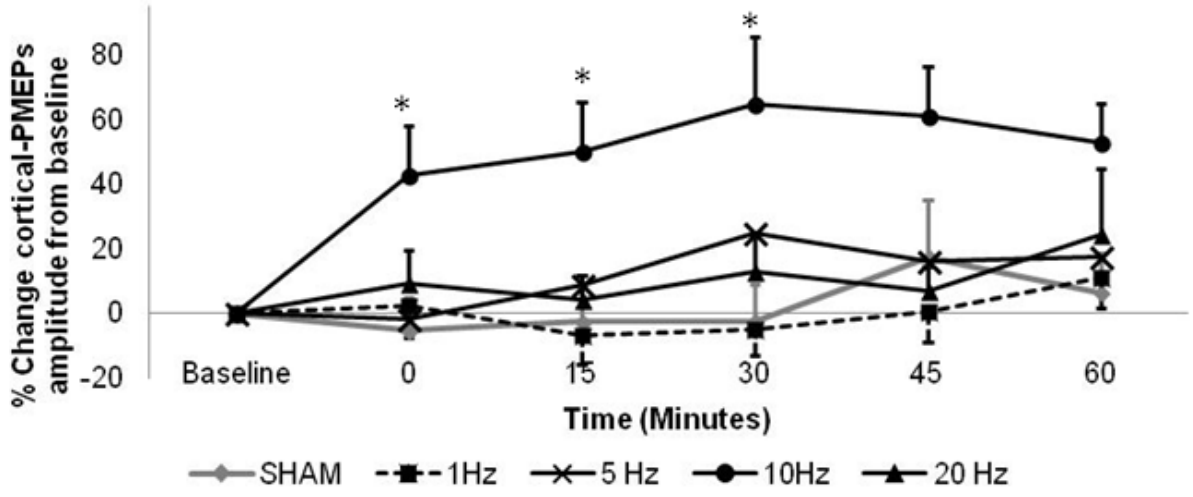


Figure 2.3 (A-E): Representative pharyngeal EMG traces from an individual participant displaying MEPs at each hot-spot following each intervention. *Trace clusters for cortical sites are comprised of 10 overdrawn responses and 5 overdrawn responses for cerebellar sites. 10-Hz cerebellar rTMS bilaterally increased pharyngeal cortical excitability compared to sham and shortened cb-PMEPs latency. Baseline cerebellar responses were consistently larger on the nominated strong (ipsilateral) cerebellar site.*

Normalised cortical PMEPs amplitudes data were examined with rmANOVA with factors of: Treatment, Hemispheric Site, and Time, and revealed a significant two-way interaction between Treatment x Time. There were also significant main effects of Treatment ($F_{4,64}$=7.0, *P*<0.001) and Time ($F_{4,64}$=2.6, *P*=0.04), without main effects of Hemispheric Site ($F_{1,16}$=0.0, *P*=0.97). Given the lack of statistical evidence to support differences in the pattern of excitability between hemispheres, PMEPs from both cortical hot-spots were combined and two-way rmANOVA with factors of: Treatment and Time. This gave a significant interaction between Treatment x Time and main effects of Treatment following 10-Hz ($F_{1,16}$=17.4, *P*=0.001, adjusted for multiple comparisons *P*=0.007 (Bonferroni)) but not for the other frequencies (1-Hz ($F_{1,16}$=0.1, *P*=0.79), 5-Hz ($F_{1,16}$=0.9,

P=0.37) or 20-Hz ($F_{1,16}$=0.7, *P*=0.43), Figure 2.4A). Subsequent one-way ANOVAs with within-subject factor of Treatment (sham, 1-Hz, 5-Hz, 10-Hz and 20-Hz) on the combined hemispheric data revealed significant overall effects of Treatment at the following time-points; immediately ($F_{4,80}$=3.6, *P*=0.01), 15 minutes ($F_{4,80}$=5.4, *P*=0.001), 30 minutes ($F_{4,80}$=5.1, *P*=0.001) and trended towards significance at 45 minutes ($F_{4,80}$=4, *P*=0.06) post-intervention. Post-hoc analyses with Bonferroni's correction confirmed significant effects compared to sham only after 10-Hz intervention that were present immediately (*P*=0.01); at 15 minutes (*P*=0.003) and peaked 30 minutes post-intervention (mean difference +67%, *P*=0.003).

A)



B)

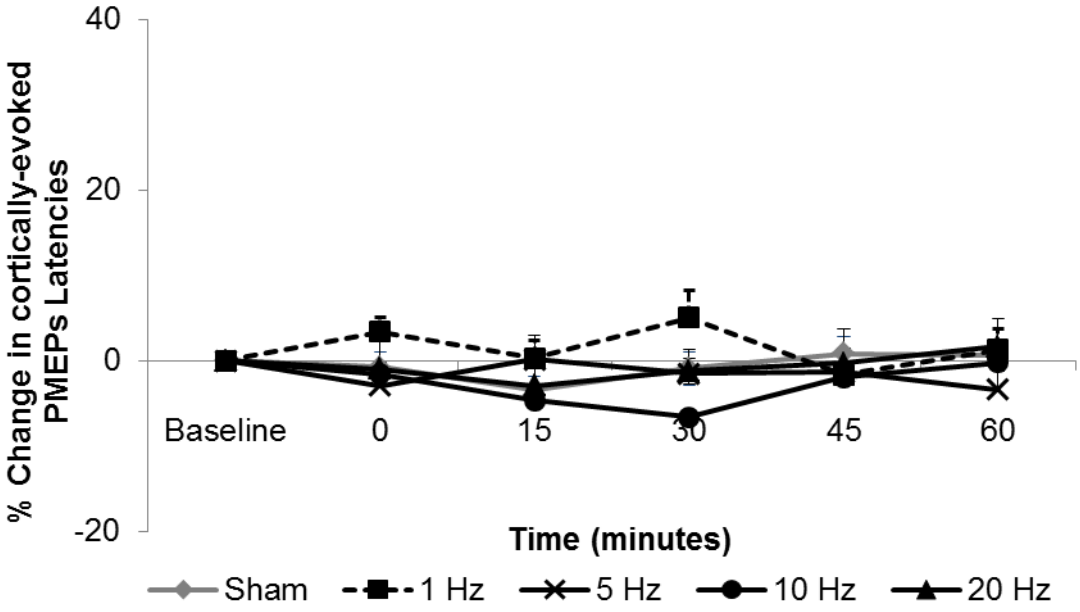


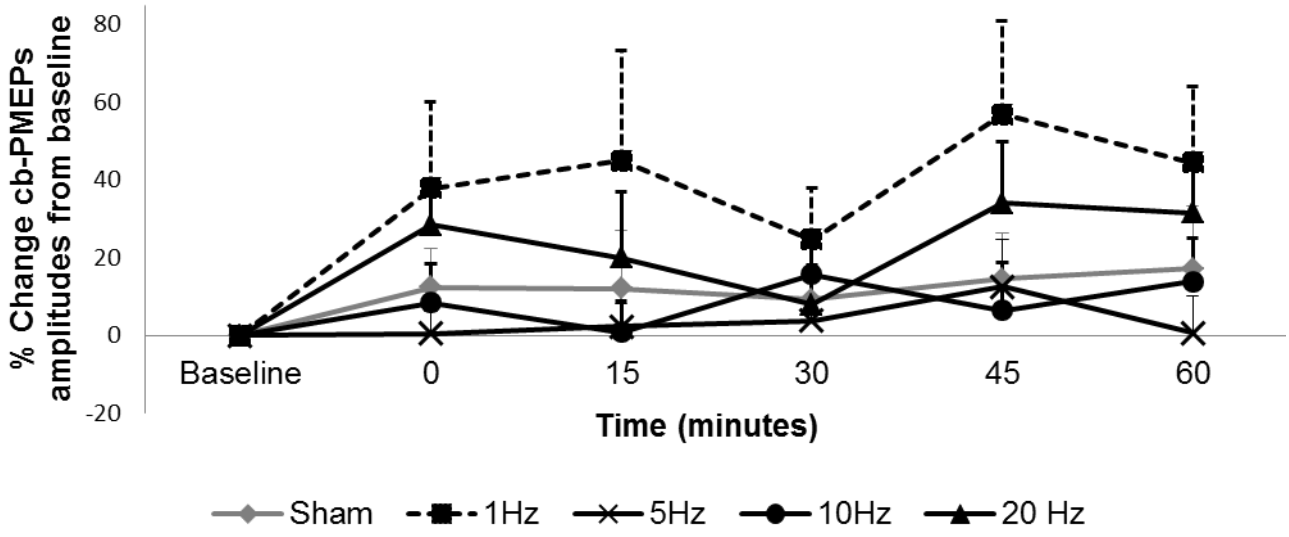
Figure 2.4: Effects of cerebellar rTMS on group mean (± standard error of the mean (SEM)) cortical-PMEPs; A) amplitudes B) latencies (combined data from both hemispheres). *10-Hz cerebellar rTMS increased cortical excitability compared to sham (P=0.007 (Bonferroni), with effects peaking 30 minutes post-intervention (*P<0.05) without altering cortical PMEPs latencies.*

Cortical PMEPs latencies were similarly compared, combining data from both pharyngeal hemispheres and examined with rmANOVA with factors of: Treatment and Time. There were no interactions between Treatment x Time ($F_{6,95}$=2.0, *P*=0.07) or main effects of factors (Treatment; $F_{4,64}$=1.4, *P*=0.24, Time; $F_{4,64}$=0.4, *P*=0.82), therefore further analyses were not considered (Figure 2.4B).

The effects on cerebellar excitability were compared using rmANOVA with factors of: Treatment, Cerebellar Site, and Time. There were no significant interactions between or main effects of factors (Treatment ($F_{3,38}$=1.8, *P*=0.17), Cerebellar Site ($F_{2,28}$=.64, *P*=0.54) or Time ($F_{4,56}$=.8, *P*=0.53)). Amongst the active interventions, only 1-Hz ($F_{1,14}$=3.15, *P*=0.09) displayed trend towards an effect of Treatment in increasing cerebellar excitability compared to sham (Figure 2.5A).

By comparison, cb-PMEPs latency data showed a significant Treatment x Cerebellar Site x Time interaction. Additionally, there was a significant main effect of 10-Hz Treatment ($F_{1,12}$=6, *P*=0.03), however this was not apparent after adjusting for multiple comparisons (mean difference -6.9 ± 2.8%, Bonferroni: *P*=0.30). There were also main effects of Time ($F_{4,48}$=3.0, *P*=0.03) but no effect of cerebellar site ($F_{2,24}$=2.1, *P*=0.15). Given the lack of evidence to support an effect of cerebellar site, we combined latencies from the three cerebellar sites, performing two-way rmANOVA with factors of: Treatment and Time. This did not reveal any significant Treatment x Time interaction or any main effects of Treatment ($F_{4,60}$=1.2, *P*=0.32) or Time ($F_{4,60}$=.8, *P*=0.49), therefore further analyses were not considered, Figure 2.5B.

A)



B)

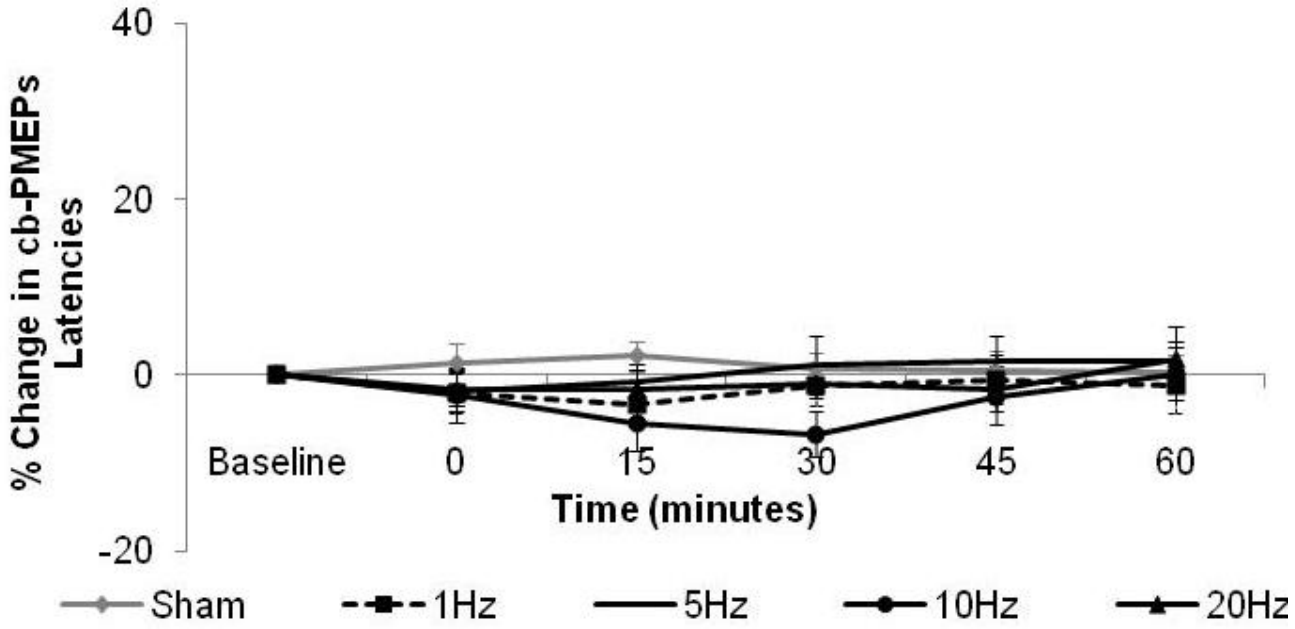


Figure 2.5: Effects of cerebellar conditioning frequency on group mean (± SEM) cb-PMEPs (combined data from all cerebellar hot-spots; A) amplitudes) B) latencies. *No interventions significantly altered cb-PMEPs compared to sham*

Network-specificity of cortical effects compared to sham were examined using rmANOVA with factors of: Treatment, Cortical Site (pharyngeal cortical sites-combined, Thenar) and Time revealed significant interactions between Treatment x Time and Site x Time and significant main effects of Treatment ($F_{4,64,}$=4.3, *P*=0.004). Subsequent two-way rmANOVA with factors of: Treatment and Time on TMEPs were non-significant (Treatment x Time; $F_{7,114}$=0.9, *P*=0.58, Treatment; $F_{2,40}$=0.8, *P*=0.51, and Time; $F_{4,64}$=0.7, *P*=0.63). This indicated that almost all of the effect was driven by changes in pharyngeal motor cortical output. Similarly, TMEPs latencies (compared the same way) did not reveal any interactions between Treatment x Time ($F_{16,24}$=0.5, *P*=0.94) or main effects of Treatment ($F_{4,60}$=0.6, *P*=0.68) or Time ($F_{4,60}$=0.26, *P*=0.9).

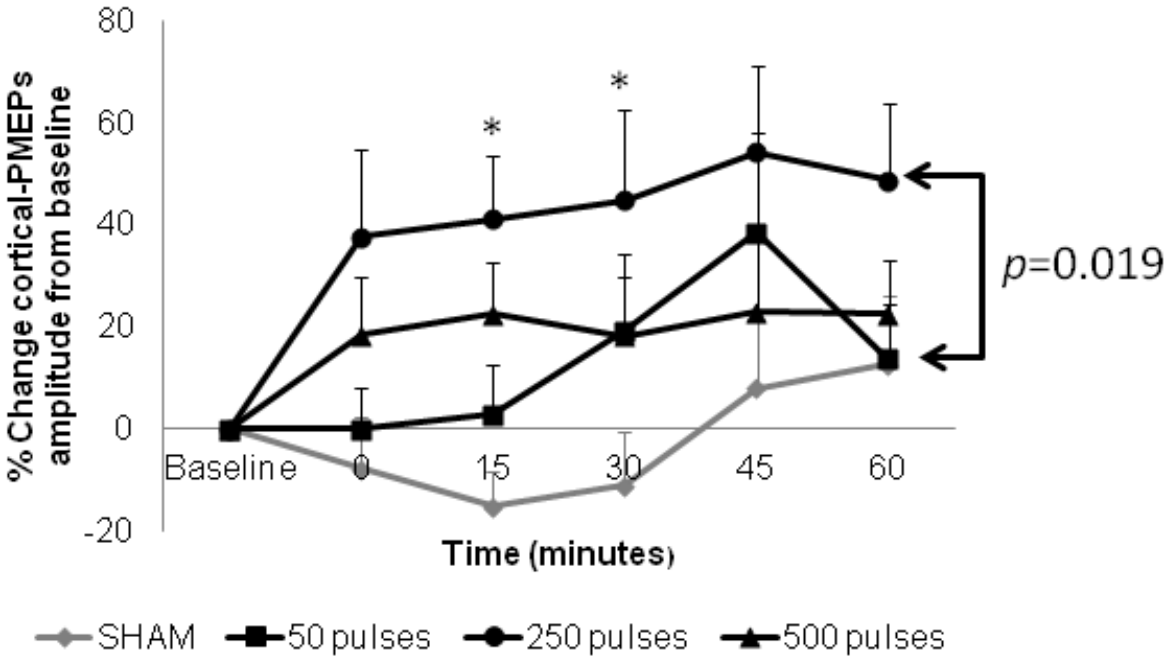
2.6.2 Protocol 2: The effects of varying cerebellar rTMS duration on PMEPs

For this component of the study, the mean baseline cortical PMEPs amplitudes were; ipsilateral 130 ± 18 μV and contralateral 121 ± 12 μV (with mean latencies 8.5 ± 0.1 and 8.7 ± 0.1 milliseconds respectively). Mean TMEPs amplitudes were 699 ± 64 μV with latency 22 ± 0.2 milliseconds. Mean cb-PMEPs amplitudes; strong hemisphere 155 ± 12 μV, midline 74 ± 9 μV and weak hemisphere 95 ± 9 μV with mean latencies; 7.9 ± 0.1, 8.3 ± 0.1 and 8.1 ± 0.1 milliseconds respectively.

The trains of 10-Hz cerebellar rTMS were delivered at similar mean intensities (50-pulses: 55 ± 3%, 250-pulses: 53 ± 2% and 500 pulses: 58 ± 2%) of stimulator output.

The effects of varying train-lengths of 10-Hz cerebellar rTMS on combined PMEPs amplitudes from both hemispheres were compared to sham using rmANOVA with factors of: Treatment (sham, 50, 250 or 500-pulses) and Time. Whilst there were no significant interactions between factors, there were overall significant main effects of Treatment ($F_{3,33}$=4.2, *P*=0.013) and Time ($F_{2,27}$=3.2, *P*=0.05). Only 250-pulses intervention significantly increased excitability compared to sham (mean increase: 48 ± 13 %, $F_{1,11}$=14.0, *P*=0.003, multiple comparisons correction *P*=0.019 (Bonferroni)). There was also a trend towards Treatment effect following the longest (500-pulses; mean increase: 21 ± 9%, $F_{1,11}$=4.5, *P*=0.058) but not with the shortest intervention (50-pulses; 15 ± 9%, $F_{1,11}$=1.7, *P*=0.21), Figure 2.6. Subsequent one-way ANOVAs with a within-subject factor of Treatment (sham, 50, 250, 500-pulses) on the combined hemispheric data revealed that overall effects of Treatments built up within the first 30 minutes after intervention (immediately ($F_{3,44}$=2.8, *P*=0.05), 15 minutes ($F_{3,44}$=6, *P*=0.002), 30 minutes ($F_{3,44}$=2.7, *P*=0.059)). Post-hoc tests (Bonferroni) confirmed that these significant Time effects over the first 30 minutes were driven by the 250-pulse intervention (immediately (*P*=0.06); at 15 minutes (*P*=0.001) and 30 minutes post-intervention (*P*=0.04)).

A)



B)

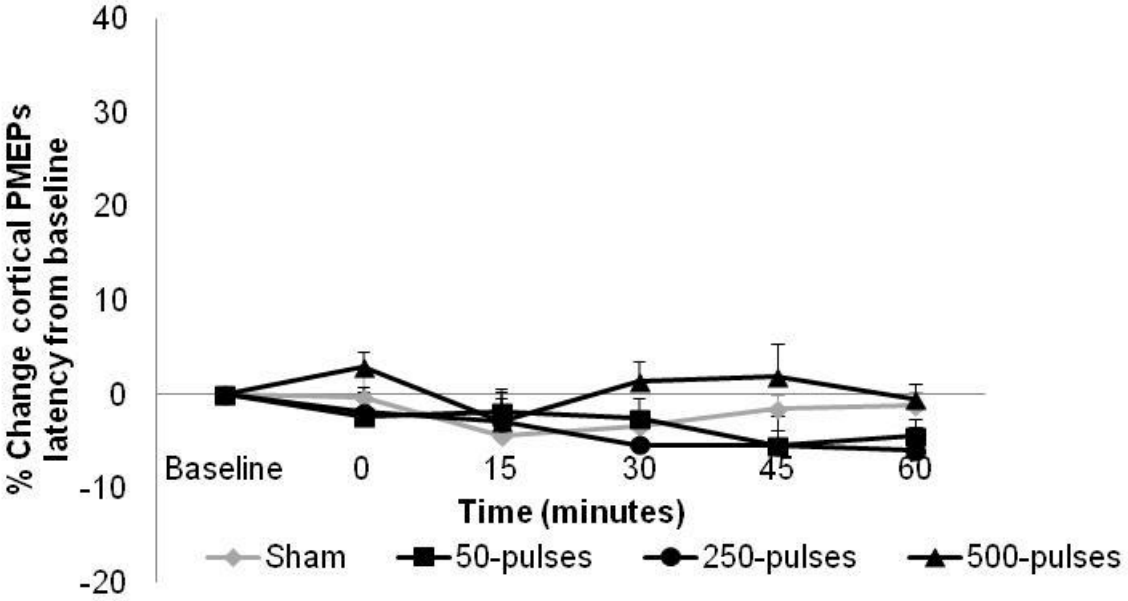


Figure 2.6: Group mean (±SEM) cortical PMEPs A) amplitudes and B) latencies following short (50-pulses) and longer trains (250 and 500-pulses) of 10-Hz cerebellar rTMS (combined data from both hemispheres). *250-pulses train-length optimally increased cortical PMEP amplitudes compared to sham (P=0.019) without altering latency.*

As before, compared to sham, none of the 10-Hz interventions altered cortical PMEPs latencies (Treatment x Time; $F_{12,120}$=0.8, *P*=0.67; Treatment $F_{3,30}$=1, *P*=0.43; Time; $F_{4,40}$=2.2, *P*=0.09,Figure 2.6B).

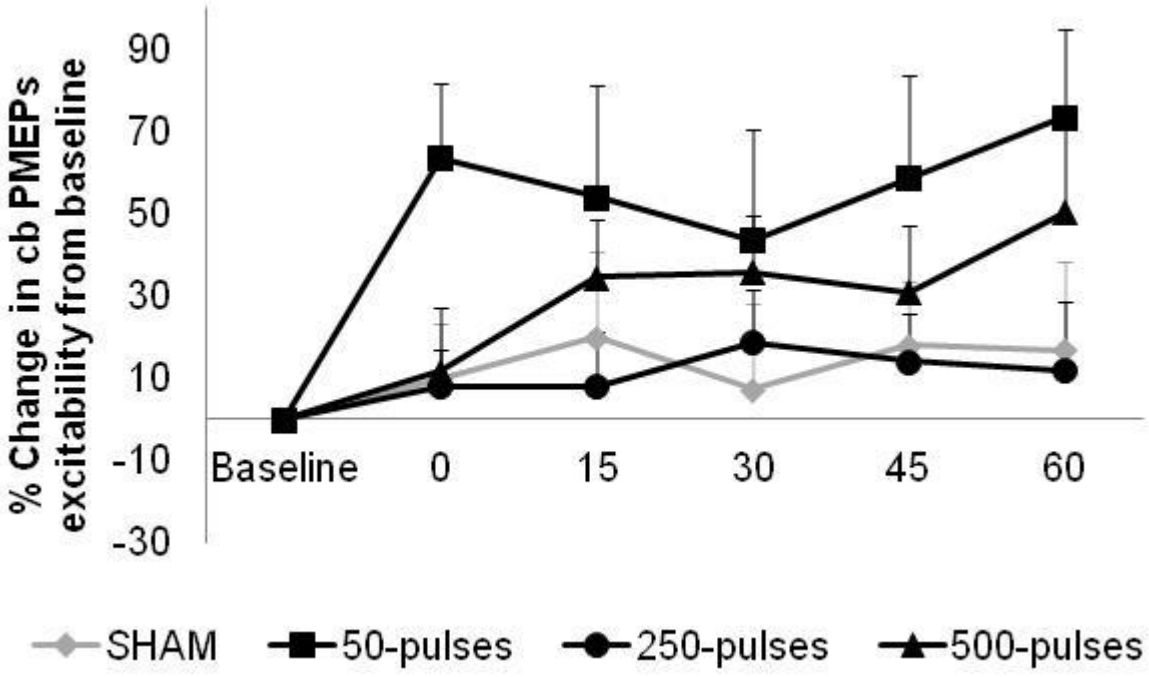
None of the train-lengths of 10-Hz rTMS altered cerebellar excitability compared to sham, with no significant interactions or main effects of factors (Treatment: $F_{3,27}$=1.9, *P*=0.15; Cerebellar Site: $F_{2,18}$=1.9, *P*=0.18; Time: $F_{4,36}$=1.1, *P*=0.38; Figure 2.7A).

Three-way rmANOVA on cb-PMEPs latencies revealed significant interactions of Treatment x Cerebellar Site x Time and Treatment x Time but without main effects (Treatment: $F_{3,18}$=1.5, *P*=0.26; Cerebellar Site: $F_{1.2,6.9}$=5.1, *P*=0.06; Time: $F_{4,24}$=0.9, *P*=0.50). The significant interactions were followed-up with a two-way rmANOVA with factors of Treatment and Time on combined data from the three cerebellar sites, which again revealed significant interaction between Treatment x Time without main effects. Subsequent one-way ANOVA on cb-PMEPs latency data suggested time-point specific effects ($F_{3,45}$=3.1, *P*=0.04) but post-hoc analyses (Bonferroni) did not reveal any statistically significant time-points after any intervention (Figure 2.7B).

<u>TMEPs</u>

As per protocol 1, we examined network-specificity of cortical effects using three-way repeated measures ANOVA with factors: Treatment, Cortical Site (Pharyngeal (combined) and Thenar) and Time. This confirmed significant interaction of Site x Time without main effects in of any the factors. Subsequent two-way repeated measures ANOVA on TMEPs (i.e. Thenar Site) did not reveal significant interactions between Treatment x Time or main effects of factors (Treatment: $F_{3,30}$=0.09, *P*=0.97, Time; $F_{4,40}$=1.0, *P*=.40) indicating that none of the train-lengths of 10-Hz cerebellar rTMS interventions altered Thenar cortical excitability. Similarly, there were no significant interactions of Treatment x Time or any main effects of factors (Treatment $F_{3,30}$=0.5, *P*=0.69; Time;$F_{4,40}$=1.4, *P*=0.25) on TMEPs latencies.

A)



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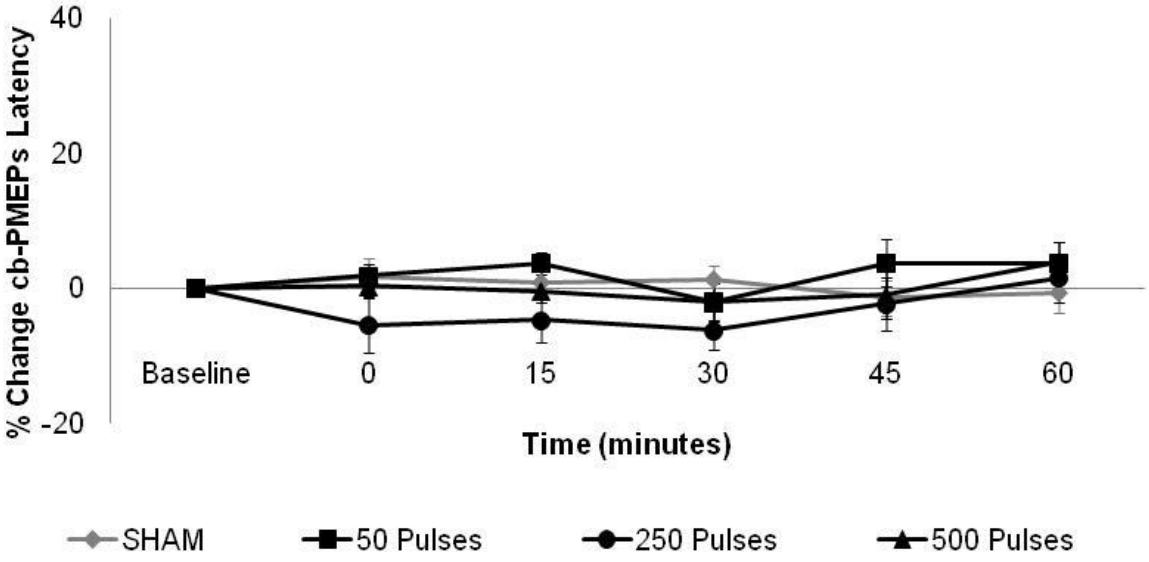
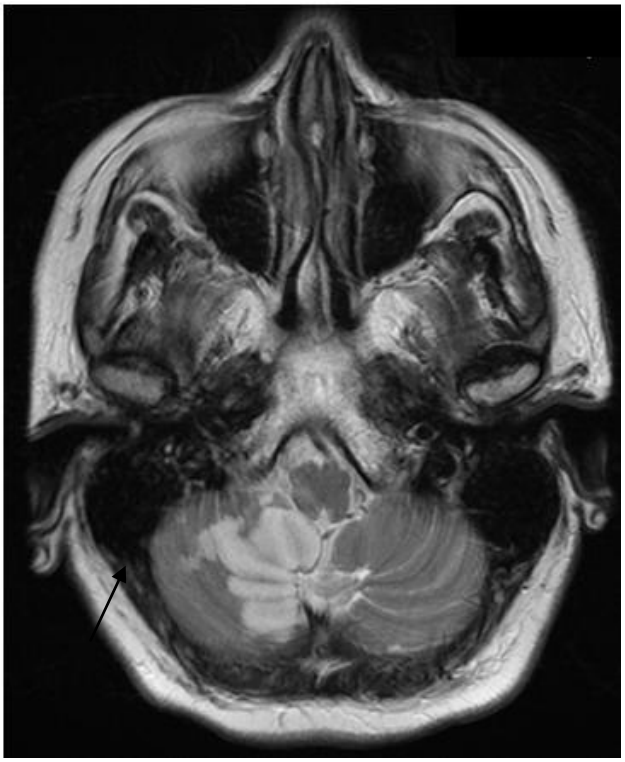


Figure 2.7: Group mean change (± SEM) in cerebellar PMEPs A) amplitudes and B) latencies (combined data from all cerebellar hot-spots) following short (50-pulses) and longer trains (250 and 500-pulses) of 10-Hz cerebellar rTMS. *None of the three train-lengths of 10-Hz rTMS produced differences in cb-PMEPs compared to sham.*

2.6.3 Protocol 3: Randomised controlled case-study of optimal parameters (10-Hz, 250-pulses) cerebellar rTMS in chronic post-stroke dysphagia

The patient's MRI brain scan confirmed extensive right cerebellar injury, whilst baseline VFS (PAs 5) confirmed oropharyngeal dysphagia (Figure 2.8 A & B). Cerebellar rTMS was delivered to the undamaged, left cerebellar hemisphere (at 47% stimulator output). Active intervention visibly increased cortico-pharyngeal excitability immediately post-intervention without any cortico-thenar changes (Figure 2.8C). Moreover, compared to sham, active intervention appeared to also improve swallowing safety during VFS post-intervention (Figure 2.8D).

A)



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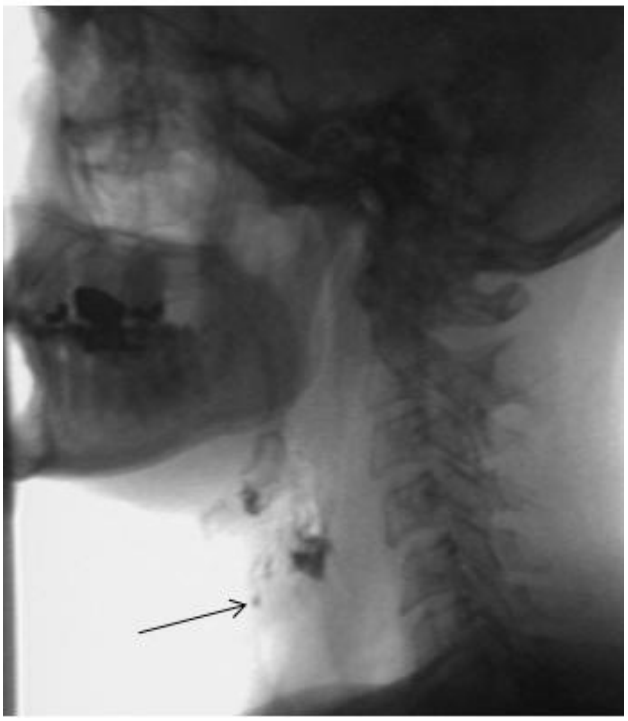
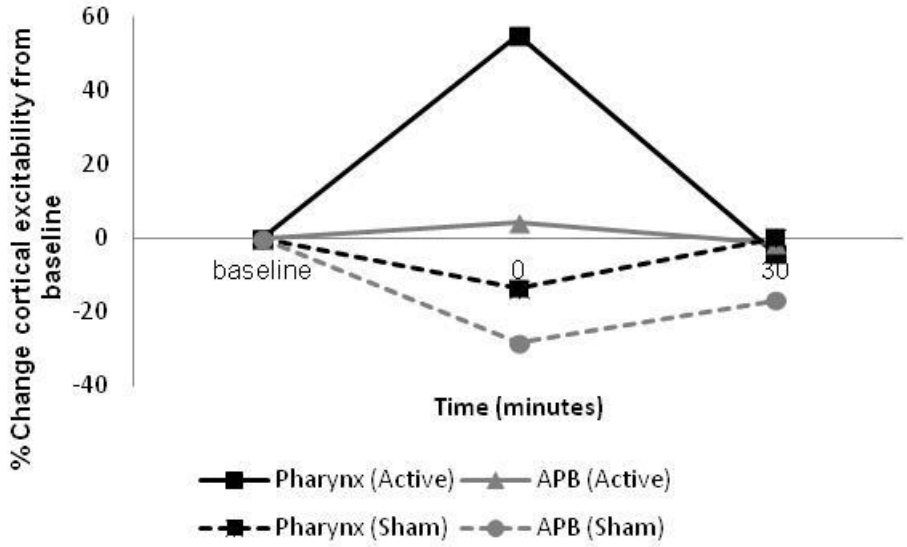


C)


60
40
20
0
-20
-40
% Change cortical excitability from baseline
baseline
0
30
Time (minutes)
Pharynx (Active)
APB (Active)
Pharynx (Sham)
APB (Sham)

D)

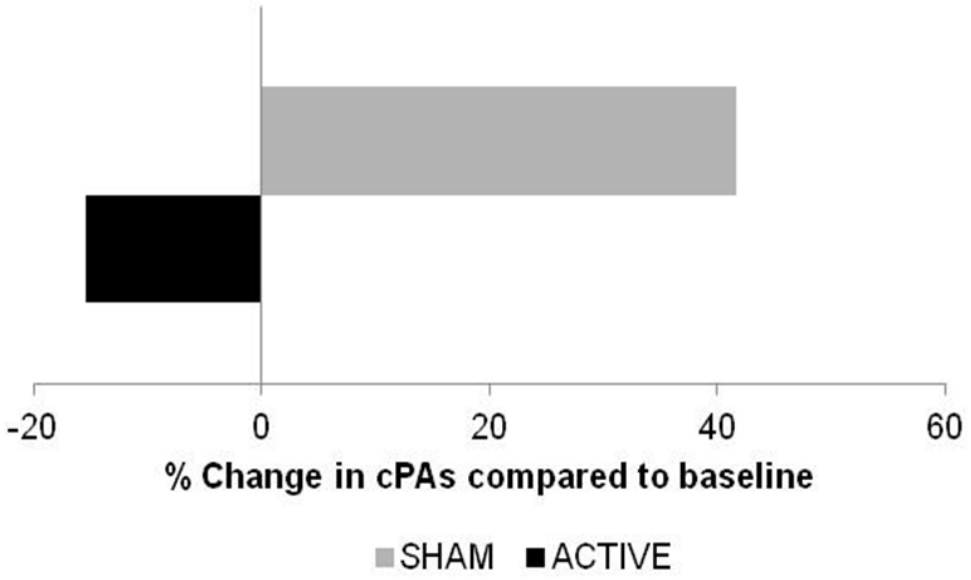


Figure 2.8: Sham-controlled case study of a patient with chronic dysphagia post posterior circulation stroke. A) MRI brain scan showing infarction of right medulla and cerebellum. B) VFS image showing entry of contrast into the airway (arrow) contacting vocal cords with failure to be ejected (PAs=5). The effects of cerebellar rTMS on; C) cortical motor excitability (both pharyngeal hot-spots combined) and D) cPAs on VFS were worse after sham, improving after active intervention.

2.7 DISCUSSION

I examined the effects of differing frequencies of cerebellar rTMS on pharyngeal cortical and cerebellar excitability. The findings supported my hypothesis that high-frequency cerebellar rTMS can robustly produce clinically relevant effects on the excitability of corticobulbar projections to the pharynx. Of interest, these effects were frequency specific, with the effects of 10-Hz cerebellar rTMS not extending to thenar cortex indicating system specificity within the pharyngeal cortex. Of further interest, our neuronavigated study provides further anatomical information about pharyngeal motor representation in the cerebellum. These findings may be important in understanding dysphagia after cerebellar injury, the role of the cerebellum in swallowing and the potential role of cerebellar conditioning in post-stroke dysphagia rehabilitation and are discussed further.

2.7.1 Effects of high-frequency cerebellar conditioning on pharyngeal cortical excitability

Based on previous work which demonstrated that paired-pulses of cerebellar and cortical TMS delivered at intervals of 50, 100 and 200ms can facilitate pharyngeal motor pathways, I hypothesised that trains of cerebellar rTMS may induce long-term plasticity in the pharyngeal motor system (53). My data now confirm that only 10-Hz cerebellar rTMS increased excitability of the corticobulbar projections to the pharynx compared to sham. These observations using cerebellar conditioning are in contrast to peripheral (pharyngeal electrical)(4) and cortical (rTMS) stimulation(7), where only 5-Hz interventions increased pharyngeal corticobulbar excitability. In the case of peripheral and cortical neurostimulation, specificity of the 5-Hz interventions can be explained by the 200 millisecond ISIs between consecutive stimuli providing adequate time for pharyngeal sensory input to reach the cortex (60-80ms), interaction of co-incident input at the level of the sensorimotor cortex and dissipation of refractory repolarisation allowing greater 'wind-up' of the swallowing system (60). Our current data may suggest that stimuli delivered to the pharyngeal cerebellar region may have a shorter conduction pathway to cortex (half the duration) possibly indirectly via the brainstem, thus favouring a shorter ISIs of 100ms between consecutive stimuli. Further research would be necessary to confirm the conduction latency for cerebello-cortical sensory evoked potentials and test this hypothesis. An alternative explanation could be that if the cerebellum has a tonic facilitatory effect on the cortex/brainstem, then some properties associated with 10-Hz stimulation may act locally in the cerebellar cortex to modulate this tonic level of drive to the motor cortex.

In terms of high-frequency intervention parameters, previous work with cortical rTMS has shown that excitatory effects are train-length but not intensity dependent, with 250-pulses having superior efficacy compared with a 100-pulse and 1000-pulse intervention (8). Recently, a small clinical trial in post-stroke dysphagia reported therapeutic benefit using a set 500-pulse intervention without confirming its effectiveness on cortical excitability (21). In the present study, the effects of 250-

pulses train length compared to sham were stronger than either shorter (50-pulses) or longer (500-pulses) train-lengths, suggesting a ceiling effect, with no additional excitatory benefit of increasing the number of stimuli beyond 250-pulses.

2.7.2 Effects of low-frequency cerebellar conditioning on pharyngeal cortical excitability

One-hertz cerebellar rTMS did not alter pharyngeal motor excitbaility, contrasting the effects of direct cortical 1-Hz stimulation using the same train-length. One explanation for this finding could be that cerebellar inputs to the central cortical swallowing system have little or no inhibitory functions. Therefore attempting to induce inhibition in the cortical system with (presumed) inhibitory paradigms to the cerebellum may be inherently impossible. However, if we assumed that the input from the cerebellum was tonic and facilitatory, another explanation could be that the delievered intensities of 1-Hz rTMS (limited to 90% of cerebellar rMT to confirm safety and tolerability) were not high enough to induce significant inhibition in the cortical system through indirect pathways. This would mirror the situation with cortical rTMS, where only high-intensity 1-Hz interventions (120% pharyngeal rMT) could sufficiently suppress cortical-PMEPs(69). Future studies examining swallowing neurophysiology and behaviour may therefore compare the effects of higher intensities or duration of 1-Hz rTMS, targeting cerbellum either ipsilaterally, contralaterally or even bilaterally.

2.7.3 Pharyngeal representation of the cerebellum and site specificity of cortical effects

A recent mapping study demonstrated that topographically organised motor responses can be evoked from cerebellar cortex (191) and previous work from our group has shown that cb-PMEPs can be evoked using single-pulse TMS (53). With the advantage of neuronavigation I have now been able to confirm pharyngeal representation in the cerebellum is consistently ~4.3cm lateral to the inion for cerebellar hemispheres but optimal sites were lower (~2.5cm) than in the previous non-navigated study,(53) potentially explaining why baseline cb-PMEP amplitudes were larger and

more comparable in magnitude to cortical-PMEPs with slightly lower cerebellar rMTs in the present study.

Specifically targeting the pharyngeal motor representation in the cerebellum may explain the lack of effects of our interventions on thenar excitability. This is in keeping with the evidence that that the hand musculature has previously been described to be represented more rostrally within hemispheric lobule VI of the cerebellum (191). Whilst I observed bilateral pharyngeal cortical excitation after cerebellar conditioning, another possible explanation for the lack of effect on thenar excitability may be that we did not record TMEPs bilaterally and therefore may have missed an opportunity to study the effects of cerebellar rTMS on the opposite thenar motor cortex. Cerebellar rTMS hand literature have largely focussed on assessing the excitability of the contralateral M1 following 1-Hz rTMS, often using higher intensities(192) and longer stimulation duration (192-193) than our study, with some authors reporting an increase in intracortical M1 excitability(194) but others reporting a decrease (192-193). However this remains a contentious issue due to conflicting data using differing parameters and measures of M1 excitability (195).

2.7.4 Effects of cerebellar rTMS interventions on cerebellar excitability

None of the cerebellar interventions trialled significantly altered direct cerebellar excitability compared to sham. Limitations of only recording five traces at each site per timepoint and performing cb-PMEPs at rMT +10% might have contributed to less stable responses (67). Another limitation may be use of a figure-of-eight coil in our cerebellar recordings. A recent study suggests superiority in terms of stimulation depth and consistency of MEP responses at lower intensities using a double-cone TMS coil for cerebellar stimulation (196). Interestingly, our data showed a visible but non-significant increase in cerebellar activity following inhibitory (1-Hz) rTMS accompanied by a trend to reduction in cortical excitability. Conversely, 10-Hz rTMS had the lowest cerebellar activity of the interventions trialled and yet produced the highest cortical

excitability. Whilst these data should be interpreted with caution, they suggest some correlation between cerebellar excitability and the detected changes in cortical excitability, implicating possible modulation of cerebellar excitability with some (bidirectional) influence on intercurrent cortical excitability. This data may infer that cerebellar rTMS might produce effects on corticobulbar projections by modulating both pharyngeal motor cortex and brainstem, whereas cb-PMEPs may only be affected locally by changes in brainstem excitability.

2.7.5 Clinical utility

As a novel treatment approach, cerebellar stimulation may have advantages compared to other neurostimulation techniques. For example, it may be considered less invasive than pharyngeal stimulation, given that intraluminal catheter intubation is not required. Additionally, safety data suggest much lower risk of seizures as an adverse event following cerebellar rTMS(189) compared to motor areas (145-146). My controlled case-study data demonstrates that clinical effects may be provoked by stimulating the unaffected cerebellum in a patient with a posterior circulation infarction. The observed reduction in aspiration scores after cerebellar rTMS are comparable with the effects of other neurostimulation interventions in chronically dysphagic stroke patients (6). Our data also suggests that cerebellar neurostimulation could be beneficial in dysphagia after cortical-stroke. Further work should therefore focus on applying the optimal parameters of cerebellar rTMS in healthy subjects after focal cortical inhibition to the pharyngeal motor cortex and thence a randomised controlled trial in post-stroke dysphagia.

2.7.6 Conclusion

In conclusion, my findings show that cerebellar rTMS can modulate pharyngeal corticobulbar excitability with long-lasting effects, in a frequency and duration dependent manner that could have future therapeutic potential for the treatment of dysphagia.

CHAPTER 3

TRANSCRANIAL DIRECT CURRENT STIMULATION REVERSES NEUROPHYSIOLOGICAL AND BEHAVIOURAL EFFECTS OF FOCAL INHIBITION OF HUMAN PHARYNGEAL MOTOR CORTEX ON SWALLOWING

Dipesh H Vasant,

Satish Mistry, Emilia Michou, Samantha Jefferson, John C Rothwell and Shaheen Hamdy.

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3.1 KEY POINTS SUMMARY

- Cortical control of swallowing exhibits functional asymmetry with brain lesions involving the strongest projection being implicated in the pathophysiology of dysphagia after unilateral stroke.
- Swallowing recovery is associated with neuroplastic adaptation in the unlesioned hemisphere, a process which can be facilitated by excitatory neurostimulation techniques including transcranial Direct Current Stimulation (tDCS).
- Unilateral suppression of the strongest pharyngeal motor projection using 1-Hz repetitive Transcranial Magnetic Stimulation (rTMS) can disrupt swallowing neurophysiology and behaviour making it a useful model for trialling novel neurostimulation interventions in healthy subjects.
- In this healthy participant study we examined the effects of tDCS after unilateral pre-conditioning with 1-Hz rTMS to determine its ability to restore swallowing neurophysiology and behaviour.
- We show that application of optimised parameters of tDCS (anodal stimulation, 1.5mA, 10 minutes) over the unconditioned hemisphere reverses the brain and behavioural consequences of inhibitory pre-conditioning, supporting the use of tDCS in clinical trials.

3.2 ABSTRACT

The human cortical swallowing system exhibits bilateral but functionally asymmetric representation in health and disease as evidenced by both focal cortical inhibition (pre-conditioning with 1-Hz repetitive Transcranial Magnetic Stimulation (rTMS)) and unilateral stroke where disruption of the stronger (dominant) pharyngeal projection alters swallowing neurophysiology and behaviour. Moreover, excitatory neurostimulation paradigms capable of reversing the disruptive effects of focal cortical inhibition have demonstrated therapeutic promise in post-stroke dysphagia when applied contralaterally. In healthy participants (n=15, 8 males, mean age 35±9 years), optimal parameters of transcranial Direct Current Stimulation (tDCS) (anodal, 1.5mA, 10 minutes) were applied contralaterally after 1-Hz rTMS pre-conditioning to the strongest pharyngeal projection. Swallowing neurophysiology was assessed in both hemispheres by intraluminal recordings of pharyngeal motor evoked responses (PMEPs) to single-pulse TMS as a measure of cortical excitability. Swallowing behaviour was examined using a pressure-based reaction time protocol. Measurements were made before and for up to 60 minutes post-interventions. Subjects were randomised to active or sham tDCS after 1-Hz rTMS on separate days and data were compared using repeated measures ANOVA. Active tDCS increased PMEPs bilaterally ($F_{1, 14}$=7.4, p=0.017) reversing the inhibitory effects of 1-Hz rTMS in the pre-conditioned hemisphere ($F_{1, 14}$ =10.1, p=0.007). Active tDCS also enhanced swallowing behaviour, increasing the number of correctly timed challenge swallows compared to sham ($F_{1, 14}$ =6.3, p=0.025). Thus, tDCS to the contralateral pharyngeal motor cortex reverses the neurophysiological and behavioural effects of focal cortical inhibition on swallowing in healthy individuals and has therapeutic potential for dysphagia rehabilitation.

3.3 INTRODUCTION

Deglutition is an essential gastrointestinal function with its motor control being bilaterally represented in the cerebral cortex (38, 45, 79). Evidence from studies of hemispheric stroke has highlighted the relevance of functional asymmetry in the swallowing motor network, with lesions affecting the "dominant" (stronger pharyngeal representation) hemisphere leading to oropharyngeal dysphagia (57, 61, 118-120). Furthermore, re-organisation with increased pharyngeal representation in the non-dominant or weaker (unlesioned) hemisphere appears to be associated with recovery of swallowing function (61, 119-120). Indeed, the swallowing motor network has been shown to be adaptable to both peripheral and cortical stimuli and exhibits remarkable plastic change (2, 7, 10). Recently there has been much interest in both peripheral and cortical neurostimulation techniques to drive this neuroplastic process by targeting the contralesional cortex (4, 10, 12-13, 17, 21). Development of an inhibitory pre-conditioning paradigm in the pharyngeal motor system has facilitated significant advances, allowing "preclinical", first-in-man application of these neurostimulation techniques in a controlled environment to assess the efficacy of these interventions in a disrupted system before progressing to patient trials (3, 8, 12, 69). Using this method, the investigator can focally inhibit the strongest pharyngeal corticobulbar projection; an intervention which has been shown to induce transient suppressive effects on swallowing neurophysiology and alter swallowing behaviour for up to 45 minutes, giving a window of opportunity to trial novel neurostimulation techniques (69). Moreover, it has recently been shown in healthy subjects that the application of this intervention can induce short-term effects on swallowing physiological measurements on videofluoroscopy, reminiscent of deficits after stroke (140).

Transcranial Direct Current Stimulation (tDCS) is a relatively new, non-invasive brain stimulation modality, in which a small direct current is applied via scalp electrodes to polarise neurones in the underlying cortex (147-148). Data from the stroke literature suggests that tDCS may have a role in

expediting recovery of motor behaviour and procedural learning (149-150, 197-198). TDCS has translational advantages compared to other cortical neurostimulation-based treatments that have been trialled in dysphagia rehabilitation; including its portability, ease of use, low costs and a less invasive intervention which in itself does not actually require pharyngeal intubation. These practical points make tDCS an attractive option for delivery at the bedside. Indeed, studies of anodal tDCS, when applied at either 1mA for 20 minutes or 1.5mA for 10 minutes (identified as the parameters which produced the largest effects at 60 minutes post-intervention (9), have been able to increase ipsilateral pharyngeal motor cortex excitability with effects comparable to other promising forms of neurostimulation such as Pharyngeal Electrical Stimulation (4) and rTMS (8). Against this background, three small clinical studies using tDCS in post-stroke dysphagia have provided tantalising evidence for a useful role in dysphagic stroke but have been hampered by methodological inconsistencies including; hemisphere selected for stimulation, interventional parameters and swallowing behavioural outcome measures. A pilot study by Kumar et al. (19) provided preliminary evidence for immediate clinical effects of active contralateral tDCS on clinical severity of dysphagia scores, but used parameters previously untested in the pharyngeal system with limited measurable effects on swallowing behaviour. The other two clinical trials (18, 20) used evidence-based parameters of tDCS to stimulate the injured (lesioned) hemisphere. Only one of these studies included swallowing behavioural measurements and reported effects that took 3 months post-intervention to build up (18). In summary, there is now a pressing need to perform studies based on robust methodological practice that will provide more information as to whether tDCS can be a useful therapeutic tool in the rehabilitation of dysphagia after stroke.

Given these clinical uncertainties, the aim of this study was to determine whether optimised parameters of contralateral tDCS are able to reverse the neurophysiological and behavioural effects of inhibitory pre-conditioning with 1 Hz rTMS applied to the strongest pharyngeal projection in healthy volunteers, as a prelude to applying this novel intervention in dysphagic stroke patients.

3.4 METHODS

3.4.1 Subjects

Sample size calculation based on previous studies using the inhibitory pre-conditioning model within our department (3, 8, 12) revealed that 12 subjects would be required to achieve a power of 80% and statistical significance of 5% (with standard deviation of 2.5). We therefore chose to recruit a minimum of 14 subjects to allow for drop-out.

Fifteen healthy volunteers (8 males, age range 21-61 years, mean 35±9 years) completed the study. All subjects were in good health, our exclusion criteria being: history of epilepsy, cardiac pacemaker, previous brain surgery, previous swallowing problems, use of medication which acts on the central nervous system or implanted metal. This trial was ethically approved by Greater Manchester South Research Ethics Committee. Written informed consent was obtained from each volunteer prior to participation.

3.4.2 Experimental Procedures

3.4.2.1 Pharyngeal Motor Evoked Potentials (PMEPs)

Volunteers were required to pass a 3.2mm diameter intraluminal catheter (Gaeltec Ltd, Dunvegan, Isle of Skye, Scotland), either transnasally or transorally depending on their preference. The catheter houses a pair of bipolar platinum ring electrodes that were positioned in the pharynx to record electromyographic (EMG) traces. An earth was connected to a skin electrode sited over the upper portion of one of the sternocleidomastoid muscles in the neck.

3.4.2.2 Thenar Motor Evoked Potentials (TMEPs)

As a control, thenar EMG from the abductor pollicis brevis (APB) muscle contralateral to the hemisphere giving the largest PMEP were also recorded by TMS over the hand motor cortex. This was achieved using gel electrodes (H69P, Tyco Healthcare, Gosport, UK) placed 1cm apart. An additional earth was connected to a skin electrode sited over a bony prominence on the wrist.

The catheter electrodes, thenar electrodes and the earths were all subsequently connected via a preamplifier (CED 1902; Cambridge Electronic Design, Cambridge, UK) with high and low pass filter settings of 200 Hz and 2 kHz, respectively, via connecting cables. Response signals were processed through a 50/60Hz noise eliminator ('HumBug'; Quest Scientific, North Vancouver, Canada) to remove any unwanted electrical interference collected through a laboratory interface (CED micro 1401) at a sampling rate of 5kHz and recorded using Signal software (v4.0, CED) running on a personal computer.

3.4.2.3 Single-pulse Transcranial Magnetic Stimulation (TMS)

Single TMS pulses were delivered using a figure-of-eight coil with an outer diameter of 7cm, which produces a maximum output of 2.2 Tesla (Magstim 200; The Magstim Company, Whitland, Wales, UK). The coil handle was held in antero-posterior direction at an angle of 45º tangential to the scalp as previously described (38).

3.4.2.4 Inhibitory pre-conditioning using repetitive Transcranial Magnetic Stimulation (rTMS)

A Magstim super rapid stimulator (The Magstim Company) was used to deliver trains of stimuli through a figure-of-eight coil with a maximum output of 1.8 Tesla. The Signal application software (CED) was programmed to deliver, 1-Hz rTMS at 120% of pharyngeal resting motor threshold (rMT); limited to a maximum of 100% of stimulator output for 10 minutes (600 pulses in total) over the hemisphere which produced the largest amplitude PMEPs (strongest pharyngeal cortical projection) (Mistry et al., 2007).

3.4.2.5 Transcranial Direct Current Stimulation (tDCS)

TDCS was delivered using a custom made device (Department of Medical Physics, Salford Royal NHS Foundation Trust). The polarity, intensity and duration settings of tDCS were based on the optimal excitatory regime defined by Jefferson et al. (1.5mA of anodal tDCS for 10 minutes) given that these parameters produced the largest increase in cortical excitability at 60 minutes post-intervention (9). Interventions were delivered via two 25cm^2 rectangular surface electrodes (current density 0.06mA/cm^2). To ensure optimal contact with the scalp, both electrodes were placed in

water-soaked pads (neuroConn GmbH, Ilmenau, Germany) and held in place by adjustable rubber straps. The anodal electrode was placed over the “unconditioned” (see inhibitory pre-conditioning above) pharyngeal motor cortex and the other overlying the contralateral supraorbital ridge. For active tDCS, the current was slowly ramped up to 1.5mA over 10 seconds, eliciting a transient tingling sensation. Impedance was monitored whilst stimulation continued for 10 minutes before being slowly turned off over 10 seconds. For sham tDCS, the current was turned off after 30 seconds, thus producing the same sensation as the active treatment but without significantly stimulating the cortex (9, 199).

3.4.2.6 Swallowing reaction task

The effects of tDCS and sham stimulation on swallowing behaviour were studied using an established experimental model as previously described by Mistry et al. (69). For these experiments, a pharyngeal catheter incorporating a single solid-state pressure transducer (Gaeltec Ltd) was used. The catheter was connected to the interface, preamplifier and into the personal computer. Three millilitre boluses of water were infused directly into the subject’s oral cavity via a catheter connected to a hand held syringe. A cutaneous electrical cue was generated using an electronic pulse generator (Digitimer, Welwyn Garden City, UK) connected to surface electrodes attached to the dorsum of the volunteers hand. ‘Normal swallow’ reaction time was determined by asking participants to swallow at a normal pace after the cutaneous trigger. ‘Fast swallows’, required the volunteer to swallow as fast as possible after the cue. The latency from the electrical cue to the onset of the pharyngeal swallow, with consequent change in pharyngeal pressure signal, gave the reaction time measurement. From the recorded normal and fast swallowing reaction times, a challenge swallowing time window was calculated as described by Mistry et al. (69). This challenge swallowing task is a visually cued, 150ms time window on the laboratory desktop computer, within which a swallow must be initiated to be successful.

3.4.3 *Experiment 1*– Effects of contralateral anodal tDCS on swallowing neurophysiology after pre-conditioning with 1-Hz rTMS to the stronger pharyngeal motor representation

Volunteers were randomised to receive active and sham tDCS interventions on two separate visits to the laboratory (Figure 3.1), at least one week apart, using a randomisation programme (Stats Direct, v2.7.8, StatsDirect Ltd., Cheshire, UK).

During each session subjects were seated in a comfortable, reclining chair with the pharyngeal catheter in-situ. The cranial vertex was marked on the scalp as a reference point. Single-pulse TMS was used at the start of each study to determine the strongest pharyngeal cortical projection and determine the optimal coil positions for recording PMEPs (the resting motor hot spots) over both hemispheres as well as the hand motor cortex in the stronger pharyngeal hemisphere. These sites were also marked on the scalp and the pharyngeal resting motor threshold (rMT) for each hemisphere was identified by using single pulses of stimulation to achieve evoked potentials of at least 20μV on 50% of occasions. The pharyngeal motor cortex which produced the largest amplitude of PMEPs, at the lowest threshold, was defined as the "stronger" pharyngeal hemisphere. Single-pulse TMS was then used to elicit TMEPs and determine thenar rMT on the side with the strongest pharyngeal representation.

Baseline measurements of cortical excitability at all three sites (stronger and weaker pharyngeal cortex and hand (thenar) motor cortex) were made by delivering 10 pulses of single-pulse TMS at rMT +10% stimulator output and 10 pulses at rMT + 20% (60 stimuli in total). Following baseline measurements, volunteers all received inhibitory pre-conditioning to the strongest pharyngeal hemisphere, as described earlier. Either active or sham tDCS, dependant on the randomisation, was then delivered immediately after the completion of 1-Hz rTMS, to the contralateral hemisphere as detailed above on two separate visits. Cortical excitability was then measured in the same way as with baseline, immediately and then repeated every 15 minutes for 1 hour post-intervention. Cortical excitability measurements post-intervention were compared to baseline.

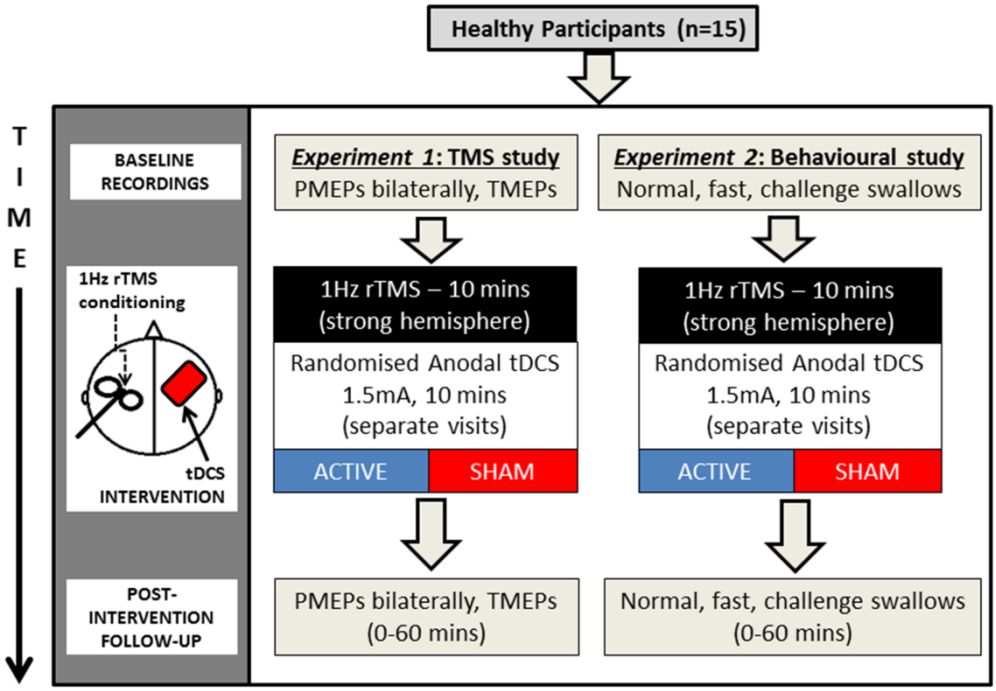


Figure 3.1: Flow chart summarising experimental protocols. (Abbreviations; PMEPs= pharyngeal motor evoked potentials and TMEPs= thenar motor evoked potentials).

3.4.4 *Experiment 2* – Effects of contralateral anodal tDCS on swallowing behaviour following pre-conditioning with 1-Hz rTMS to the stronger pharyngeal motor representation

The swallowing behavioural studies were also conducted over two separate sessions (Figure 3.1), with the same 15 subjects randomised to active and sham tDCS interventions at least one week apart. Volunteers were seated as per *Experiment 1* with the catheter housing the pressure sensors in-situ. TMS was performed identically to *Experiment 1* in order to determine the PMEP hot spots and the strongest pharyngeal hemispheric projection. Baseline PMEP data were also recorded before proceeding to behavioural measurements. Volunteers performed 10 normal swallows, followed by 10 fast swallows at baseline, with the volunteer swallowing 3ml water each time. A challenging time window was then calculated via the software, with the volunteer required to perform the challenge task on 10 occasions. The volunteer's baseline challenged swallows score (number of correctly time swallows out of 10) was subsequently recorded. Each subject then received inhibitory pre-conditioning (as in *Experiment 1*) to the strongest pharyngeal projection. Immediately after 1-Hz rTMS conditioning, each volunteer received either active or sham tDCS to

the unconditioned pharyngeal motor cortex as pre-determined by randomisation. Latencies for normal swallows and fast swallows as well as the number of successful challenge swallows were measured: immediately, 5, 10, 15, 30 and 60 minutes post-tDCS intervention and compared to baseline.

3.5 DATA ANALYSIS

Experiment 1: The mean latencies and amplitudes of PMEPs and TMEPs were determined from each group of 10 EMG traces (for each site and intensity). In order to minimise variability, data were then normalised to baseline for each subject and expressed in the results as a percentage change from baseline. *Experiment 2:* The swallowing reaction time was defined as the interval between the onset of the stimulus to the hand and the time at which the pharyngeal pressure crossed a pre-determined threshold. The results for each set of normal and fast swallows were then averaged and normalised to baseline. The percentage change of correctly timed challenge swallows at each time point was also calculated by comparing the number of swallows where the pressure crossed the threshold within the set time window (out of 10) to baseline.

3.6 STATISTICAL METHODS

All data were analysed separately using a standard statistical software package (SPSS 20.0, SPSS Inc, Chicago, Illinois, USA). Initially, raw baseline MEP data from both experiments for the two interventions were compared separately using non-parametric (Wilcoxon Signed Rank) tests to exclude any bias resulting from the studies being conducted on separate days. Then, based on previous studies (3, 8-10, 12, 69) normalised (percentage change from baseline) MEP data from *Experiment 1* were compared using a general linear model repeated-measures analysis of variance (ANOVA), with factors of treatment (active or sham tDCS), hemisphere (conditioned or unconditioned) and time (immediately, 15, 30 and 60 minutes post-intervention). In *Experiment 2*, normalised (percentage change from baseline) swallowing behavioural data were also compared using a general linear model repeated-measures ANOVA, with factors of Treatment (active or sham tDCS), and Time (immediately, 5, 10, 15, 30 and 60 minutes post intervention). In both

experiments, when significant effects were present, these were followed up with post-hoc analysis including adjustment of *p*-values for multiple comparisons (Bonferroni) to explore the strength of the main effects. Non-sphericity was corrected using Greenhouse-Geisser where necessary. The above analyses were also performed for the MEP latency data using the raw values which displayed a normal distribution. *P*-values of < 0.05 were taken as a measure of statistical significance, and data are expressed as mean (± standard error of the mean (SEM)) unless stated otherwise.

3.7 RESULTS

In all 15 healthy volunteers; both TMS and rTMS were tolerated well with no adverse effects. Anodal tDCS (1.5mA) for 10 minutes was also well tolerated and impedance was maintained below 8kOhms in all subjects.

3.7.1 Cortical hotspot mapping, resting motor thresholds and baseline TMS

During single-pulse TMS mapping, 8/15 subjects were found to have stronger pharyngeal hemisphere representation on the left hemisphere whilst the other 7 subjects had stronger right hemispheric pharyngeal projections. The mean distance from the cranial vertex to the motor hot spots were: strong pharyngeal hemisphere 3.2 ± 0.2cm medio-lateral and 4.1 ± 0.2cm antero-posterior, weaker pharyngeal projection 3.1 ± 0.2cm medio-lateral and 4.2 ± 0.3cm antero-posterior and thenar motor cortex representation 3.5 ± 0.2cm lateral and 4.0 ± 0.2cm anterior. Mean rMT for strong pharyngeal hemisphere was 68 ± 3 % stimulator output and 70 ± 3% stimulator output in the weaker pharyngeal hemisphere. Mean rMT for thenar motor cortex was 42 ± 2% stimulator output. The mean baseline PMEP amplitudes were 83 ± 5μV for strong pharyngeal projection and 55 ± 4μV over the weaker pharyngeal hemisphere. The mean baseline TMEP amplitudes were 772 ± 78μV. There was no significant difference in baseline MEP data across the separate days (Wilcoxon Signed Rank Tests: strong pharyngeal projection $Z=-1.14$, $p=0.26$, weaker pharyngeal projection $Z=-0.51$, $p=0.61$ and APB $Z=-0.22$, $p=0.83$). Figure 3.2 shows representative pharyngeal and thenar MEP data from one participant during the study.

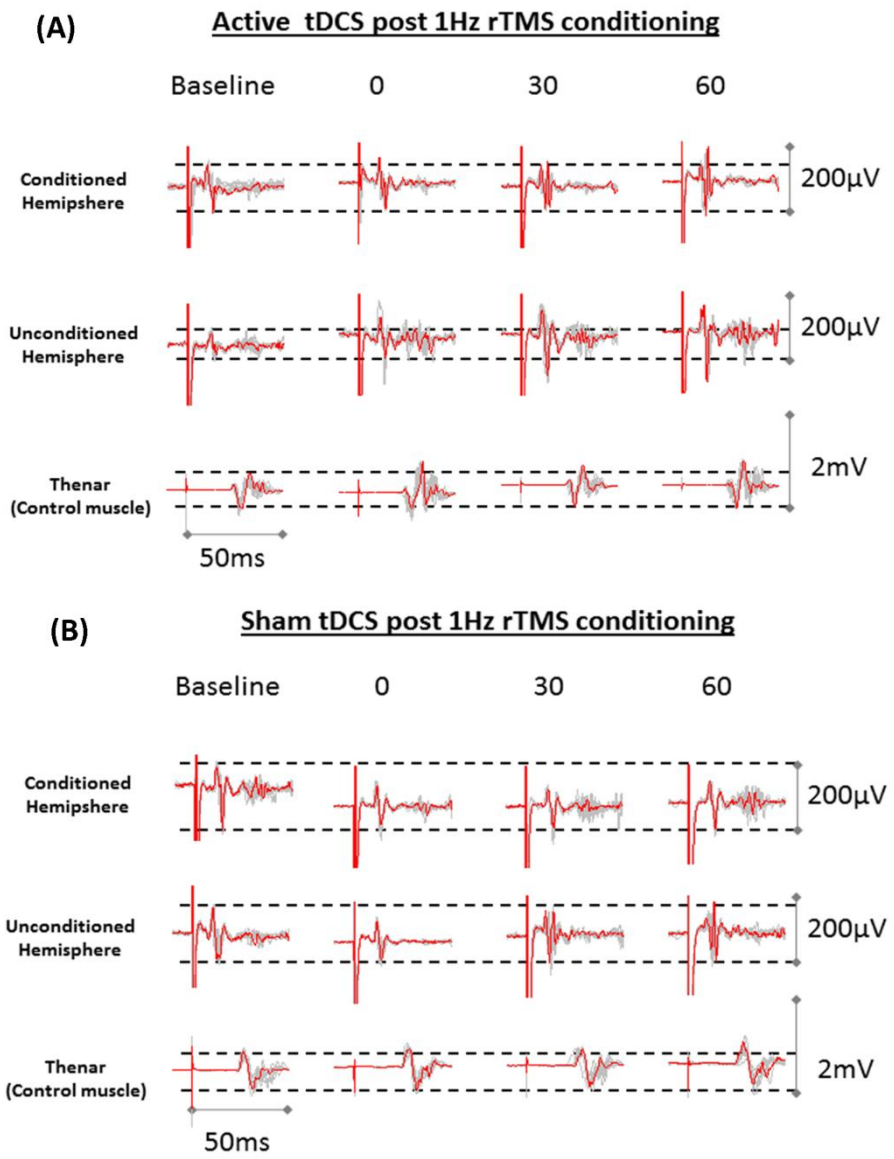


Figure 3.2: *Experiment 1* - Representative PMEPs and TMEPs data traces from an individual participant for all muscle groups after; (A) Active tDCS post pre-conditioning with 1-Hz rTMS increased PMEP amplitudes bilaterally and (B) Sham tDCS post pre-conditioning with 1-Hz rTMS, suppressed PMEPs on the conditioned hemisphere. TMEPs were not affected by either tDCS intervention. For visual purposes, responses from the intermediate time points 15 and 45 minutes post-tDCS have been removed. Trace clusters for each recording site are comprised of 10 overdrawn responses.

3.7.2 *Experiment 1:* Effects of contralateral anodal tDCS on swallowing neurophysiology after pre-conditioning the strong pharyngeal motor cortex with 1-Hz rTMS

One Hz rTMS over the strong pharyngeal projection was tolerated well by all subjects with no adverse effects and was delivered at an average intensity of 96 ± 1% of rTMS output. Inhibitory pre-conditioning with 1-Hz rTMS, followed by contralateral sham anodal tDCS, suppressed cortical

excitability in the conditioned hemisphere for the duration of the study (Figure 3.3), with a decrease in PMEP amplitude of up to -13 ± 9%. However, inhibition in the unconditioned hemisphere was shorter (15 minutes), with a decrease in PMEP amplitude of only -2 ± 8%. By contrast, active tDCS post inhibitory pre-conditioning increased PMEPs bilaterally (Figure 3.3), by a maximum of 30 ± 17% in the conditioned hemisphere and 38 ± 17% in the unconditioned.

A three-way repeated-measures ANOVA on normalised MEP data with factors of Treatment (active and sham tDCS), Site (conditioned pharyngeal hemisphere, unconditioned pharyngeal hemisphere and thenar cortex) and Time (immediately, 15, 30, 45 and 60 minutes post-treatment) revealed a significant interaction of Treatment x Site x Time factors ($F_{1, 14}$ =7.72, p=0.015) and a significant effect of Treatment ($F_{1, 14}$ = 6.57, p=0.023). A further three-way repeated measures ANOVA, this time with factors of Treatment (active and sham tDCS), Pharyngeal Hemispheres (conditioned and unconditioned) and Time (immediately, 15, 30, 45 and 60 minutes post-treatment) confirmed significant effects of Treatment (mean difference in PMEPs of 30±11%, 95% confidence interval of 6 to 53, $F_{1, 14}$ =7.38, p=0.017; adjusted for multiple comparisons: Bonferroni) on PMEPs but without differences in the pattern of excitability between Pharyngeal Hemispheres ($F_{1, 14}$ =1.06, p=0.32), implying that the significant effects of Treatment on PMEPs were bilateral. There were no significant effects of Time ($F_{4, 56}$ =0.89, p=0.48) and no other significant interactions were found.

When considering only the focally inhibited hemisphere, two-way repeated measures ANOVA with factors of Treatment and Time demonstrated a strong reversal effect by Treatment (Mean difference in PMEPs of 35 ±11%, 95% confidence level 11 to 58, $F_{1, 14}$ = 10.1, p=0.007; adjusted for multiple comparisons: Bonferroni, Figure 3.3).

The neurophysiological effects of contralateral tDCS did not however extend to the thenar motor cortex (two-way repeated measures ANOVA; no significant effects of Treatment ($F_{1, 14}$ = 0.83, p=0.38), Time ($F_{2, 29}$ = 1.56, p=0.23) or Treatment x Time ($F_{4, 56}$ = 0.79, p=0.54), therefore no further analyses were considered for the thenar data.

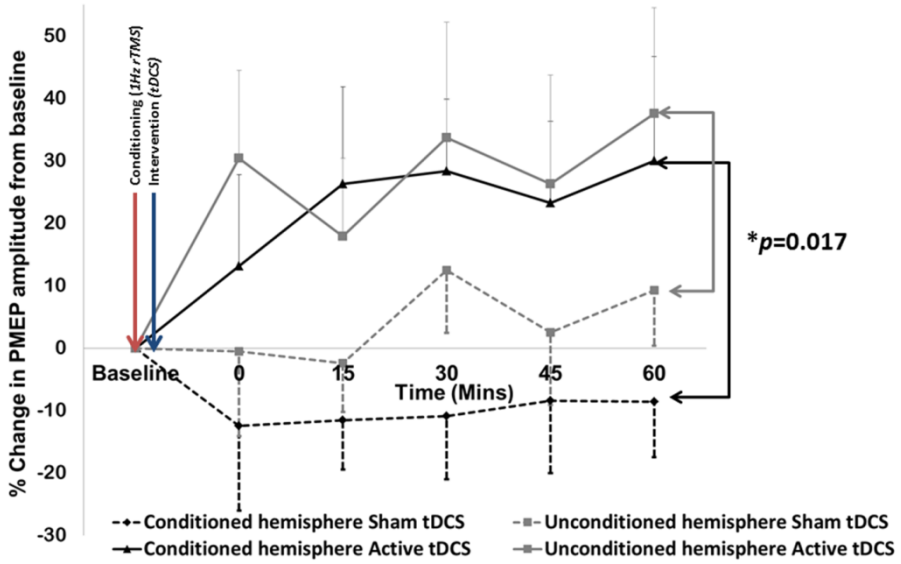


Figure 3.3: *Experiment 1* - Group mean effects (± SEM) of contralateral tDCS interventions on swallowing neurophysiology after pre-conditioning with 1-Hz rTMS. The dashed lines in this figure show the inhibitory changes induced by 1 Hz rTMS after sham tDCS. Active tDCS increases pharyngeal cortical excitability bilaterally (*p=0.017).

3.7.3 *Experiment 1* - The effects of tDCS on PMEP and TMEP latencies

The mean response latencies at baseline and each time point for the PMEPs and TMEPs following tDCS are shown in Figure 3.4. Wilcoxon Signed Rank Tests comparing the raw baseline PMEP response latency values for each of the treatments (active tDCS and sham tDCS) for conditioned hemisphere (Z=-0.50, p=0.62) and unconditioned hemisphere (Z=-0.54, p=0.59) did not reveal any significant differences across the study days. There was also no significant difference in baseline TMEP latencies on the separate study days (Z=-0.94, p=0.35).

Three-way repeated measures ANOVA did not reveal any significant effects of Treatment ($F_{1, 14}$ = 0.06, p=0.81), Site ($F_{1, 14}$ = 3.2, p=0.26) or Time ($F_{5, 70}$ = 1.3, p=0.21) on PMEP latencies. Two-way repeated measures ANOVA revealed that there were also no significant effects of Treatment ($F_{1, 14}$ = 0.7, p=0.20) or Time ($F_{5, 70}$= 2.2, p=0.07) on TMEP latencies.

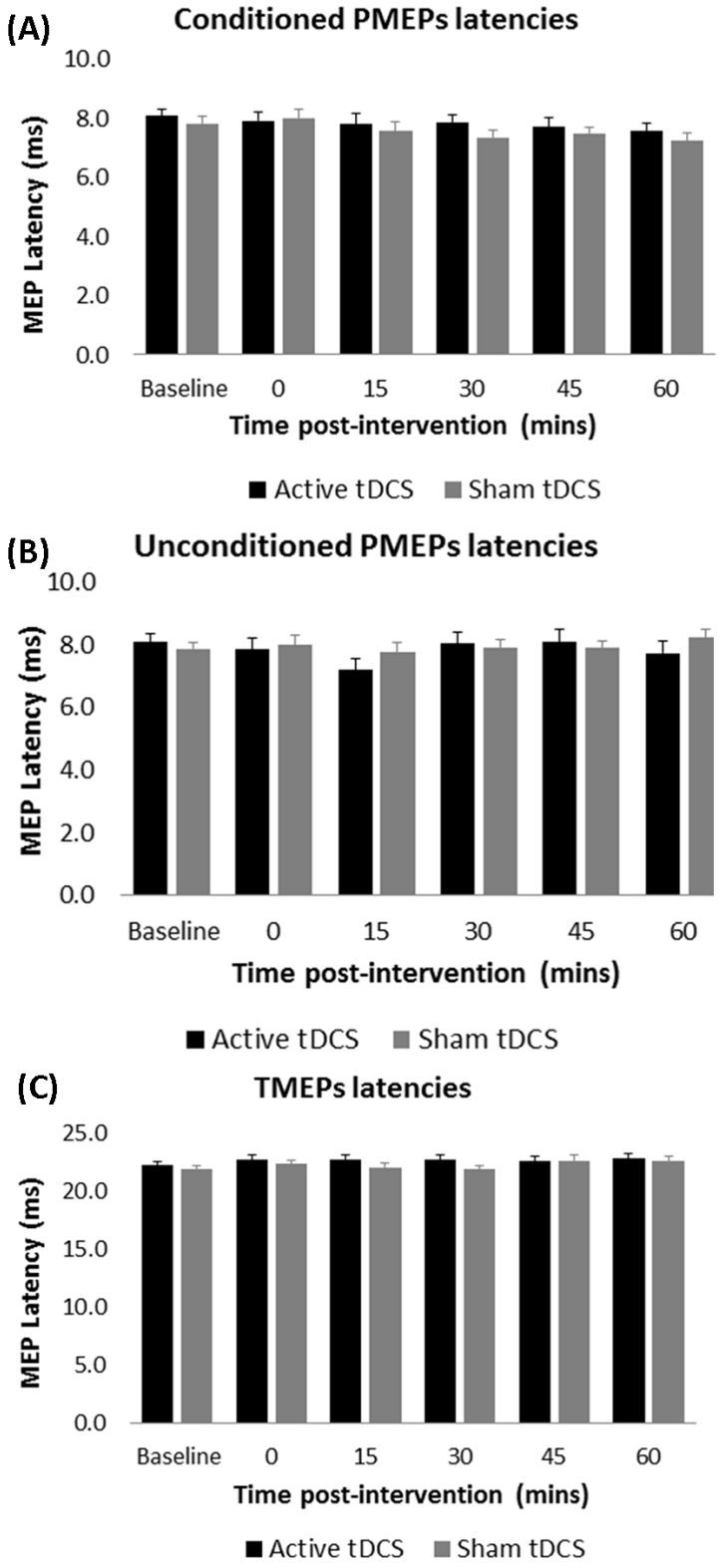


Figure 3.4: *Experiment 1*- Group mean (±SEM) pharyngeal and thenar MEP response latencies post-interventions. No significant effects of interventions on; (A) conditioned hemisphere PMEPs latencies, (B) unconditioned hemisphere PMEPs latencies or (C) Thenar MEPs latencies.

3.7.4 *Experiment 2*: Effects of contralateral anodal tDCS on swallowing behaviour after pre-conditioning the strong pharyngeal motor cortex with 1-Hz rTMS

Baseline TMS data collected prior to any interventions in *Experiment 2* confirmed that there was no difference in baseline PMEP amplitudes before receiving either active or sham tDCS on separate days (Related-Samples Wilcoxon Signed Rank Test; strong pharyngeal projection Z=-0.09, *p*=0.93, weaker pharyngeal projection Z=-0.89, *p*=0.37). There was no significant difference in baseline swallowing behavioural measures between the two separate sessions (Related-Samples Wilcoxon Signed Rank Tests; Normal Swallows Z=-1.13, *p*=0.26, Fast Swallows Z=-0.79, *p*=0.43 and Challenge Swallows Z=-1.79, p=0.07). Grand mean (from both sessions) baseline reaction times for normal, fast swallows and challenge swallows data are displayed in Figure 3.5.

Normal and fast swallow latencies (expressed as percentage change from baseline) were analysed using a three-way repeated measures ANOVA with factors of Treatment (active or sham tDCS), Behaviour (normal or fast swallows) and Time (immediately, 5, 10, 15, 30 and 60 minutes post-intervention). This revealed a significant effect of Treatment (mean change in reaction time: -5±2%, 95% confidence interval -9 to -0.5, $F_{1, 14} = 5.62$, *p*=0.03; adjusted for multiple comparisons: Bonferroni, Figure 3.6). However, there were no significant effects of Behaviour (mean change in reaction time: -0.2 ± 1.7%, 95% confidence interval -4 to +3.5, $F_{1,14} = 2.2$, *p*=0.93) and no significant effects of Time when adjusting for multiple comparisons (Bonferroni) without any other significant interactions.

Active tDCS also improved the accuracy of the challenge swallow reaction time task with a mean improvement in correctly attempted swallows of +3.0 ± 0.6 out of 10 trials (+174% above baseline, Figure 3.6C) at 60 minutes post-intervention. In contrast, following sham tDCS there was virtually no improvement at the same time-point, +0.3 ± 0.6 out of 10 swallows (only +29% above baseline, Figure 3.6C). Two-way repeated measures ANOVA on challenge swallow data (percentage

change from baseline), revealed a significant effect of Treatment (Mean difference: 119±48%, 95% confidence interval 17 to 221, $F_{1, 14} = 6.3$, $p = 0.025$; adjustment for multiple comparisons: Bonferroni) but no significant effects of Time ($F_{5, 70} = 1.31$, $p = 0.27$) or Treatment x Time ($F_{5, 70} = 0.92$, $p = 0.47$).

There was no correlation between findings in either experiment and relative inter-hemispheric asymmetry in pharyngeal projection (see supplementary data).

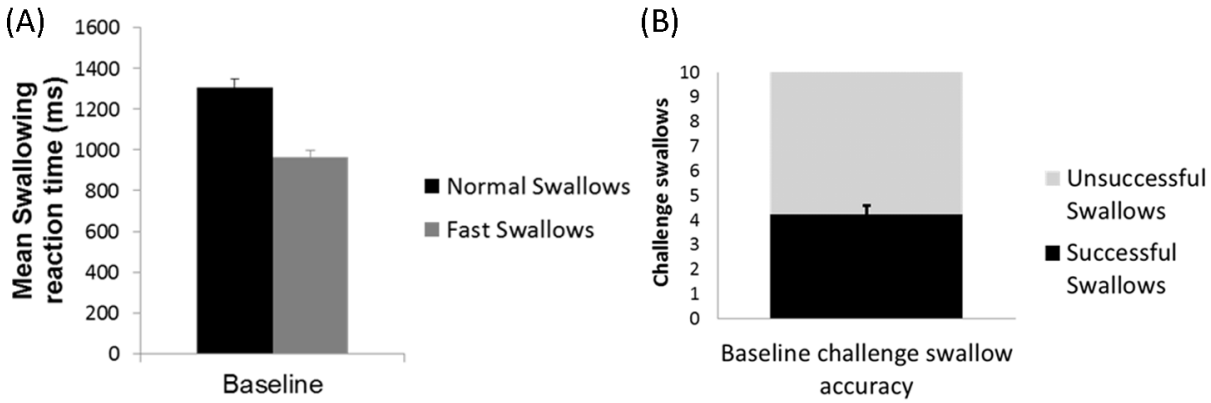


Figure 3.5: *Experiment 2* - Baseline swallowing behavioural data.
(A) Group mean (± SEM) normal and fast swallowing reaction times (both visits);
(B) Group mean (± SEM) number of correctly timed challenge swallows (both visits)

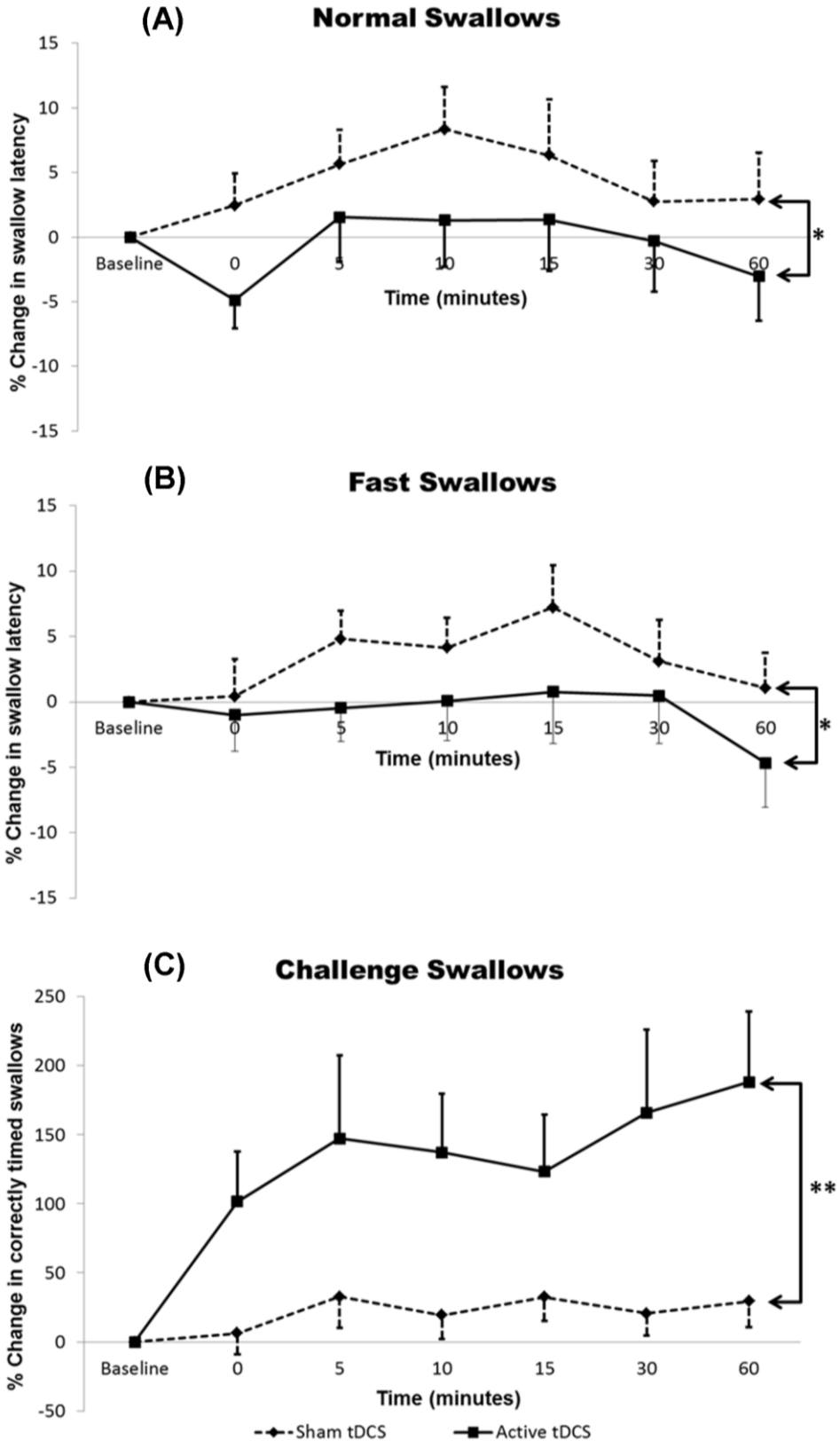


Figure 3.6: *Experiment 2* - Swallowing behavioural effects. Graphs showing group mean percentage change from baseline (±SEM) in (A) normal swallow reaction times (B) fast swallow reaction times (*p=0.03) and (C) correctly timed challenge swallows over 60 minutes post-intervention. Active anodal tDCS significantly improved challenge swallow behaviour (**p=0.025)

3.8 DISCUSSION

Our experiments examined the effects of tDCS on swallowing neurophysiology and behaviour after inhibitory pre-conditioning in an established model of brain suppression and swallowing disturbance in healthy participants. This model resembles the situation in unilateral stroke, where patients with lesions affecting the strongest hemisphere often develop dysphagia. Interestingly, a sub-analysis of our data (see supplementary data) similarly suggests that subjects with a greater degree of hemispheric asymmetry have greater neurophysiological and swallowing behavioural disruption after inhibitory pre-conditioning to the stronger pharyngeal projection. The observed effects of the active tDCS intervention to the unconditioned pharyngeal motor cortex were localised, with excitability of the hand motor cortex remaining unaltered.

3.8.1 Bilateral reversal of focal cortical inhibition post anodal tDCS

Previous work from our group has shown that sham tDCS over the pharyngeal motor cortex does not alter cortical excitability in an unconditioned system (9). Hence, as expected, sham tDCS following conditioning with 1-Hz rTMS to the strongest pharyngeal projection, resulted in ipsilateral suppression of PMEPs that persisted throughout the 60 minutes of follow-up during *Experiment 1*. By contrast, following active contralateral tDCS, there is a clear reversal in the direction of pharyngeal motor cortex excitability in the conditioned hemisphere. These sustained, bilateral excitatory effects peaked 60 minutes post-stimulation. The increase in pharyngeal motor cortex excitability in the conditioned hemisphere implies that in an inhibited system, contralateral tDCS can more effectively produce transcallosal excitation. This is in contrast to the situation in an undisrupted system, where the same parameters of tDCS did not modulate pharyngeal cortical excitability in the opposite (unstimulated) hemisphere (9). Previous studies suggest differing inter-hemispheric interactions in the bilaterally represented pharyngeal motor system, where both hemispheres appear to synergistically co-ordinate swallowing (albeit with functional asymmetry) (69, 129), compared with the unilaterally innervated hand motor system, where transcallosal inhibition has been demonstrated (200). Following stroke affecting the hand motor areas,

maladaptive increases in transcallosal inhibition from the unlesioned hemisphere have prompted investigators to attempt to counteract this with inhibitory tDCS paradigms over the unlesioned hemisphere and applying anodal tDCS over the lesioned hemisphere in rehabilitation trials (201-203). Given the lack of transcallosal inhibition in the pharyngeal system, the hand-stroke restorative model was not appropriate for the present study. We therefore targeted the unconditioned hemisphere as per previous inhibitory pre-conditioning studies of brain stimulation in the pharyngeal system (Jefferson et al., 2009a; Michou et al., 2012). Similar to our findings, the previous studies also demonstrated excitatory effects on the conditioned hemisphere, thereby increasing excitability of cortical projections to pharynx from both hemispheres. Our findings of increased excitability following contralateral tDCS in the hemisphere pre-conditioned with inhibitory rTMS are in accordance with previous work describing the phenomenon of homeostatic plasticity in the human motor cortex (204-206). These studies demonstrated a strong shift in the direction of cortical excitability, when interventions ordinarily unable to enhance cortical excitability were preceded by inhibitory stimulation (205-206). Therefore, we propose that in a similar fashion, inhibitory (1-Hz) rTMS sensitised the conditioned cortical neurons before transcallosal spread of excitation via tDCS from the unconditioned hemisphere, provoking the reversal in direction of pharyngeal cortical excitability. During the early phase of recovery after stroke, similar homeostatic shifts in brain excitability have been described in lesioned areas with associated neurophysiological deficits (Murphy & Corbett, 2009; Carmichael, 2012). The same homeostatic shifts in brain excitability may therefore contribute to the measureable improvements in swallowing function seen in clinical trials of ipsilesional tDCS in post-stroke dysphagia (Yang et al., 2012; Shigematsu et al., 2013), whereby excitatory effects of lesioned hemisphere tDCS may be transmitted transcallosally to the unlesioned hemisphere, that being the hemisphere more closely implicated in swallowing recovery according to post-stroke dysphagia literature (Hamdy et al., 1998b; Li et al., 2009; Kumar et al., 2011; Teismann et al., 2011; Michou et al., 2012; Park et al., 2013).

One limitation of *Experiment 1* is that we did not measure pharyngeal cortical excitability between inhibitory pre-conditioning with 1 Hz rTMS and the contralateral tDCS intervention. Considering that a previous study has confirmed sham tDCS does not alter pharyngeal cortical excitability (Jefferson, Mistry et al. 2009), our sham tDCS data in the present study indirectly demonstrate evidence for the inhibitory effects of 1 Hz rTMS. Inclusion of the additional time point between protocols may have provided further evidence for induction of focal cortical inhibition, homeostatic interactions and served as a secondary control for response variability between conditioning protocols. Another limitation of *Experiment 1* is that we did not re-investigate the effects of anodal tDCS in an unconditioned system, where previously, anodal tDCS has already been demonstrated to increase pharyngeal motor excitability in the stimulated hemisphere only (Jefferson et al., 2009b). By contrast, in our study in a pre-conditioned system after 1 Hz rTMS we demonstrated bilateral effects of contralateral tDCS on pharyngeal excitability. These findings imply transcallosal spread of excitation to the opposite hemisphere. As discussed, the most plausible explanation for these findings is homeostatic plasticity after inhibitory pre-conditioning. Repeating the unconditioned experiments (ie. after sham 1-Hz stimulation and anodal tDCS) in the present study would have strengthened our conclusions by enabling more direct (within-subject) comparisons of facilitation patterns between pharyngeal projections following anodal tDCS in pre-conditioned and unconditioned systems.

3.8.2 Effects of reversing focal cortical inhibition by anodal tDCS on swallowing behaviour

In *Experiment 2*, active tDCS following inhibitory pre-conditioning reduced both normal and fast swallowing reaction times. The physiological significance of this small but statistically supported reduction in normal and fast swallow latencies is unclear. Given that the increased velocity of these reaction time tasks was accompanied by a more accurate performance in the more complex challenged swallows, there is suggestion that either the overall speeding up effect itself is beneficial or perhaps the by-product of a more co-ordinated and efficacious swallow post active

tDCS. Using our pharyngeal pressure-based measures of normal and fast swallowing reaction times it is not possible to determine precisely which component of the swallow was influenced by reversal of focal cortical inhibition by tDCS. A videofluoroscopic study post 1-Hz rTMS to the stronger oropharyngeal projection has previously shown that focal cortical inhibition has differential effects on oral transit time (speeded up) and swallowing response times (delayed), without alteration in pharyngeal transit time or laryngeal closure duration (140). Our timings of normal and fast swallows would only capture the oral transit and the transitional phase between the oral and pharyngeal swallow (swallowing response time). Our findings therefore suggest that reversal of focal cortical inhibition may have improved the control and efficacy of the oral phase or reduced the 1-Hz rTMS induced delay between the oral and pharyngeal phases of swallowing. Behavioural data from an unconditioned system in healthy subjects (Suntrup et al., 2013) which found no effects of anodal tDCS on normal and fast swallows imply that our findings result from reversal of the inhibitory pre-conditioning. As per the limitations of the neurophysiological experiment, we did not re-examine the behavioural effects of anodal tDCS in an unconditioned system which would have helped confirm this in the same group of subjects. A future study incorporating videofluoroscopic swallowing studies both in conditioned and unconditioned systems would help elucidate precisely which specific components of deglutative behaviour and timings are affected by anodal tDCS.

Compared to the normal and fast swallowing reaction time tasks, the challenge swallows are a more complex motor task, requiring processing of sensory cues and co-ordination of pharyngeal muscular activity within the 150ms time window. After active tDCS, our data clearly show positive effects on swallowing behaviour, with a significant improvement in the number of correctly timed challenge swallows compared to sham. Given the progressive improvement in swallowing accuracy over time with maximum effects at the end of follow-up, these data demonstrate consolidation of motor learning and skill acquisition with repetition over time. Our behavioural data

in a disrupted/conditioned system are in accordance with recently published findings in an unconditioned system in healthy subjects, where active tDCS combined with an oral motor and sensory task improved challenged swallow behaviour (11). In the present study, each subject's first exposure to the swallowing reaction time paradigm (and indeed their only training) was the 10 trials of each task during baseline recordings. TDCS stimulation was then administered 'offline', i.e. without any swallowing training taking place during stimulation. Our observations are in keeping with the studies of hand motor tasks where both 'online' (207-210) and 'offline' (211) anodal tDCS has been shown to enhance performance.

3.8.3 Mechanism of action of tDCS

Mechanistic studies to date suggest that anodal tDCS-induced increases in excitability result from depolarisation of cortical neurones and subsequent changes in resting membrane potential (212). Pharmacological studies have demonstrated that anodal tDCS-induced increases in MEPs are dependent on synaptic sodium and calcium conductance and suggest that the long-lasting after-effects on cortical excitability may be dependent on Glutamatergic N-Methyl-D-aspartate (NMDA) receptors (Liebetanz et al., 2002; Nitsche et al., 2003a). Additionally, one magnetic spectroscopic study suggests decreases in GABAergic inhibition following anodal tDCS (213). Functional magnetic resonance imaging studies have shown that tDCS to the motor cortex induced changes in neuroplasticity that can alter functional connectivity within the human brain (214-215). Therefore when tDCS is specifically applied to the pharyngeal motor cortex, our findings lead us to hypothesise that the increased cortical excitability in the pharyngeal motor areas may facilitate strengthening of task related synapses in the swallowing motor network by enhancing functional coupling between the various cortical regions involved in swallowing. Recently published Magnetoencephalography (MEG) data in healthy subjects provides further evidence for this, showing increased activity of several cortical regions involved in the planning, initiation and execution of swallowing following tDCS to pharyngeal motor cortex (Suntrup et al., 2013). The authors paired swallowing training and sucking flavoured lollipop interventions with tDCS in an

undisrupted system and reported bilateral increase in swallow-related brain activation on MEG after tDCS (Suntrup et al., 2013). This is contrary to tDCS without swallowing training, which only increases ipsilateral cortical excitability as measured by TMS in an undisrupted system (Jefferson et al., 2009b) and suggests that there may be added benefits of synergistic swallowing training with tDCS. Future TMS studies in both undisrupted and disrupted systems examining the neurophysiological effects of swallowing training alone, compared to tDCS alone and tDCS with training would therefore be of value to test this hypothesis and further optimise tDCS interventions. Recent evidence from animal literature suggests that cortical tDCS can have a facilitatory effect in subcortical structures (216-217). With respect to the level of facilitation in the swallowing motor system to tDCS, there is some evidence from our data (and previous studies (9)) that anodal stimulation effects are predominantly due to intracortical neuronal excitation rather than at the brainstem level. Firstly, if the effects of active tDCS on PMEPs were due to increased excitability of bulbar motoneurones, then we would have expected a shortening of cortico-pharyngeal latency reflecting the excited motoneurones being nearer to threshold. However, in the present study there were no differences in cortico-pharyngeal latency following active and sham tDCS. Secondly, a previous study in an uninhibited system has shown that tDCS only increased MEPs ipsilaterally in the pharyngeal motor system (9) and if these changes were at the motoneurone level we would expect the MEP effects be the same bilaterally as bulbar neurones receive input from both hemispheres. These observations make it unlikely that tDCS directly affected the brainstem, but in the absence of intra-brainstem recording, this assertion remains uncertain.

3.8.4 Conclusion

In conclusion, we have demonstrated that optimised parameters of anodal tDCS (without swallowing training) over the unconditioned hemisphere can restore swallowing physiology and behaviour to a disrupted system after inhibitory pre-conditioning to pharyngeal motor cortex. These results are of physiological and clinical relevance and suggest that 10 minutes of anodal tDCS at 1.5mA has therapeutic potential as an adjunctive treatment for dysphagia post-

hemispheric stroke when applied contralesionally and supports its application in future randomised clinical trials using these parameters. We have demonstrated that tDCS is a safe modality and is well tolerated at these parameters in healthy participants. Indications from small clinical trials of anodal tDCS, despite varying stimulation sites (18, 20) and parameters (19) also suggest safety of this intervention in post-stroke dysphagia patients. A future clinical trial applying 1.5mA anodal tDCS for 10 minutes contralesionally in post-stroke dysphagia patients will be required to confirm this. Further unanswered questions requiring investigation include the optimal number of treatment sessions required to facilitate recovery of swallowing function in a post-stroke dysphagia patient population. Therefore a randomised controlled dose-response study incorporating videofluoroscopic swallowing studies in a methodologically robust protocol would be an important step in determining the optimal dosage of contralesional tDCS. Data in the present study suggest that tDCS may enhance motor memory acquisition resulting in improved swallowing behaviour therefore future trials in healthy participants and patients may explore the role of standardised swallowing training during active tDCS intervention compared to active tDCS without training and sham tDCS with training.

3.9 SUPPLEMENTARY MATERIAL

3.9.1 Calculation of relative inter-hemispheric asymmetry between pharyngeal projections and correlation with neurophysiological and behavioural outcomes

In order to quantify relative right-left asymmetry in pharyngeal projection we calculated a laterality index (LI) for each subject (n=15) based on mean baseline PMEPs amplitudes using the formula: (Right mean PMEPs – Left mean PMEPs)/ (Right mean PMEPs + Left mean PMEPs). The overall direction of laterality (plus = right, minus = left) was ignored for the purpose of this analysis.

For both *Experiment 1* data (conditioned and unconditioned PMEPs) and *Experiment 2* data (challenge swallows) we calculated mean post-tDCS percentage change using data from each post-intervention time point. Using Pearson bivariate correlation coefficient we tested for correlation between LI scores and percentage changes post-tDCS in; conditioned hemisphere PMEPs after active tDCS and sham tDCS; unconditioned hemisphere PMEPs after active and sham tDCS and challenge swallows after active and sham tDCS.

3.9.2 Subdivision of subjects into Laterality groups

Based on the median LI score we divided our subjects into a lateralised and an unlateralised group. Using mean percentage change post-tDCS PMEPs data we then calculated a repeated measures ANOVA with factors of Treatment (active or sham tDCS), Pharyngeal Hemisphere (conditioned or unconditioned) with Laterality (Lateralised or Unlateralised) as a between-subjects factor. Similarly, for *Experiment 2* data we calculated repeated measures ANOVA on challenge swallows data with factors of Treatment and Laterality as a between-subjects factor.

3.9.3 Relative asymmetry between pharyngeal projections and lack of correlation with neurophysiological and behavioural outcome measures

The mean LI score was 0.22 ± 0.03 (range of 0.07 to 0.51) and a median of 0.2.

There was no significant correlation between LI scores and mean post tDCS percentage change in PMEPs (n=15) in the conditioned hemisphere after active (Pearson correlation -0.3, p=0.2) or sham tDCS (Pearson correlation -0.4, p=0.16). There were also no significant correlations with LI scores and unconditioned hemisphere PMEPs after active (Pearson correlation 0.3, p=0.9) or sham tDCS (Pearson correlation -0.3, p=0.4)

Similarly, there were no significant correlations between LI scores and mean post tDCS improvement in challenge swallows (Active tDCS Pearson correlation: 0.1, p=0.7, Sham tDCS Pearson Correlation: -0.4, p=0.1).

3.9.4 Comparison of outcome measures between laterality groups

Based on median LI score, we subdivided our subjects into those with LI>0.2 (lateralised n=7) and LI≤0.2 (n=8). Repeated measures ANOVA on mean post tDCS PMEP data with laterality as a between-subjects factor once again confirmed significant effects of Treatment ($F_{1, 13}$=6.8, p=0.02), but without significant effects of Pharyngeal Hemisphere ($F_{1, 13}$=0.99, p=0.33), or any Treatment x Laterality ($F_{1, 13}$=0.07, p=0.80), Hemisphere x Laterality ($F_{1, 13}$=0.004, p=0.95), or Treatment x Hemisphere x Laterality interactions (($F_{1, 13}$=0.38, p=0.55). On challenge swallows data, again we found a significant effect of Treatment ($F_{1, 13}$=6.5, p=0.02), but no significant Treatment x Laterality interaction ($F_{1, 13}$=0.64, p=0.44).

3.9.5 Comparing the effectiveness of 1-Hz rTMS pre-conditioning in disrupting swallowing neurophysiology and behaviour between laterality groups

Compared to unlateralised subjects, sham tDCS following 1-Hz to the strong pharyngeal projection produced greater neurophysiological deficit to the pre-conditioned hemisphere (Mann-Whitney U

test; K=516, median difference (PMEP % change from baseline)= -22.9, *U*=386, *p*=0.0009, Figure 3.7A) and greater disruption to challenged swallowing behaviour (Mann-Whitney-*U* test; K=766, median difference (% change challenge swallows from baseline)= +25, *U*=749.5, p=0.04 Supplementary Figure 3.7B) in lateralised subjects.

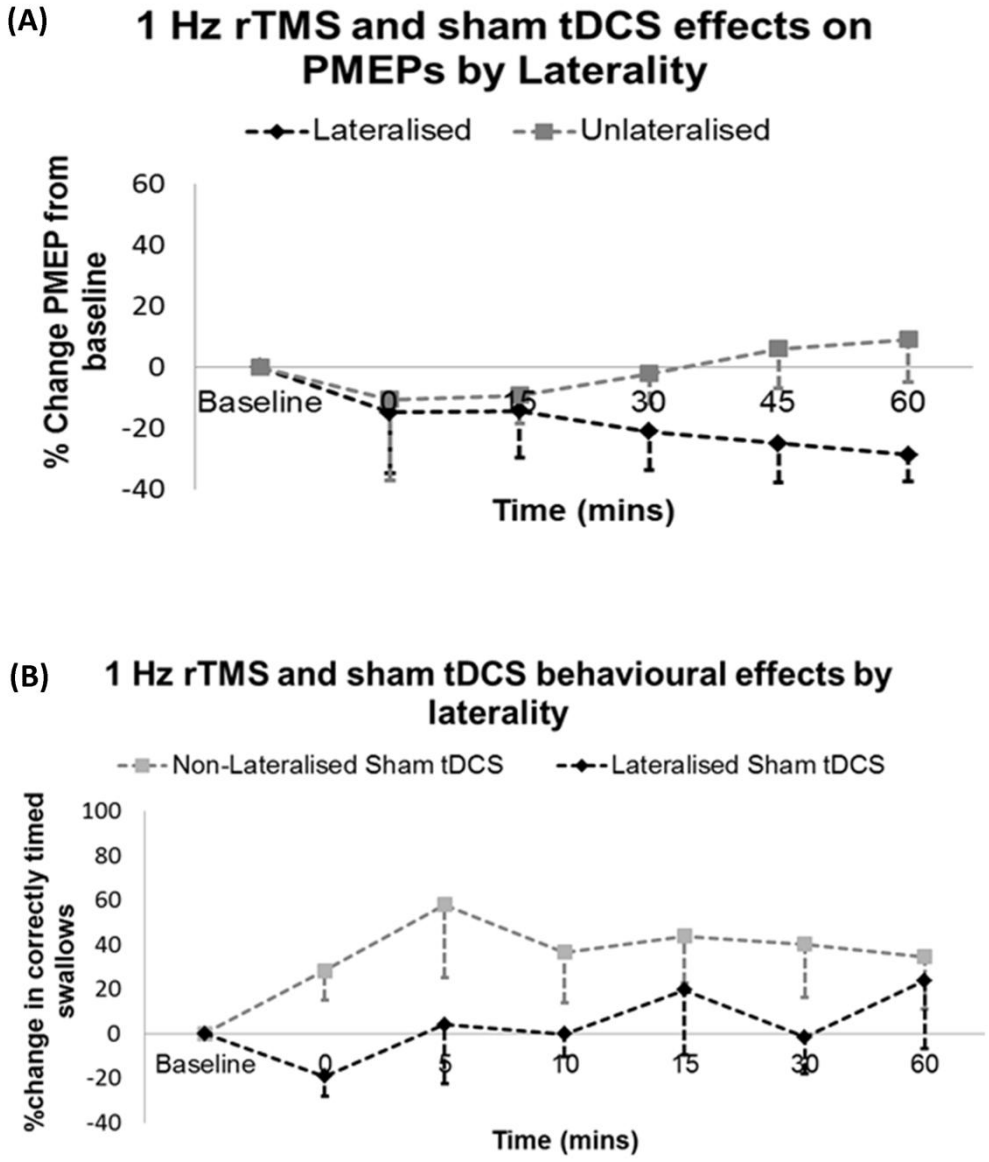


Figure 3.7: Group mean effects (±SEM) of 1Hz pre-conditioning following sham tDCS were stronger in subjects with a Laterality Index >0.2 on;

A) Conditioned hemisphere PMEPs (*p*<0.01)

B) Challenged swallow behaviour (*p*=0.04)

CHAPTER 4

PHARYNGEAL ELECTRICAL STIMULATION (PES) IN DYSPHAGIA POST-ACUTE STROKE: A DOUBLE-BLIND, RANDOMISED TRIAL

4.1 ABSTRACT

Background & Aims: Pharyngeal electrical stimulation (PES) promotes plasticity in the pharyngeal motor cortex with promising therapeutic effects in post-stroke dysphagia observed 2-weeks post intervention. Our aim was to provide further pragmatic evidence with 3-months follow-up.

Methods: Dysphagic patients recruited within 6-weeks of stroke, received active or sham PES in a double-blinded randomised design via an intraluminal pharyngeal catheter, left in situ for 10 minutes, once-daily for 3 days. Outcome measures included; feeding status using Dysphagia Severity Rating (DSR) scale (by independent, blinded speech therapists, ≤4=no/mild dysphagia, ≥4=moderate/severe dysphagia), times to hospital discharge and feeding tube removal. Data were analysed under an intention to treat principle using logistic regression. Additionally, a sub-group of patients were genotyped for Brain Derived Neurotrophic factor (BDNF) to assess if the presence of a common Val66Met polymorphism could predict non-response to PES.

Results: 36 patients (median 71years; 61% male) were recruited**.** At 2-weeks, 11/18 (61%) in the active group had DSR<4 (responders) compared with 9/18 (50%) in the sham group: OR (95% CI) = 2.5 (0.5 to 14.6). Patients in the active group also had shorter times to hospital discharge (39 vs. 52 days, HR (95% CI) of 1.2 (0.6, 2.6)) and removal of nasogastric feeding tubes (8 vs.14 days, HR (95% CI) of 2.0 (0.5, 7.9)). By 3-months, however, all but 3 patients in each group had responded: OR (95% CI) = 1.0 (0.13 to 7.02). Of 16/36 genotyped patients, the frequency of Val66Met BDNF polymorphisms was higher in those with DSR≥4 (non-responders) at 2-weeks (71%) compared to responders (44%). This distribution was maintained in the Active PES group, where the Val66Met frequency was 75% in non-responders but only 40% in responders.

Conclusions: The observed differences are consistent with the hypothesised effect of PES in accelerating swallowing recovery over the first 2-weeks following treatment, with some preliminary suggestions that BDNF polymorphisms may be predictive of response to PES. Lower than desired recruitment prevents definitive answers from this study but study design experience and outcome data reported here are essential to inform a definitive, multi-centre randomised trial.

4.2 INTRODUCTION

Oropharyngeal dysphagia is a major complication of stroke (detected in up to 78% of patients (93)) and is considered a major adverse prognostic factor with increased risks of pulmonary aspiration (93), malnutrition, increased lengths of hospital stay and death (218). Current rehabilitative strategies, including behavioural therapies have limited evidence base (219). However, with recent developments in our understanding of the role of neuroplasticity and cortical re-organisation in swallowing recovery (57, 61, 120), there is now increasing interest that neurostimulation techniques can drive this natural recovery process (157). Intraluminal pharyngeal electrical stimulation (PES) is one such neurostimulation technique which has shown promising effects over the last decade in a series of studies and has been shown to promote this type of plasticity in healthy subjects and achieving clinically meaningful improvements in dysphagic stroke patients (2-4, 6, 60). Importantly, PES mechanisms explored with Transcranial Magnetic Stimulation (TMS) and functional brain imaging have confirmed sustained increases in pharygeal motor cortex excitability and re-organisation of the pharyngeal motor cortex (increasing pharyngeal representation area) (2, 4-5), with suggestion that these responses may be predicted by Brain Derived Neurotrophic Factor (BDNF) genotype (117). Furthermore, the optimal stimulation parameters (5-Hz frequency, 10 minutes duration and 75% of maximum tolerated intensity) have been defined (4). In further translational work, these optimal parameters of PES have been shown to reverse the neurophysiological and behavioural effects of experimental brain lesions (1 Hz rTMS (69)) and a dose-response study confirmed the optimal regimen in dysphagic stroke patients; once daily stimulation for 3 days (3). Moreover, in randomised clinical studies PES has been shown to improve swallowing safety on videofluoroscopy (VFS) within an hour post-intervention in both acute (4) and chronic (6) dysphagic stroke. Finally, data from a phase II randomised controlled trial in acute stroke patients (n=28) demonstrated positive effects of 3 days of active PES, which at 2-weeks post intervention significantly improved feeding status and improved penetration-aspiration scores (PAs) on VFS (3). Active PES also reduced the length of

hospital stay in this stroke group (3) while in a randomised controlled trial of PES in neurogenic dysphagia secondary to multiple sclerosis, active stimulation produced longer-term (1 month) clinical improvements in swallowing with little change in the sham arm (220).

The aim of this phase IIc double-blinded randomised control trial was to provide further pragmatic evidence to support the hypothesis that PES can improve swallowing in post-stroke dysphagia and provide longer-term follow up (3 months post-interventions).

4.3 METHODS

4.3.1 Power calculation

Sample size calculation was based on demonstrating a difference in the intended primary outcome measure of PAs at VFS at 2 weeks. Using data from the preliminary randomised trial of PES (3), a mean improvement (±SD) of 1.8 (±1.77) in the number of aspirative swallows out of six (swallows that scored >3 on the PAs (190)) in the treated group, compared with a deterioration of 0.6 (1.56) swallows in the controls (3). Therefore a sample size of 50 per group would have provided 80% power at the 5% significance level to detect a difference between groups of 1 swallow, based on an estimated common SD of 1.75. Unfortunately, due to unforseen difficulties with obtaining research VFS at one of the major recruiting sites, prior to unblinding of the data, the dysphagia severity rating scale (DSR) at 2-weeks (3) was upgraded to be the primary outcome measure.

4.3.2 Screening and recruitment

Stroke patients in three participating Greater Manchester hospitals (Salford Royal Foundation Trust, University Hospital of South Manchester and Trafford District General Hospital) who fulfilled inclusion criteria were identified during screening and were approached on the stroke units. All recruited participants either had the capacity to consent and provided informed written consent or if they lacked capacity as a result of stroke, were recruited if a named ‘consultee’ after reading the information declared that in their opinion, the patient would not be thought to object to participation in the trial.

Inclusion criteria included all patients with dysphagia that presented for the first time following acute anterior cerebral circulation or brainstem stroke, within 6 weeks of onset. There was no age limit but recruited patients were medically stable at the time of entry.

Exclusion criteria included advanced dementia, other neurological conditions that may explain dysphagia, previous history of dysphagia, presence of cardiac pacemaker of implanted cardiac defibrillator, a diagnosis other than stroke is suspected (e.g. brain tumour), any severe concomitant chronic medical condition that compromises cardiac or respiratory status (severe emphysema or heart failure that may render the insertion of the throat unsafe), and significant structural abnormalities of the mouth or throat. Patients requiring oxygen treatment were also excluded at point of entry.

4.3.3 Randomisation

Following consent and baseline assessment, patients were randomised through a concealed programme provided by the trial statistician. Allocation was blocked in randomly permuted sizes and stratified by centre and feeding status (presence/absence of artificial feeding) to optimise balance.

4.3.4 Procedures

4.3.4.1 Bedside screening swallowing test: Independent to swallowing assessments conducted by members of the supervising clinical team, all recruited patients had a standardised bedside swallowing test using the validated Toronto Bedside Swallowing Screening Test (TOR-BSST)(221) conducted by a trained research practitioner at entry into the study. During this test the patient's tongue movement and voice quality during phonation was assessed, and the patient's ability to swallow up to ten teaspoons of water and an additional 50ml bolus, whilst the examiner observes changes in voice quality before and after each swallowing trial. Any abnormality observed in any of the items tested results in a failed TOR-BSST. Subjects failing TOR-BSST proceeded to baseline VFS (where available) and when this was not possible the subjects were directly randomised into

the trial. By contrast, patient's that successfully passed all items of TOR-BSST were classed as 'non-dysphagic' and were excluded from further participation in the trial.

4.3.4.2 Videofluoroscopy: Subjects fit enough for baseline VFS had a standardised videofluoroscopic assessment of their swallow supervised by the research team. The research VFS examination comprised 6 swallows of 5ml boluses of liquid barium (60% w/v, EZ-HD®, E-Z-EM Limited, UK) with lateral view images (Siemens Fluorospot® H SIRESKOP SX Unit, Germany), carried out at the radiology department of the recruiting site. Images were captured digitally on digital video disc to be reviewed off line frame by frame for evidence of ingress of material into the airway (190) independantly by two blinded speech and language therapists with special interest in dysphagia, who were blinded to study allocation.

Patients with normal swallowing at baseline VFS (six swallows each with PAs <3) were excluded from recieving their randomised interventions, whereas those with evidence of swallow safety compromise (PAs ≥3) on videofluoroscopy recieved intervention as per their randomisation (Active or Sham).

4.3.4.3 Dysphagia Severity Rating scale (DSR):

Based on detailed clinical bedside assessments performed by independant (blinded) trained speech and language therapists (SALTs) involved in the patients care, feeding recommendations were incorporated to calculate the patients dysphagia severity rating scale (3) at; baseline, 2 weeks and 3 months post-interventions. These scores reflect the feeding status acheived by the patient across three domains; fluids, diet and level of supervision required for feeding (Table 4.1).

Table 4.1 Dysphagia severity rating (DSR) Scale (3) - The DSR scale assigns a score to the feeding status achieved by the dysphagic patient depending on the categories of feeding stage for fluid and dietary consumption in addition to the level of dependency required for feeding. The score for each category from 0-4, and is added to give a composite score. These scores are calculated based on clinical recommendations from an independent speech and language therapist with special interest in neurogenic dysphagia.

Score	Fluids	Score	Diet	Score	Supervision
4	No oral fluids	4	No oral feeding	4	No oral feeding
3	Pudding consistency	3	Puree	3	Therapeutic feeding (SALT/trained staff)
2	Custard consistency	2	Soft, moist diet	2	Feeding by third party (untrained)
1	Syrup consistency	1	Selected textures	1	Eating with supervision
0	Normal fluids	0	Normal	0	Eating independently

4.3.4.4 Pharyngeal Electrical Stimulation (PES): PES was delivered at the patient's bedside. The patient was intubated with the intra-luminal pharyngeal stimulation catheter (Gaeltec, Dunvegan, Isle of Skye) housing bipolar electrodes connected to stimulator box (Model DS7; Digitimer, Welwyn-Garden City, Herts, UK) via a trigger generator (Neurolog System, Digitimer) and stimuli were delivered (0.2 ms pulses, max 280 V) at the previously defined optimal parameters (5Hz frequency and an intensity (current) 75% of the maximum tolerated) (4). The maximum tolerated intensity was determined from each patient's perception and pain thresholds, these values were calculated from an average of three consecutive measurements on each of the 3 days. Group 1 received three sessions of PES for 10 minutes on three consecutive days. Group 2 received sham stimulation (catheter in-situ with stimulator turned off) for the same period. Both groups continued to receive standard swallowing treatments as decided by the clinical speech and language therapist of the respective hospitals. Study interventions were delivered by a trained member of the research team independently of the clinical team.

4.3.4.5 BDNF genotyping: As an additional component of the study, patients were asked to provide a saliva sample (2-3ml in Oragene-250 self-contained DNA collection kits; DNA Genotek

Oragene Inc, Ontario, Canada) for BDNF genotyping to see if presence of the Met66 allele could be a predictor of non-response to PES interventions. Specific consent was obtained for this optional component of the study. Following collection, the saliva kits were hand-delivered to the Centre for Integrated Genomic and Molecular Research (CIGMR) at the University of Manchester for analysis and longer term storage in suitable freezers. The samples were processed to extract DNA, normalisation and polymerase chain reaction using standard operating procedures (1, 117). Single nucleotide polymorphisms (SNPs) of BDNF at codon 66 were genotyped using the Sequenom MassARRAYR system (Sequenom Inc., Hamburg, Germany). All the analysis was carried out at the CIGMR, University of Manchester, by independant researchers that were blinded to the treatment status of the patients.

4.4 DATA ANALYSES AND STATISTICAL METHODS

Primary analyses were performed in an intention to treat framework by independent medical statisticians at the University of Manchester using a standard statistical software package (R Core Team. R: A Language and Environment for Statistical Computing, Vienna, Austria). In the primary analyses data were therefore analysed according to whether patients were allocated to active or sham treatment, not according to whether they actually received either or none. If a patient withdrew from the study, we endeavoured to obtain their hospital records for the period during the study and use any appropriate and relevant outcome data.

4.4.1 Primary outcome: DSR at 2 weeks post-interventions

Separate analysis of previously published data of an independent patient group with similar characteristics to those in this study showed the distribution of scores on the DSR to be highly non-normal, bi-modally distributed at the extremes and with a median DSR 3.5 (3). Thus DSR scores at 2 weeks were dichotomised from a 13 point severity scale (0-12) into a binary outcome, one value indicating mild or no dysphagia (scores from 0 to 3) the other moderate to severe dysphagia (scores from 4 to 12). Thus the binary DSRS score at 2 weeks was analysed as the response in a generalised linear model with treatment allocation as the factor of interest and

including feeding method (natural or artificial), age (under 75 or 75 and over), and treatment centre as covariates, as these were the stratified randomisation factors. In this case, compared to the control group, an odds ratio less than 1 would indicate a worse outcome for the treatment group and an odds ratio greater than 1 would indicate a favourable outcome for the treatment group.

4.4.2 Secondary outcomes

DSR at 3 months was analysed similarly to DSRS scores at 2 weeks, by adjusting for feeding method, age, and treatment centre and where an odds ratio for active treatment significantly greater than 1 would indicate a significant positive treatment effect.

The time from randomisation until medically fit for hospital discharge (days): was analysed using a Cox proportional-hazards model including feeding method, age, and treatment centre as covariates. A hazard ratio significantly greater than 1 for the treatment factor would indicate shorter times to discharge and thus a positive treatment effect.

For those patients with either a nasogastric (NG) or a percutaneous endoscopic gastrostomy (PEG) feeding tube inserted, the time from randomisation until tube removal or the end of the study, whichever was earlier, was analysed using a Cox proportional-hazards model including treatment arm as a factor. Again, a hazard ratio significantly greater than 1 for time to tube removal for the treatment factor would indicate shorter times to tube removal and thus a positive outcome. The proportional hazards assumption of these survival models was assessed by visual inspection of plots of the scaled Schoenfeld residuals over time (222).

VFS measures were also analysed, in PAs (scoring of the degree of airway compromise on the PAs from 0 to 8, Table 4.2). At baseline, 2 week and 3 month assessments 6 swallows were evaluated using the Penetration-Aspiration Scale. The number of swallows PAs≥3 (aspirative swallows) were recorded. A significantly lower number of adverse scores in the active treatment arm compared to those of the control arm at 2-week and 3-month assessments would indicate a significant positive treatment effect. Counts were assessed by a two sample t-test assuming unequal variance in arms.

The number of adverse events: chest infections and death before study end were also evaluated and compared between groups.

In genotyped patients, the prevalence of Val66Met genotype was compared between responders (DSR<4) and non-responders (DSR≥4) by Chi-square using a standard statistical software package (SPSS 20.0, SPSS Inc, Chicago, Illinois, USA).

Table 4.2 Penetration Aspiration scale (190).

PA score	**Description**
1	Material does not enter the airway
2	Material enters the airway, remains above the vocal cords, and is ejected from the airway
3	Material enters the airway, remains above the vocal cords, and is not ejected from the airway
4	Material enters the airway, contacts the vocal cords, and is ejected from the airway
5	Material enters the airway, contacts the vocal cords, and is not ejected from the airway
6	Material enters the airway, passes below the vocal cords, and is ejected into the larynx or out of the airway
7	Material enters the airway, passes below the vocal cords, and is not ejected from the trachea despite effort
8	Material enters the airway, passes below the vocal cords, and no effort is made to eject the material

4.5 RESULTS

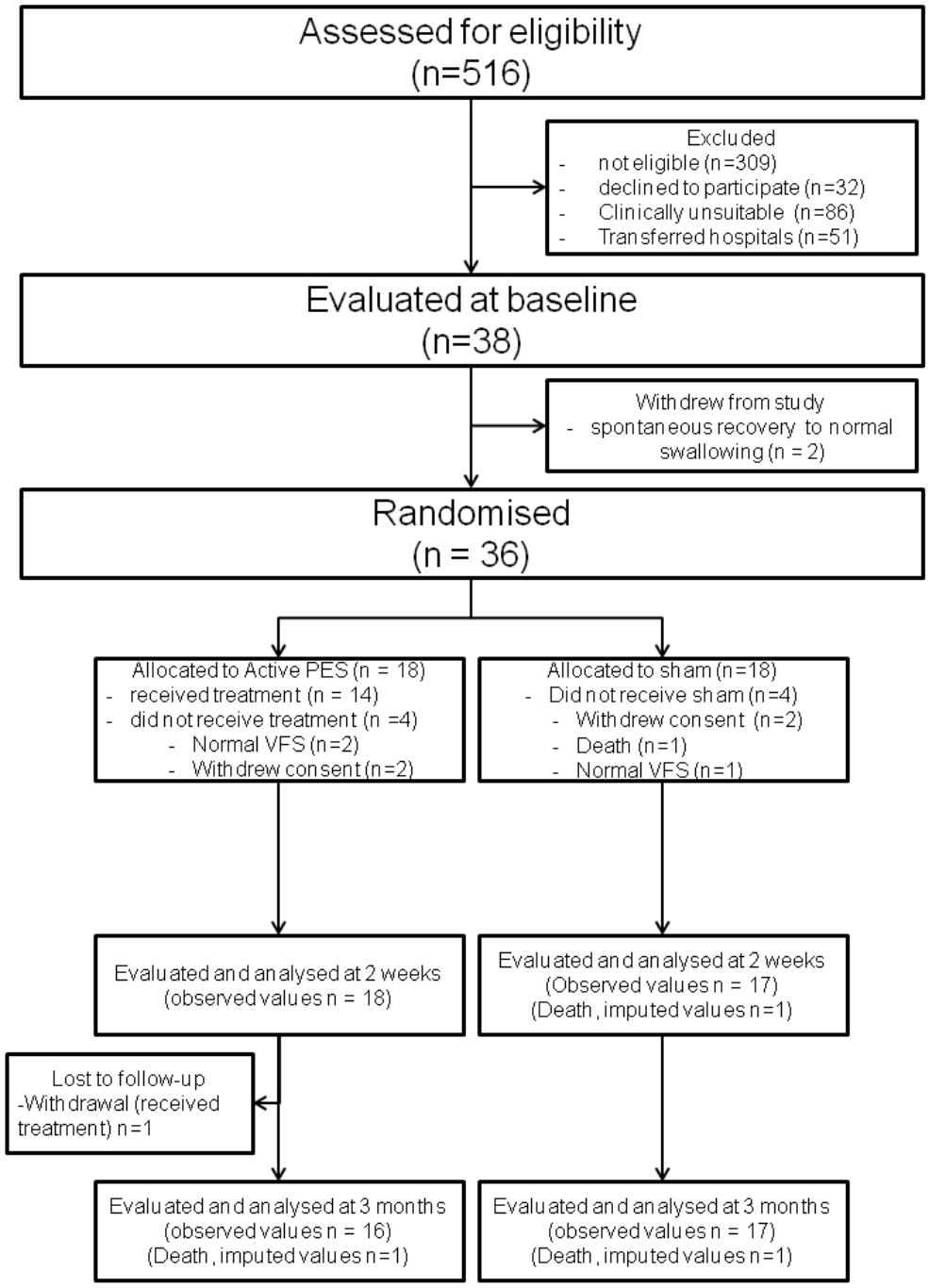


Figure 4.1 Data flowchart: numbers and reasons for dropout. PES: pharyngeal electrical stimulation, DSR: dysphagia rating scale, VFS: videofluoroscopy.

The study is summarised in Figure 4.1. Briefly, 36 patients were randomised; 18 to the treatment arm and 18 to the sham arm. One patient withdrew from the trial for reasons independent of their health and in whom further data could not be obtained. Two patients died and their missing outcomes were imputed as the worst possible values for all analyses. Patient and baseline characteristics are detailed and summarised in tables 4.3 and 4.4.

Table 4.3: Patient demographics at study entry

A) Group 1: randomised to active PES

Case	Age, Y	Sex	Oxford classification	Stroke type	NIHSS	Barthel	MRS	DSR	Baseline VFS/FEES (Worst PAs)	Received intervention	BDNF genotype
1	51	M	Left PICH	Bleed	9	5	4	4	VFS (5)	Y	Val/Val
2	85	M	Right PACS	Infarct	7	27	4	7	VFS (8)	Y	NA
3	83	F	Right TACS	Infarct	4	36	3	10	None	Y	Val/Val
4	69	M	Right TACS	Infarct	21	15	4	3	None	Y	NA
5	88	F	Right TACS	Infarct	5	23	4	4	VFS (5)	N	NA
6	52	F	Right TACS	Infarct	19	23	5	12	VFS (8)	Y	Val/Met
7	71	M	Bilateral PACS	Infarct	17	20	4	12	VFS (8)	Y	NA
8	79	F	Right PICH	Bleed	15	30	4	10	VFS (1)	N	NA
9	74	F	Right TACS	Infarct	5	56	4	5	VFS (5)	Y	NA
10	89	F	Right PACS	Infarct	10	2	4	10	VFS (5)	Y	Val/Met
11	66	F	Right TACS	Infarct	22	26	5	12	None	Y	Val/Val
12	56	F	Left TACS	Infarct	19	18	5	4	VFS (5)	N	NA
13	76	M	Bilateral PICH	Bleed	10	51	4	6	VFS (5)	N	NA
14	43	M	Right TACS	Infarct	6	70	2	12	FEES (8)	Y	Val/Met
15	70	M	Right TACS	Infarct	5	0	3	5	None	Y	Val/Met
16	79	F	Left TACS	Infarct	19	0	4	12	FEES (8)	Y	NA
17	57	M	Left TACS	Infarct	13	20	4	8	None	Y	Val/Val
18	47	M	Left POCS	Infarct	1	99	1	12	None	Y	Val/Met

B) Group 2: randomised to sham PES

Case	Age, y	Sex	Oxford Classification	Stroke type	NIHSS	Barthel	MRS	DSR	Baseline VFS/FEES (Worst PAs)	Received intervention	BDNF (rs6265) Genotype
19	74	F	Right PACS	Infarct	10	2	2	9	FEES (5)	Y	Val/Met
20	58	M	Left TACS	Infarct	12	45	4	7	None	N	NA
21	77	M	Right PACS	Infarct	8	34	4	4	VFS (5)	Y	Met/Met
22	58	M	Left PACS	Infarct	16	17	3	4	VFS (8)	Y	Val/Met
23	65	M	POCS (Pontine)	Infarct	11	25	4	3	None	Y	NA
24	85	F	Right TACS	Infarct	10	40	3	11	VFS (6)	Y	NA
25	61	M	Right TACS	Infarct	8	80	1	6	VFS (7)	Y	Val/Val
26	62	M	Left TACS	Infarct	17	19	4	9	VFS (6)	Y	NA
27	60	M	Left POCS	Infarct	6	100	2	12	VFS (3)	N	NA
28	85	M	Right TACS	Infarct	22	0	4	12	VFS (8)	Y	NA
29	62	M	Right TACS	Infarct	15	20	-	12	None	N	NA
30	73	M	Left TACS	Infarct	9	51	3	5	VFS (6)	Y	Val/Val
31	54	M	Right TACS	Infarct	20	4	4	8	None	Y	Val/Val
32	86	F	Right TACS	Infarct	22	0	4	4	VFS (3)	N	NA
33	74	M	Right TACS	Infarct	7	55	3	3	FEES (3)	Y	NA
34	89	F	PICH (Brainstem)	Bleed	16	1	4	12	FEES (8)	Y	NA
35	68	M	Right LACS	Infarct	13	5	4	12	FEES (8)	Y	NA
36	78	F	Left TACS	Infarct	26	5	5	8	VFS (4)	Y	Val/Met

Abbreviations: BDNF; Brain Derived Neurotrophic Factor, DSR; dysphagia severity rating scale, FEES; fiberoptic endoscopic evaluation of swallowing, LACS; Lacunar stroke, MRS; modified Rankin scale, NG; nasogastric, PACS; partial anterior circulation stroke, PAs; penetration-aspiration scores, PICH; primary intracereberal haemorrhagic stroke, POCS; posterior circulation stroke, TACS; total anterior circulation stroke.

Table 4.4 Summary of patient characteristics by arm and overall. All values are medians (interquartile range) except for those relating to proportions.

	Sham	Active	Overall
Sex, m/f (%)	13/5 (72/28)	9/9 (50/50)	22/14 (61/39)
Baseline age, years	71 (61, 78)	71 (56, 79)	71 (60, 79)
Tube type, NG/PEG/none	9/5/4	11/5/2	20/10/6
Centre, Salford/Trafford/ Wythenshawe	12/5/1	12/5/1	24/10/2
Time post stroke, days	11 (7, 17)	16 (9, 23)	13 (7, 19)
Baseline National Institutes of Health stroke scale	12.5 (9.2, 16.8)	10.0 (5.2, 18.5)	11.5 (7.7, 17.5)
Baseline DSR	8.0 (4.2, 11.8)	9.0 (5.0, 12.0)	8.0 (4.7, 12.0)
Baseline Barthel score	19.5 (3.5, 46.5)	23 (12.5, 39.75)	21.5 (5, 43.75)
Baseline Modified Rankin Scale	4 (3, 4)	4 (3.75, 4)	4 (3, 4)

Stimulation Intensity

PES interventions were delivered at mean intensities (mA); day 1: 20.4 ± 1.8, day 2: 17.9 ± 1.5 and day 3: 16.3 ± 1.3.

4.5.1 Primary outcome

Thirty-six patients were included in analysis for the primary outcome at 2 weeks; 18 in the active treatment arm and 18 in the control arm (one patient had died and their DSRS binary severity was imputed as moderate/severe). At the 2 week follow-up, 11 patients (61%) had no/mild dysphagia in the active treatment group and 9 patients (50%) had no/mild dysphagia in the sham group. Only one patient in the sham group had a DSRS worse at 2 week follow-up than at baseline assessment (from a score of 9 to 10) and no patients in the active treatment group had worse DSRS than baseline at 2 week follow-up. The treatment effect, of the relative presence of no/mild

dysphagia (by DSRS) at 2 weeks, was estimated by an odds ratio (95% C.I.) of 2.52 (0.52, 14.56), not significant by the likelihood ratio test (P=0.26). Figure 4.2 illustrates the observed DSRS scores in both arms over the study duration.

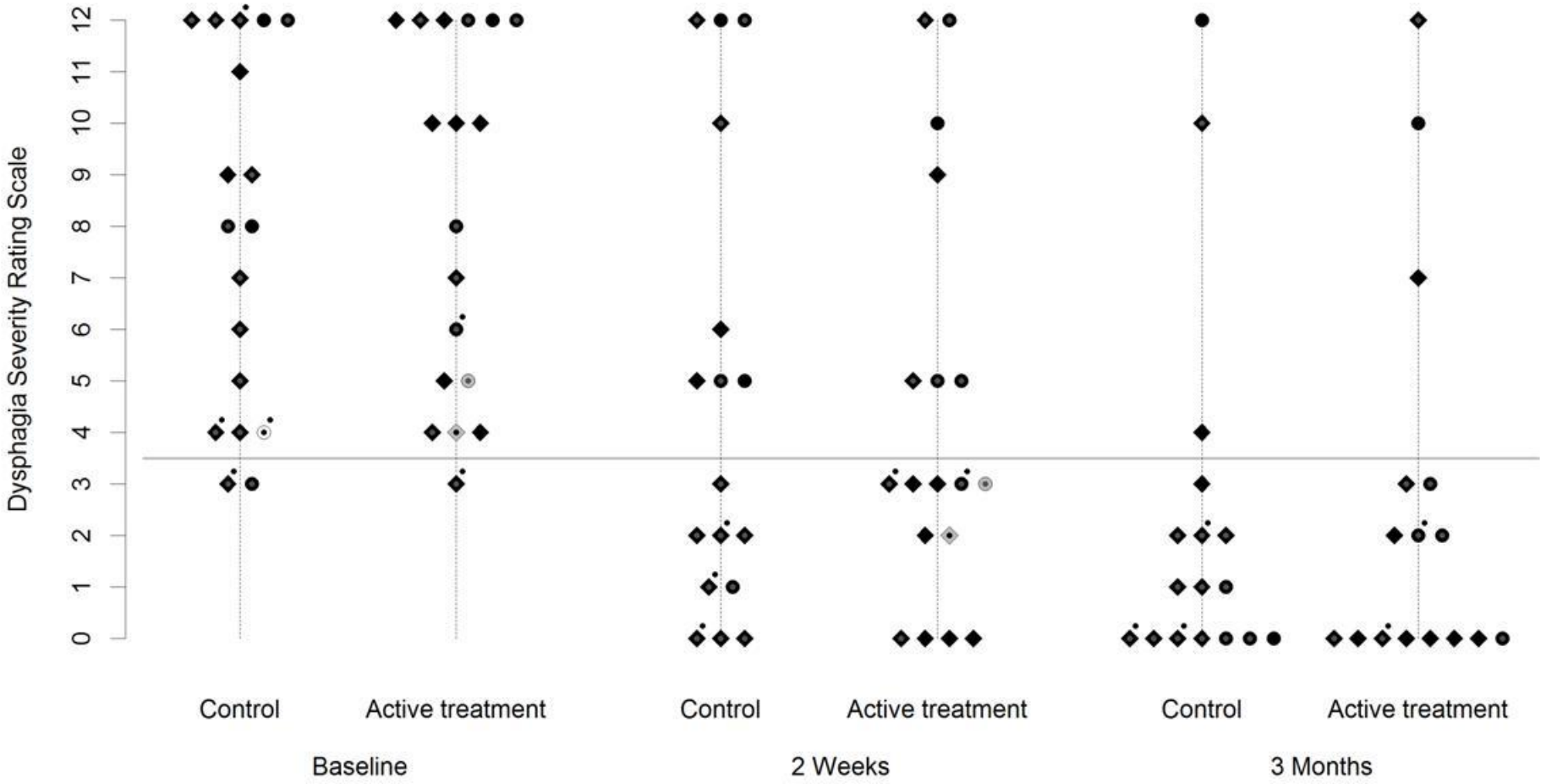


Figure 4.2 Observed DSRS measurements in each trial arm and at each time point. White and grey-filled points indicate patients withdrawing from the trial before the 2 week and 3 month evaluations respectively. Diamonds indicate patients treated at the primary centre (Salford) and circles those treated outside (Trafford/Wythenshawe). Grey outlines indicate males, black outlines indicate females. Dots over points indicate patients feeding naturally, all others were feeding by either NG or PEG feeding tubes.

Per-protocol analysis (excluding withdrawn subjects that did not receive PES interventions)

Of the patients in the study, 8 did not receive treatment (either sham or active treatment), 4 in the control group and 4 in the active treatment group (Table 4.3 and Figure 4.1). In a per-protocol analysis only the data of the other 28 patients were included in the assessment of the primary outcome – in a logistic regression model as described. In this analysis with baseline DSR as a covariate, the treatment effect, of the relative presence of no/mild dysphagia (by DSR) at 2 weeks, was estimated by an odds ratio (95% C.I.) of 4.90 (0.56, 89.09), not significant by the likelihood ratio test (P=0.16). The summary of patient baseline and demographics for these 28 patients are described in Table 4.5 and the mean change in dysphagia severity over time is shown in Figure 3.

Table 4.6 Numbers, baseline and demographic characteristics of only patients receiving intervention by arm and overall (per-protocol). All values are medians (interquartile range) except for those relating to proportions.

	Sham	Active
Sex, m/f (%)	10/4 (71/29)	8/6 (57/43)
Age, years	74 (63, 78)	70 (53, 78)
Tube type, NG/PEG/none	7/5/2	8/5/1
Centre, Salford/Trafford/Wythenshawe	9/4/1	9/4/1
Time post stroke, days	10 (6, 17)	12 (7, 21)
Baseline National Institutes of Health stroke scale	12.0 (9.2, 16.8)	9.5 (5.2, 18.5)
Baseline DSR	8.0 (4.3, 10.5)	10.0 (5.5, 12.0)
Baseline Barthel score	18 (3.5, 42.8)	21.5 (4.3, 41)
Baseline Modified Rankin Scale	4 (3, 4)	4 (3, 4)

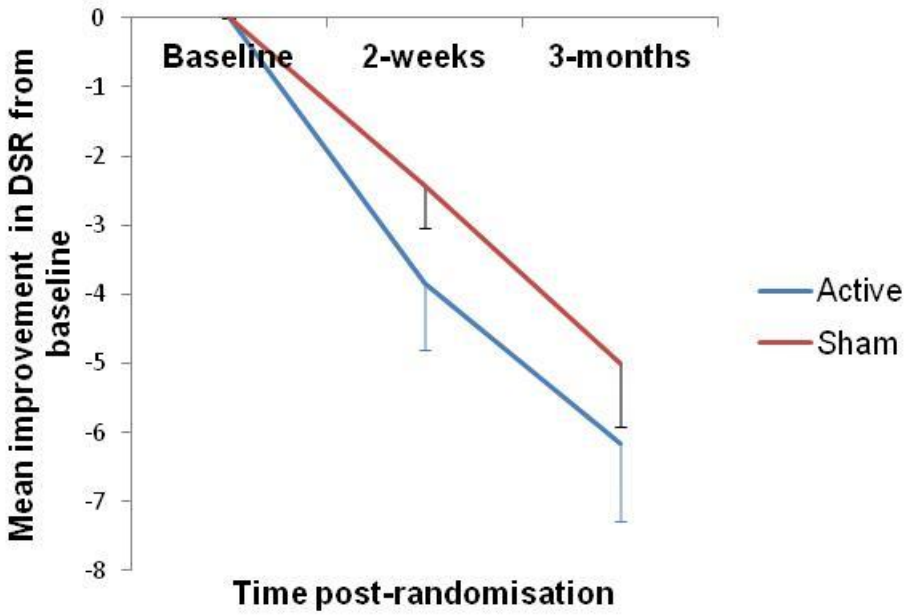


Figure 4.3 Group mean change (error bars showing standard error of the mean (SEM) in Dysphagia severity (DSR) from the date of randomisation in subjects that received interventions (n=28). The grand mean presented here includes 1 patient that died by 3-months in the active group that had the worst possible imputed DSR score.

4.5.2 Secondary outcomes

There were 35 patients included in analysis for secondary outcomes at 3 months; 18 in the control arm and 17 in the active treatment arm (one patient in each group had died and their DSRS binary severity was imputed as moderate/severe), with one patient in the active arm having been lost to follow-up. No patients in either arm had an observed DSRS score which increased between 2 week and 3 month follow-up assessments. At the 3 month follow-up, 14 patients (78%) had no/mild dysphagia in the control group and 13 patients (76%) had no/mild dysphagia in the active treatment group. The treatment effect of the relative presence of mild to no dysphagia (by DSRS) at 3 months was estimated by an odds ratio (95% C.I.) of 0.97 (0.13, 7.02), not significant by the likelihood ratio test (P=0.97).

The time from randomisation until hospital discharge again trended towards shorter time in the active compared to sham group (Table 4.6). A Cox proportional hazards analysis of time from the date of randomisation until hospital discharge estimated a hazard ratio (95% CI) of 1.19 (0.55, 2.57). No statistically significant difference between arms was observed by the stratified log-rank test (P=0.62). Figure 4.3 shows the observed survival probabilities (probability of not being discharged) for patients in each study arm.

A total of 21 patients had a feeding tube inserted at the time of randomisation, 11 NG and 10 PEG tubes. At study completion, all 11 NG tubes had been removed whereas only 2 PEG tubes (20%) had been removed. There was a trend towards earlier removal of NG tubes after active treatment compared to sham (Table 4.6 and Figure 4.4) with a Cox proportional hazards analysis of time for removal of patients NG feeding tube (if inserted) from the date of randomisation estimated a hazard ratio (95% CI) of 2.01 (0.51, 7.93) However, this was not statistically significant by the stratified log-rank test (P=0.33).

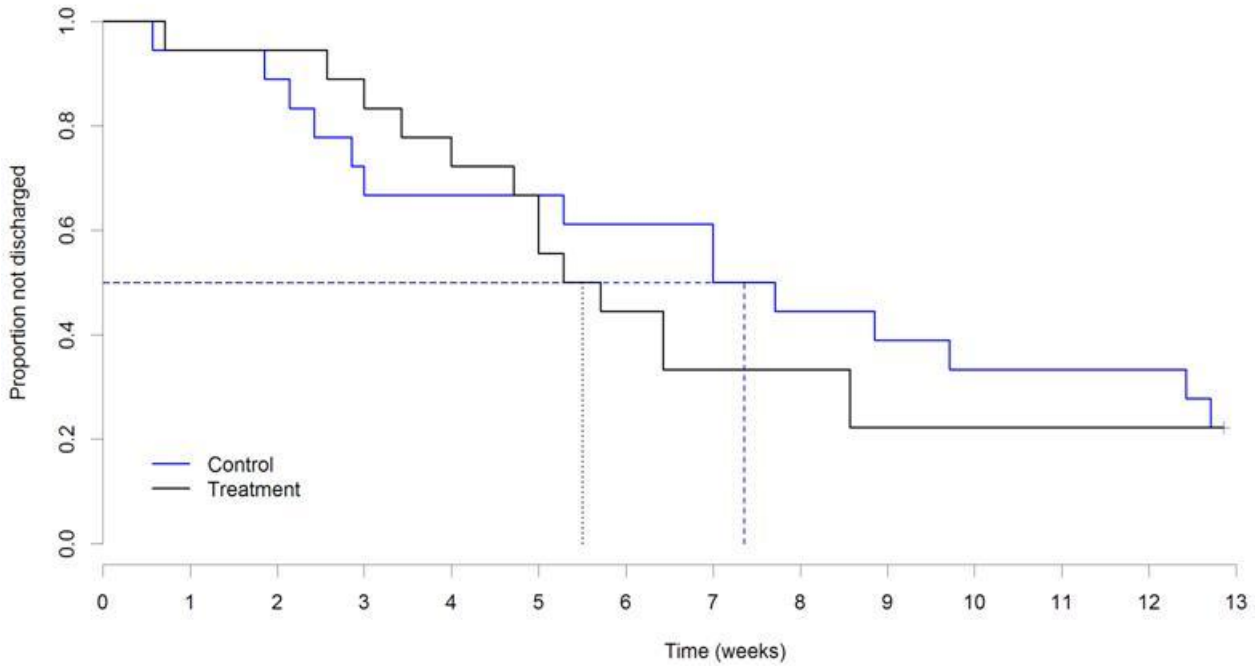


Figure 4.4 Kaplan-Meier estimate of survival curves for time discharge from hospital: proportion over time since randomisation of a patient in either the control or active treatment arms. Median times to discharge for each group are indicated by the dashed lines (see Table 4.6).

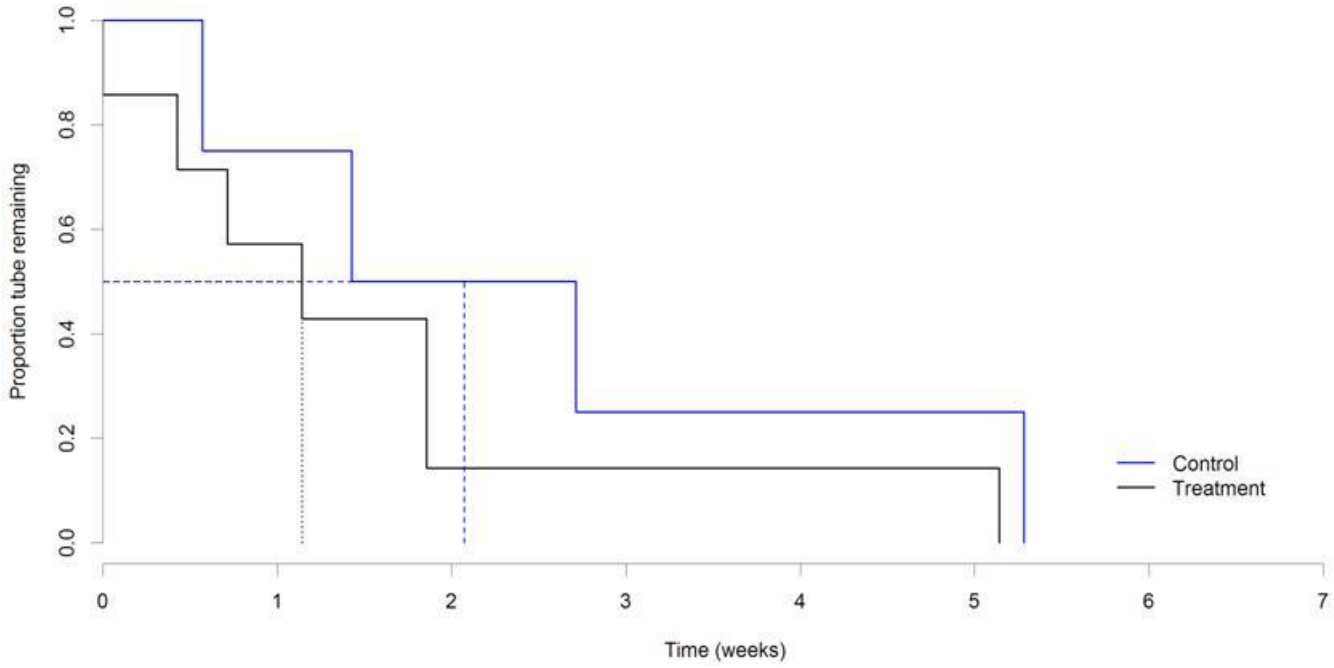


Figure 4.5 Kaplan-Meier estimate of survival curves for time to feeding tube removal: proportion over time since randomisation of a nasogastric feeding tube remaining in patients in either the control or active treatment arms. Median times to discharge for each group are indicated by the dashed lines (Table 4.6).

Table 4.7 Number and median survival times of hospital discharges, and of feeding tube removal from randomisation until 90 days post-randomisation.

Time to discharge	Sham	Active	Overall
Patients discharged by 90 days	14 (78%)	14 (78%)	28 (78%)
Median time to discharge (days)	52	39	45
Hazard ratio for treatment (95% CI)		1.19 (0.55, 2.57)	
Time to tube removal (NG only)			
Patients with tube removed by 90 days	4 (100%)	7 (100%)	11 (100%)
Median time to tube removal (days)	14	8	10
Hazard ratio for treatment (95% CI)		2.01 (0.51, 7.93)	
Time to tube removal (PEG only)			
Patients with tube removed by 90 days	0 (0%)	1 (25%)	1 (20%)

The majority of patients (26/36 (72%), Table 4.3) had instrumental swallowing evaluation (VFS or fibreoptic endoscopic evaluation of swallowing (FEES)) to confirm oropharyngeal dysphagia (i.e. PAs ≥3) prior to intervention.. VFS data that was available showed that 8 (61%), 7 (54%) and 2 (15%) patients in the sham arm had valid PAs at baseline, 2 weeks and 3 months respectively. Corresponding figures for the active treatment arm were 9 (69%), 7 (54%) and 7 (54%). Overall, At 2-weeks there was a mean reduction in the number of aspirative swallows (PAs≥3) on VFS, estimated treatment effect (95% C.I.) at 2 weeks: -0.95 (-3.3, 1.4), not statistically significant (P=0.4), Figure 4.6. However at 3-months, the difference in the number of aspirative swallows between the two groups was minimal, estimated treatment effect (95% C.I.): 0.14 (-3.2, 3.5), P=0.9.

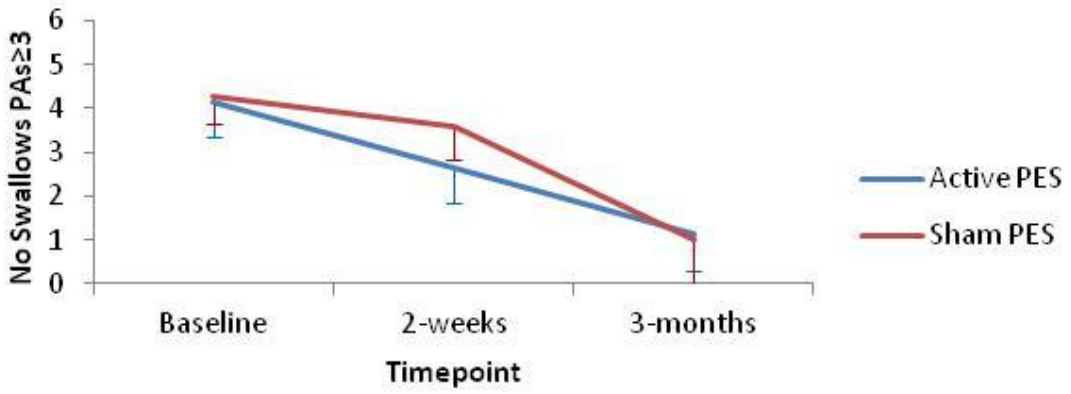


Figure 4.6 The effect of PES on airway aspiration at videofluoroscopy. At 2-weeks, Active PES reduced the group mean (±SEM) number of aspirative swallows (PA≥3) out of six trials but this was not statistically significant.

One patient suffered a single chest infection during the study follow-up period, in the active treatment group approximately 2-months after randomisation. The patient responded to a five-day course of antibiotics.

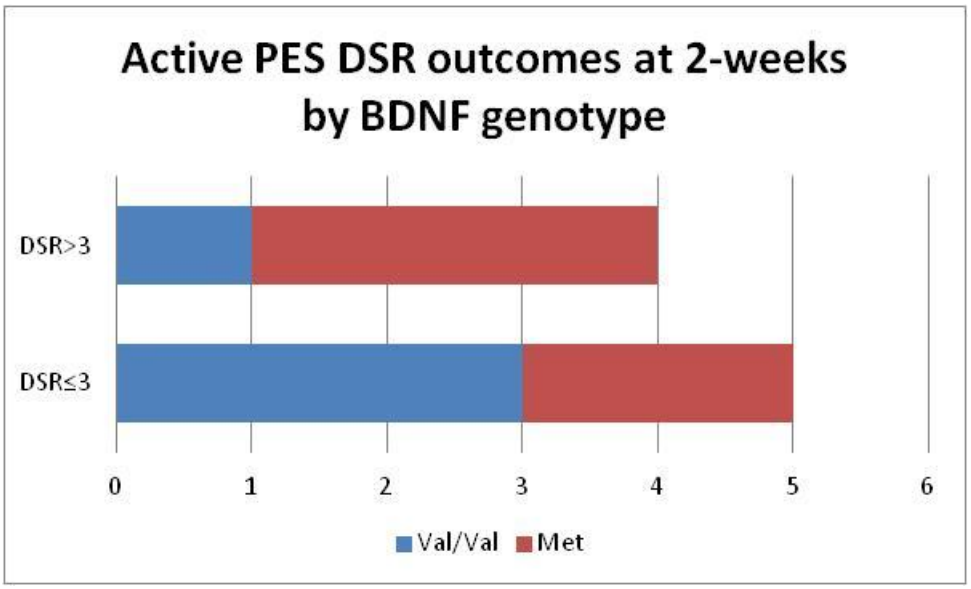
One patient from each arm died within the duration of the study, the patient in the sham group (Case 32, Table 4.3) was withdrawn from the study before application of sham PES and died 2 weeks post randomisation (from aspiration pneumonia). The patient in the active treatment group (Case 15, Table 4.3) had self-discharged from hospital against medical advice (42 days after randomisation) and died 11 days later from an unknown cause. These patients had baseline DSR scores of 4 and 5 respectively.

4.5.3 BDNF genotypes

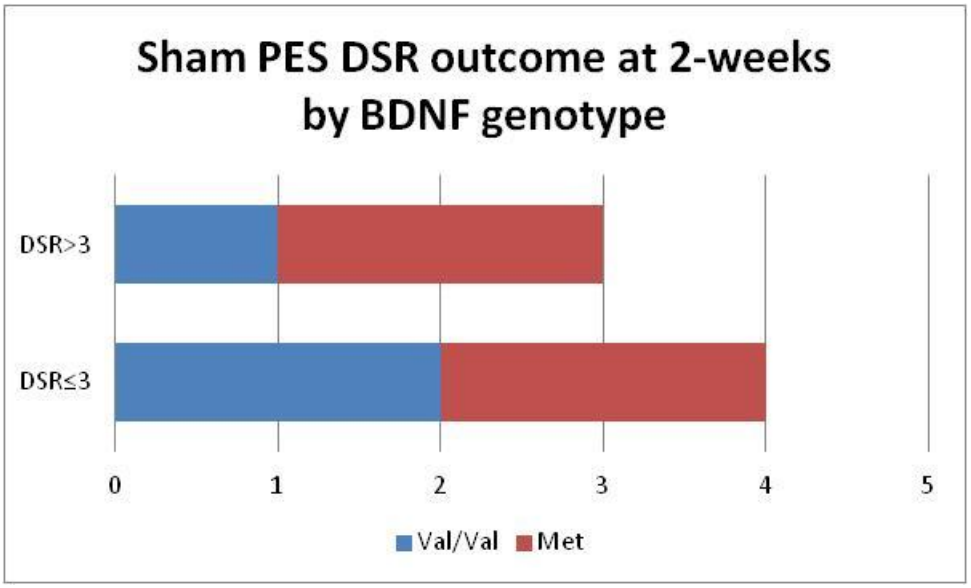
Twenty patients provided saliva samples for genotyping. Unfortunately, only 16/20 samples were of sufficient quality to be processed. Nine of the 16 processed samples were from the active group, with the remaining 7/16 coming from the sham group. The overall incidence of BDNF Met66 allele was 9/16 (56%). The baseline demographics for the 16 genotyped subjects are detailed in Table 4.3.

Overall (N=16/36), 7/18 in the active arm and 9/18 in the sham arm had DSR>3 at 2-weeks post-randomisation (i.e. were non-responders, Figure 4.2). When considering the 16 non-responders only at 2-weeks, 7/16 were genotyped with an incidence of Met66 BDNF allele of 71% vs. 44% Met66 BDNF allele in responders (Chi-square: 1.2, *p*=0.28). By contrast, when sub-divided into interventional groups, responders to active PES had an incidence of the Val66Val genotype of 60% compared to an incidence of Val/Val of only 25% in non-responders to active PES (Chi-square: 1.1, *p*=0.29). Interestingly by 3-months, all but 1 genotyped non-responder at 2-weeks had recovered (DSR<3) and another had died, both patients received active PES and were Met66 allele carriers. The BDNF genotype frequencies are illustrated in Figure 4.7, correlated with dysphagia (DSR) outcomes following both Active and Sham PES.

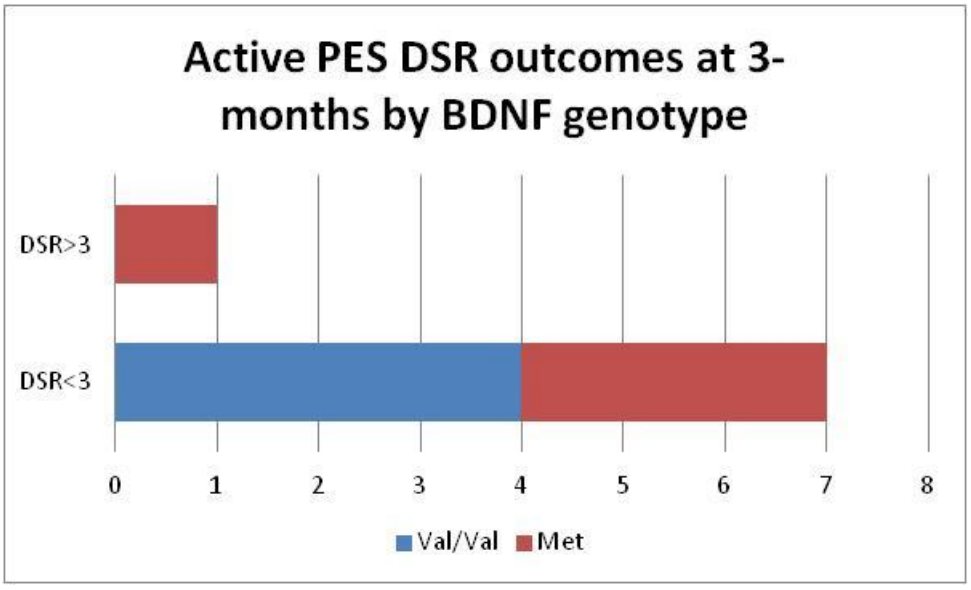
A)



B)


Sham PES DSR outcome at 2-weeks by BDNF genotype
DSR>3
DSR≤3
0
1
2
3
4
5
Val/Val
Met

C)


Active PES DSR outcomes at 3-months by BDNF genotype
DSR>3
DSR<3
0
1
2
3
4
5
6
7
8
Val/Val
Met

D)

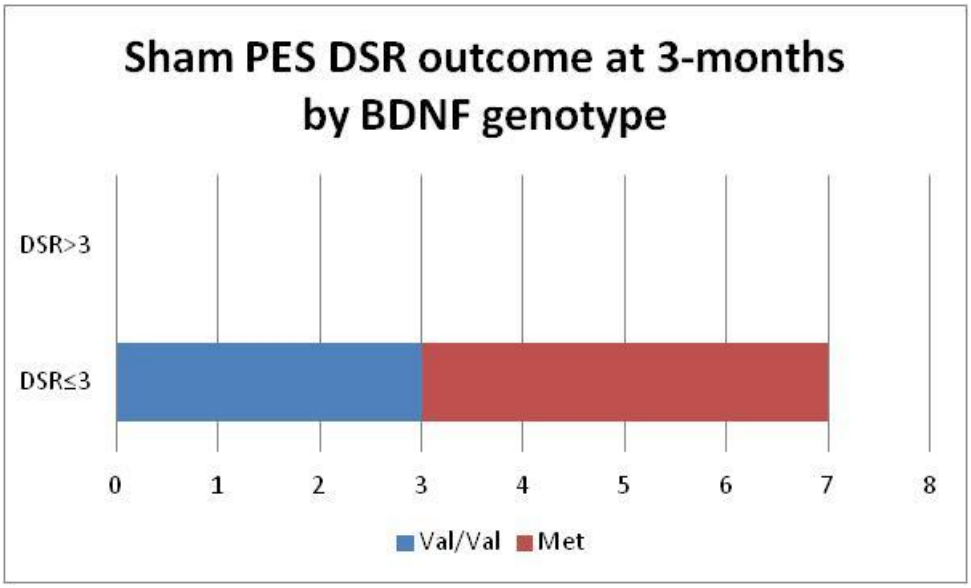


Figure 4.7 The frequency of BDNF genotypes by DSR outcomes at 2-weeks after; A) active PES and B) sham PES and at 3-months after; C) active and D) sham PES. The overall incidence of Met66 BDNF allele was higher in non-responders (DSR>3) at 2-weeks (N=5/7), particularly in non-responders to active PES (3/4, 75%). By 3-months, all genotyped patients except one non-responder to Active PES and another who died (both with Met66 genotype) had DSR<3.

4.6 DISCUSSION

From this study, given the smaller than planned recruitment target, one cannot conclude with certainty whether PES is having an effect on dysphagia recovery in stroke patients. Despite this, there are several indications of a favourable outcome to treatment, as indicated by the consistency of the direction of the estimated effects relating to dysphagia severity at 2-weeks, the time to feeding tube removal, the time to discharge (odds ratio and hazard ratios are all >1) and from the limited VFS data available which also trended towards improvement in swallowing safety after active PES. Additionally, PES was well tolerated, with no adverse effects related to the therapy. These findings taken together would be consistent with my hypothesis and

adds to the evidence-base including the previously demonstrated effects of PES in expediting the recovery of post-stroke dysphagia (3, 6).

Importantly, despite a similar sample size, we were unable to replicate the significant effects of PES on DSR at 2-weeks and time to hospital discharge as seen in the first randomised control trial (3). There are some relevant differences in the baseline characteristics in the two studies which may help account for some of the contrasting observed outcomes. In the present study, the data presented in Table 4.4 suggest a higher stroke and dysphagia severity as evidenced by higher median NIHSS and DSR scores (both parameters approximately 2 points higher than in the study by Jayasekeran et al., at baseline in both interventional groups) (3). This could produce differences in the rehabilitation needs and resultant length of stay (which would be influenced by factors independent of swallowing status). The differences in baseline stroke severity also partially account for the incompleteness of VFS data from the centres where this was available, with many subjects being physically unable to sit in a chair and unable to follow commands for VFS examination due to either impaired cognitive function and/or disabilities secondary to stroke severity. Secondly, in the present study there was more improvement in the sham group (presumably related to natural swallowing recovery) during the first 2-weeks as one would expect, albeit at a slower mean rate than that seen following active PES (Figure 4.3). This slow but expected natural recovery was not so evident following sham PES in the study by Jayasekeran et al., where there was an observed deterioration in VFS PAs and only minimal improvement in DSR at 2-weeks (3). The present cohort of patients may represent a more realistic population than the former study, bearing in mind that the lower baseline DSR (5.6 in sham group (3)) may have offered these subjects less room for improvement over time compared to subjects in the present study.

At the end of 3-months follow-up in the present study, only 17% of subjects had moderate/severe dysphagia, with no difference in the prevalence between the two interventional groups. This suggests the main effects of PES may be in early expedition of the natural swallowing recovery process, rather than influencing the long-term swallowing outcome. Nonetheless, expedition of swallowing recovery seemed to be in line with other clinically relevant outcomes, given that our data also showed a trend towards earlier nasogastric tube removal and a suggestion to shorter lengths of inpatient stay (a median difference of 13 days between groups). Dysphagia is known to impact on lengths of hospital stay (218, 223-224) and considerably increase medical expenses in stroke patients (225-226), therefore our data suggest that PES treatment may also have important socio-economic benefits. In addition to the trend towards earlier removal of nasogastric feeding tubes, the only PEG removal during the study was also after active PES. Indeed data from the hyper-acute setting would support the notion that early interventions in acute stroke whereby the need for feeding tubes could be reversed may potentially have prognostic significance by reducing the risk of serious complications including respiratory infections (223, 227). In terms of serious complications in the present study, there was only a single respiratory infection and one death in each interventional group; such end points usually require many hundreds if not thousands of patients to demonstrate an effect so it is not surprising that PES interventions could not be shown influence these particular secondary outcome measures.

One individual patient of interest in the trial that warrants further discussion is subject 7 (Table 4.3), a patient that had a bi-hemispheric infarction. This patient was the only subject in the trial that did not have any clinical improvement in terms of dysphagia

severity from baseline to 3-months following active PES (Figure 4.2, DSR 12/12 throughout the study). There is indeed recent evidence from the largest retrospective study of patient outcomes to date, that bi-hemispheric infarction is an independent prognostic factor for persistent dysphagia and PEG feeding at the time of hospital discharge (123). Given that natural swallowing recovery after cortical stroke appears to relate to compensatory neuroplastic changes in the contralateral (intact) hemisphere (57, 61, 120) and that the effects of PES therapy have been shown to drive this process (2-4), one might argue that such patients (without an intact hemisphere) may have limited scope for rehabilitation by this mechanism and may not be good candidates for these types of interventions.

As described above, positive effects of PES interventions appear to be predominantly within the first fortnight after intervention. By the 2-week stage it is therefore interesting to note that 7/18 (39%) of subjects could be classed as non-responders to active PES based on DSR. Similar to our findings, Jayaskeran et al. described a distinct group of non-responders to this form of neurostimulation (based on both healthy control and patient data) and the authors speculated whether other factors including genetics may dictate brain plasticity to PES (3). In the cortical plasticity neurostimulation literature there is now evidence that genetic variance is indeed an important factor in the neuroplastic response to neurostimulation interventions (228) and in particular presence of a single nucleotide polymorphism (substitution of Methionine for Valine) at codon 66 in the BDNF gene appears to influence responses which may help to explain inter-individual differences in brain plasticity (114-116, 229-232). We attempted to genotype a small number of these patients for presence of the BDNF Val66Met polymorphism. Whilst the numbers are small, it is striking that there is a higher incidence of the BDNF Met66 allele in our

dysphagic patients compared to expected incidence of 33% reported in healthy subjects (233). Our observations here are clearly preliminary but appear to suggest that Val66Met polymorphism may have negative influences on plasticity and swallowing recovery during the first two weeks and may be consistent with the hypothesis that BDNF Val66Met may be a predictor of poorer response to PES interventions. These findings are consistent with the findings of Jayasekeran et al., where Val66Met healthy subjects had significantly less PES induced plasticity compared to Val66Val subjects. These findings whilst of interest, would require replication in a larger dysphagic stroke population and in larger clinical trials of PES and other brain stimulation techniques such as rTMS, which based on neurophysiological studies we hypothesise may have more favourable outcomes in patients with the Met66 BDNF genotype (117).

In terms of limitations, this study was powered to detect a clinically important difference in a VFS related outcome. However, due to reasons unrelated to patient outcomes, VFS measurement was not possible in all patients. As such, given that the study was powered using a VFS outcome the statistical power to detect a clinically important treatment effect on the DSR was going to be difficult. The protocol specified 50 patients per arm; in the final study we have only 18 patients per arm. Thus the trial recruitment has reached only 36% of its targeted number of patients. It is possible that the current outcome measure may require even more patients; our estimate is that it is closer to 100 patients per arm rather than 50 patients. This also diminishes the ability of randomisation to achieve balance on important prognostic factors. There are several reasons why use of the DSR as an outcome measure may be less sensitive than VFS. Firstly, DSR scores are largely based on observations from bedside clinical assessments of swallowing by trained

SALTs rather than being based on instrumental examinations (VFS/FEES). It is recognised that bedside testing would not detect silent aspiration and there is evidence that without direct visualisation of the swallowing mechanism, subtle abnormalities can be missed at bedside examination (234). Secondly, calculated DSR ratings were based on the most up-to-date SALTs assessments as part of routine clinical care at the specified time-point (i.e. the patients' recommended safe oral intake at 2-weeks and 3-months). It could be argued that using assessments from impartial SALTs, blinded to the intervention as part of routine clinical care as opposed to a member of the research team would be a strength, providing a more 'real-life' (pragmatic) situation, given that detectable clinical differences here would be based on the way patients are routinely assessed at the bedside. However, at all three trial sites, it was unfortunately noted that SALTs did not review patients every day and it was not uncommon that their assessments would only be carried out as infrequently as several times per week. Therefore it is probable that some changes in DSR scores may have been missed here due to a SALT assessments being several days old at the time of DSR calculation. Another limitation of the primary analysis data is that many patients did not receive their first intervention on the day of randomisation itself as there was occasionally several days delay until baseline VFS could be performed in the radiology departments. However, under the intention to treat analysis, DSR values used are strictly 2-weeks post randomisation (as per protocol) rather than 2-weeks post intervention. Once again this may miss some effects on DSR at 2-weeks post treatment as the 2-weeks post randomisation date may be slightly premature compared to when the treatment was actually delivered.

In summary, the data presented here give further indications of a possible favourable outcome after active PES compared to sham intervention at 2 weeks which by 3

months is neutral. However, due to the listed limitations above, including lower than desired recruitment and unexpected change in primary outcome measure, we acknowledge that this is not a definitive study. The important lessons learned have helped inform an independent definitive, multi-centre international trial of this intervention which is now ongoing (235).

CHAPTER 5

DISCUSSION

5.1 SUMMARY OF CHAPTERS

Chapter 1 introduced the rationale for a proposed five-step translational model in developing neurostimulation techniques for post-stroke dysphagia (prior to randomised clinical trials). The chapter discusses recent advances in understanding the neural control of swallowing, techniques used to study swallowing neurophysiology and swallowing function in health and disease before discussing key developments in our understanding of swallowing recovery after hemispheric stroke. The latter part of the chapter discussed the potential of neurostimulation techniques to facilitate this natural recovery process in post-stroke dysphagia before summarising the neurophysiological and behavioural data available for each stimulation technique and highlighting which translational steps have been followed for each modality, identifying gaps in the literature.

Chapter 2 involved a series of studies where I investigated the effects of non-invasive brain stimulation targeted to the pharyngeal representation of the human cerebellum, this approach has never previously been attempted in the swallowing literature. In a series of studies in healthy subjects (steps 1 and 2 of my proposed translational model), I confirmed lasting increases in pharyngeal motor cortical excitability could be achieved via cerebellar stimulation and determined the optimal frequency and durational parameters of rTMS (10-Hz, 250-pulses). In a pilot proof of concept application in a dysphagic stroke patient I was then able to confirm that increases in pharyngeal motor excitability could be induced with this intervention with some behavioural effects on swallowing in a patient with cerebellar stroke.

In Chapter 3, I performed a 'step 3' study (Figure 1.1) using the 'virtual-lesion' model in healthy subjects. Optimal parameters of a different neurostimulation modality (anodal tDCS, 1.5mA for 10 minutes) were targeted to the pharyngeal motor cortex

contralateral to the 'virtual-lesion'. The rationale for this approach was based on evidence from previous similarly designed studies (8, 12) and the post-stroke swallowing recovery literature (57, 61, 120). Differences between the unilaterally innervated hand system and bilaterally represented swallowing system are discussed in this chapter and the implications for cortical stimulation in terms of selecting excitatory vs. inhibitory interventions and targeting lesioned vs. unlesioned hemisphere. Whilst the earlier tDCS study in an undisrupted system showed that anodal tDCS could only increase cortical excitability ipsilaterally(9), our data in a 'virtual-lesion' model of inhibitory pre-conditioning confirmed bilateral enhancement in cortical excitability compared to sham tDCS, changes that are likely due to homeostatic plastic changes which may also occur in stroke patients. In addition to favourable neurophysiological changes, active tDCS also improved the accuracy of the swallowing reaction time task whilst influencing the speed of normal and fast swallowing reaction time tasks.

In Chapter 4, at the other end of the spectrum in terms of translational development, I performed a phase IIc randomised controlled trial of PES. PES is a technique which has followed the translational model steps 1-5 and the next challenge was a larger randomised control study with longer-term follow-up. This study indicated that the favourable effects of PES likely in expediting swallowing recovery occur within the first two weeks following intervention with these effects compared to sham neutralising by 3 months. Unfortunately due to unforeseen circumstances the outcome measures of this trial were changed to an outcome measure for which the trial was not powered for prior to data analysis. Despite the study being underpowered for final primary outcome measure there are trends towards a favourable outcome in terms of improvement in dysphagia severity, length of hospital

stay, time to nasogastric tube removal and number of aspirative swallows on videofluoroscopy at 2-weeks. Lessons learned from this study will be important in informing future trials of this and indeed other neurostimulation techniques for post-stroke dysphagia.

In this thesis I have developed three different neurostimulation techniques in studies covering the entire spectrum of the translational model. Given that each chapter has its own discussion, the aim of chapter 5 is to bring together the results from each section, providing an overview of the work in this thesis. The discussion includes suggestions for future research in developing of these neurostimulation techniques further and how they may be utilised clinically in post-stroke dysphagia.

5.2 OVERVIEW OF DISCUSSION POINTS IN THESIS

5.2.1 Novel findings

Early translational phase work (steps 1-2)

Previous work from our group confirmed PMEPs can be evoked by single-pulse TMS over the cerebellum and that cerebellar-cortical TMS pulses in rapid succession can facilitate the pharyngeal motor cortex(53). I have now demonstrated for the first time that conditioning the cerebellum with excitatory non-invasive brain stimulation can produce sustained bilateral increases in pharyngeal motor excitability. As with cortical and peripheral stimulation, the optimal effects were found to be dependent on the frequency and duration (10-Hz, 250-pulses) of stimulation. This series of studies is also the first in the swallowing literature to adopt frameless stereotaxy to co-localise the TMS motor 'hot-spots' with the subjects anatomical MRI brain scan. Experience from this study shows that 'hot-spots' could be targeted to within 2mm of

the same spot from previous sessions. The cerebellum could be consistently targeted using this approach which obviated any difficulties which may arise in cranial anatomy when targeting a structure such as the cerebellum.

'Virtual-lesion' study (step 3)

Anodal tDCS is one of the most attractive neurostimulation modalities due to its relatively low cost, portability, tolerability and an excellent sham intervention. Several parameters were compared by Jefferson et al. and both 1mA for 20 minutes or 1.5mA for 15 minutes were found to be effective (9). However the therapeutic potential of tDCS has been clouded by uncertainty due to clinical studies using parameters without confirmed corticobulbar effects and using of the hand restorative model (i.e. targeting the lesioned) instead of the unlesioned hemisphere (18, 20). Using the 'virtual-lesion' model, targeting the unlesioned hemisphere with anodal tDCS, I have confirmed that evidence based parameters of tDCS can reverse the neurophysiological effects of 1-Hz rTMS bilaterally and improve swallowing behaviour. This work supports that this intervention has therapeutic potential and confirms progression of tDCS using these parameters to the patient stages of the translational model (steps 4 and 5) would be appropriate.

Testing cortical and behavioural effects in a dysphagic stroke patient (stage 4)

In a proof-of-concept application, administered on separate sessions a week apart, I applied real and sham cerebellar rTMS at optimal parameters to the unlesioned cerebellum in a patient with posterior circulation stroke involving the right cerebellar hemisphere. Interestingly this intervention produced an increase in pharyngeal motor cortical excitability bilaterally immediately post intervention. Whilst the cortical excitatory effects had worn off by 30-minutes, this intervention appeared to improve

swallowing behaviour at VFS by similar magnitude (~15% reduction in PAs) to central and peripheral neurostimulation interventions in the same setting (6).

Randomised clinical trial in post-stroke dysphagia (advanced translational phase study)

Despite methodological limitations of this study, there were indications of a favourable treatment effect of PES in improving feeding status at 2-weeks, earlier discharge and feeding tube removal and trend to improvement in the number of aspirative swallows on the limited VFS data that was available. New observations in this study include that by 3-months the recovery end-point was almost identical in both groups, suggesting the beneficial effects of PES are in expediting swallowing recovery in the first fortnight after stimulation. Following on from earlier healthy subject work which suggested the role of a common single nucleotide polymorphism in the BDNF gene have altered stimulus dependent plasticity in the pharyngeal motor cortex (117), I genotyped a subset of patients for this polymorphism. Interestingly, the incidence of the val66met polymorphism in the BDNF gene was much higher in our dysphagic patients (56%) than reported in the general population (~33% (233)). Statistical interpretation of genetic data was limited by small sample size, however it was noticeable that 75% of 'non-responders' to PES in the active group had the val66met genotype, a finding which may be relevant future neurostimulation studies.

5.2.2 General discussion

My thesis, whilst covering the entire spectrum of development, has studied three different neurostimulation approaches taking each forward by careful small steps. During my time of study, neurostimulation has become increasingly recognised internationally in the field of neurogenic oropharyngeal dysphagia as a promising

therapeutic avenue based on strong scientific evidence-base. This has been reflected by numerous sessions where it has been topical at European and North American international meetings of dysphagia societies (Dysphagia Research Society and European Society for Swallowing Disorders) and neuromotility sections of the Gastroenterology associations (United European Gastroenterology and American Gastroenterology Association) over the past year. The increased interest has also been reflected in the increasing number of publications in recent years (Table 1.3), particularly using cortical stimulation techniques (rTMS and tDCS) from interested research groups in the North America, Korean Republic, Japan, France, Germany and Egypt. Given the multidisciplinary nature of post-stroke dysphagia management, it has attracted welcome interest from researchers from diverse clinical backgrounds including those from neurology, gastroenterology, speech and language therapy, stroke and rehabilitation medicine. Some of these researchers have successfully applied these cortical stimulation techniques outside of the pharyngeal motor cortex in stroke studies, targeting the lesioned hemisphere with excitatory stimulation (236-237) and used these experiences as rationale to target the same approach in post-stroke dysphagia with similar parameters. These studies (17-18) and others which have used the limb rehabilitation model (15-16, 20) have disregarded the strong evidence-base for targeting the unlesioned hemisphere in the special circumstance of the bilaterally innervated pharyngeal motor system based on swallowing recovery data (57, 61, 63, 120), experience from lesioned vs. unlesioned hemisphere 'virtual-lesion' studies (12) and post-neurostimulation brain imaging and TMS cortical excitability effects correlated with swallowing behavioural improvements at VFS in patients (4, 6). Future cortical stimulation studies in post-stroke dysphagia therefore need to uniformly target the hemisphere based on the pathophysiology of

the system being studied. I have presented further evidence in this thesis of the importance of specificity of parameters for producing increased cortical excitability in the pharyngeal motor system. Lessons need to be learnt from the cortical stimulation experience in dysphagia studies to date, which have not only been heterogeneous in terms of the hemisphere targeted but parameters selected for rTMS and tDCS have varied widely in intensity, duration and the number of doses making data incomparable and confusing. Given the significant co-morbidity of stroke patients with dysphagia, it is important that stimulation parameters are optimised in healthy subjects, with confirmed cortical effects of the parameters selected. Following the proposed translational model in this thesis will ensure that only evidence-based parameters progress to clinical trials.

There are still some unanswered questions in the utility of neurostimulation interventions in post-stroke dysphagia which we will only be able to answer sensibly when we have comparable data from larger clinical studies using the correct parameters, at the same stimulation site, using an evidence-based dosage. These questions include timing of intervention (i.e. acute vs. chronic phase of rehabilitation), which modality works best and for which patient (peripheral, cortical, cerebellar or combined peripheral and brain stimulation (PAS)). Whilst most of the interventional patient data we have to date has focussed on acute stroke, there is evidence that neurophysiological and behavioural effects of interventions can be reproduced in the chronic phase (6, 12), but longer-term effects and dosing data are lacking in this population. From experience acute stroke patients are more accessible than chronic patients for such studies and whilst both groups may benefit from such interventions, completing the translational model in the acute population may help focus definitive studies in the chronic patients at a later stage.

In terms of assessing which of the neurostimulation approaches discussed in this thesis have the best potential for adoption as a therapy, one must consider what factors would make a desirable clinical tool. In addition to clinical efficacy, important factors in determining the ideal neurostimulation technique would be cost-effectiveness, deliverability at the bedside, portability, safety profile and the technique requiring minimal specialist skills to administer. Patient choice may be another important factor which could be considered. Pharyngeal stimulation involves intubation with a fine pharyngeal catheter, which is very well tolerated by the majority of patients, although patients unable to tolerate nasogastric feeding may prefer cortical stimulation over PES if given the choice, as this group may consider this intervention less invasive. Currently there is no qualitative data of patient views on the different modalities and this type of study may be helpful in the further development of these techniques but patient choice may be another reason to have different modalities in the neurostimulation armoury. As previously eluded to, the preliminary suggestions from genotypic data in chapter 4 hint that presence of BDNF val66met polymorphism may be a predictor of non-response to PES and this hypothesis would be consistent with healthy volunteer TMS cortical excitability data findings (117). If this is proven in a larger population, then the healthy volunteer data would suggest that these patients may benefit from cortical rather than peripheral stimulation (117), making a further case for having both approaches and this will be the subject of future research.

Whilst the healthy subject data for all modalities show similar neurophysiological efficacy, PES and tDCS would appear to fulfil more of the criteria of the 'ideal' intervention (listed above), rather than techniques involving rTMS. For example rTMS requires bulky, less portable and more expensive equipment, with a reported

risk of seizures (albeit exceptionally rare with parameters used) and requirement of a skilled experienced operator to ensure proper delivery and coil placement, making it more difficult to use outside of the research setting. Both PES and tDCS have the potential to be delivered at the bedside potentially by nurses or other health professionals after minimal training. Combining the two modalities, pairing cortical or cerebellar anodal tDCS with peripheral pharyngeal electrical stimuli would be an interesting approach which has not been studied before. In theory the tDCS/PES combination should have additive benefits to either modality used alone in terms of neuroplastic effects, although this would require a full work-up through the translational model.

Finally, an important practical consideration that has recently been raised includes the practicalities of delivering these interventions to patients if they are to be adopted in clinical practice and how strategic guidelines and frameworks may be required to plan for the anticipated revolution in treatment (157). Such considerations include the impact on the roles of multidisciplinary clinicians looking after patients with post-stroke dysphagia. Training programmes for professionals such as SALTs and other interested health professionals incorporating TMS/tDCS/PES techniques and how neurostimulation could be included in undergraduate or post graduate training with consideration of the roles of the physicians and SALTs in prescribing such interventions for patients (157). Interestingly at the 2013 conference of the European Society for Swallowing disorders (ESSD) held in Malmo Sweden, there was a dedicated training workshop arranged for this purpose where subscribing delegates received training in tDCS, PES and NMES.

5.3 DIRECTIONS FOR FUTURE RESEARCH

This thesis has studied three different neurostimulation approaches (cerebellar, cortical and peripheral stimulation) at different translational stages in their development for post-stroke dysphagia. Therefore here I will discuss how each of these techniques may be developed further with future research.

5.3.1 Neuronavigated TMS

Neuronavigated TMS is expensive as it requires all subjects to have had an anatomical MRI brain scan. In chapter 2, I demonstrated that this technique could be used to target specific brain regions repeatedly and were able to optimise hot-spots based on MEP amplitudes and anatomical position which appeared to add validity to our data particularly confirming cerebellar coil positioning. A further study comparing PMEPs responses from navigated and un-navigated cortical 'hot-spots' and responses to cortical neurostimulation interventions at navigated vs. un-navigated 'hot-spots' would be important before recommending this technique for all TMS swallowing studies in the future.

5.3.2 Cerebellar stimulation

Whilst I have confirmed the optimal frequency and duration of cerebellar rTMS, I have not compared it to the effects of a higher intensity regime (120% of thenar rMT) as we based our parameters on the findings from cortical stimulation studies where 90% rMT intervention (8) was optimal for high-frequency stimulation. It is possible that cerebellar rTMS may respond better to higher intensity stimulation than cortical stimulation and this could be tested in a future study.

The next logical phase of studies using cerebellar rTMS would be an application of it after a 'virtual-lesion' using the same methodology as chapter 3. Given that this

intervention excited the pharyngeal cortex bilaterally, it appears to have therapeutic potential for cortical stroke as well as posterior circulation stroke and therefore the proposed study would be beneficial before progressing to further patient work.

Given the translational issues raised about the potential to develop cerebellar rTMS as a clinical therapy, one might argue that given that targeting the cerebellum has been confirmed to be a viable target for exciting pharyngeal cortex, developing cerebellar tDCS becomes a more attractive proposition, rather than progressing with further cerebellar rTMS studies. A study in healthy subjects could address this by examining the effects of anodal cerebellar tDCS comparing cortical parameters (1.5mA for 10minutes) with higher intensities at the cerebellum and sham to evaluate whether the cortical effects seen with cerebellar rTMS could be reproduced with this technique.

Other important mechanistic work would involve investigating the neuroplastic mechanisms of cerebellar rTMS applied to pharyngeal regions using functional brain imaging techniques.

5.3.3 Cortical stimulation

As discussed earlier cortical tDCS is a very promising approach for which I have confirmed therapeutic potential of in a 'virtual-lesion' model using optimised parameters. Previous work using rTMS (238) and PAS (14) have demonstrated additional effects on cortico-pharyngeal excitability of a 'booster' second dose of intervention 90 minutes after the initial dose, however the effects of a second dose of tDCS have not yet been studied and could be the subject of a future study. The next phase of studies in developing this technique would be a dose-response study in post dysphagic stroke patients. I have written a protocol and obtained a favourable ethical opinion for this series of studies for which funding has now been secured and

is due to commence shortly. This study will use anodal tDCS 1.5mA for 10 minutes to the unlesioned hemisphere in acute dysphagic stroke patients and patients will be randomised to five days therapy of either once daily or twice daily active tDCS or once daily sham tDCS. In a similar study, the Kumar et al. group intend to use untested parameters (2mA for 20 minutes) again to the unlesioned hemisphere, using similar outcome measures which will at least allow direct comparison between these two studies(153). A cortical excitability TMS study evaluating their parameters (2mA for 20 minutes) in healthy subjects would be useful in interpreting and comparing data from these two studies.

Cortical rTMS was not utilised in this thesis apart from applying the 'virtual-lesion' in chapter 3. Future studies needed to develop this technique would require a dose-response study in patients, similar to the tDCS studies outlined above with its optimal parameters (5-Hz, 250-pulses, contralesionally at 90% thenar rMT).

Finally recent uncontrolled case-series have provided anecdotal evidence for bilateral cortical stimulation in post-stroke dysphagia (135, 143). This approach requires validation in a comparison in healthy subjects between optimal parameters of 5-Hz unilaterally, bilaterally and sham stimulation randomised to separate days in a TMS cortical excitability study before progressing further.

5.3.4 Pharyngeal (peripheral) electrical stimulation

Unfortunately my study in Chapter 4 could not provide definitive conclusions about this treatment due to the limitations explained. However, based on the evidence for this technique, which has completed all the translational steps, a customised medical device (Phagenyx®, Phagenesis, Manchester, UK), has been developed to deliver PES with a CE-marked license which is now available commercially. An independent, multi-centre randomised controlled trial across Europe in patients

(n=140) using this device including VFS and the other end-points in Chapter 4, has now completed recruitment(235). The data from this potentially definitive trial is eagerly anticipated.

5.4 CONCLUSIONS

In this thesis I have discussed and provided further evidence for neurostimulation approaches including peripheral, cortical and cerebellar stimulation and how they may have the potential to revolutionise the treatment of post-stroke dysphagia by driving the natural swallowing recovery process. Based on evidence from development of PES over the past 20 years and the examples of less successful techniques, a translational pathway has been described. Adoption of these interventions will require a sound methodological, mechanistic approach and a considerable amount of patience before progressing into patient studies. Large clinical trials using evidence-based parameters, which are difficult to conduct and require considerable resources, are required before these devices can be adopted in routine clinical practice.

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